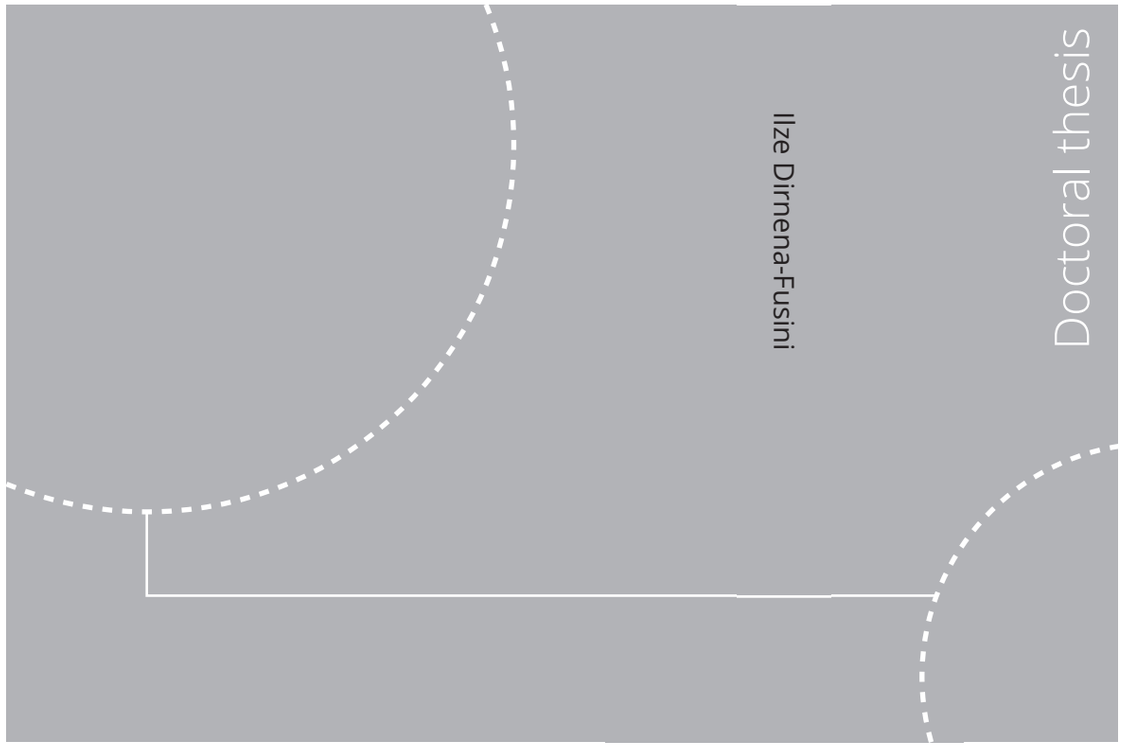
ISBN 978-82-326-6322-4 (printed ver.)
ISBN 978-82-326-5734-6 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (electronic ver.)



Doctoral theses at NTNU, 2021:334

Ilze Dirnena-Fusini

Intraperitoneal insulin administration - superior choice for an artificial pancreas?

NTNU
Norwegian University of
Science and Technology

Doctoral theses at NTNU, 2021:334

NTNU
Norwegian University of
Science and Technology
Thesis for the degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

NTNU
Norwegian University of
Science and Technology

Ilze Dirnena-Fusini

Intraperitoneal insulin administration - superior choice for an artificial pancreas?

Thesis for the degree of Philosophiae Doctor

Trondheim, October, 2021

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of Philosophiae Doctor

Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

ISBN 978-82-326-6322-4 (printed ver.)
ISBN 978-82-326-5734-6 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (electronic ver.)

Doctoral theses at NTNU, 2021:334

Printed by Skipnes Kommunikasjon AS

Intraperitoneal insulin administrasjon – det optimale valget for en kunstig bukspyttkjertel?

Sammendrag

Hovedfokuset for denne avhandlingen er å utforske det intraperitoneale rom som sted for administrering av insulin. Vi antar at intraperitoneal administrering av insulin er mer fysiologisk korrekt ettersom denne administrasjonsmetoden etterligner endogen insulinsekresjon og teoretisk sett skal oppnå overlegen glykemisk kontroll sammenlignet med subkutant tilført insulin. Et ytterligere fokus i avhandlingen er å undersøke intraperitoneal administrering av glukagon. Glukagon gis i tilfeller med alvorlig hypoglykemi og blir undersøkt for bruk i en bihormonell kunstig bukspyttkjertel. Vi antar at intraperitoneal administrasjon av glukagon, som ved insulin, etterligner endogen sekresjon bedre enn når hormonet gis subkutant.

Som en del av avhandlingen utførte vi en metaanalyse av data fra tilgjengelig litteratur hvor vi utforsket en rekke fysiologiske effekter og sammenlignet kontinuerlig intraperitoneal insulininfusjon med kontinuerlig subkutan insulininfusjon hos pasienter med diabetes mellitus type 1 (**Paper I**). I den andre artikkelen undersøkte vi farmakokinetikken og farmakodynamikken til forskjellige insulinboluser gitt intraperitonealt og sammenlignet med boluser gitt subkutant i anesteserte griser (**Paper II**). I den tredje artikkelen undersøkte vi farmakodynamikken til glukagon etter intraperitoneale og subkutane injeksjoner hos rotter (**Paper III**).

Avhandlingen omhandler de to viktigste bukspyttkjertelhormonene som påvirker glukosehomeostasen, insulin og glukagon. Derfor, i kapittel 2 "Bakgrunn", er endogent insulin og glukagonsyntese, sekresjon og effekter beskrevet. Relevante tilgjengelige eksogene insulin-analoger er også beskrevet.

I tillegg er somatostatin beskrevet, fordi somatostatinanaloger ble brukt i dyreforsøkene for å undertrykke endogen sekresjon av insulin og glukagon.

Forskningsgruppen Artificial Pancreas Trondheim (APT) har som hovedfokus å utvikle en bihormonal kunstig bukspyttkjertel, dvs. et lukket sløyfesystem for intraperitoneal insulin- og glukagoninfusjon. Denne avhandlingen bidrar med ny kunnskap om farmakodynamikken og farmakokinetikken til intraperitonealt administrert insulin og farmakodynamikken til intraperitonealt administrert glukagon som kan brukes til å utvikle algoritmer for en intraperitoneal kunstig bukspyttkjertel. Derfor inneholder kapittel 2 en evaluering av potensielle fordeler og ulemper ved kontinuerlig intraperitoneal insulininfusjon sammenlignet med kontinuerlig subkutan insulininfusjon. Tilgjengelig informasjon om mulige fordeler og ulemper ved en intraperitoneal kunstig bukspyttkjertel og bi-hormonell kunstig bukspyttkjertel er også oppsummert i avhandlingen.

Denne avhandlingen demonstrerer de potensielle fordelene ved intraperitoneal insulininfusjon og glukagonadministrasjon som en del av en kunstig bukspyttkjertel. Mitt håp er at denne avhandlingen vil være ett skritt nærmere en kunstig bukspyttkjertel som normaliserer eller nær normaliserer glukosenivået hos pasienter med diabetes mellitus type 1.

Navn kandidat: *Ilze Dirnena-Fusini*

Institutt: *Institutt for klinisk og molekylær medisin, NTNU*

Veileder(e): *Sverre Christian Christiansen, Sven Magnus Carlsen, Anders Lyngvi Fougner*

Finansieringskilde: *Finansiert av* Forskningsrådet

Ovennevnte avhandling er funnet verdig til å forsvares offentlig

For graden Ph.D. i medisin og helsevitenskap

Disputas finner sted digitalt via Zoom

Onsdag 27. oktober 2021, kl. 10:15

Summary

The main focus of this thesis is to explore the intraperitoneal (IP) cavity as the location for insulin administration. We hypothesized that IP insulin administration is more physiologic as it mimics endogenous insulin secretion and theoretically should achieve superior glycaemic control compared to subcutaneous (SC) delivered insulin. An additional focus was on IP glucagon administration, as glucagon is administered in a case of severe hypoglycaemia and is explored for use in a bi-hormonal artificial pancreas (AP). We hypothesized that IP glucagon administration, as IP insulin administration, mimics endogenous glucagon secretion closer than SC glucagon administration.

In the work of the thesis, we performed a systematic review where we explored a variety of physiological effects in a comparison between continuous IP insulin infusion (CIPII) and continuous SC insulin infusion (CSII) in patients with diabetes mellitus type 1 (**Paper I**). In the second paper, we contrasted the pharmacokinetics and pharmacodynamics of various IP insulin boluses in anaesthetised pigs to that of SC insulin (**Paper II**). In the third paper, we investigated pharmacodynamics of glucagon after IP and SC injection in rats (**Paper III**).

The thesis involves the two most important pancreatic hormones affecting glucose homeostasis, insulin and glucagon. Therefore, in the chapter 2 'Background', endogenous insulin and glucagon synthesis, secretion and effects are described. Relevant available exogenous analogues are also described.

Additionally, somatostatin is also mentioned as somatostatin analogues were used in the animal trials to supress endogenous insulin and glucagon secretion.

The Artificial Pancreas Trondheim (APT) research group has its main focus towards the development of bi-hormonal AP system, i.e., a closed-loop IP insulin infusion system. In this thesis we obtained information of insulin pharmacodynamics and pharmacokinetics and glucagon pharmacodynamics that will be used to develop an algorithm for an IP AP. Therefore, chapter 2 includes an evaluation of potential benefits and disadvantages of CIPII compared to CSII. Also, available information on possible advantages and disadvantages of IP AP and bi-hormonal AP development are summarized.

This thesis demonstrates the potential benefits of IP insulin and glucagon delivery as part of an AP. My hope is that this thesis will be one more step toward an AP that normalizes or close to normalizes glucose levels in patients with DM1.

Kopsavilkums

Šī promocijas darba tēma ir izpētīt vēderplēves dobuma izmantošanu insulīna ievadīšanai pacientiem ar pirmā tipa cukura diabētu. Darbā tika izvirzīta hipotēze, ka fizioloģiskajai insulīna sekrēcijai tuvāks process būtu papildus ievadītā insulīna uzsūkšanās no vēderplēves dobuma nevis no zemādas audiem. Papildus tika salīdzināta glikagona uzsūkšanās spēja pēc ievadīšanas vēderplēves dobumā un zemādas audos, ka arī noteikts glikozes līmenis asinīs.

Darba gaitā tika veikts sistemātisks literatūras izpētes un salīdzināšanas darbs. Pirmajā rakstā, kurā publicēti iegūtie rezultāti no veiktās sistemātiskās literatūras izpētes, tika salīdzināti dažādi fizioloģiskie faktori laika posmos kad insulīns tika ievadīts vēderplēves dobumā (1.5 – 36 mēneši) un kad insulīns tika ievadīts zemādas audos (vismaz 3 mēneši) pacientos ar pirmā tipa cukura diabētu. Nākamajā rakstā, tika prezentēti rezultāti par insulīna uzsūkšanās ātrumu un ietekmi uz glikozes līmeni asinīs, pēc insulīna ievadīšanas vēderplēves dobumā un zemādas audos cūkās, kā modeļorganismā. Pirms insulīna injekcijām, cūkām tika ievadīti somatostatīna analogi (oktreotīds un pasireotīds). Pēdējā rakstā, kas iekļauts šajā darbā, tika prezentēti rezultāti par glikagona uzsūkšanās ātrumu un ietekmi uz glikozes līmeni asinīs, pēc glikagona ievadīšanas vēderplēves dobumā un zemādas audos. Kā modeļorganisms tika izmantotas baltās žurkas, kurām pirms glikagona injekcijas tika ievadīts somatostatīna analogs (oktreotīds).

Ņemot vērā to, ka promocijas darba fokuss bija svarīgākie aizkuņģā dziedzera sekrēcijas hormoni – insulīns, glikagons un somatostatīns, ievaddaļā apkopoti minēto hormonu metabolisma apraksti, ar papildus informāciju par eksogēno analogu izmantošanu un pieejamību klīniskajā praksē.

Darbs tika izstrādāts kā pētījums kopā ar pētnieku grupu “Artificial Pancreas Trondheim”, un darba iegūtie rezultāti tiks izmantoti mākslīgā aizkuņģā dziedzera izveidē. Tādēļ galvenais uzsvars šājā darbā bija iespējamie ieguvumi no insulīna ievadīšanas vēderplēves dobumā salīdzinot ar tā ievadīšanu zemādas audos pirmā tipa cukura diabēta pacientos.

Darbs parāda pozitīvos ieguvumus no insulīna ievadīšanai vēderplēves dobumā: (i) pazemināts insulīna līmenis perifērajā asinsritē, (ii) ātrāka insulīna uzsūkšanās, un (iii) pazemināts HbA1c līmenis.

Promocijas darbs izstrādāts: *Norvēģijas Zinātnes un Tehnoloģiju Universitātes (NTNU) Medicīnas un Veselības zinātņu fakultātes Klīniskās un Molekulārās medicīnas zinātņu katedrā*

Darba vadītāji: *Asociētais profesors Sverre Christian Christiansen, Profesors, Medicīnas doktors Sven Magnus Carlsen un asociētais profesors Anders Lyngvi Fougner.*

Darba recenzenti: *MD, PhD Peter Ruben Van Dijk un Asociētā profesore Elisabeth Qvigstad*

Darbs aizstāvēts: *2021. gada 27. Novembrī, Trondheimā, Norvēģijā.*

‘The joy of discovery is certainly the liveliest that the mind of man can ever feel’

Claude Bernard

Preface

This thesis is a product of my doctoral studies carried out at the Department of Clinical and Molecular Medicine (IKOM), The Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU) under the supervision of associate professor Sverre C. Christiansen and co-supervision of professor Sven M. Carlsen and associate professor Anders L. Fougner. During my Ph.D. studies, I was part of the research group "Artificial Pancreas Trondheim", which has a long-term aim to develop a robust closed-loop glucose control system, i.e., an artificial pancreas (AP) for patients with diabetes mellitus type 1 and type 2 and for intensive-care patients. The work was funded by the Research Council of Norway (Project no 248872/070) and is part of The Centre for Digital Life Norway through the Double Intraperitoneal Artificial Pancreas (DIAP) project. The animal experiments were conducted at the Comparative medicine Core Facility (CoMed) at NTNU. CoMed is funded by the Faculty of Medicine at NTNU and Central Norway Regional Health Authority.

Acknowledgments

This Ph.D. has been a great and long journey for me, with many ups and downs along the way and plenty of inspiration for my self-development. I have met many amazing, friendly and helpful people, who have enriched and enlightened my life.

First of all, I would like to thank my supervisors, Professor Sven Magnus Carlsen, Dr. Sverre Christian Christiansen and Associate Professor Anders Lyngvi Fougner for giving me the opportunity to work on this project and to be creative in my working environment, and for showing support in everything I intended to do. Thank you also for the many productive discussions which helped me improve my research, and thank you for the incredible support in preparing the systematic review.

I also have to thank my closest colleague Marte Kierulf Åm, with whom I shared office for almost five years. You gave me priceless ideas, suggestions, opinions, experience through the many conversations during lunch breaks, coffee breaks, experiments and in our free time, of which we had very little. I learned so much from you!

I also have to thank the colleagues at the Animal facility, especially Oddveig Lyng. Thank you for your priceless help and suggestions with the animal trials and for the interesting

and sometimes hilarious conversations in the lab: without you my time in the lab would have been plain and tedious.

I would also like to thank the Artificial Pancreas Trondheim (APT) research group, especially Patrick Christian Bösch, Silje Skeide Fuglerud, and Reinold Ellingsen, for the interesting conversations and encouragements during all APT meetings and conferences.

Finally, I would like to thank my family, my mum and dad, my brothers and sisters, and especially my little sister Baiba, who helped unconditionally through the toughest times, babysat my kids, and provided constructive criticism on my research and life in general. It was not easy to move away from you guys, to change country, language, and friends, but your support and encouragement gave me strength: I love you all, no matter what. And I would like to thank my beloved husband Lorenzo, my strongest and most passionate supporter: thank you for being my strong shoulder after long days at work, and for encouraging me in low periods, giving opinions in times of hard decisions and support when needed. And most importantly, thanks for being a loving husband and patient dad to our sons.

Ilze Dirnena-Fusini

Table of Contents

List of figures

List of tables

Abbreviations

Adc	Adenylyl cyclase
AIA	Anti-insulin antibodies
AP	Artificial pancreas
ATP	Adenosine triphosphate
BG	Blood glucose
BCH	2-amino-2-norbornanecarboxylic acid
BMI	Body mass index
BW	Body weight
CAD	Coronary artery disease
cAMP	cyclic Adenosine monophosphate
CGM	Continuous glucose monitoring
CII	Continuous insulin infusion
CIPII	Continuous intraperitoneal insulin infusion
CSII	Continuous subcutaneous insulin infusion
DKA	Diabetic ketoacidosis
DM1	Diabetes mellitus type 1
DM2	Diabetes mellitus type 2
ECs	Endothelial cells
ECM	Extracellular matrix
ELISA	The enzyme-linked immunosorbent assay
FFA	Free fatty acid
FOR	Free oxygen radicals
GDM	Gestational diabetes mellitus
GH	Growth hormone
GI	Gastrointestinal
Gnas	G-protein alpha subunit
GCGR	Glucagon receptor
GSIS	Glucose-stimulated insulin secretion
GSSS	Glucose-stimulated somatostatin secretion
HbA1c	Glycated haemoglobin A1c
HGP	Hepatic glucose production
HGS	Hepatic glycogen synthesis
IC	Intracellular
IKD	Intracellular kinase domain
IM	Intramuscular

IP	Intraperitoneal
IPII	Intraperitoneal insulin infusion
IR	Insulin receptor
LADA	Latent autoimmune diabetes in adults
MD	Mean difference
MDI	Multiple daily injections
MODY	Maturity-onset diabetes of the young
NTNU	Norwegian University of Science and Technology
OGTT	Oral glucose tolerance test
PID	Proportional integral derivative
PKA	Protein kinase A
RAI	Rapid-acting insulin
RER	Rough endoplasmic reticulum
RHI	Regular human insulin
RevMan	Review Manager (Software)
RRP	Readily releasable pools
RTK	Receptor tyrosine kinase
SC	Subcutaneous
SCAT	subcutaneous adipose tissue
SD	Standard deviation
SHBG	Sex hormone binding globulin
SMBG	Self-monitoring of blood glucose
SR	Systematic review
SSA	Somatostatin analogue
SST	Somatostatin
SSTR	Somatostatin receptor
TG	Triglyceride
TSH	Thyroid-stimulating hormone
VAT	Visceral adipose tissue
WHO	World Health Organization

Definitions

2-amino-2-norbornanecarboxylic acid – (BCH) – amino acid, an inhibitor of system L amino acid transporters, BCH suppresses mTORC1 signalling that drives DNA synthesis and cell proliferation [1].

Brittle diabetes – (also called labile diabetes) an uncommon variant of DM1, in which patients' lives are affected by glycaemic instability, i.e., frequent hypoglycaemia, hyperglycaemia or both [2].

DM1 – (previously known as insulin-dependent, type 1 diabetes, juvenile or childhood-onset diabetes) is characterized by absence or low endogenous insulin production and requires daily administration of exogenous insulin. The ultimate cause of DM1 is unknown, and it is not preventable with current knowledge [3].

Diabetes mellitus type 2 – (formerly called non-insulin-dependent or adult-onset diabetes) insulin is present, but accessibility is reduced, i.e., insulin resistance or insulin production is decreased [3].

Endothelial cells – cells that line all blood vessel walls and are exposed to the mechanical forces of blood flow which modulate their function and play a role in vascular regulation, remodelling and disease [4].

Free oxygen radicals (FOR) – unstable molecules that contain oxygen and can efficiently react with other molecules in the cell. The increased amount of FOR in cells can cause damage to DNA, RNA, proteins and may cause cell death [5].

Gestational diabetes mellitus – defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies whether insulin or only diet modification is used for treatment and whether or not the condition persists after pregnancy [6].

Glycocalyx – proteoglycan, glycosaminoglycan and plasma protein layer on the external surface of the plasma membrane of the epithelial cells. Identifier and distinguisher between original body's cells and external organisms, cells or tissues. Contribute to cell-to-cell recognition [7].

HbA1c – glycated haemoglobin – develops when haemoglobin, a protein within red blood cells that carries oxygen throughout a body, joins with glucose in the blood. HbA1c can be used to clinically reflect the blood glucose level with at least 8 – 12 weeks intervals. In nondiabetic individuals HbA1c levels are < 6.0 %. In prediabetic individuals 6.0 % – 6.4 %. In diabetic individuals HbA1c levels are > 6.5 % [8].

Hyperglycaemia – fasting blood glucose levels ≥ 7.0 mmol/L or oral glucose tolerance test – 2 hours value ≥ 10 mmol/L [9] with additional symptoms: dry mouth, increased thirst, weakness, headache, blurred vision, frequent urination.

Hypoglycaemia – blood glucose levels < 3.9 mmol/L [10] with clinical symptoms: sweating, sleepiness, pallor, lack of coordination, irritability, hunger.

Insulin resistance – is a condition in which a person`s body tissues shows a lowered level of insulin response [11].

Intramuscular – within the substance of a muscle.

Intraperitoneal – the potential space between the parietal and the visceral peritoneum.

Intravenous – within a vein.

Latent Autoimmune Diabetes in Adults – the slow onset of the disease, where patients being affected by an autoimmune DM1 not requiring insulin at the present state of diagnosis [9].

Ketoacidosis (diabetic) – the accumulation of ketone bodies in the blood, which results in metabolic acidosis, caused by hyperglycaemia. DKA can progress to diabetic coma [12].

Multiple daily injections – the administration of 2 or more insulin injections/day. MDI include one or two injection of medium or long-acting insulins (24-hour active) and injections of rapid or short-acting insulin preceding meals [13].

Obesity – body mass index (BMI) calculated by dividing a person`s weight in kilograms by a square of his height in meters. Obesity is BMI 30 – 40 kg /m^2 [14].

Proportional integral derivative – a control loop system that based on the feedback from the system continuously calculates an error value as the difference between a desired set-point and a process variable and applies a correction based on proportional, integral and derivative terms [15].

Severe hyperglycaemia – increased blood glucose levels. Glucose is utilized by the body cells; the body cells must use ketones as sources of energy; develops ketoacidosis [16].

Severe hypoglycaemia – such low blood glucose levels that the patient requires assistance from another person [17].

Subcutaneous – beneath the layers of the skin.

1. Introduction

1.1. Motivation

Around 422 million adults worldwide have diabetes, which equates to 1 of 11 persons [18]. In Norway, with 5.4 million inhabitants, there are approximately 375 000 people with diabetes which implies a prevalence of 6.9 % [19]. Diabetes is one of the main causes of death worldwide; 1.6 million deaths are directly attributed to diabetes each year [18].

Around 10 % of all people with diabetes have diabetes mellitus type 1 (DM1) [20]. Based on The Norwegian Diabetes Association data, in Norway, it is 7.5 % or 28 000 people living with DM1 [19].

Hyperglycaemia or symptoms related to hyperglycaemia, are the first signs that indicate DM1. Due to hyperglycaemia, patients experience well-known classical symptoms such as feeling tired and sick, polyuria, excessive thirst, hunger, and weight loss [9]. Symptoms of DM1 can develop suddenly (over days or weeks) in previously healthy children or adolescents (DM1) or can develop gradually over months or years in adults (Latent Autoimmune Diabetes in Adults, LADA) [21].

DM1 is a chronic disease that, if not treated accordingly, in the long term, can lead to a numbers of life-threatening complications such as renal failure, heart disease, stroke, and blindness [22]. Furthermore, psychological health is affected, with disease fatigue [23] and fear of hypoglycaemia [24]. Thus, it is vital to manage hyperglycaemia efficiently and in a safe manner to prevent complications of the disease and improve patient's quality of life.

In patients with DM1, every day's therapy implies blood glucose (BG) measurements and insulin injections. Exogenous insulin administration is preferably tailored to mimic endogenous insulin secretion. In DM1, insulin injection is necessary every day, before every meal and before bedtime, and when patients suspect hyperglycaemia [25]. The most practised method for insulin delivery is subcutaneous (SC) via a syringe, insulin pen or insulin pump. However, most patients do not achieve their glucose level targets by SC insulin administration, i.e., they experience regular hypo- and/or hyperglycaemic events, and poor results regarding HbA1c. HbA1c is a measure of average glucose control for the previous 2-3 months.

Diabetes research aims to reduce the burden to DM1 patients by establishing more manageable, faster and more painless technologies for blood glucose monitoring and insulin delivery. For decades an artificial pancreas (AP), i.e., a fully automated closed-loop

system controlling the insulin delivery by mimicking the endogenous insulin secretion, has been a distant goal. At present, the goal seems to be within reach, and in recent years, hybrid SC APs have been introduced to clinical practice [26-28]. The main focus in the current AP approach is on algorithm-steered CSII, the commonly used route of insulin delivery as SC tissue is readily assessable. However, SC insulin delivery comes with a relatively slow absorption, compared to insulin secretion from the pancreas in healthy people [29]. Animal trials show that SC insulin induces hyperinsulinemia in the systemic circulation and subnormal insulin concentration in the pancreas portal circulation that delivers the blood entering the liver [30].

1.2. Scope

Insulin absorption should be fast, effective and predictable, in order to provide optimal benefits from the administrated hormone. Intraperitoneal insulin infusion (IPII) seems to offer these benefits. However, insulin pump implantation for IPII is an invasive, costly and burdensome procedure. Therefore, it is essential to verify the current benefits of IPII. Insulin dynamics in the IP space and its effect on BG levels been sparsely studied. Moreover, new insulin analogues are being developed, and new faster-acting and more concentrated insulins are in the pipeline towards the market.

Some research groups focus on bi-hormonal APs, which preferably combine insulin and glucagon delivery. This approach allows a more aggressive way of insulin delivery as glucagon is used to counteract and/or prevent hypoglycaemia.

The overall scope of this thesis is to examine the potential benefits of continuous IP insulin infusion (CIPII) as part of an IP AP. **Paper I** provides a systematic review and meta-analyses of some of the effects of CIPII compared to CSII in DM1 patients.

Further, we explored the pharmacokinetics and pharmacodynamics of IP insulin delivery in anaesthetised pigs in **paper II**.

In clinical practice, glucagon is used for severe hypoglycaemia and has also been used in bi-hormonal AP studies. Thus, we explored the glucose response to IP delivered glucagon in rats in **paper III**.

1.3. List of papers

Paper I

Physiological effects of intraperitoneal versus subcutaneous insulin delivery in patients with diabetes mellitus type 1: A systematic review

Ilze Dirnena-Fusini, Marte Kierulf Åm, Anders Lyngvi Fougner, Sven Magnus Carlsen, Sverre Christian Christiansen.

Published in PLoS ONE, 2021 Apr 13;16(4): e0249611
DOI: 10.1371/journal.pone.0249611
PMID: 33848314

Paper II

Intraperitoneal insulin administration in pigs: Effect on circulating insulin and glucose levels

Ilze Dirnena-Fusini, Marte Kierulf Åm, Anders Lyngvi Fougner, Sven Magnus Carlsen, Sverre Christian Christiansen.

Published in BMJ Open Diabetes Research & Care, 2021 Jan;9(1): e001929.
doi: 10.1136/bmjdrc-2020-001929.
PMID: 33452058 PMCID: PMC7813410

Paper III

Intraperitoneal, subcutaneous and intravenous glucagon delivery and subsequent glucose response in rats: a randomized controlled crossover trial

Ilze Dirnena-Fusini *, Marte Kierulf Åm *, Anders Lyngvi Fougner, Sven Magnus Carlsen, Sverre Christian Christiansen.

(*shared first authorship)

Published in BMJ Open Diabetes Research & Care, 2018 Nov 9;6(1): e000560.
doi: 10.1136/bmjdrc-2018-000560.
PMID: 30487972 PMCID: PMC6235059

Conference posters

Results from conference posters are included in the main articles:

Dirnena-Fusini I, Åm MK, Christiansen SC, Fougner AL, Carlsen SM.
Physiologic effects of intraperitoneal vs. subcutaneous insulin delivery in patients with DM1: A systematic review.
Presented as poster at the conference Advanced Technologies & Treatments for Diabetes (ATTD 2017) Paris, France, February 2017.

Dirnena-Fusini I, Åm MK, Christiansen SC, Fougner AL, Carlsen SM.
Intraperitoneal, subcutaneous and intravenous glucagon delivery in rats: Effect on glucose levels.
Presented as poster at the conference Advanced Technologies & Treatments for Diabetes (ATTD 2018) Vienna, Austria, February 2018.

Dirnena-Fusini I, Åm MK, Carlsen SM, Fougner AL, Christiansen SC.
Intraperitoneal insulin administration in pigs: Effect on circulating insulin and glucose levels.
Presented as poster at the conference Advanced Technologies & Treatments for Diabetes (ATTD2019) Berlin, Germany, February 2019.

Dirnena-Fusini I, Åm MK, Carlsen SM, Fougner AL, Christiansen SC.
The metabolic effects of continuous intra-peritoneal insulin infusion, a systematic review.
Presented as poster at the conference Advanced Technologies & Treatments for Diabetes (ATTD, 2020), Madrid, Spain, February 2020.

2. Background

To properly understand exogenous insulin absorption from the IP cavity, it is essential to comprehend endogenous insulin biogenesis, signalling pathways, and hormones that can affect insulin absorption and interfere with metabolic reactions, such as glucagon and somatostatin.

Therefore, in this chapter, I present a brief review of general concepts of insulin biosynthesis and the importance of insulin in homeostasis. I briefly describe hormones that are affected or can be affected by insulin production and presence in the circulation. I briefly explain the most common complications and the struggle patients with DM1 encounter. I touch on insulin analogues and insulin administration routes, their advantages and disadvantages. At the end of the chapter, I introduce the IP cavity as a potential site of insulin administration, as it is a well-known insulin administration route; however, not commonly used in clinical practice. And at the very end, I briefly discuss glucagon as a potential hormone for avoiding hypoglycaemia during automated insulin administration (closed-loop system) in patients with DM1.

2.1. Pancreas

The pancreas was first described by the Greek anatomist and surgeon Herophilus (335–280 BC) [31]. The pancreas (from Greek “πάγκρεας”, literally means “all-flesh”) abide on the posterior wall of the abdominal cavity. Theoretically, we can separate it into different parts: head, neck, body and tail. The pancreas is one of the most complex tissues in the body. It is composed of a mixture of endocrine and digestive exocrine cell components.

In 1869, the German pathologist, physiologist and biologist Paul Langerhans (1847–1888) reported that the pancreas has two systems of cells [31]. Islets of Langerhans contain α, β and δ cells. They are responsible for maintaining homeostasis as insulin secretion is heterogeneous and dependent on cell-to-cell contact, i.e., insulin secretion is increased from β cells that have direct contact with α cells [32]. The islets are dispersed within the pancreas instead of forming a solid endocrine gland-like most other endocrine tissues. The distribution may reflect the function of the islets. Based on physical law, many small spheres' surface is larger than the body of the same volume of the object condensed into a single sphere [33]. Therefore, in the islets, hormone secretion from the islets' cells is more effective than from a single solid endocrine gland.

As widely known, β cells are responsible for insulin synthesis and secretion to decrease glucose levels. In contrast, α cells act counterregulatory and secrete glucagon in the time

of hypoglycaemia. Not so well known, there are δ cells responsible for somatostatin secretion. This hormone inhibits the release of almost all endocrine and exocrine secretions of the pancreas, gut and gallbladder [34].

The endocrine pancreas consists of the islets of the Langerhans that secretes hormones to the blood. The total number of islets varies as much as between 3.6 and 14.8 million, and the highest number of islets are in the pancreas body. The total islet volume is between 0.5 to 1.3 cm^3. Furthermore, the cellular composition of the islets differs with ~ 60 % for β cells, ~30 % for α cells, whereas the remaining 10 % contains δ cells and γ cells (pancreatic polypeptide secretion) [35].

The enzyme-producing cells (produce pancreatic juice) form pancreatic acini and are known as the exocrine pancreas (Fig 1). The intercalated ducts connect the acini to the intralobular ducts. These ducts drain to the interlobular ducts making the pancreatic duct system. The juice contains water, bicarbonate ions and various enzymes, i.e., trypsinogen, chymotrypsinogen, carboxypeptidases, elastase, lipase, phospholipase A, amylase, DNAse and RNAse). The pancreatic juice is excreted to the duodenum via the pancreatic duct [36].

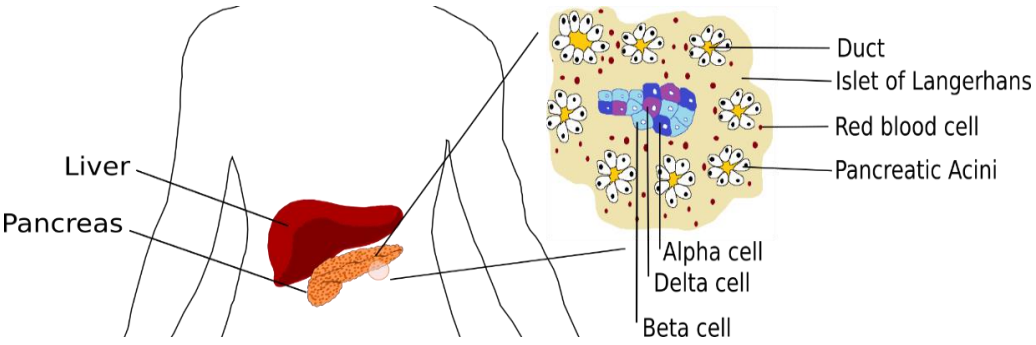


Figure 1. Exocrine pancreas and structure of Islet of Langerhans. Schematic drawing made by Ilze Dirnena-Fusini.

2.2. Insulin and its metabolism

2.2.1. Proinsulin and its structure

Insulin biogenesis starts with the synthesis of preproinsulin in the rough endoplasmic reticulum (RER). In RER, preproinsulin is transformed into proinsulin (Fig 2). Proinsulin contains an amino acid A-chain, a B-chain linked together by two disulphide bonds, and a C-chain with extra amino acids (n = 4) that connects the C-chain with the A- and B-chains (Fig 3). Proinsulin is packed in the Trans-Golgi Network (TGN) and sorted into immature

secretory granules. From these immature granules via the constitutive (unregulated) pathway, proinsulin can be transported to the plasmatic membrane and, during exocytosis, fuse into the cytoplasm. However, proinsulin has the only weak binding ability to insulin receptors (IR) and is released via proteolytic processes [37].

2.2.2. Insulin biogenesis

During the secretory (regular, i.e. usual pathway) pathway, before the formation of granules, proteins in the lumen of the TGN accumulates in a mildly acidic, high Ca^{2+} concentration environment, and the accumulated proteins directly interact with lipid membrane cholesterol, which leads to reorganization of cholesterol-rich microdomains. Subsequently, immature granules are produced; during the maturation process and insulin formation by excision of the C-peptide, the acidity level increases in the granule. The insulin dense-core granules are generated via Ca^{2+} and zinc-dependent condensation processes. The insulin granules contain readily releasable pools (RRP), responsible for the initial (first phase) insulin secretion, and a second reserve pool, more prolonged (second phase) insulin secretion [38]. RRP links with the plasma membrane and prepares for acute Ca^{2+}-dependent release of insulin. Secretion from the reserve pool requires granule trafficking to the plasma membrane (Fig 2) [38].

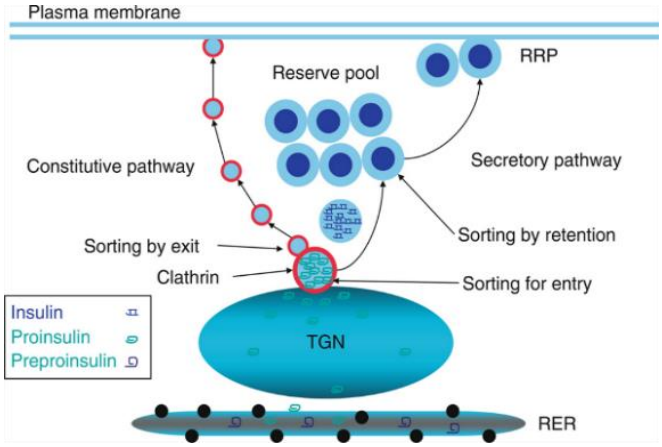


Figure 2. Insulin biogenesis. Preproinsulin is produced in RER, where it transforms into proinsulin. Proinsulin enters TGN where it is packed into immature granules. The constitutive pathway: proinsulin is packed into small transport vesicles, directly transferred to and fused with the plasma membrane. The secretory pathway: immature granules shift to the acidic state via adenosine triphosphate (ATP)-dependent proton pump where proinsulin undergo proteolysis by endoproteases, with separation of C-terminal by carboxypeptidase E. It results in the formation of mature, dense core insulin (A- and B-chain) granules and C-peptide (C-chain). Reused from June Chunqiu Hou, Le Min, Jeffrey E. Pessin. Vitamins & Hormones [38]. This figure is licensed under an Elsevier and Copyright Clearance Center. No modifications have been made. Abbreviations: RRP, readily releasable pools; TGN, *trans*-Golgi network; RER, rough endoplasmic reticulum; VDCC, voltage-dependent Ca^{2+} channels; ER, endoplasmic reticulum; cAMP, cyclic adenosine monophosphate; EPAC, exchange protein activated by cAMP; GLP-1R, glucagon-like peptide-1 receptor.

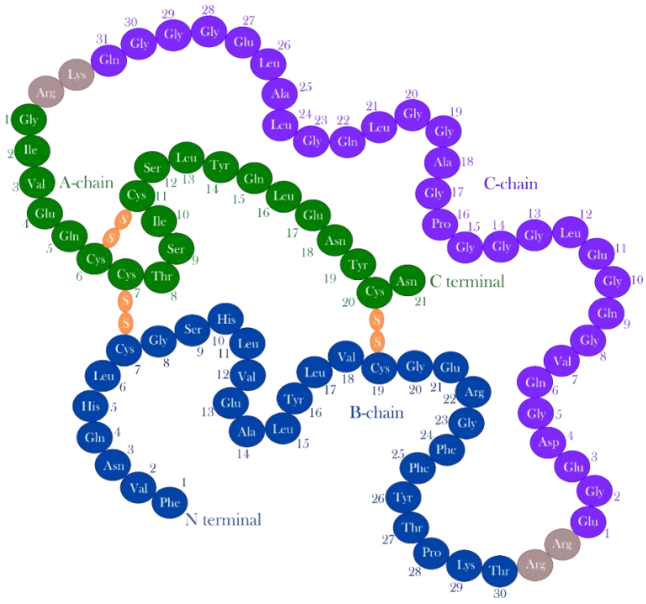


Figure 3. Structure of human proinsulin. Schematic drawing made by Ilze Dirnena-Fusini.
Abbreviations: Ala, alanine; Asp, aspartic acid; Arg, arginine; Asn, asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; S, sulfide; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

2.2.3. Insulin structure

Insulin is a polypeptide hormone made by two peptide chains: A-chain and B-chain. Human insulin is composed of 51 amino acids: 21 amino acids in the A-chain and 30 amino acids in the B chain linked together by two disulphide bonds (Fig 4). The molecular weight of the human insulin monomer is 5808 Daltons with a hydrodynamic diameter of 2.69 – 5.50 nm (27 – 55 Å) [39]. Some of the amino acids are structurally functionally important and essential for insulin binding to the IR, including A_1Gly, A_2Ile, A_3Val, A_{19}Tyr, B_6Leu, B_{12}Val, B_{23}Gly, B_{24}Phe, and B_{25}Phe [40] and are present in most, if not all animal species [41-45].

At increased monomer concentration, insulin fuses into dimer structures. In the presence of zinc ions, further insulin associates into hexamers [46]. Hexamers are produced during the maturation process when six insulin molecules stabilize around two zinc ions to form hexamers. These insulin hexamer granules are inactive and are too bulky, with a size of about 36 kiloDaltons (~300 – 400 nm diameter; compared to small transport vesicles (~50 nm diameter)) to be transported via the plasmatic membrane [38].

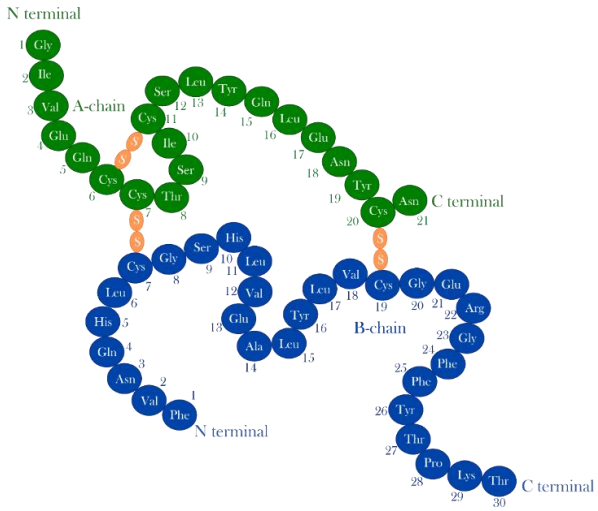


Figure 4. Human insulin structure. Amino acids A_6-A_{11}, A_7-B_7 and A_{20}-B_{19} are connected by disulphide bonds. Schematic drawing made by Ilze Dirnena-Fusini.
Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; S, sulphide; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

2.2.4. β-cell signalling pathway and insulin secretion

Glucose is the primary regulator of insulin biosynthesis and secretion [47]. Glucose-stimulated insulin secretion (GSIS) is promoted by glucose stimulating ATP production, inducing closure of ATP-sensitive potassium channel, which results in depolarization of the β cell and increases Ca^{2+} entry via voltage-dependent Ca^{2+} channels. Rise of intracellular Ca^{2+} triggers insulin release from RRP (Fig 5) [48].

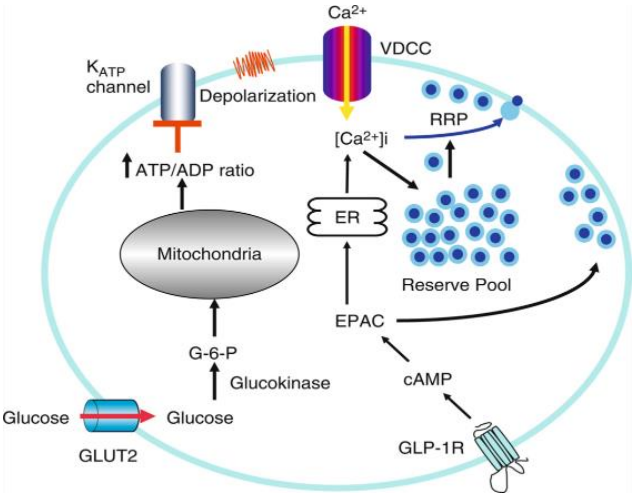


Figure 5. β cell signalling pathways. Via the glycolysis and mitochondrial ATP energy production the ATP/ADP ratio increases that leads to the closure of the ATP-sensitive potassium channels (K_{ATP}). The subsequent cellular depolarization activates voltage dependent Ca^{2+} channels resulting in extracellular Ca^{2+} influx and fusion of insulin granules with the plasma membrane. The incretin hormone GLP-1 acts on its receptor at β cell plasma membrane to activate adenylyl cyclase and increase intra cellular cAMP levels. Consequently, cAMP binds and activates protein kinase A and EPAC. EPAC increase intra cellular Ca^{2+} level from intra cellular Ca^{2+} stores in the ER, thereby reserve pool insulin granules are fused closer to the plasma membrane and transformed to the RRP.
Reused from June Chunqiu Hou, Le Min, and Jeffrey E. Pessin. Vitamins & Hormones [38]. This figure is licensed under an Elsevier and Copyright Clearance Center. No modifications have been made. Abbreviations: RRP, readily releasable pools; VDCC, voltage-dependent Ca^{2+} channels; ER, endoplasmic reticulum; cAMP, cyclic adenosine monophosphate; EPAC, exchange protein activated by cAMP; GLUT2, glucose transporter 2; GLP-1R, glucagon-like peptide-1 receptor.

Insulin secretion

Insulin secretion is stimulated by hyperglycaemia [49], increased levels of specific amino acids [50-52], and proteins [53, 54]. The exact mechanism behind insulin release from RRP is unsettled. There are three theories:

1) The membrane of dense-core hexamer insulin granule completely fuses with the plasma membrane resulting in the emptying of the granule contents and complete

integration of granule content (membrane lipids, proteins and all granule content) with the plasma membrane [55].

2) A kiss-and-run type mechanism, where transient pores open between the granule membrane and the plasma membrane allowing for a partial or complete release of the granule content, followed by the closure of the plasma fusion pore [56].

3) A kiss-and-run type mechanism, called cavicapture, where only selective components of the granule membrane and granule content undergo exocytosis followed by the closure of the plasma fusion pore [55].

During hyperglycaemia, Ca^{2+} levels in the β cells increases [49] due to the closure of ATP-sensitive potassium (K_{APT}) channels. Increased Ca^{2+} levels stimulate exocytosis of insulin RRP granules (first phase secretion) [57]. Increased Ca^{2+} promotes the insulin granule mobilization in the reserve pool and enables them for release once the Ca^{2+} level increases to exocytotic levels (second phase secretion) [58]. In an experiment with pseudo-islet, increased insulin secretion was observed during the presence of additional potassium chloride (KCl) but increased minimally during glucose boluses. Surprisingly, higher insulin production was observed in 32 °C, compared to 37 °C and 22 °C [59].

In experiments with mice pancreatic β cells, exocytosis of ∼35 – 40 granules was seen during the first-phase insulin secretion compared to exocytosis of 120 – 130 granules by mimicking the second-phase insulin secretion via the stimulation of GSIS [58]. Mobilizing insulin granules from the reserve pool that contains most of the insulin granules requires a series of Ca^{2+}, ATP, time and temperature-dependent processes [38].

In individuals without diabetes, endogenous insulin secretion follows in two steps: (1) a rapid insulin increase in the bloodstream with a peak after 30 to 45 minutes after the meal (post-prandial), and its return to basal levels after one to three hours; and (2) a constant insulin secretion at a lower rate (basal) [60].

Post-prandial endogenous insulin level increase depends on the number of carbohydrates consumed during the meal. Though, basal endogenous insulin is released continuously at low rates (5 – 15 µU/mL) in response to hepatic glucose production to retain stable glucose levels (4 – 5 mmol/L) [25]. Insulin in interaction with glucagon regulates BG levels that are described in section 2.3. In healthy individuals, post-prandial BG concentration can increase till 11.1 mmol/L, and due to endogenous insulin release, it fast returns to basal BG levels. This endogenous regulation system manages BG levels in a narrow range (3.5 – 7.5 mmol/L) (Fig 6) [25].

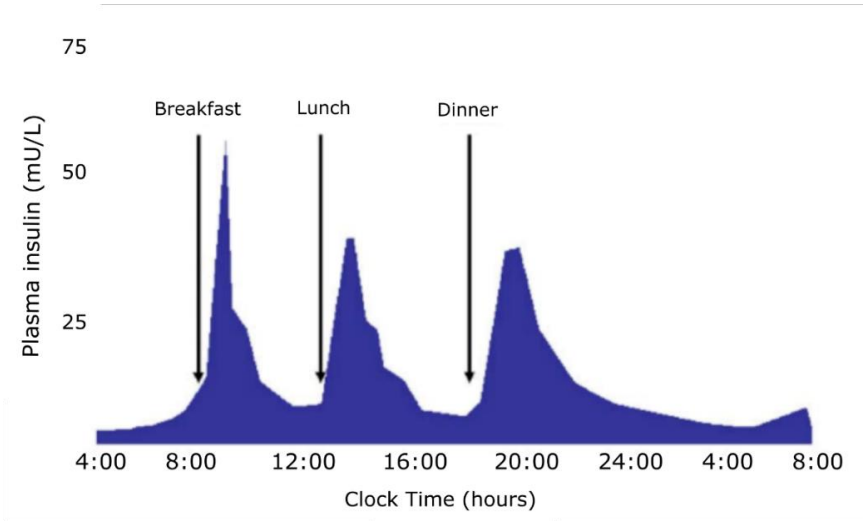


Figure 6. Diagram of endogenous insulin secretion in the healthy individual during the 24-hour profiling. This figure is licensed under an Elsevier and Copyright Clearance Center. Reused without modifications from Thompson et al. [25].

2.2.5. Insulin receptor and role in metabolism

In the form of a bioactive monomer, insulin binds to a specific insulin receptor (IR) on the cell's surface to promote metabolic processes [61]. This action activates a cascade of intracellular signalling processes that regulate essential biological processes such as glucose and lipid metabolism, gene expression, protein synthesis and growth, division and survival of cells [62]. The IR also play an essential role in the modulation of pancreatic α cell functions via intra-islet regulation [63].

The IR is a receptor tyrosine kinase (RTK), a heterotetrameric membrane protein that contains two monomers linked by disulphide bonds. Each monomer contains an α-subunit which is an insulin binding subunit and a β-subunit which includes the extracellular part, a membrane-spanning transmembrane domain and an intracellular kinase domain (IKD). In an inactive state, the α-subunit binds to the β-subunits extracellular region and inactivates IKD [64]. Adipocyte and liver plasma membrane IR α-subunits links with other α-subunits through disulphide bridge [65]. In the active state, when insulin binds to the IR α-subunit, it activates derepression of the IKD in the β-subunit following a trans-phosphorylation process of the β-subunit and a conformation change that increases IKD activity [66]. RTKs coordinate a variety of cellular functions such as growth (insulin-like growth factor 1 (IGF-1)), survival, differentiation, metabolism and inflammatory responses [67]. In particular, insulin RTK stimulates the synthesis and storage of carbohydrates, lipids and proteins (Fig 7).

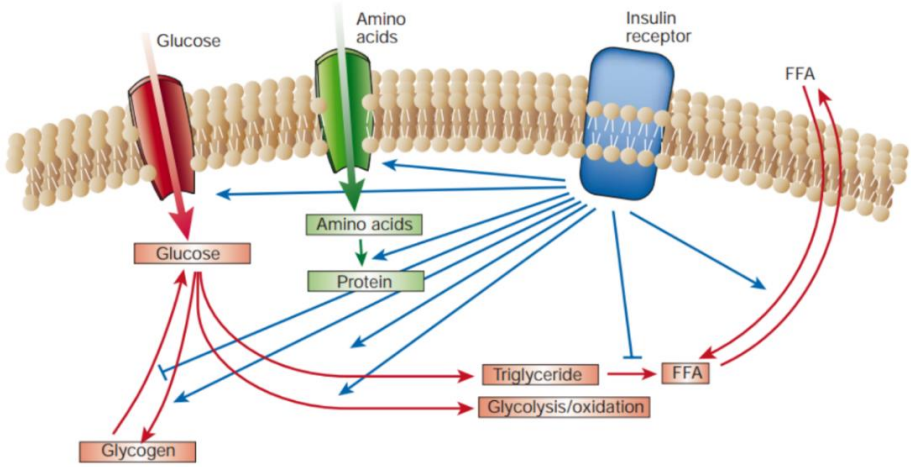


Figure 7. The regulation of metabolism by insulin. Insulin is the most critical anabolic hormone known and stimulates the synthesis and storage of carbohydrates, lipids and proteins due inhibiting their degradation and release into the circulating system. Insulin triggers the uptake of glucose, free fatty acids (FFA) and amino acids into the cells and increases activity of enzymes that catalyse glycogen, lipid and protein synthesis and deters the activity of catalysing degradation. Reused without modifications from Saltiel et al. [66].

Primary, insulin regulates hepatic glucose uptake and production (HGP) by directly binding to hepatic IRs [68], the hepatic uptake of insulin is 40 – 80 % of total body insulin removal, and uptake increases with increasing insulin infusion rate [69].

Secondary, insulin binds to skeletal and cardiac muscle and adipose tissue IRs and stimulates glucose, free fatty acids and amino acids uptake into the tissues where they are assimilated and stored as glycogen, lipids and protein and, consequently, increases cell growth (Table 1) [41, 70]. In patients with DM1, fatty acid metabolism is reduced [71].

Another insulin interaction is with endothelial surface glycocalyx, where insulin increases glycocalyx exposure to circulating blood; thus, glycocalyx can effectively dispose of glucose from circulation by transcapillary transport [72]. In patients with DM1, blood flow in peripheral arterial and microvascular circulation is reduced compared to healthy individuals [73], and peripheral capillary blood vessels thicken, occasionally progresses to complete occlusion [74]. Thus, insulin distribution in peripheral tissues does not happen evenly and reduced glycocalyx permeability for glucose.

Adipose tissue
Increased glucose entry
Increased fatty acid synthesis
Increased glycerol phosphate synthesis
Increased triglyceride deposition
Activation of lipoprotein lipase
Inhibition of hormone-sensitive lipase
Increased K+ uptake
Muscle
Increased glucose entry
Increased glycogen synthesis
Increased amino acid uptake
Increased protein synthesis in ribosomes
Decreased protein catabolism
Decreased release of gluconeogenic amino acids
Increased ketone uptake
Increased K+ uptake
Liver
Decreased ketogenesis
Increased protein synthesis
Increased lipid synthesis
Decreased glucose output due to decreased gluconeogenesis, increased glycogen synthesis, and increased glycolysis
General
Increased cell growth

Table 1. Effects of insulin on various tissues. Table reproduced from Ganong`s review of medical physiology (without modifications) [41].

2.2.6. Hyperinsulinemia

In non-diabetic people, the insulin concentration may rise 14-hold in the portal vein compared to peripheral venous concentration after IV glucose infusion [75]. Hyperinsulinemia can result from insulin resistance (see section 2.6.5.) or iatrogenic peripheral hyperinsulinemia that is a result of, for instance, SC insulin administration [76]. In both cases, hyperinsulinemia may increase the possibility of coronary artery diseases (CAD) [77].

In obese individuals, glucose extraction from the circulation is reduced due to large adipocytes with reduced metabolic activity and an altered balance towards more fat and less glucose entering the cells. An increased circulating BG level triggers insulin secretion with consecutive systemic hyperinsulinemia [78], which among others, leads to hypertension [14] and atherosclerosis [77].

Noteworthy (however, not related to this thesis) is overexpressed endogenous insulin secretion from the pancreatic β cells leading to a severe hypoglycaemia. Cardiometabolic disease risk (hyperlipidemia, hyperinsulinemia, hypertension, elevated C-reactive protein) is clinically related to illicit drug use [79] and genetic abnormalities in specific genes [80]. Another reason for hyperinsulinemia is insulin resistance caused by obesity [81, 82] and polycystic ovary syndrome (PCOS) [83].

2.3. Glucagon and its role in metabolism

2.3.1. Glucagon structure and its role in metabolism

Glucagon is a polypeptide hormone-containing 29 amino acids (Fig 8). It has a molecular weight of 3485 Daltons [84] and is 4.8 nm in length [85]. Glucagon is mainly produced in α cells of the islets of the Langerhans with additional production in small and big intestine L-cells [86]. Glucagon is a counterregulatory hormone that is produced and secreted in response to hypoglycaemia. Upon production, glucagon binds to its receptors in the liver, hence stimulating hepatic glycogenolysis and gluconeogenesis, enhancing the hepatic output of glucose and subsequently increasing the circulating glucose levels [87]. However, during normoglycaemia, increased glucagon level does not necessarily increase BG level [87]. On the contrary, glucagon deficiency in mice does not lead to hypoglycaemia [88]. Glucagon is an essential hormone in amino acid homeostasis by stimulating hepatic amino acid breakdown [87]; and lipid metabolism, where glucagon activates lipolysis and inhibits lipid synthesis [89].

Figure 8. Human glucagon structure. Schematic drawing made by Ilze Dirnena-Fusini.
Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Gln, glutamine; Gly, glycine; His, histidine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

2.3.2. Glucagon biogenesis

Glucagon processing from proglucagon to glucagon differs between the pancreas and intestines. In pancreatic α cells, mainly glucagon is produced but with two additional glucagon-like peptides (GLP) (GLP-1 and GLP-2). In the intestinal L-cells of the mucosa, mainly GLP-1 and GLP-2 are produced with an additional glucagon production [86]. Neurons in the brain stem cells and hypothalamus also synthesize a small amount of glucagon [90].

Proglucagon protein hormones are first sorted into specific intracellular (IC) compartments with consecutive sorting, processing, and storage of peptide hormones. Peptide hormones first undergo formation into prohormones and are selectively targeted to the regulated secretory pathway via the TGN. There, similar to insulin formation, prohormone glucagon is sorted and packaged into budding immature secretory granules. Prohormones then undergo endoproteolysis to form their constituent peptide hormones, which are then sorted into mature, dense-core secretory granules until exocytosis [91].

2.3.3. Glucagon secretion

Glucagon is secreted in response to various metabolic changes such as a decrease in BG levels in combination with other paracrine factors [92, 93]; increase in certain amino acids, such as arginine and alanine [94], increase in gastrointestinal peptides, such as ghrelin and oxyntomodulin [95, 96], and stimulation of sympathetic nervous system such as stress (Fig 9) [97, 98].

In healthy individuals without DM1, glucagon secretion is in the picomolar range, and glucagon concentration can vary from 5 pmol/L during the OGTT, 10 pmol/L during the fasting state till 20 pmol/L during the meal tolerance test to keep BG levels in the normal range (3.5 – 7.5 mmol/L) [99]. This thesis will shortly look into some of the glucose-dependent glucagon secretions.

The α and β cells contain ATP-sensitive potassium (K_{APT}) channels, which signalize variation in the extracellular glucose concentration due to changes in the membrane potential [100]. These changes in the membrane potential inhibit glucagon production and stimulate insulin production and *vice versa* [101]. During hypoglycaemia, the intracellular glucose level decreases with a subsequent reduction in glycolysis-produced ATP in the cell's mitochondria. [102]. The decreased level of ATP closes K_{ATP} channels, and the intracellular K^+ concentration increases, which depolarizes the cell membrane, and thereby opens voltage dependent Ca^{2+} channels allowing an influx of Ca^{2+}. Increase in intracellular Ca^{2+} concentration triggers secretion of glucagon via exocytosis (Fig 10) [103].

Glucagon production is inhibited by β cell produced amylin, γ-aminobutyric acid, and zinc. Delta cell produced somatostatin also inhibits glucagon production. A high concentration of glucagon is a glucagon production inhibitor by itself, as well as fatty acids and glucose inhibit glucagon production [97].

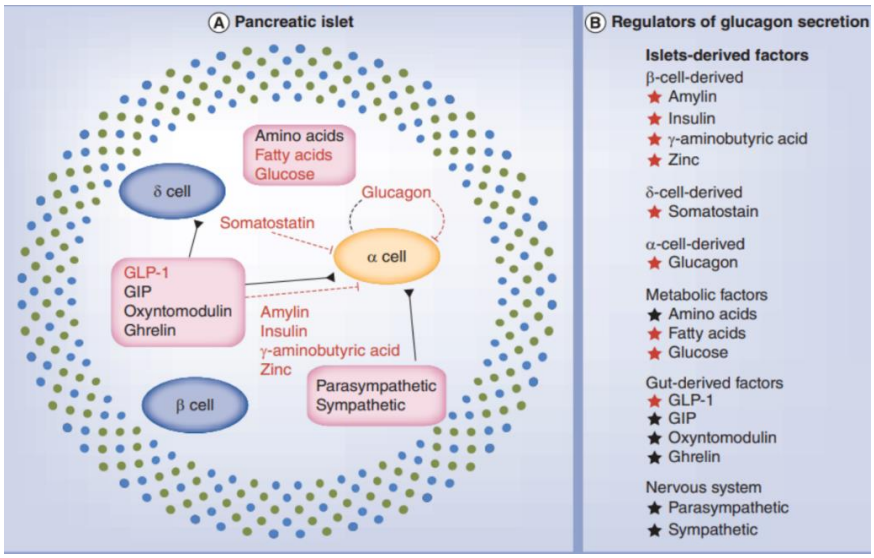


Figure 9. Regulation of glucagon secretion. Glucagon secretion inhibitors (red, red star), glucagon production stimulators (black, black star). Reused without modifications from Wewer Albrechtsen et al. [97]. With permission from Future Medicine Ltd. Abbreviations: GLP1, glucagon-like peptide 1; GIP, gastric inhibitory polypeptide (glucose-dependant insulinotropic polypeptide).

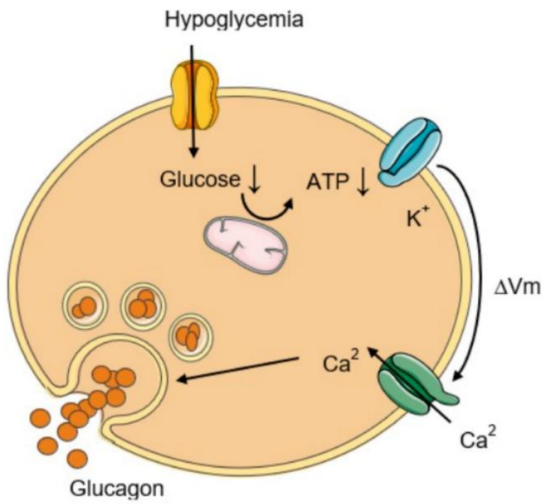


Figure 10. Glucagon secretion. During hypoglycaemia intracellular glucose concentration decreases, reducing ATP levels in the cell, this closed K_{ATP} channels and IC K+ concentration increase which depolarizes the cell membrane and opens Ca^{2+} channels increasing Ca^{2+} levels in the cell which activates glucagon secretion.

 Legends: delta Vm, change in membrane potential.

2.3.4. Glucagon receptor and signalling pathways

Glucagon receptors (GCGRs) are membrane proteins that contain an extracellular and an intracellular part. When glucagon binds to the extracellular part of the receptor, conformational changes in the receptor occurs and causes activation of a G-protein. That causes the G-protein to attach to the intracellular part of the receptor [104, 105]. GCGRs are expressed in several tissues but mainly in the liver and kidneys [105].

GCGRs are most important in the activation of the gluconeogenesis pathway. For example, GCGR knockout mice are resistant to diet-induced obesity, and after the destruction of β cells, they are also resistant to hyperglycaemia [106].

Signalling pathways

The binding of glucagon triggers conformational changes in the GCGR leading to an intermediate state of the protein. The combined action of the peptide and heterotrimeric G-proteins (Gnas) rearranges the extracellular and intracellular subunit and connections and activates the GCGR [107]. Gnas signalling activates adenylyl cyclases (Adc), resulting in the production of cAMP and subsequent activation of the protein kinase A (PKA) pathway. PKA inhibits glycolysis and glycogen biosynthetic pathways. PKA activates gluconeogenesis and glycogen degradation pathways and activate gluconeogenic genes expression via phosphorylation (Fig 11) [105].

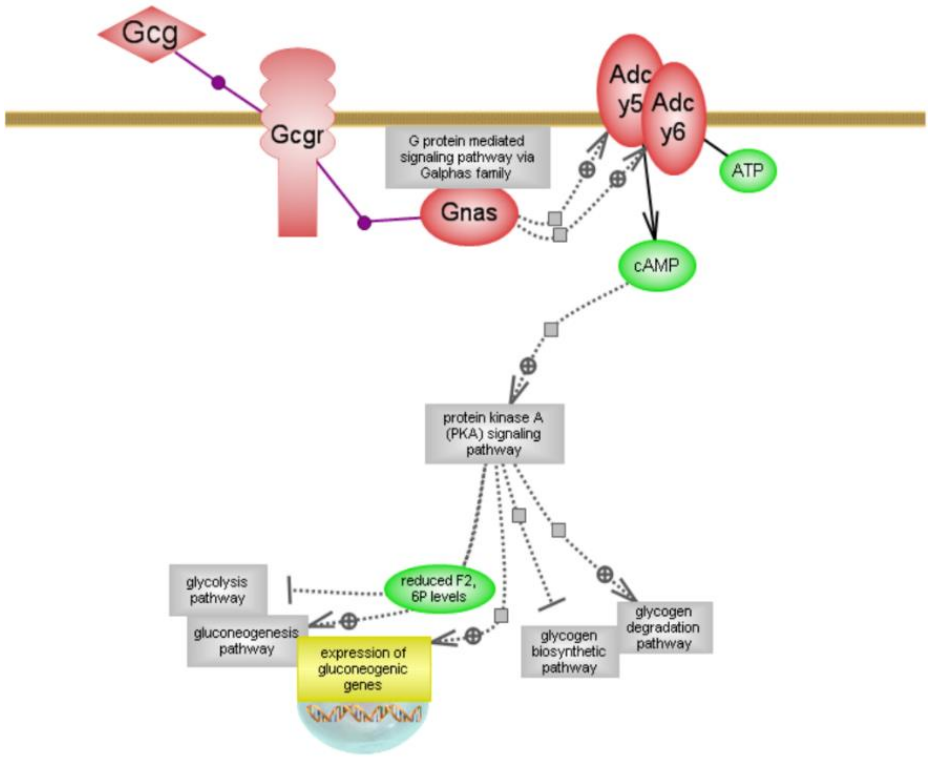


Figure 11. Glucagon signalling pathway. Binding of glucagon (Gcg) triggers conformational changes in the glucagon receptor (Gcgr) leading to activation of G proteins (Gnas) alpha subunit. Gnas signalling activates adenylyl cyclases (Adc) resulting in the production of cAMP and subsequent activation of protein kinase A (PKA) pathway. PKA inhibits glycolysis and glycogen biosynthetic pathways and activates gluconeogenesis and glycogen degradation pathways, and activates gluconeogenic genes expression. Diagram from the Rat Genome Database (https://rgd.mcw.edu), generated using Elsevier/Ariadne Pathway Studio software, used with RGD permission [105]. No changes were made. Legends: binding (purple line and circle); regulation (grey square); direct regulation (grey circle with cross); extracellular proteins (red ellipse); small molecules (green ellipse); ligands (red rhombus); pathway (grey rectangle).

2.3.5. Glucagon administration

Exogenous glucagon administration can be made via intranasal administration in powder, IV infusion, IM, or SC injections [108]. In general, IV infusion, IM or SC glucagon injections are used in case of mild-to-moderate and severe hypoglycaemia. Intranasal glucagon can be used to treat severe hypoglycaemia with a similar effect as IM injection and also without serious side effects [109, 110]. However, all glucagon formulations may cause side effects such as nausea, vomiting, headaches, discomfort at the administration site (nasal discomfort, injection place reaction) [111]. A recent study in patients with postbariatric hypoglycaemia, showed that a closed-loop SC glucagon system with additional CGM does not produce rebound hyperglycaemia after delivery of up to 2 doses of glucagon (300/150 µg) [112]. A bi-hormonal closed-loop SC system using a liquid stable glucagon and insulin

demonstrated reduced hypoglycaemia during and after exercises compared to single closed-loop insulin injection, however, time spent in target range (3.9 – 10.0 mmol/L) was not different. Moreover, time in hyperglycaemia was increased compared to a single hormone closed-loop SC insulin system (28 % vs 25 %) [113].

A challenge for glucagon injection or infusion is glucagon`s poor dissolving properties in pH neutral solutions [114]. In acidic pH solutions (pH 2.5 – 5), glucagon encounters physical and chemical degradation from where peptide cleavage occurs on the C terminal part of aspartic acid (Asp_{21}, Asp_{15} and Asp_{9}). Deamination can form other amino acids. For example, glutamine (Gln_{24}) can convert to glutamic acid (Glu_{24}) [115]. In basic pH (pH 9 and 10), glucagon solutions, deamination can be observed as well as isomerisation and oxidation of amino acids [116].

For medical injections (as glucagon is preserved in a solid powder state), glucagon needs to be dissolved in pH from 6.5 to 9.0 – 10.0; unfortunately, the chemical stability is reduced [114]. The solubility of the glucagon in the pH neutral solutions can be increased by lowering the isoelectric point of the hormone via replacing asparagine-28 with aspartic acid or making modifications in the C-terminal part of the molecule [114].

A standard glucagon kit contains a syringe with dilute acid or sterile water and 1 mg glucagon. Its injection should be immediately after the combination of the powder and the dilute. Nevertheless, its high concentration in this standard kit carries a risk of persistent post-injection hyperglycaemia. Mini-dose glucagon (150 µg) can prevent hypoglycaemia during intense physical activities without any subsequent major hyperglycaemia [117]. Therefore, preferably a tailored glucagon dose, based on individual requirements, should be used.

2.4. Glucose – regulator of cellular mechanisms

Blood glucose concentration is strongly linked to the grade of secretion of insulin and glucagon. Glucose enters the β and α cells through the glucose transporter type 1 (GLUT1) [118], and in the mitochondria, in the presence of oxygen, it is converted to APT, CO_2 and water (Fig 12). During hyperglycaemia, intracellular levels of ATP increases while levels of ADP decreases. In the β cells, an increase of APT closes K_{APT} channels. In a chain reaction, the cell membrane is depolarized, and Ca^{2+} channels open. Thus, ultimately stimulating insulin exocytosis [119].

In contrast to the β cells, the α cells require lower APT concentration for closure of K_{APT} channels and opening of Ca^{2+} channels and glucagon release [100]. During hyperglycaemia,

K_{APT} channels depolarize the membrane to a point where Ca^{2+} and Na^{+} channels are inactive [120].

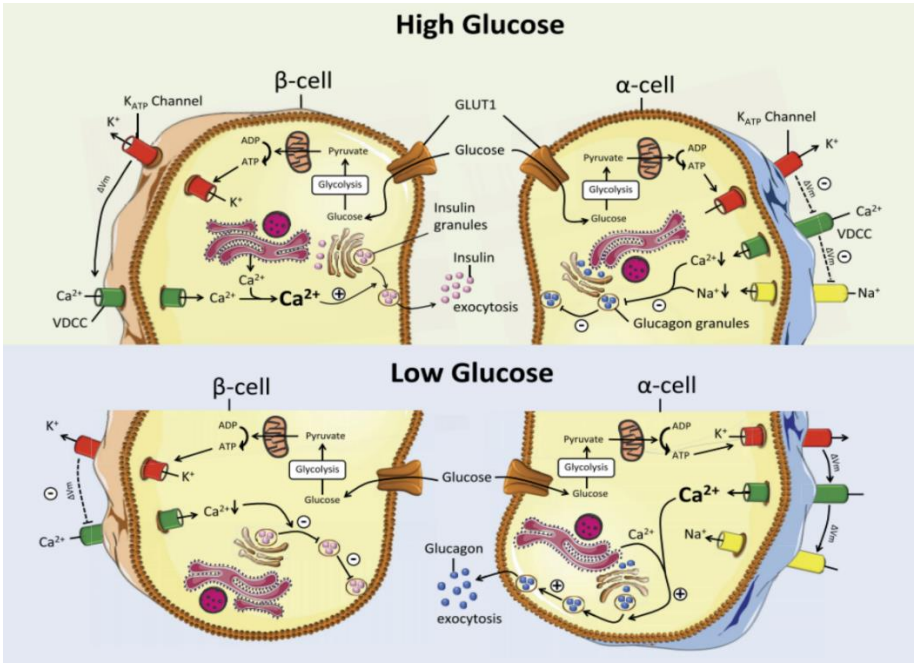


Figure 12. Schematic on the glucose-dependent regulation (high glucose levels and low glucose levels) of glucagon (α cell) and insulin secretion (β cell). Both regulations are affected by glucose levels and Ca^{2+} levels, as well as K^{+} and Na^{+} molecular volume. Reused without modifications from Müller et al. [119]. This figure is licensed under an Elsevier and Copyright Clearance Center.

2.5. Somatostatin and its role in metabolism

Somatostatin (SST) is a regulatory peptide hormone that consists of at least two bioactive forms, SST-14 and SST-28 (numbers represent amino acids in the peptide). SST is produced from neuroendocrine, inflammatory, and immune cells, mainly in gastrointestinal D cells, hypothalamus and endocrine pancreas. SST-14 and SST-28 are produced in variable amounts due to different processing from a common precursor. SST-14 predominates in the pancreatic islets and stomach [121], while SST-28 predominates in intestinal mucosa cells [122].

SST is an inhibitory peptide hormone. It regulates neurotransmission in the brain by activating SST receptors which results in an inhibition of adenylate cyclase enzyme activity, reduction in intracellular Ca^{2+} levels, and hyperpolarization of cells by inducing outward K^{+} currents. SST inhibit the secretion of growth hormone (GH), thyroid-stimulating hormone (TSH) [123], GI hormones, such as gastrin, histamine, and acid secretion from parietal cells

[124], and more importantly, for the thesis, pancreatic enzymes, such as insulin and glucagon [125].

SST inhibits the release of virtually every gut hormone that has been tested. In general, it inhibits gut exocrine secretion, i.e., gastric acid, pepsin, bile, colonic fluid, and suppresses motor activity in the GI tract [126]. On the contrary, it stimulates migrating motor complex activity [126]. SST-14 is more potent in inhibiting glucagon release, while SST-28 preferably inhibits insulin release [127].

2.5.1. Somatostatin secretion

Pancreatic somatostatin secretion is induced by membrane depolarisation [126]. Nutrients exert tissue-specific effects on pancreatic SST release, for example, stimulated by BG level increase via membrane potential-dependent and independent pathways [128]. Gut SST secretion is stimulated by luminal but not by circulating nutrients (circulating BG levels) [129]. Glucagon is one of the potent stimulators of SST release from cells [129]. Insulin, however, stimulates hypothalamic SST release but has an inhibitory effect on the release of the islet and gut SST [126]. In rat study, measured SST-like immunoreactivity was 65 % in the gut, 25 % in the brain, 5 % in the pancreas and 5 % in the remaining organs [130].

2.5.2. Somatostatin receptors

Somatostatin receptors (SSTRs) contain seven transmembrane domain G-protein-coupled receptors that comprise five subtypes (SST 1 – 5) with isoforms SSTR 2a and SSTR 2b in humans [131]. The five receptor subtypes bind the natural SST peptides – SST 14 and SST 28. Somatostatin analogue (SSA) octreotide bind well to three of the subtypes – 2, 3 and 5 [126], while pasireotide bind to four of the subtypes – 1, 2, 3 and 5 [132]. SSTRs are expressed in many tissues, frequently as multiple subtypes in the same cells [126].

Some of the subtypes are coupled to inward rectifying K^+ channels (SSTR 2, 3, 4 and 5), to voltage-dependent Ca^{2+} channels (SSTR 1 and 2a [133]), and Na^+/H^+ exchange channels (SSTR 1) [126]. Activated SST 2 and SST 5 receptor combinations inhibit insulin synthesis, and SST 1 and SST 2 receptor combinations inhibit glucagon synthesis [134]. Activated SSTRs blocks cell secretion by inhibiting intracellular cAMP and Ca^{2+} channels [126]. However, cAMP is a potent activator of the secretion of STT [135]. Thus, SSA works by blocking the secretion and release of hormones instead of inhibiting the action of hormones [136].

In islet cells, receptors SST 1 and 5 are strongly expressed in β cells, with lower expression of SSTR 2 and minimal expression of SSTR 3 and 4. SSTR 2 is strongly expressed in α cells,

with minimal expression of other SST receptors. SSTR 5 is strongly expressed in δ cells, with minimal expression of other SST receptors [137].

2.5.3. Somatostatin analogues

SST is secreted locally to the binding site with a short half-life; thus, decrease unnecessary systemic effects [126]. When injected IM or SC, SST analogues need to have a property of long-acting release (LAR) for clinical purposes. However, as SSTRs are located on different cells, including lymph nodes, tonsils, appendix, spleen, and thymus [138], SST analogues affect various aspects of the metabolism before reaching the target cells (in this case, α and β cells). However, SSTRs are differentially sensitive to the SST analogues [127]. Due to somatostatins wide variety of inhibition of secretory properties, its analogues are used in diverse clinical situations. Octreotide [139] and lanreotide [140] are used in patients with neuroendocrine tumours as these tumours have an increased amount of somatostatin receptors, i.e., STTR 2a in meningioma [141], and their metastases are somatostatin receptor-positive. Thus, SST analogues suppress the growth rate of tumorous cells [142]. Octreotide and pasireotide are used in the treatment of patients with acromegaly [143].

Another ability of somatostatin analogues is their inhibition of pancreatic enzyme secretion [136]. Accordingly, octreotide and pasireotide are widely used in scientific experiments as insulin suppressive drugs, because pasireotide has a high binding affinity for SSTR 2, 3 and 5 [144]. Octreotide has a strong glucagon secretion inhibitory effect that is not observed during the use of pasireotide [144].

2.6. Diabetes mellitus

The first description of diabetes is cited back in 1552 BC, while the first connection between diabetes and the pancreas was made as late as in the 1870s [31]. Later on, different types of diabetes have been identified.

Based on clinical care, there are four main types of diabetes:

Diabetes mellitus type 1 (DM1), that is a chronic autoimmune disease characterised by insulin deficiency following hyperglycaemia.

Diabetes mellitus type 2 (DM2), when insulin is produced, but the effect on glucose homeostasis is reduced (insulin resistance), or insulin production is decreased [18]. DM2 comprises 90% of people with diabetes around the world and is to a substantial degree related to physical inactivity and excess body weight [3], and a high carbohydrate diet.

Gestational diabetes mellitus (GDM) can occur during pregnancy when some of the produced hormones show a blocking effect on insulin and increase insulin resistance [6]. This type of DM is a temporary condition that resolves after pregnancy.

Maturity-onset diabetes of the young (MODY) is diabetes caused by a monogenic disorder with a mutation in the autosomal dominant gene disrupting insulin production [145].

The thesis is focused only on patients with DM1 and its current treatment methods.

2.6.1. Diabetes mellitus type 1

DM1 patients suffer from an auto-immune disorder that destroys the pancreas β cells, and the patients are subsequently depleted of insulin, a hormone essential for the regulation of BG levels [146]. Typical symptoms of DM1 are the so-called *3-P*: Polyuria (excessive micturition), Polydipsia (excessive thirst) and Polyphagia (excessive appetite). When a severe insulin deficiency is present, the assimilation and storage of glucose in muscle, adipose tissues, and the liver are extremely diminished. Non-existing glycogenesis induces an accumulation of glucose in the blood, and, consequently, the extracellular osmolarity increases [147]. In response to this osmotic pressure, intracellular water is depleted, and osmotic diuresis is promoted [148]. In the end, if untreated, life-threatening diabetic ketoacidosis will develop [149]. Therefore, it is crucial to diagnose DM1 and start treatment (insulin administration) instantly.

DM1 is a lifelong disease, with additional possible complications such as microvascular diseases, nephropathy [150], retinopathy [151], coronary artery diseases [152], tissue stiffness [153], and increased mental stress [154].

One of the crucial goals is to stabilize and maintain normal range BG levels. The most used method for self-monitoring is capillary blood testing of blood glucose (SMBG). In recent times, continuous glucose monitoring (CGM) or flash glucose monitoring has become available for adolescent [155] and adult [156] patient's use in addition to or instead of SMBG [157]. These last generation CGM systems are less painful and provide continuous real-time information on actual BG levels.

2.6.2. Glycaemic control and complications

In general, glucose is the only fuel for the brain and red blood cells. Other tissues are able to utilise other fuel sources. However, reduced or elevated BG levels can create permanent tissue damage. Therefore, to keep glucose levels in the desired normal range during the day (3.9 – 7.5 mmol/L) is crucial to avoid long term adverse effects of DM1 [158].

Sudden BG level elevation, e.g. during oral glucose load (meal), IV glucose administration, or intraportal glucose administration, activates hepatic glycogen synthesis (HGS) [159]. The most effective stimulation of HGS is by oral glucose administration because the flow in the portal and hepatic vein do only increase after oral glucose administration [160]. Thus, the

most appropriate test to measure body response to increased BG levels is an oral glucose tolerance test (OGTT).

In DM1, BG levels are not automatically corrected via a physiological release of endogenous insulin from the pancreas, and BG levels increase and do not return to the basal levels after 2 hours. In patients with DM1, endogenous insulin production is missing, while hepatic glucose production and release are present, which leads to hyperglycaemia [68]. Thus, additional BG level measurements are compulsory, and additional exogenous insulin administration necessary for correction. Considering necessary manipulations, maintaining constant BG level is challenging.

Hyperglycaemia, diabetic ketoacidosis

Hyperglycaemia with BG levels above 10 mmol/L two hours after a meal, over time, can induce a toxic effect on β cells and produce free oxygen radicals (FOR). Prolonged hyperglycaemia can, by increased levels of FOR, induce damage in inside organs, such as the liver, kidneys, and brain, and can increase cardiovascular and neurological complications [150, 161].

In the absence of insulin in DM1 patients, glucose levels will increase and lead to diabetic ketoacidosis (DKA) within a few hours [162]. DKA can be predeceased by insulin pump failure of any situation with under-delivery of insulin [163] or illicit drug use or increased need of insulin caused by infection [164]. New onset and yet undiagnosed DM1 is also a classic cause of DKA [165].

DKA can be diagnosed by three criteria: D – high glucose concentration (BG > 11 mmol/L); K – the presence of ketones in serum or urine; and A – confirmation of acidosis (pH < 7.3, and serum bicarbonate > 10 (not measured in all countries) [162]. Untreated DKA can lead to hypokalaemia [165], cerebral oedema [166], pulmonary oedema [167] or damage of organs due to loss of fluids [168]. If untreated, cardiac arrest and death from DKA are inevitable.

Sometimes in DM1 patients, but more common in patients with DM2, high BG levels can induce a hyperglycaemic hyperosmolar state (HHS) with severe hyperglycaemia and mild or no ketosis [169]. HHS induces glycosuria, resulting in hyperosmolarity and dehydration, and can cause diabetic coma and death [170].

Hypoglycaemia

A common definition of hypoglycaemia is not established as in most cases, self-monitored hypoglycaemia is in general obtained based on symptoms [171]. This late observation can, in a short time, lead to severe hypoglycaemia [172].

Hypoglycaemia with BG level < 3.9 mmol/L is a common and a potential side effect of intensive treatment of DM1 in order to achieve the best possible glucose control. It has been estimated that 4 % to 10 % of deaths of patients with DM1 are associated with hypoglycaemia [173]. In one six-day study, three-quarters of the participants (n = 153) experienced asymptomatic hypoglycaemic events due to reduced hypoglycaemia awareness [174]. Signs of hypoglycaemia can be neuroglycopenic symptoms, such as concentration problems, confusion, sweating; or adrenergic symptoms, such as shakiness, fast heartbeats, hunger, dizziness, anxiety or nervousness or headache, irritability or moodiness [175].

Severe hypoglycaemia

Severe hypoglycaemia is when a person cannot take care of themselves due to the low BG level, and the help of a third person is needed. If not attained, it can develop into a hypoglycaemic coma, cardiac arrest and death [172]. Also, severe hypoglycaemia increases the risk of cardiovascular diseases [176] and all-cause mortality, especially during subsequent months after the event [177]. Noteworthy is that predictive factors of severe hypoglycaemia are decreased HbA1c in the last three months and prior severe hypoglycaemia episodes [178]. Common signs of severe hypoglycaemia are muscle weakness, clumsiness, inability to eat or drink, slurred speech, confusion, drowsiness, convulsion, unconsciousness or death [175], and hypothermia [179].

2.6.3. Exogenous insulin and analogues

History

The administration of insulin as a treatment of diabetic started on January 23, 1922, when in Canada Frederick Banting and Charles Best, after intense experimental work and purification of insulin, injected bovine insulin from pancreatic extractions in a 14-year-old boy close to dying from diabetes in a Toronto hospital. After multiple daily injections, BG level decreased, and glycosuria was reduced. In April 1922, the Toronto team published the paper and gave the name ‘insulin’ to the substrate that was injected [180].

On August 19, 1922, Eli Lilly and Co started the first shipments of extracts of pork pancreases. With the permission of the University of Toronto, by the end of 1923, the non-profit Nordisk Insulin laboratory was in the production of insulin [181]. From 1922 to 1972, the only available insulins were purified from porcine and bovine pancreases [180]. However, during the same period, insulin administration was in fast development. In 1949 the first standardized insulin syringes became available, and in 1963 the first insulin pumps were introduced.

In 1975, by using recombinant DNA technique, human insulin was produced by bacteria [31]. With genetic engineering, it became possible to produce human insulin analogues instead of insulin produced or extracted from other species. After these breakthroughs, exogenous insulin analogue production is expanded by producing different acting insulin analogues (Table 2).

Insulin	Onset	Peak (h)	Duration (h)
Rapid-acting			
Regular, human	30 – 60 min	2 – 4	8 – 10
Semilente	30 – 60 min	4 – 6	8 – 12
Lispro (Humalog)	5 – 15 min	1 – 2	4 – 6
Rapid-acting analogues			
Aspart (Novolog)	5 – 15 min	1 – 2	4 – 6
Glulisine	5 – 15 min	90 min – 2 h	~8
Intermediate-acting			
Lente	2 – 4 h	6 – 10	12 – 24
Isophane/NPH	1 – 2 h	4 – 8	10 – 20
Long-acting			
Ultralente	2 – 4 h	Unpredictable	16 – 36
Protamine zinc	3 – 4 h	14 – 20	24 – 36
Long-acting analogues			
Glargine	1 – 2 h	No pronounced peak	~24
Detemir	2 h	No pronounced peak	20 for dose > 0.4 U/kg
Ultralong-acting analogues			
Degludec	30 – 90 min	No pronounced peak	~24
Premixed human insulin			
NPH/R 70/30	30 – 60 min	2 – 12	24
NPH/R 50/50	30 – 60 min	2 – 12	24
Premixed analogues			
Protamine aspart/aspart 70/30	15 min	1 – 3	24
Protamine lispro/lispro 75/25	15 min	0.5 – 1.5	24

Table 2. Pharmacokinetics of available insulins. For use in CSII systems or SC injections. Adapted from Davidson JA et al. 2004, with permission from Taylor & Francis and Copyright Clearance Center's Rights Link service. With additional information from Hilgenfeld et al. [182]. Abbreviations: NPH, Neutral protamine Hagedorn; R, regular.

Insulin analogues

Regarding exogenous insulin administration in patients with DM1, it is essential to understand endogenous insulin secretion, explained in section 2.2. Ideally, exogenous insulin analogues should mimic physiological endogenous insulin secretion. Essential amino acids that are critical for the stability and functionality of insulin were mentioned in section 2.2.3. Amino acids B_{26} – B_{30} are not particularly crucial to the insulin structure and are not particularly essential for IR recognition and binding to it. Thus, they can be used for structural modification to change insulin's pharmacokinetics and pharmacodynamics (Table 2, Fig 13, Fig 14) [40]. The downside is that amino acid region B_{26} – B_{32} in the insulin molecule is vital for IGF-1R binding [183].

As demonstrated in Table 4, there are significant pharmacokinetic differences between available insulins. In general, rapid and long-acting insulins are used together: rapid-acting insulin (RAI) is preferably provided before meals, while long-acting insulin covers a continuous basal rate of insulin. Regular insulin can increase hypoglycaemic events due to late peak time (2 – 4 hours) [184]. However, all pharmacokinetics are measurements from SC insulin administration and do not imply information about pharmacokinetics from IP insulin administration. We set out to confirm the pharmacokinetics of IP delivered insulin in one of the papers included in the thesis (**Paper II**).

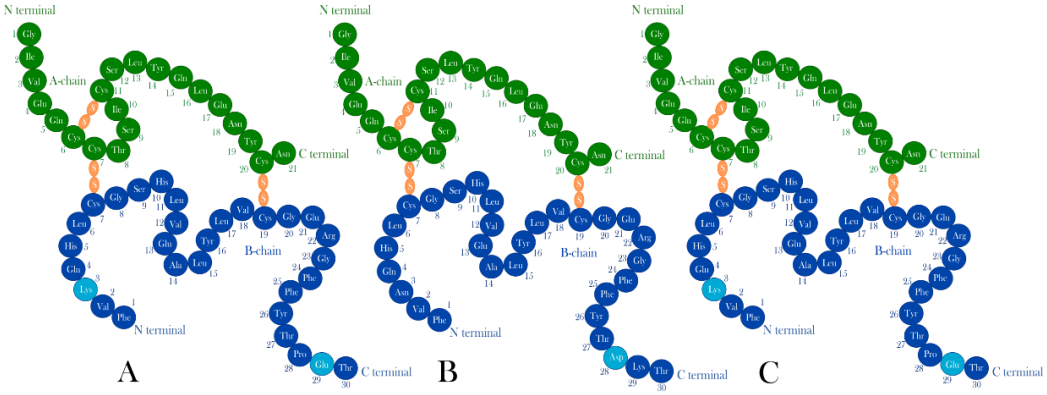


Figure 13. Fast-acting insulin analogues. Lispro (A), aspart (B), glulisine (C) structure. Schematic drawings made by Ilze Dirnena-Fusini. Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

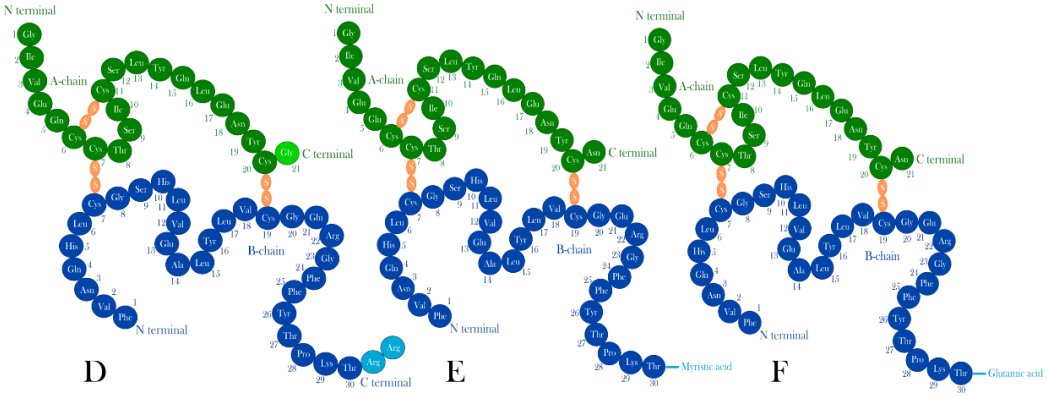


Figure 14. Long-acting insulin analogues. Glargine (D), detemir (E), degludec (F) structure. Myristic acid – a saturated long-chain fatty acid with a 14-carbon backbone. Schematic drawings made by Ilze Dirnena-Fusini. Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

2.6.4. Insulin administration

Intravenous

In case of hyperglycaemia, as an acute treatment for ketoacidosis, insulin administration can be administered intravenously to reassure its direct bioavailability in an urgent insulin depleted clinical situation [185]. However, intravenous (IV) insulin administration is too complicated on a daily basis and is not used for the long-term treatment of patients with DM1.

Intramuscular

Intramuscular (IM) insulin administration can be used as an alternative if other insulin administration routes are not effective [186]. IM insulin administration is associated with increased pain during the administration [187]. However, one study found that IM insulin injection can lead to faster insulin absorption without increased pain level [188]. It can be speculated that some patients with DM1 and low body mass index (BMI) occasionally inject their insulin (via insulin pen or syringe) IM and not into the SC tissues [189].

Subcutaneous

The SC insulin administrations are widely used and by far the most commonly practised administration route, with administration in the abdomen, upper arm and thigh [189]. There are two ways to administer insulin via the SC route:

1) By multiple daily (SC) injections (MDI) using syringes or insulin pens.

2) By continuous SC insulin infusion (CSII) by an insulin pump (open-loop insulin delivery system) or an automated closed-loop insulin delivery system (based on SC glucose sensing and SC insulin delivery).

CSII has a significant advantage compared to MDI regarding a lower HbA1c and a lower risk of severe hypoglycaemia [190]. Insulin pump systems provide accurate insulin delivery with more flexible insulin administration than syringes and insulin pens [191].

Many patients using CSII calculate carbohydrates that will be consumed during the next meal and subsequently calculate the next insulin dose to be administrated via an insulin pump. Thus, insulin dose is tailored according to patient input. CSII is mainly used in developed countries due to the costs of maintenance and regular change of insulin catheters [156]. Rapid insulin pump development and supportive government actions worldwide increase the opportunity to provide better healthcare for children [155] and adults with DM1 [156].

The goal of an automated closed-loop insulin delivery system, known as a fully automated AP, is to mimic endogenous insulin secretion and to achieve and maintain non-diabetic BG levels (3.9 – 7.5 mmol/L). Currently, hybrid closed-loop controllers with wireless SC glucose sensors and insulin pumps are available (Fig 15). The challenge with this concept for an AP is that patients need to count carbohydrates and provide input regarding planned specific activities [192]. Fully automated APs are automated insulin delivery systems that calculate insulin dose based on received data from CGM and adjust basal insulin infusion rate accordingly via a specific algorithm for patients with DM1. A closed-loop system has advantages over an open-loop system by increasing BG time spend in the target range [29, 193]. However, at present, the target range BG levels in APs is far broader than in non-diabetics – from 3.9 to 10.0 mmol/L [194].

CSII carries a slight but relevant risk of ketoacidosis, mainly due to malfunction of insulin pump and/or catheter occlusion [195]. In recent years, the technology of insulin pumps and infusion sets has improved [196]. However, after SC insulin administration, insulin forms depots in the SC tissues [197] and increase insulin molecule aggregation. This influences the rate of insulin absorption with a day-to-day variation in the same subject and variation between patients. In cases of high volumes of boluses, administration can be painful [198]. The major disadvantage of SC insulin administration is the slow and sometimes unpredictable glucose-lowering effect of insulin due to inconsistent pharmacokinetics [199].

In summary, pharmacokinetics and pharmacodynamics of SC insulin delivery are affected by volume, concentration and additives of administered insulin solution, as well as differences in anatomical regions, tissue locations and local factors at the site of injection (see section 2.6.5.).

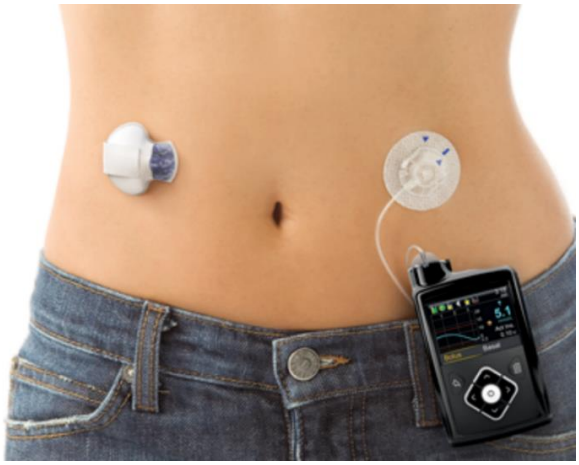


Figure 15. CSII system and CGM. Insulin pump (this particular is MINIMED® 640G) is wirelessly connected to CGM; thus, helps to ensure more accurate dosing by calculating dose based on circulating insulin levels, blood glucose levels, planned meal and personal insulin settings. Picture is reused from Medtronic website: https://www.medtronic-diabetes-mena.com/en/life-with-diabetes/managing-hypoglycaemia/insulin-pump-potential-solution with permission from Medtronic.

Intraperitoneal

The continuous IP insulin infusion (CIPII) is mainly used in patients with brittle DM1 if SC insulin administration does not improve and stabilise BG levels. It is also the therapy of choice in DM1 patients with severe SC insulin resistance.

Two ways for the location of the insulin pump system have been used:

1) Externally attached insulin pump with a catheter inserted via an abdominal port into the IP space for insulin delivery.

2) Implantable pump system inserted into a pocket of tissue directly under the skin and delivers insulin via an attached catheter into the IP space (Fig 16, Fig 17).

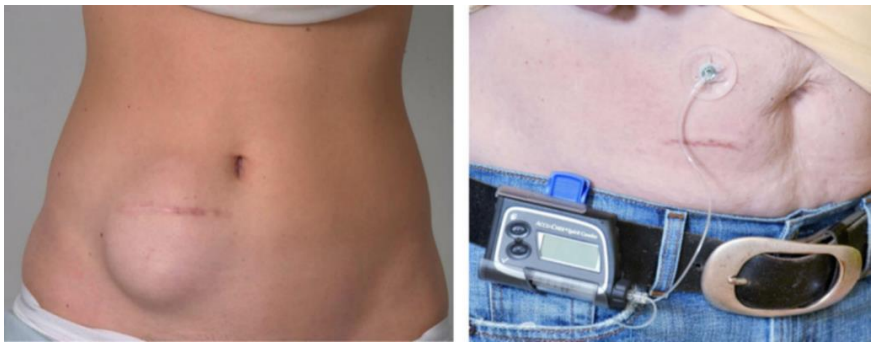


Figure 16. CIPII using an implantable pump (left) and the externally attached CIPII system (right). Figure reused without modifications from van Dijk et al. [200].

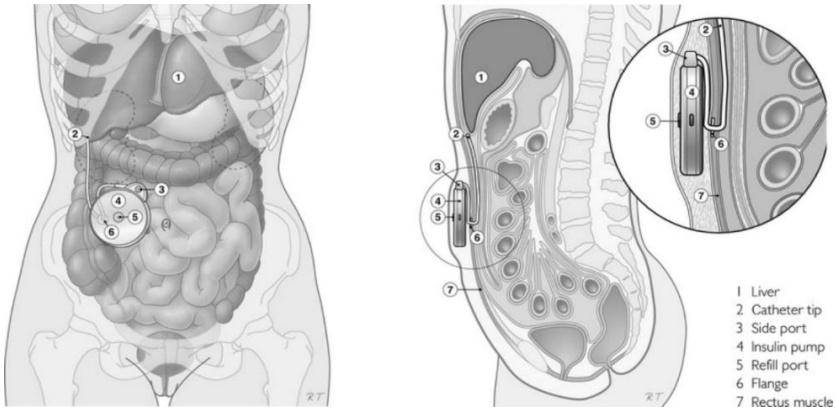


Figure 17. Illustration of the implantable pump system with catheter inserted into the IP space. Figure reused without modifications from van Dijk et al. [200].

IP insulin infusion provides many benefits compared to SC insulin infusion. For example, after a change of insulin type and infusion route, closed-loop fully automated IP AP (Insuman, regular U 100 insulin) increases time spent in the BG target range compared to closed-loop SC AP system (Humalog, RAI analogue) [201]. The use of two different types of insulins can produce a bias compared to two insulin administration systems. Because, after regular insulin is administered subcutaneously, post-prandial BG levels and HbA1c levels are higher compared with the use of RAI analogue [202].

With the CIPII system, insulin is infused in the IP space, where it is absorbed via the capillaries of the visceral peritoneum into the portal vein [203, 204]. IP insulin pharmacokinetics depend on the volume, concentration of the dose, and injection time [205]. In one study, the insulin peak into the portal vein was measured within 20 minutes after administration [204]. Another study measured insulin peak into the systemic circulation within 30 minutes after insulin bolus (1.92 U, 3 mL) and within 40 minutes after smaller volume (1.92 U, 0.6 mL) insulin bolus [205]. After insulin infusion over 30 minutes (1.92 U, 3 mL), the insulin peak into systemic circulation was reached at 50 minutes [205]. Due to the absorption into the portal vein, there is a higher uptake into the liver and higher first-pass liver insulin extraction. Thus, lower systemic insulin levels are reached compared to the SC insulin administration [203, 206].

Intraperitoneal insulin administration is an invasive treatment, and the risk of obtaining infection is significant, most likely due to skin microflora [207]. In the last decade, due to improved technologies and clinical equipment, a reduction in complications during CIPII treatment has been achieved; the most common complications are catheter occlusion, pump dysfunction, pain at the pump site and infection [208].

2.6.5. SC Insulin absorption

As discussed in the previous section, the most common route for insulin delivery is the SC route, where insulin is injected or infused through the skin into the SC layer.

The skin is made of three layers: epidermis, dermis and hypodermis (SC fat layer) [209].

The epidermis (transdermal layer), the site for SC insulin delivery, contains four cell-type layers:

1) Squamous cells that constitute the outermost layer, also called a stratum corneum (keratin layer). It serves as a protective outer layer.

2) Stratum granulosum, a granular cell layer that contains several layers of lipid-rich granule cells. In this layer, cells become mitotically inactive as they lose their nuclei before they enter the stratum corneum.

3) Stratum spinosum, the thickest layer of all epidermis. It contains several layers of keratinocyte cells connected by desmosomes; thus, allowing tight bounding between cells.

4) Stratum basale, basal cells that are located under the keratin layer are mitotically active, contain melanocytes, i.e., produce melatonin, and stem cells [210].

The dermis (intradermal layer) is the middle layer of the skin. It contains blood vessels, lymph vessels, hair follicles, sweat glands, collagen bundles, fibroblasts, nerves, and sebaceous glands. The dermis is supported by the protein collagen that provides flexibility and strength to the skin. The structure of the dermis offers skin support and protection of the deeper anatomical structures.

The hypodermis (SC layer) is the deepest layer of the skin. It is located between two skin layers and muscles. It consists of collagen and adipose tissue composed of fat lobules, elastin, glycosaminoglycans, and blood and lymph vessels (Figure 18).

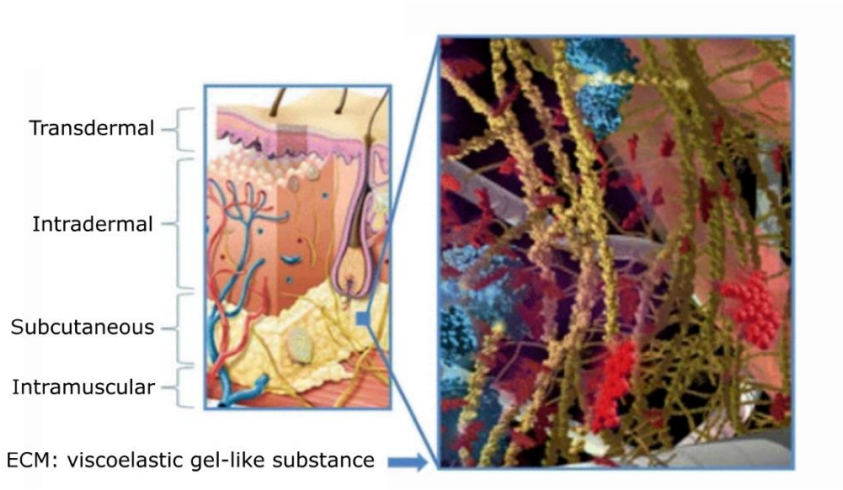


Figure 18. Human skin layers and extracellular matrix. Reused with minor modifications from Wasserman et al. [209] with permission from Springer Link. Extracellular matrix (ECM) is located in the SC tissues and is a barrier to SC insulin delivery.

An insulin SC injection can be inaccurate, as it can be injected in the deepest layers of the dermis, in the SC tissues or the muscle. Insulin absorption and activity from SC injections can be delayed due to obesity [211]. Adipose tissues are supported by collagen in the extracellular matrix (ECM). An increased amount of SC adipose tissue (SCAT) increases collagen expression and reduces the number of capillaries [212].

Insulin injected in SC tissue is distributed between the layers of the fat cells and ECM. Fat cell size vary between non-obese and obese individuals and within each individual. Obese individuals have larger fat cells than non-obese individuals. The size of the fat cells can vary in different SC fat depots [213]. The fluid between fat cells is 10 % of the volume of the SC tissue [214]. Injected insulin does not necessarily spread isotropically in the fat tissue. However, in most cases, it is a preferred direction. Insulin distribution varies from time to time, and as cellular density is similar in the dermis and SC tissues, it can spread in the dermis as well; thus, increasing variability of the insulin activity [215].

Another factor influencing the SC insulin absorption is insulin oligomers injected. As mentioned before, insulin`s active form is a monomer. To increase insulin stability, insulin is injected mainly as hexamers where zinc needs to be present, as well as phenol and /or phenol-like substances [214]. When insulin is injected into SC tissue, lipophilic substances such as zinc, phenol, and phenol-like substances disperse away from insulin into the adipose tissue; thus, allowing insulin to dissociate into dimers and monomers [199].

In DM1, SC insulin delivery can be affected by various factors: SC blood flow, injection site (abdomen, arm, thigh, buttocks), administration route (SC or IM), BG levels,

lipohypertrophy, skin temperature, local degradation, local massage, diabetes comorbidities and complications (oedema), obesity (insulin resistance), exercises and activity level (exercises accelerate insulin absorption), smoking (delays absorption), and body position (sitting position delays absorption) (summarised in [199]).

Peritoneum

The peritoneum is much thinner than skin and provides better fluid movement through the tissues. More details read in section 2.7.

2.7. Peritoneal cavity

The peritoneal cavity is classified into three parts: an intraperitoneal, retroperitoneal and subperitoneal part. Organs that line the intraperitoneal cavity are usually mobile compared to the usually fixed (to the posterior abdominal wall) organs that line the retroperitoneal cavity [216]. The subperitoneal part lines all the abdominal pelvic organs and their associated vessels, lymphatics, and nerves, and it is separated from the peritoneal cavity by the peritoneum [217]. The peritoneal cavity does not contain organs and is a potential space between the visceral and parietal layers of the peritoneum [217, 218].

2.7.1. Peritoneum

2.7.1.1 Anatomy

The peritoneum is an extensive serous membrane, which is essential for maintaining intra-abdominal homeostasis and protection of the abdominal organs. The visceral peritoneum covers visceral organs, e.g. the stomach, spleen, liver, first and fourth part of duodenum, jejunum, ileum, colon transversum, and colon sigmoideum, while the parietal peritoneum lines the abdominal wall (Fig 19) [219]. The peritoneum is the largest serous membrane with a surface of 13 000 – 15 000 cm^2 in adults [220], approximately the surface area of skin (15 000 – 20 000 cm^2). The visceral peritoneum accounts for about 70 % of the total peritoneum surface, while the parietal peritoneum accounts for about 30 % of the peritoneum surface [221].

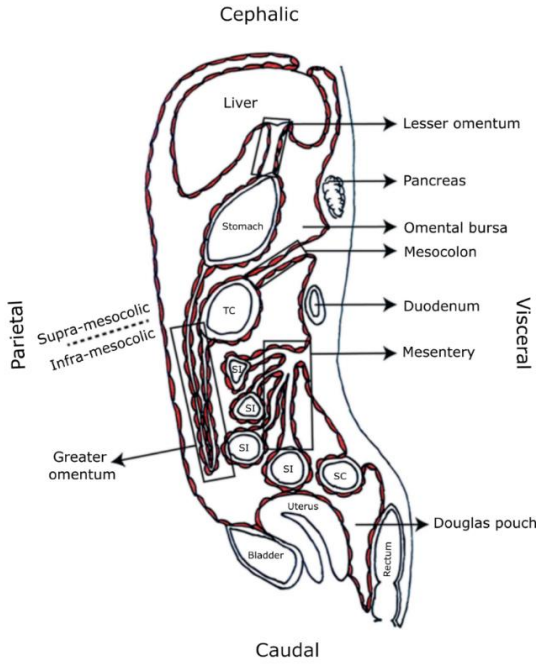


Figure 19. Sagittal view of abdominal cavity. TC, transverse colon, SI, small intestine, SC, sigmoid colon. Red marks line all the peritoneum. Reused with minor modifications from Isaza-Restrepo et al. [222].

The peritoneum is a 3-layer structure: a mesothelial cell layer, a basal lamina and submesothelial stroma (Fig 20). Sometimes peritoneum is defined as a monolayer of mesothelial cells [223]. In some descriptions, it is structured into the 3-layers [224]. In this thesis, it is important to understand peritoneal fluid circulation into and from the peritoneal cavity. Therefore, the 3-layer structure definition is used to describe the peritoneum.

The mesothelial cells are metabolically active cells that are crucial in maintaining serosal homeostasis [225]. The mesothelium is the innermost monolayer of the peritoneum [225]. The mesothelial cells possess a system of intracellular vesicles, including the formation of granules containing secretory products [226]. At the apical surface of mesothelial cells, i.e., pointing into the peritoneal cavity, a considerable amount of microvilli and occasional cilia are present [226], coated with glycocalyx [225]. Thus, this monolayer provides a slippery non-adhesive surface through the production of phospholipids and surface glycocalyx to protect surface during intracoelomic movement [227]. During peritoneal dialysis, mesothelial cells may be mechanically damaged, and during an extended period of time, it will promote peritoneal fibrosis [228].

Mesodermal cells have a mesodermal origin and possess both epithelial and mesenchymal features [218]. These cells can lose their epithelial characteristic and become more mesenchymal, for instance, in the presence of tumour cells [229]. The boundaries between mesothelial cells are often overlapping each other. Thus, they have well developed cell-cell junctional complexes. Tight junctions are essential for developing cell surface polarity and establishing and maintaining a semipermeable diffusion barrier. Adherent junctions are thought to form the cell layer's structural and adhesive support, and gap junctions are aqueous intercellular channels [227].

The basal lamina supports mesothelial cells. It consists of a layer of extracellular matrix and is less than 100 nm thick [226].

The peritoneum is supported by the submesothelium, which consists of connective tissue [220]. The submesothelium varies in its density, being thinner in the areas with possible expansion (e.g. a lower abdominal wall) and thicker in more fixed areas (e.g. pelvic fascia) [224].

The submesothelial stroma consists of the extracellular matrix made up of different types of collagen, glycoproteins, glycosaminoglycans and proteoglycans. Vascular structures and lymphatic vessels are located in the subserous space. Diffusion and resorption of fluid move freely through the mesothelium and submesothelial stroma [219].

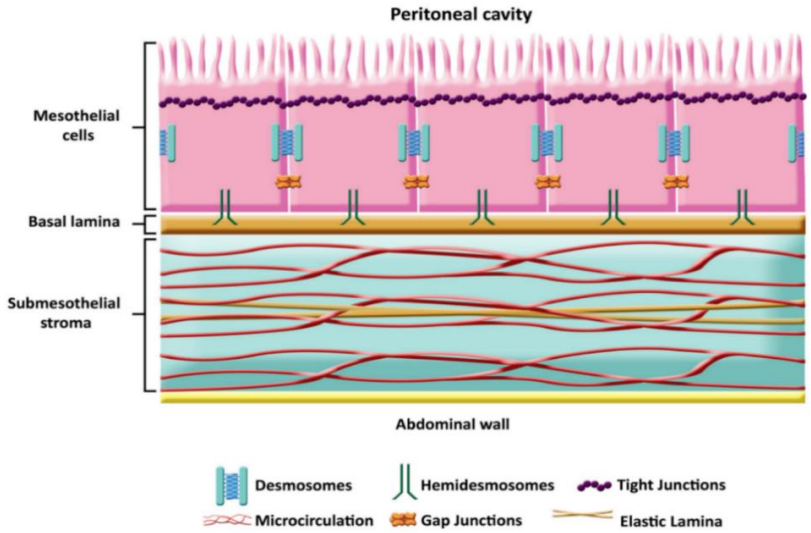


Figure 20. Schematic representation of the peritoneum. Mesothelial cells are linked by intercellular junctions, including tight junctions, gap junctions and desmosomes. Basal lamina supports mesothelial cells is < 100 nm thick and consist of an extracellular matrix (collagen type IV and laminin). Submesothelial stroma provides support to the mesothelial cell layer. Reused without modifications from Kastelein et al. [230], licensed under an Elsevier and Copyright Clearance Center.

The peritoneum forms a double-layered fold – the omentum – connecting the stomach to adjacent organs and supporting a structure within the peritoneal cavity [218]. The peritoneum folds form the greater and lesser omenta. The greater omentum is composed of a double layer of peritoneum that loosely hangs down from the greater curvature of the stomach and the proximal part of the duodenum before curving back superiority to attach to the colon transversum [216]. The lesser omentum, made of two contiguous components called the gastrohepatic and hepatoduodenal ligaments, attach the stomach and proximal duodenum to the liver [218, 231]. The submesothelial stroma in the omental peritoneum contains adipose tissue, capillaries, and isolated bundles of large vessels [232].

The peritoneum forms another double-layered fold – mesentery – it contains multiple mesenteries: the small-bowel mesentery, mesoappendix, transverse and sigmoid mesocolon, and mesorectum [223]. These mesenteries are interrelated and represent a single large contiguous peritoneal structure that attaches the intestines to the posterior abdominal wall [233]. The mesentery contains blood vessels, lymphatics and nerves that supply and drain the intestines [218].

The inferior part of the parietal peritoneum covers the anterior abdominal wall, below the umbilicus, and is raised in many varieties of folds: the median, medial and lateral folds [230]. In men, the peritoneal cavity is closed, while in women, it forms a deep supportive parallel fold over the entire length of the fallopian tubes [226] and connects with the extraperitoneal pelvis exteriorly through the fallopian tubes, uterus, and vagina [218].

2.7.1.2 Physiology

A small volume of fluid separates the peritoneum's parietal and visceral layers. In the peritoneum, small sections with extra fluid can be found in healthy people [234]. The mesothelium acts as a semipermeable membrane that regulates fluid and cells transport across the peritoneum [225]. The peritoneal microcirculation is located in the submesothelial layer; blood flow to the parietal peritoneum is provided by arteries of the abdominal wall and pelvic parietal, while blood flow to the visceral peritoneum comes from the mesenteric, coeliac and visceral pelvic arteries. Venous blood from the parietal peritoneum drains into the inferior vena cava, whereas venous blood from the visceral peritoneum drains into the portal vein [230]. The parietal peritoneum is sensitive to pressure, pain, temperature, and laceration, while the visceral peritoneum is not exposed by these sensations but is sensitive to stretch and chemical irritation [235].

2.7.2. Peritoneal fluid

There is no common knowledge about the volume of peritoneal fluid. In the literature the volume vary between 5 – 20 mL [226], 50 – 100 mL [234], and to 100 mL [217]. Some

authors state that the exchange of peritoneal fluid occurs at a rate of approximately 5 mL/24 hours [224], while other literature states that approximately one litre of peritoneal fluid is produced daily [226, 236]. For certain, it is known that peritoneal fluid contains water, electrolytes, immunoglobulin, complement, coagulation factors, proteins and cells [224]. Peritoneal fluid is transferred through a specific intra-abdominal circulation from the lower abdomen to the upper abdomen and then returns to the lower abdomen. This circulation is driven by respiratory movements, resulting in an upwards flow and gravity, resulting in the downward flow [226].

2.7.2.1. Transperitoneal particle transfer

Particle transfer occurs from the peritoneal cavity through the mesothelium into the submesothelium and capillaries distributed in the interstitium surrounding the cavity [237]. There are six regions of resistance to moving solutes and water across the peritoneum from capillaries to the peritoneal fluid: 1) the capillary fluid film, 2) the capillary endothelium, 3) the endothelium basement membrane, 4) the interstitium, 5) mesothelium, and 6) fluid film (Fig 21).

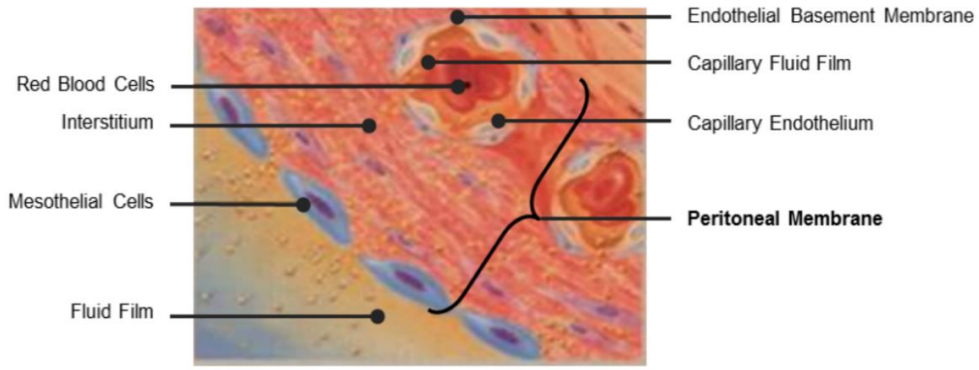


Figure 21. Six layers of the peritoneum. Capillary fluid film: Overlays the endothelium of the peritoneal capillaries; Capillary endothelium: Thin layer of cells that lines the interior wall of the capillary; Endothelial basement membrane; Interstitium: A gel-like matrix containing the peritoneal capillaries, some lymphatics, collagen and other fibers; Mesothelium: contains microvilli and produces a thin film of lubricating fluid; Fluid film: Stagnant fluid that overlies the peritoneal membrane. Figure courtesy of the Advanced Renal Education Program®. https://advancedrenaleducation.com/wparep/article/anatomy-of-the-peritoneum/ Downloaded February 2021.

Fluid movement from the peritoneal cavity to the capillaries and then into the veins is described as a three-pore theory of membrane permeability (Fig 22) [238]:

1) Transcellular pores (Aquaporin 1; water channels), with pore radius less than 0.8 nm (< 8 Å), compose less than 1 – 2 % of all pores; Water filtration happens with dominantly osmotic pressure.

2) Small pores, with a radius of 4.0 – 6.0 nm (40 – 60 Å) compose 90 – 93 % of the total pore area. Particles smaller than 2.5 nm (25 Å), such as glucose, urea, creatinine, sodium, potassium, in association with water, cross the peritoneal membrane with the force of hydrostatic and osmotic pressure.

3) Large pores, with a radius of 20.0 – 40.0 nm (200 – 400 Å) compose 5 – 9 % of the total pore area. Particles more than 4.0 nm (40 Å), such as glucagon, insulin and other proteins, cross the membrane with, dominantly, hydrostatic pressure [237, 238].

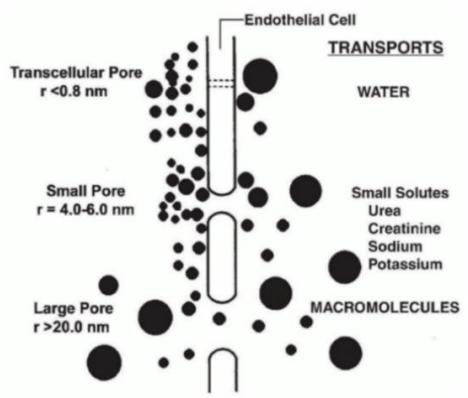


Figure 22. Simplified representation of the three-pore model of peritoneal membrane. This figure is licensed under an Elsevier and Copyright Clearance Center. Reused without modifications from Flessner MF [237].

2.7.3. IP hormone delivery

Regarding treatment, the IP cavity is mainly used for peritoneal dialysis, i.e., exchange of solutes and water between blood in the peritoneal capillaries and the intraperitoneal fluid, where the peritoneum is used as dialysis surface [239]. Peritoneal dialysis is driven by the additional osmotic force from glucose [240] or icodextrin concentration [241].

In the last decades, the IP cavity also is used to infold broad-spectrum drugs whose target organs are located in the vicinity of the peritoneal cavity, e.g. drugs for cancer treatment [242], gonadal drugs [243], glucagon [244] and insulin [245] for diabetes treatment, and diabetes-inducing streptozotocin [246].

CIPII is used as the last treatment option for patients with DM1 who struggle to manage BG levels in the desired range and who has repeated severe hypoglycaemic and/or severe hyperglycaemic events due to unawareness of hypoglycaemia and hyperglycaemia, with HbA1c levels above 8 % [201, 247] or SC insulin resistance, lipoatrophy or skin disorders associated with SC insulin delivery [248]. The insulin pump itself, IP catheter insertion, and maintenance (filling and rinsing) are expensive. In the year 2010, it was 11 000 EUR per year (approximately 111 000 NOK) [249]. In 2021, the new Accu-Chek Diaport system, with

maintenance included, cost 250 000 NOK per two years if no additional complications occur during the period (information acquired from personal communication). CIPII treatment, provided by the DiaPort system specifically for IP insertion, is used by a small number of patients and in most cases, patients are included in trials. The CIPII carry a risk of hyperglycaemic events due to insulin under-delivery, pump pocket infection, and skin erosion [247]. However, not all studies observe pump or catheter malfunctions [250]. Overall, most studies observe a lowering of HbA1c levels and improved pharmacokinetics and pharmacodynamics of insulin. The benefits of the CIPII will be discussed in the Discussion, as well as in the **Paper I**.

IP Glucagon administration is explored only in a few rat [251, 252] and mice [253] studies, including some animal trials performed by the APT research group, described in this (**Paper III**) and a previously published thesis [254]. Before our experiments, we experienced a lack of information regarding IP exogenous insulin pharmacokinetics. Previously published reports contained wide time gaps between measurements. Thus, crucial information, such as time till maximum concentration, minimum time of decline, etc., was missing.

Loxham et al. observed advantages of insulin absorption from the IP cavity, for instance, more rapid hepatic glucagon extraction as compared to SC delivered insulin. The authors agreed that their first measured time point (20 min) probably not represented the correct blood glucose decrease which theoretically could be even larger [251]. This limitation was not mentioned in the study by Zlotnik et al. [252], and possibly, their first measured BG level at 30 minutes did not represent actual glucagon pharmacodynamics. Summarising all available information, we did not have valid information to compare with our data. Therefore, we decided that our animal studies in rats (**Paper III**) and pigs [255] should form the basis for our future research of IP glucagon pharmacokinetics and pharmacodynamics.

2.7.4. IP insulin absorption

Preliminary insight into the insulin absorption from the peritoneal cavity was presented in section 2.7.2.1. The present section will focus on the mechanism of IP insulin absorption and factors that can affect it.

The mesothelial layer contains three different kinds of pores that allow particles to circulate in and out of the peritoneum. Insulin in the submesothelial layer experience similar challenges as in SC tissue. Thus, both ECM and adipocytes affect insulin absorption into the capillaries located in the submesothelium. Preadipocytes in the submesothelium differentiates into large adipocytes, while SC preadipocytes differentiate into small and large adipocytes [256]. The distribution between small and large adipocytes are influenced by obesity. Small adipocytes are more insulin sensitive while large adipocytes are insulin

resistant [213]. Thus, obesity has an extensive impact on insulin absorption in IP submesothelium and less in SC tissues.

2.7.5. Insulin resistance

In abdominal obesity, fat is located in two separate sites, in SCAT and visceral adipose tissue (VAT). VAT, compared with SCAT, is more vascular and contains more inflammatory and immune cells and more large adipocytes [78]. VAT adipocytes are located in the greater and lesser omentum and mesenteric fat [257] and comprise 20 % of total body fat mass [258]. The IP (VAT) and retroperitoneal fat constitute about 11 % and 7 % of the total fat in humans with average weight, respectively, while SC abdominal fat constitutes about 21 % [259]. VAT is more metabolically active, hyperlipolytic and resistant to the anti-lipolytic effect of insulin than SCAT [258]. In obese individuals, VAT releases excessive amounts of FFA and glycerol from adipocytes into the circulation and drain directly into the liver via the portal vein [257]. An FFA increase in the liver will increase hepatic glucose production, reduce hepatic insulin clearance and ultimately lead to insulin resistance [258]. VAT carries a more significant predictor of total mortality than SCAT [260].

2.7.6. Intraperitoneal AP

I previously presented several advantages of open-loop CIPII compared to open-loop CSII, i.e., faster insulin absorption and action, more reproducible insulin effect (less day-to-day variation), and more physiologic gradient between portal and systemic insulin levels [261].

In the study, comparing IP and SC closed-loop insulin delivery systems with identical SC CGM and Zone Model Predictive Control (ZMPC) algorithm, time in target range (3.9 – 10.0 mmol/L) improved to 66 % in IP insulin delivery system, compared to 44 % during the SC insulin delivery. During these identical 24-hour trials, insulin doses were higher during the IP insulin delivery (43 U) compared to the SC insulin delivery (32 U) [201].

Experience with a closed-loop CIPII system with an implanted insulin pump (MiniMed-Medtronic) and IV BG measurements in the jugular vein, with SC connecting lead between the insulin pump and glucose sensor was reported by Renard et al. [261]. The authors noted that, during closed-loop CIPII, 80 % of the time was spent in their specified rather wide normoglycaemic range (4.4 – 13.3 mmol/L). Another study, with short-term closed-loop CIPII from an implanted pump driven by an SC CGM via a PID algorithm, demonstrated the potential for an AP. The mean time spent in normoglycaemia (4.4 – 6.6 mmol/L) improved by 39 % during closed-loop CIPII compared to 28 % during open-loop CIPII [262].

Burnett et al. found that IP sensor measurement is twice as fast as SC sensor measurements (mean time constant of 5.6 min for IP vs 12.4 min for SC) [263].

	Subcutaneous	Intraperitoneal
Insulin absorption peak	50–60 min [264]	20–25 min [265]
Insulin residence time	6–8 h [264]	1–4 h [265]
Sensor measurement time constant	12.4 min [263]	5.6 min [263]
Device placement	External, placed on skin with adhesive patches and tubing [266, 267]	Implanted, no components attached to skin [263]
Device lifetime	Replace sensor every 7 days and pump infusion set every 2–3 days [266, 267]	Implanted pumps last years, with transcutaneous insulin refills every few months [268]
Device invasiveness	Minimally invasive [266, 267]	Requires surgery [263, 268]
Device availability	Commercially available [266, 267]	In development [263, 269]

Table 3. Summary of differences between subcutaneous and intraperitoneal insulin pumps and glucose sensors. Reused with minor changes from Huyett et al. [270].

Potentially, a closed-loop CIPII with IP insulin administration and IP glucose sensing, i.e., a double IP approach for an AP, would provide a more rapid insulin absorption and faster glucose-sensing than a double SC approach for an AP (Table 3) [271].

Most of the closed-loop CIPII (IP–IP) studies are in *in-silico* experiments. Before starting *in silico* experiments, basic data of drug effects, i.e., insulin pharmacokinetics and pharmacodynamics are needed to construct new mathematical models and formulate the algorithm for a double IP closed-loop system. A new mathematical model based on IP insulin and glucose-sensing is more effective than previously reported models (SC–SC, IP–SC) [272] and increase time spent in clinically acceptable BG range to 97 % from previously reported 90 % [270]. However, it should be mentioned that all these results are published by one single research group (F.J Doyle 3rd, E Dassau, H.C Zisser).

Bi-hormone intraperitoneal AP

Bi-hormone (insulin and glucagon) double SC AP systems has been shown to increase BG time spend in the target ranges 3.9 – 10.0 mmol/L or 3.9 – 9.8 mmol/L, compared to insulin only double SC APs [193, 273]. Possible concerns regarding glucagon stability over the time used in bi-hormone AP were clarified in studies by Ward et al. [274, 275]. During the seven days after the glucagon solution was made, the glucagon effect on BG levels did no diminish.

The goal of the APT research group is to make an AP that normalises or close to normalises glucose levels, i.e., keep them in the non-diabetic range, without risk of hypoglycaemia and a daily intervention of the patients. To achieve this goal, APT is working on a bi-hormonal double IP AP. The present thesis exploring the IP delivery of insulin and glucagon is part of this work.

3. Aim of the thesis

3.1. Overall aim

The main aim of this thesis was to investigate the potential of intraperitoneal insulin administration to improve glucose control in DM1 patients. IPII is still at the clinical research level, it is only occasionally used in clinical practice and there is minimal clinical evidence to conclude on the efficiency of IPII.

3.2. Secondary aims

Secondary aim I

Review and perform meta-analyses of reported physiological effects of continuous IP insulin infusion compared to continuous SC insulin infusion (**paper I**).

Secondary aim II

Explore the pharmacokinetics and pharmacodynamics of IP insulin administration and compare that to SC administration (**paper II**).

Secondary aim III

Compare the blood glucose response following intraperitoneally and subcutaneously administered insulin boluses (**paper II**).

Secondary aim IV

Explore the pharmacodynamics of IP glucagon administration and compare that to SC and IV administration (**paper III**).

4. Materials and methods

4.1. Ethics

4.1.1. Replacement, reduction and refinement: The 3R`s.

Since 1959, when the first 3R`s were introduced by Russell and Burch`s *The Principles of Humane Experimental Technique*, not much is changed. However, new technologies and new approaches have been invented. Therefore, further clarifications in definitions and purposes are frequently suggested [276]. The 3R`s implies that we, as researchers, need to use replacement alternatives to the animals whenever possible, we should reduce the number of animals used, whenever it is possible (reduction), and we need to minimize the stress and pain provided before, during and after the procedures (refinement). Importantly, animal research should only be conducted if the results have the potential to provide beneficial scientific information. It is important to follow guidelines to produce valuable protocols, studies and scientific articles, with precise details about included animals, size of groups, procedures followed during the studies, and data and statistical analysis used.

The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendation for Excellence) guidelines for planning animal experiments [277] and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting animal experiments [278] complement each other and are used in good practice animal research.

In the work of this thesis, experiments with animals could not be replaced by cell cultures or *in vitro* experiments. As neither insulin nor glucagon dynamics could be studied using previously mentioned techniques. The number of animals included in the experiments was considered based on a resource equation (**paper III**) [279]. In order to avoid unnecessary animal use in the experiments and reduce the number of animals needed, the aim of every experiment was to collect as many and as good data and samples as possible. In Norway, the use of laboratory animals is governed by the Regulations Relating to the Use of Animals in Research, which follows from the Animal Welfare Act. The European Economic Area (EEA) Agreement obliges Norway to implement EU Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes. As by Norwegian law, all animal experiments included in this thesis were approved by the Norwegian Food Safety Authority (Mattilsynet). Gas anaesthesia was used for animals during the experiments, therefore, ensuring minimal additional pain and stress to the pigs and rats.

4.2. Systematic review and meta-analysis (Paper I)

4.2.1. Search strategy

The protocol followed the PRISMA and Cochrane Handbook guidelines and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on the 30th of June 2016 (registration number CRD42016040124).

Systematic searches were performed in PubMed, EMBASE (Medline/Ovid), The Cochrane Library's CENTRAL database (Wiley Online Library), and Scopus. A person with knowledge of information retrieval in science assisted in developing the search strategy (Table S1). Searches for trial protocols registered with ClinicalTrials.gov and the International Standard Randomized Controlled Trial Number (ISRCTN) registry were also performed. Furthermore, the International Clinical Trials Registry Platform Search Portal was used to search for ongoing or recently completed trials. Dissertation Abstracts, Electronic Thesis Online Service (EthOS) and Network Digital Library of Theses and Dissertations database were additionally searched. For all relevant material, all references were checked to identify additional material (grey literature). The last search was performed on the 10th of July 2020.

All abstracts and titles of articles from the systematic search were uploaded to Distiller SR software. Two reviewers independently screened the reports and abstracts based on predefined inclusion and exclusion criteria. During the data evaluation, we observed that CSII significantly reduced HbA1c levels compared to MDI. This reduction could be a possible bias in the results when SC insulin delivery was compared to CIPII. To avoid this bias, we decided to restrict the results to the effects of CSII and CIPII exclusively. When any disagreement occurred during the data evaluation or extraction, two consultants with expertise in endocrinology independently evaluated the material.

4.2.2. Included studies

All reports and abstracts from studies addressing physiological effects of CIPII versus CSII in DM1 patients were included, i.e., controlled trials, observational studies, case series (> 1 case), case reports (single case), as well as abstracts from clinical and scientific conferences (Fig 23).

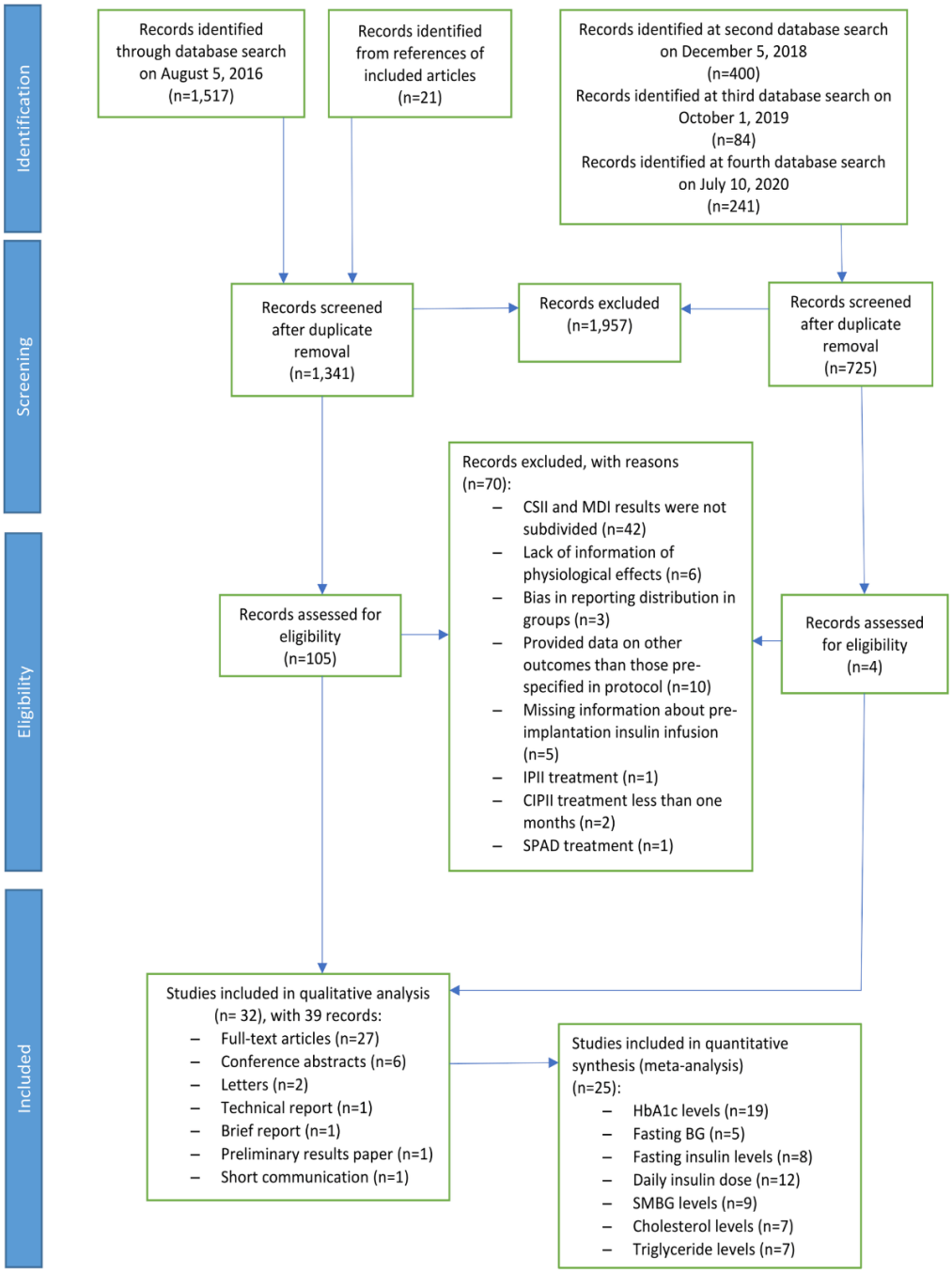


Figure 23. Flow chart of literature search and selection of reports for systematic review. CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections; IPII, intraperitoneal insulin infusion; CIPII, continuous intraperitoneal insulin infusion; SPAD, subcutaneous peritoneal access device, SMBG, self-monitoring blood glucose.

4.2.3. Participants and measurements

Studies were determined to be eligible if CIPII treatment was compared to CSII treatment in DM1 patients. The CIPII treatment had to exceed one month in duration (including the wound healing period after establishing the abdominal port for insulin delivery). The minimum follow-up for the evaluation of HbA1c levels was set to three months, as HbA1c reflects the average glucose levels of the previous 120 days (the average erythrocyte life span) [280]. Consequently, as the follow-up was less than three months in two studies, they were excluded from the HbA1c analyses [281, 282].

Any outcome reported in any of the included studies was included in **paper I**.

The primary outcomes included the following: (1) glycaemic control (HbA1c levels, fasting blood glucose (fasting BG) levels, hypoglycaemia, and hyperglycaemia); and (2) insulin levels (fasting insulin levels, time to reach peak insulin concentrations, maximum insulin levels, and time until insulin levels return to the basal level) and the mean daily insulin dose.

The secondary outcomes included any reported variable other than those listed in the primary outcomes. These included the following: (1) glycaemic control (self-monitoring of blood glucose (SMBG), mean daily BG levels, time spent in normoglycaemia, and glucose variability); (2) intermediate metabolites (triglycerides, cholesterol, free fatty acids, lactate, ketone bodies, and apolipoproteins); (3) counterregulatory hormones and other hormones (glucagon, catecholamines, growth hormone, insulin-like growth hormones, and binding proteins); (4) other metabolic outcomes (levels of anti-insulin antibodies (AIA), sex hormone-binding globulin (SHBG), and plasminogen activator inhibitor-1 (PAI – 1)); and (5) any technical and/or physiological complications reported during CIPII treatment.

4.3. Animal studies

4.3.1. Paper II

Pigs as model organisms for human diseases is widely used, as they resemble anatomic and physiologic characteristics of humans [283]; and the pig immune system resembles 80 % of the human immune system genes [284]. In comparison, mice resembles 10 % [284].

In the animal trial for intraperitoneal insulin pharmacodynamics and pharmacokinetic described in **paper II**, separated blood samples for BG levels and insulin levels were needed; thus, pigs were chosen as the model organism. All in all, 11 juvenile, non-diabetic crossbred domestic pigs (*Sus scrofa*, breed TN 70, mix between Norwegian Landswine and Yorkshire) approximately three months of age were provided by a local farmer who is the

exclusive provider of pigs to the animal research facility of NTNU. Another reason to choose pigs was the animals' size as it allows for extensive blood sampling for the glucose and insulin analysis.

Approximately one week before the trial's pigs were transported to the animal facility and acclimatized to the staff and new environment. Pigs were kept in groups in a common stall with a concrete floor covered with wood chips. In every stall, heat lamps were provided, and the day-night light period was maintained at +22 °C. The pigs were fed standard food, and fresh water was available *ad libitum*. Food was removed 17 hours before the start of the trial, while water was available until anaesthesia was initiated.

4.3.2. Paper III

IP, SC, and IV glucagon delivery were studied using a rat model (*Rattus norvegicus*). Ten male Sprague Dawley rats were used in the pilot study to refine the experimental protocol and determine the glucagon dose to be used in the main study with an additional 20 rats. Rats were kept in groups of three in plastic solid bottom cages (515 × 381 × 256 mm, Tecniplast, Italy) filled with sawdust as a floor cover. The rats were acclimatized to the animal facility and maintained on a 12-hour light – 12-hour dark photoperiod at +20 – +24 °C and relative humidity of 55 % ± 5 %. They were fed expanded pellets (Special Diets Services RM1 for rats, UK), and fresh water was available *ad libitum*. The rats were trained to accept general handling and use of a restrainer (Harvard Apparatus, Holliston, USA) for three weeks prior to the start of experiments. Thus, reduce stress and the possible effect of stress on glucose levels. In practice, my colleague and I visited animals 3 – 4 times per week, held them in our hands, petted them and trained them to use restrainers; after that, rats got a treat (Multigrain Cheerios). During the trials, rats were kept in restrainer's awake while blood samples were taken. During the incision of the tails, anaesthesia was provided.

4.4. Surgery and equipment

4.4.1. Paper II

Before removal from the stall, pigs were sedated. After sedation, pigs were carried into the operation room, weighted, and put on the operation table. A cannula was inserted in an ear vein, and anaesthesia was induced by IV infusion. During additional mechanical ventilation, pigs were intubated. Via incision in the neck, an intra-arterial line was placed in the left carotid artery for blood sampling and monitoring of physiological parameters. An IV line was placed in the left internal jugular vein for glucose and fluid infusions. Both catheters were inserted through the same surgical incision.

With a low laparoscopic incision, the bladder was catheterised. The catheter for IP delivery by an Animas Vibe insulin pump (Animas Corp., West Chester, PA, USA) was inserted through a 2 – 3 cm long caudal-umbilicus incision in the abdominal wall. The tip of the catheter was inserted IP in the upper right region with a pair of long forceps and externally fixed with tape. The tip was not fixed internally in the stationed position.

To avoid coagulation 150 IU of heparin (LEO Pharma A/S, Ballerup, Denmark) was injected into the IP space. In both the main trial and the additional trial, anaesthesia was maintained by IV infusion of midazolam (0.5 mg/kg/h) (Accord Healthcare Limited, Middlesex, UK) and fentanyl (7.5 µg/kg/h) (Actavis Group, Hafnarfjordur, Iceland) and by inhalation of isoflurane (0 – 2 %) (Baxter AS, Oslo, Norway). The anal temperature was measured every hour, and in the case of higher or lower temperatures than 37 °C, medical blankets were adjusted to stabilise the temperature. The experiments lasted for approximately eight hours, and vital sensors were continuously monitored and adjustments made if needed. At the end of all trial days, and still, under full anaesthesia, the pigs were euthanized with an IV overdose of pentobarbital (minimum 100 mg/kg) (pentobarbital NAF, Apotek, Lørenskog, Norway).

4.4.2. Paper III

On the experiment days, rats were weighted for the calculation of the appropriate drug dose. Before transportation to the operation room, rats received an SC octreotide injection. All rats were initially anaesthetised in a gas chamber with 5 % isoflurane, 95 % air with the additional possibility to use a face mask with 2 % isoflurane, 95 % air for further handling.

The first ten rats, i.e., the pilot rats, were anaesthetised using the face mask for the entire experiment. We experienced a steady increase in BG levels in these rats. After an additional literature search, we came to the conclusion that continuous anaesthesia was the reason for increasing BG levels. Thus, for the next rats, the use of anaesthesia was limited to a minimum, and consequently, the BG levels were stable. Anaesthesia was used for animal welfare, i.e., reduced pain during incisions, and for standardisation of the protocol. During the first anaesthesia period, a cut in the tail for the collection of blood samples was made. During the second anaesthesia period, an injection of glucagon or placebo was given. When required, additional anaesthesia was provided to rats showing signs of stress while kept in the restrainer.

For the blood glucose samples, a 6 – 9 mm cut was made with a straight-edged scalpel over the lateral tail vein two-thirds down the length of the tail for blood sampling. To ensure sufficient blood flow for sampling, the vein was gently stroked from the base of the tail and

toward the wound, and the first small drop of blood was removed. For the third intervention, both veins had been used for sampling at former trials, and the new cut was made proximal to the older cut. Whenever needed, the vein was carefully reopened with the tip of the scalpel to ensure sufficient blood flow for sampling.

The rats were given a non-steroid anti-inflammatory drug (Metacam vet, Boehringer Ingelheim Vetmedica) 1 mg/kg BW as a single SC injection at the end of the two first procedures. A suture to close the wound and stop the bleeding at the end of the procedure was necessary on 19 occasions. The wounds healed well after sampling regardless of the wound being sutured or not, and no wound infections were observed. After the third procedure, the rats were euthanized with an IV injection of pentobarbital (100 mg/kg) under isoflurane anaesthesia.

4.5. Endogenous insulin and glucagon suppression

One of the challenges in our animal experiments was the animal's physiological response after insulin or glucagon injection. For example, glucagon injection would increase glucose levels via increased glucose production, which would stimulate insulin secretion and *vice versa.* Therefore, the effect of the hormone after administration would reduce, and the experiment would be biased. In order to avoid this problem, the somatostatin analogues octreotide and pasireotide were used to suppress the endogenous insulin, and glucagon secretion [144] as the combination of different SSA for specific SSA receptors intensifies the inhibition of hormones [134].

4.5.1. Paper II

All the pigs received the somatostatin analogues octreotide (Sandostatin 200 µg/ml, Novartis Europharm Limited, United Kingdom, UK) and pasireotide (Signifor 0.3 mg/ml, Novartis Europharm Limited, UK).

We injected octreotide and pasireotide one hour before the first insulin bolus of the day. Subsequently, 0.4 mg octreotide was given IV, and 0.3 mg pasireotide was injected SC. Octreotide injections were repeated every hour, and pasireotide injections were repeated every three hours during the trial. Octreotide and pasireotide had the expected effect on insulin levels, as endogenous insulin levels (measured by the enzyme-linked immunosorbent assay (ELISA) Porcine insulin kit) were not detectable during the experiments (< 13.8 pmol/L). Porcine insulin had minimal cross-reaction with NovoRapid insulin analogue (3.2 %).

4.5.2. Paper III

All rats received two SC injections of 10 µg/kg body weight (BW) octreotide. The first injection was given approximately 30 min before the start of each procedure and the second at the time of glucagon/placebo injection. Octreotide was given subcutaneously in the neck but not at the exact location as the SC glucagon injection.

4.6. Intervention

4.6.1. Paper II

At the start of every trial day, new human insulin (U 100, Human insulin Novorapid, Novo Nordisk, Denmark) was inserted into an insulin pump (Animas Vibe, West Chester, PA, USA).

In the main trial, all pigs (n = 7) received three insulin boluses, 2 U, 5 U and 10 U, in the upper right IP space.

In the additional trial, insulin boluses were injected into the SC tissues in the left side of the neck. Two pigs received 10 U boluses, and one pig received a 5 U bolus.

4.6.2. Paper III

All rats (n = 20) received IP and SC injection of 5 µg/kg BW glucagon, 15 rats received placebo IP injections of 1 mL/kg BW of isotonic saline. The volume of the placebo injection (1 mL/kg BW) was similar to the IP glucagon injection (approximately 500 µL). Five rats received an IV injection of 5 µg/kg BW glucagon. Thus, to obtain information of IV delivery of glucagon. The volume of the injection was considered based on a good practice guideline [285]. There was at least one week between each test procedure on each rat.

Glucagon solutions were kept in a refrigerator and used the same day as they were made. Solutions were warmed to approximately body temperature just before administration. SC glucagon was injected at the back of the neck, and IP glucagon and placebo (an equal volume of 0.9 % NaCl) in the lower part of the abdomen, while the rat was held at an angle after its hind legs. IV glucagon was given in the lateral tail vein that was not currently used for blood sampling.

4.7. Analysis of glucose and insulin

In the experiments described in **paper II** and **paper III** BG levels were analysed on a Radiometer ABL 725 blood gas analyser (Radiometer Medical ApS, Brønshøj, Denmark).

4.7.1. Paper II

In the main trial, arterial blood samples for glucose and insulin measurements were collected 10, 5 and 1 minute prior to the first somatostatin analogue injection, every 10 minutes for the first hour after Sandostatin injection, with one minute in-between for 10 minutes after insulin bolus, and after that every fifth minute for the next 110 minutes.

In the additional trial, samples were collected 10, 5 and 1 minute prior to the first somatostatin analogue injection and before initiating glucose infusion. Subsequent blood glucose samples were collected 2 minutes after the insulin boluses and after that every 5 minutes for the next 118 minutes.

Arterial blood samples for glucose analysis were collected in heparinised syringes (LEO Pharma A/S, Ballerup, Denmark). All samples were placed on ice after extraction. Most samples were analysed immediately, but some samples were stored on ice for a maximum of 20 minutes before analysis.

In both trials, arterial blood samples for insulin analysis were collected directly in heparinised syringes (LEO Pharma A/S, Ballerup, Denmark) and stored on ice at least 10 minutes before centrifugation (10 minutes at 2.000 x rpm in a refrigerated centrifuge). Plasma was collected from the samples immediately after centrifugation, transferred to Eppendorf tubes and temporarily stored at -20 °C. At the end of each trial day, plasma samples were stored at -80 °C.

Plasma insulin was analysed as singles by Iso-Insulin enzyme-linked immunosorbent assay (ELISA) kit (10-1128-01, Mercodia, Uppsala, Sweden). Suppression of endogenous insulin secretion was verified by analyses of Porcine Insulin ELISA kit (10-1200-01, Mercodia, Uppsala, Sweden) according to the manufacturer`s protocol. The results were converted from mU/L to pmol/L by a conversion factor 6, as recommended by the manufacturer. The lowest detectable insulin concentration for Iso-Insulin ELISA kit was < 3.0 mU/L (< 18 pmol/L); for Porcine insulin ELISA kit was < 2.3 mU/L (< 13.8 pmol/L). Insulin analysis was performed in the St. Olav's hospital medical biochemistry laboratory by an experienced bioengineer.

In the main trial, all insulin samples were run in singles with a coefficient of variation (CV) < 5 %. Inter-assay CV for Porcine insulin were 4.1 %, 4.3 % and 3.3 % for 5.04, 17.6 and 55.4 mU/L standards, respectively. Inter-assay CV for Iso-Insulin were 4.9 % and 4.7 % for 9.84 mU/L and 60.7 mU/L standards, respectively.

4.7.2. Paper III

Venous blood samples for BG measurements were collected 10, 5 and 1 min prior to glucagon injection, and 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, and 60 min after glucagon or placebo injections. Samples were collected directly in heparinised capillary tubes (35 µL, Clinitubes, Radiometer Medical ApS, Brønshøj, Denmark) and stored on ice for a maximum of 30 minutes before analysis on a blood gas analyser.

4.8. Statistical analysis

4.8.1. Paper I

Continuous outcomes were measured and analysed as mean differences and 95 % confidence intervals (MD, 95 % CI); skewed data and non-quantitative data were presented descriptively [286]. A meta-analysis was performed on the primary outcomes, including HbA1c, fasting BG, and fasting insulin levels, and daily insulin dose, and the secondary outcomes, including SMBG, cholesterol, and triglyceride levels using STATA software (Stata Corp. 2019. Stata Statistical Software: Release 16. College Station, TX: Stata Corp LLC). When the outcome variables were continuous measurements, the mean difference (MD) was used as the effect size. The heterogeneity was estimated with random effects models and restricted maximum likelihood as the analysis model. The heterogeneity was estimated by the I^2 statistic and categorised as follows: a) heterogeneity that might not be important (0 – 40 %), b) may represent moderate or substantial heterogeneity (40 – 75 %), or c) considerable heterogeneity (75 – 100 %). However, the observed value's importance depends on the magnitude and direction of the effects and the strength of the evidence for heterogeneity [287]. Each study was weighted using STATA software for continuous outcome variables based on the SD and the study sample size. This weighting determined how much each individual study contributed to the pooled results estimates [288].

Subgroup analyses were performed for all studies included in the meta-analysis. The categories for the subgroup analyses were: (1) HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %), (2) study type (case-control studies and crossover studies), (3) duration of the CIPII-period (≤ 6 months and > 6 months), and (4) whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period. As an additional analysis, studies were sorted by the duration of the CIPII-period (months) to provide information about changes in the effect with time.

Evaluation of heterogeneity between studies was performed for studies reporting HbA1c levels by meta-regression and bubble plot analysis with 95 % CI and linear prediction of HbA1c levels with CIPII treatment, using CSII controls for comparison.

Heterogeneity of effects was also explored with funnel plots [289] when ten or more studies were included in the meta-analysis. Assessments for publication bias across studies were performed using graphical (funnel plot) and statistical (Egger´s test: random-effect model, t-distribution) analyses. For quantitative testing of skewness in the funnel plot, the Egger´s test was chosen to analyse the MD of continuous outcomes.

A cumulative, sequential meta-analysis of the studies was performed according to the duration of the CIPII-period.

4.8.2. Paper II

Statistics were performed with GraphPad Prism 8 Statistics. All values are given as mean ± standard deviation (SD). Differences between the groups were considered significant if $p \leq 0.05$. Delta values collected from the main trial with different insulin boluses (2 U, 5 U and 10 U) in the pigs were analysed. Two-way repeated measurement analysis of variance (ANOVA) in order to estimate possible significant differences in circulating insulin and BG levels after IP insulin boluses were performed. Treatment and time were the sources of variation. Tukey`s multiple comparisons test was used for distinguishing comparisons between different insulin boluses. All non-measurable insulin values were set as 18 pmol/L. All treatments are compared as models; therefore, comparison between unequal groups was allowed.

4.8.3. Paper III

The software package R was used to analyse the data. The relationship between BG levels and time was analysed for all interventions using a mixed linear model with the combination of time and treatment as the fixed effect. The dependent variable was defined as log glucose concentration to achieve normal distribution. To account for multiple measurement series on each rat, rat identification was included as a random effect. To account for dependence within each series, the error term for each series was specified as a first-order autoregressive process AR (1) series accounting for minutes between measurements. Mean changes in glucose concentrations from −1 min to 60 min for the four treatments were compared using the Wald test. Maximum concentration and time until a maximum concentration of the estimated model for the treatments were compared using the Mann-Whitney U test. To eliminate the effect of placebo intervention on the glucose response, the mean value of the 15 placebo interventions was subtracted from the mean value of the 20 SC and IP interventions and the mean value of the 5 IV interventions at the given time points. All interventions were compared as models. All values in the text are presented as mean ± SE of the mean unless stated otherwise. Differences between the group means were considered statistically significant when $p \leq 0.05$.

The most appropriate statistical method used for the study was discussed with associated professor Øyvind Salvesen. He also wrote the script for the statistical analysis in the statistical program R.

5. Summary of papers

5.1. Paper I

"Physiological effects of intraperitoneal versus subcutaneous insulin infusion in patients with diabetes mellitus type 1: A systematic review and meta-analysis"

The IP route of administration accounts for less than 1 % of insulin treatment regimes in patients with DM1. Despite being used for decades, a systematic review of various physiological effects of this insulin administration route is lacking.

The aim of **paper I** was to identify the physiological effects of CIPII compared to those of CSII in patients with DM1.

Systematic searches were performed in PubMed, EMBASE (Medline/Ovid), The Cochrane Library's CENTRAL database (Wiley Online Library), and Scopus. The search identified a total of 2242 records; 39 reports from 32 studies met the eligibility criteria. When performing a meta-analysis, we focused on the most relevant clinical endpoints, such as HbA1c, hypo- and hyper-glycaemia events, peripheral insulin levels, and time to peak insulin concentration. However, among the mentioned endpoints, only for HbA1c and fasting insulin levels we could perform meta-analyses; other beforehand mentioned endpoints were provided in a descriptive or non-comparative manner. Three additional meta-analyses (daily insulin dose, triglycerides, cholesterol) are described in **paper I.**

We found significantly lower MD in HbA1c levels during CIPII than during CSII (MD = -6.7 mmol/mol, [95 % CI: -10.3 – -3.1]; in percentage: MD = -0.61 %, [95 % CI: -0.94 – -0.28], p = 0.0002). In subgroup analysis according to HbA1c levels before starting CIPII treatment, significantly lower HbA1c levels were observed during CIPII treatment than during CSII treatment in the subgroup with HbA1c levels > 53.0 mmol/mol (> 7 %) and remained unchanged in the subgroup with HbA1c levels ≤ 53.0 mmol/mol (≤ 7 %) (MD = -8.1 mmol/mol, [95 % CI: -12.5 – -3.8], $p < 0.01$ and MD = -1.8 mmol/mol, [95 % CI: -5.5 – 1.9], p = 0.33, respectively; in percentage: MD = -0.74 %, [95 % CI: -1.14 – -0.35], $p < 0.01$ and MD = -0.16 %, [95 % CI: -0.50 – 0.17], p = 0.33, respectively). The difference between the two subgroups was significant (p = 0.03). There was substantial heterogeneity between the studies with HbA1c levels > 53.0 mmol/mol (> 7 %) (I^2: 70 %, $p < 0.01$) and no heterogeneity in the studies with HbA1c levels ≤ 53.0 mmol/mol (≤ 7 %) (I^2: 0 %, p = 0.72).

The frequencies of severe hypo- and hyper-glycaemia were reduced during CIPII. The fasting insulin levels were significantly lower during CIPII than during CSII (MD = -16.70 pmol/L, [95 % CI: -23.62 – -9.77], $p < 0.0001$). Compared to CSII treatment, CIPII treatment improved overall glucose control and reduced fasting insulin levels in patients with DM1.

5.2. Paper II

"Intraperitoneal insulin administration in pigs: Effect on circulating insulin and glucose levels"

The effect of IP insulin infusion has limited evidence in the literature. Also, there is a lack of studies comparing the insulin appearance in the systemic circulation after IP compared with SC insulin delivery.

The aim of **paper II** was to investigate the pharmacokinetics and pharmacodynamics of different IP insulin boluses and compare them with SC insulin boluses.

All pigs were treated with somatostatin analogues (octreotide and pasireotide). Seven pigs received 2 U, 5 U and 10 U IP insulin boluses; the last six pigs in a randomised sequence. There were at least two hours and 30 minutes between each bolus. In the additional trial, two pigs received 10 U SC insulin bolus, and one pig received 5 U SC insulin bolus. All SC insulin boluses were performed as the first bolus of the day.

We observed a faster glucose response after IP insulin boluses, with a significant decrease after 5 U and 10 U IP insulin boluses compared to the 2 U insulin boluses. However, we did not observe a significant difference between 5 U and 10 U IP insulin boluses. Interestingly, circulating insulin levels increase after 10 U insulin boluses were observed after 10 minutes; on the contrary, no significant insulin level increase was observed after 5 U and 2 U IP insulin boluses.

In the additional trial, insulin levels were increased five minutes after both 5 U and 10 U SC insulin boluses, with four to seven folds higher maximum circulating insulin increase compared to the IP insulin boluses.

Smaller IP boluses of insulin have an effect on circulating glucose levels without increasing insulin levels in the systemic circulation. By increasing the insulin bolus, a major increase in circulating insulin was observed, with a minor additive effect on circulating glucose levels. This is compatible with a close to 100 % first-pass effect in the liver after smaller intraperitoneal boluses. SC insulin boluses markedly increased circulating insulin levels.

5.3. Paper III

"Intraperitoneal, subcutaneous, and intravenous glucagon delivery and subsequent glucose response in rats: a randomized controlled crossover trial"

Hypoglycaemia is a frequent and potentially dangerous event among patients with DM1. SC glucagon is an emergency treatment to counteract severe hypoglycaemia. The effect of IP glucagon delivery is sparsely studied.

The main aim of **paper III** was to investigate and compare the glucose pharmacodynamics after IP, SC, and IV glucagon administration in octreotide treated rats.

Fifteen rats received IP and SC glucagon injection with a dose of 5 µg/kg and IP placebo injection of saline. Five rats received IP, SC, and IV glucagon injection with a dose of 5 µg/kg. The different randomized interventions were done one week apart. The rats were kept awake for most of the time of the experiments with brief anaesthesia for the incision in the tail for blood sampling and the injection of glucagon of placebo (saline).

After four minutes, we observed a significant glucose increase after IP glucagon injection compared with IP placebo injection ($p = 0.009$). In comparison, after SC and IV glucagon injection significant glucose increase was observed after eight minutes ($p = 0.002$ and $p < 0.001$, respectively). The glucose excursion after IP glucagon injection lasted shorter than after SC glucagon injection, i.e., BG levels were higher after four minutes ($p = 0.019$) and lower after 40 min ($p = 0.005$) and 50 min ($p = 0.011$) after IP glucagon injection. The maximum glucose response occurred 25 minutes after IP glucagon injection compared with 35 minutes after SC glucagon injection ($p = 0.003$). BG levels also increased in the rats after the placebo (saline) injection.

Glucagon administered intraperitoneally gives a faster glucose response compared with subcutaneously administered glucagon in rats. If repeatable in humans, the more rapid glucose response may be of importance in a bi-hormone artificial pancreas using the IP route for administration of insulin and glucagon.

6. Discussion

6.1. Methodological considerations

Human study

In **paper I,** the inclusion criteria was crucial in developing the qualitative systematic review. Many studies compared SC insulin administration with CIPII without considering the potential bias in the study. There is substantial evidence that CSII shows significant improvement in glucose control, i.e., average glucose exposure and hypoglycaemic episodes, compared to MDI treatment [290]. Therefore, to avoid this bias, we included only studies with CIPII vs CSII comparison. We hold that this approach is a major strength of **paper I**.

To establish IP insulin infusion a surgical procedure is needed that may influence insulin resistance; and thus, the daily insulin dose required for adequate glucose control. Therefore, one month of the IP period was considered an appropriate time for wound healing and observing the potential effects of CIPII. Another criterion was the effect on HbA1c levels during CIPII. We limited our inclusion to CIPII periods of three and more months. Most of the studies repeated measurements every two to three months for an extended period. Therefore, we used the last measurements of both the CSII and CIPII periods in the studies.

We encountered high heterogeneity between studies (I^2 = 68 %). Thus, we made subgroup analyses to explore possible reasons for heterogeneity. We know that duration of the treatment could affect the results. Therefore, we divided studies by duration of the CIPII period and chose six months as a breaking point (≤ six months versus > six months). During the process, we chose four criteria that could influence the results: (1) HbA1c levels before the start of the CIPII period; (2) type of the study; (3) duration of the CIPII treatment; and (4) controlled CSII treatment before starting CIPII treatment, as just intensive control of BG levels could improve HbA1c levels [268]. During the generation of subgroup analyses, we were curious about the effect of the duration of the CIPII period. Therefore, we categorized all measurements in meta-analyses by the duration of the CIPII period (from 1.5 months to 24 months).

By performing the review and meta-analyses the way we did, we hold that even when the studies included were in general of moderate and poor scientific quality, the observations that CIPII treatment is superior to CSII on several variables related to glucose control are quite trustworthy.

Animal studies

In paper II, the pig model was chosen for the study of IP insulin delivery on insulin pharmacokinetics and dynamics. This was based on similar size, anatomical features that pigs share with humans, such as similarities in peritoneum [291] and physical parameters [292]. Pigs also share similarities in blood pressure and heart rate, and pigs eat any food and drink. Pigs suffer from obesity and diabetes, and medical devices used for humans such as catheters can also be used in pigs [293].

Rats as model organisms were chosen for the animal trial identifying glucose levels change in response to glucagon injection in **paper III**. Rats possess physiological similarities to humans, such as similarities in mesothelial cells, and it's possible to develop specific conditions in rats to mimic human diseases, i.e., diabetes [294]. In general, rats and humans share a majority of their biochemical capabilities at the genome level [295]. Previously, rats have been used as model organisms for studying morphological changes of peritoneum [296]. And rats are cheap, easy to handle and easy to keep for extended periods in an animal facility.

Insulin IP injection in the pigs was done by opening the peritoneum and inserting a catheter attached to an insulin pump. Thus, we know that insulin was delivered in the IP cavity. In the rats, glucagon IP injection was done blindly, i.e., rats were anaesthetised, and glucagon was injected by holding their hind legs. Therefore, we do not know for sure that glucagon was administrated in the IP cavity. We cannot exclude that glucagon was administrated in the intestines or nearby the peritoneum. Thus, we should be a bit careful about our conclusions about glucagon absorption from the peritoneal cavity in rats. APT has repeated the glucagon study in the pigs with quite similar results as the present results in rats [255]. This paper is not included in this thesis.

Anaesthesia and intervention

During the trials, pigs were exposed to anaesthesia for a long time, i.e., seven to eight hours. Duration of anaesthesia could potentially affect the results of the insulin pharmacokinetics and pharmacodynamics that we observed. The pigs were exposed to drugs such as sedation and anaesthesia and an unnatural position on the operation table. This could potentially affect blood flow and gastrointestinal and peritoneal fluid movements. General anaesthesia and surgical incision in the abdomen reduces intestinal motility [297], and incision in the peritoneum activates an inflammatory response [298] with a potential collection of fluids. Accumulation of IP fluid was observed in some pigs during the experiment day. This side effect of our experimental procedure may have had

an influence on the IP absorption of insulin and hence on the observed pharmacokinetics and pharmacodynamics of IP delivered insulin. Therefore, we tried to interfere with the IP space as little as possible in the successive experimental days.

BG levels can be affected by sedation and anaesthesia in the absence of additional insulin delivery or suppression of endogenous insulin secretion. Out of the anaesthetics used in the pig trials, azaperone [299], thiopental [300, 301], ketamine [302, 303] and isoflurane [304] may increase BG levels. Another study observed BG level increase 30 minutes after continuous use of isoflurane [304]. A strength of our research in pigs is that we compared insulin and glucose levels with identical experiment protocols, avoiding potential biases introduced by the experimental procedures.

Rats were anaesthetised with a maximum of 5 % isoflurane. When combined, first and second anaesthesia, the period during the anaesthesia was shorter than previously mentioned 30 minutes. Thus, the risk of anaesthesia affecting BG levels was minimised.

Endogenous insulin and glucagon suppression

In **paper II,** we used the somatostatin analogues octreotide and pasireotide for suppression of endogenous insulin and glucagon secretion. In these experiments, we wanted to identify the pharmacokinetics and dynamics of exogenous IP insulin delivery. Therefore, we combined two somatostatin analogues to ensure that endogenous insulin secretion is suppressed. Pasireotide was chosen as one of the somatostatin analogues, as it has a high affinity for the SSTR 1 and SSTR 5 [305]. These receptors are strongly expressed in β cells [137]. Octreotide was used as a complimentary hormone, as it predominantly targets SSTR 2, and it is strongly expressed in α cells [137].

We only used octreotide for the suppression of endogenous glucagon and insulin secretion in the rats. Previous trials provided evidence that octreotide is sufficient for inhibition of glucagon and insulin secretion in rats [306], as it has a high affinity for the SSTR 2 [144], which is strongly expressed in the α cells [137]. It also has a high affinity for the SSTR 5 [307], which is strongly expressed in the β cells [137].

Endogenous insulin production was measured in **paper II**, where a Porcine insulin ELISA kit was used to detect possible endogenous insulin secretion. There were no detectable levels of endogenous insulin during the experiments.

Fasting of animals

Pigs fasted for the last 16 hours before the experiments took place. Possible hypoglycaemia would explain decreased endogenous insulin levels before the start of the experiments. Before the start of pig experiments, we did not observe hypoglycaemia. Moreover, fasting

insulin levels in non-diabetic pigs were comparable with fasting insulin levels in non-diabetic humans [25]. Thus, we hold that non-measurable endogenous insulin levels was not due to a prolonged fasting state but rather caused by treatment with somatostatin analogues.

In the rat study, fasting was initiated one hour before the experiments. Rats were kept in cages in groups of three. Thus, the first rat fasted for a shorter period of time than the third rat. Rats are night active animals; in the daytime period, rats are less active. Thus, the metabolic process is reduced as experiments were done during the day and rats were randomised. Thus, the fasting time difference would not affect the results.

6.2. Encountered difficulties during analysis of results and studies

Paper I

In general, healthy people have more similar fasting insulin levels than patients with DM1, where fasting insulin levels show high intra-individual variability (Fig 24, 25) [76]. As each patient represents individual results (Fig 25), studies with few participants use to produce results with a wide confidence interval. Thus, reducing the study's importance in terms of conclusions that can be drawn.

Another challenge that was encountered during the data extraction of the studies' was spare some and insufficient material and method description. It was not possible to compare some crucial parameters from the studies with an inappropriate description of insulin type, insulin pumps, and the IP or SC insulin delivery site location. Five out of ten authors responded to our request for additional information, although only two of them provided informative answers. These and other measurements (fatty tissue distribution around the infusion site, VAT and SCAT ratio, individual activity level) could give a deeper insight into the CIPII and provide some comparisons for future studies.

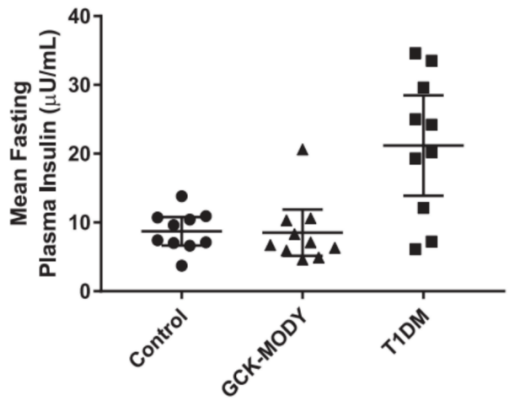


Figure 24. Arterialized plasma concentration of insulin before insulin bolus. Control, healthy subjects; GCK-MODY, glucokinase-maturity-onset diabetes of the young; T1DM, patients with diabetes mellitus type 1. Figure reused without major modifications from Gregory et al. [76].

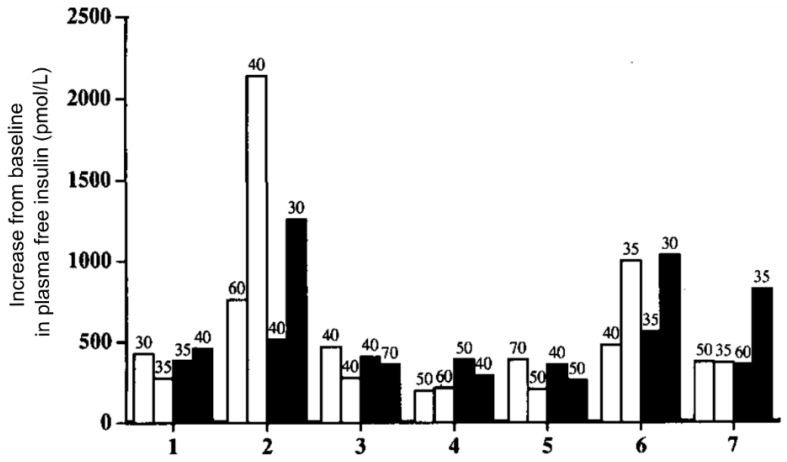


Figure 25. Increase from baseline in plasma free insulin concentration after the IP administration of 15 U of insulin a 20-min square wave infusion using the implantable device at IP 3 months (white rectangular prism) and IP 30 months (black rectangular prism). Each bar represents a patient, and time (min) to the insulin peak are reported at the top. Figure reused without modifications from Scavini et al. 1995 [308].

Animal studies

During stressed situations, the body secretes adrenaline that stimulates glucagon secretion; thus, BG levels increase [309]. To avoid this, we trained rats to accept the persons (my colleague and I) who performed the experiments, and the rats were trained to be held in restrainer to minimise their movements. During the rat studies, we observed individual stressed animals and even aggression in some observed individuals in all

intervention times. As previously mentioned, another difficulty was blind IP insulin injection, as it was not possible to make a clear and visible injection.

6.3. Discussion of main findings and comparison with other studies

6.3.1. Benefits of CIPII (Paper I)

In paper I, we observed a highly significant reduction of HbA1c levels during CIPII compared to CSII treatment. Moreover, subgroup analysis of studies with HbA1c levels > 7 % during CSII before changing to CIPII showed an even larger decline in HbA1c levels during CIPII treatment (Fig 26). During CIPII treatment, a visual pattern of gradual and significant HbA1c levels decreased with increased CIPII treatment time. Overall, long-term CIPII may be more advantageous than observed in our meta-analyses as most studies lasted for less than one year. Most studies also had close follow-up by clinicians during the CIPII period, while this follow-up in most studies seems less rigorous during CSII treatment; this is a potential source of bias.

Stabilised BG levels are a significant improvement in patients with MD1. In some studies, we observed a reduced frequency of severe hypoglycaemic events and less time spent in severe hypoglycaemia during CIPII. It is noteworthy that these effects were observed in combination with reduced HbA1c levels as the frequency of hypoglycaemias usually increase with improved glucose control evaluated by decreased HbA1c levels. Most would hold as a significant achievement that the combination of decreased glucose levels and fewer episodes of severe hypoglycaemia can be achieved only by changing the site of insulin delivery. In our mind, this underlines the potential of IP insulin delivery for the future treatment of DM1.

Paper I is the first systematic review comparing CIPII with CSII only, i.e., comparing *per se* the two different sites of insulin delivery while keeping other variables as equal as possible. We hold that it is essential to compare the effects of IP and SC insulin delivery *per se*, as other systematic reviews showed lower HbA1c levels during CSII compared to MDI in paediatric patients [310] and adults with DM1 [311].

Previously published systematic reviews in patients with DM1 using CIPII investigated IP insulin administration in patients on peritoneal dialysis [312] and CIPII with implantable insulin pumps [313]. However, the comparisons were performed in SC insulin administration in general, including both CSII and MDI.

The first systematic review observed that daily insulin dose was two-fold higher than during SC insulin delivery [312]. In our systematic review, in one out of 23 studies, the daily insulin dose increased during the CIPII compared to the CSII; other studies reported no differences

in the daily insulin dose. Increased daily insulin dose could be explained by peritoneal dialysis mechanical damage on peritoneal mesothelium and aggravation of peritoneal fibrosis leading to decrease of ultrafiltration [314] and delayed absorption of insulin. Further, the large volumes of fluid deposited in the peritoneal cavity would dilute the insulin delivered in the same site. Theoretically, instead of being dissolved in a few millilitres of fluid, the same amount of insulin would be dissolved in several millilitres of fluid. It would be strange if the absorption of insulin was unaffected by this major change in insulin concentration [205].

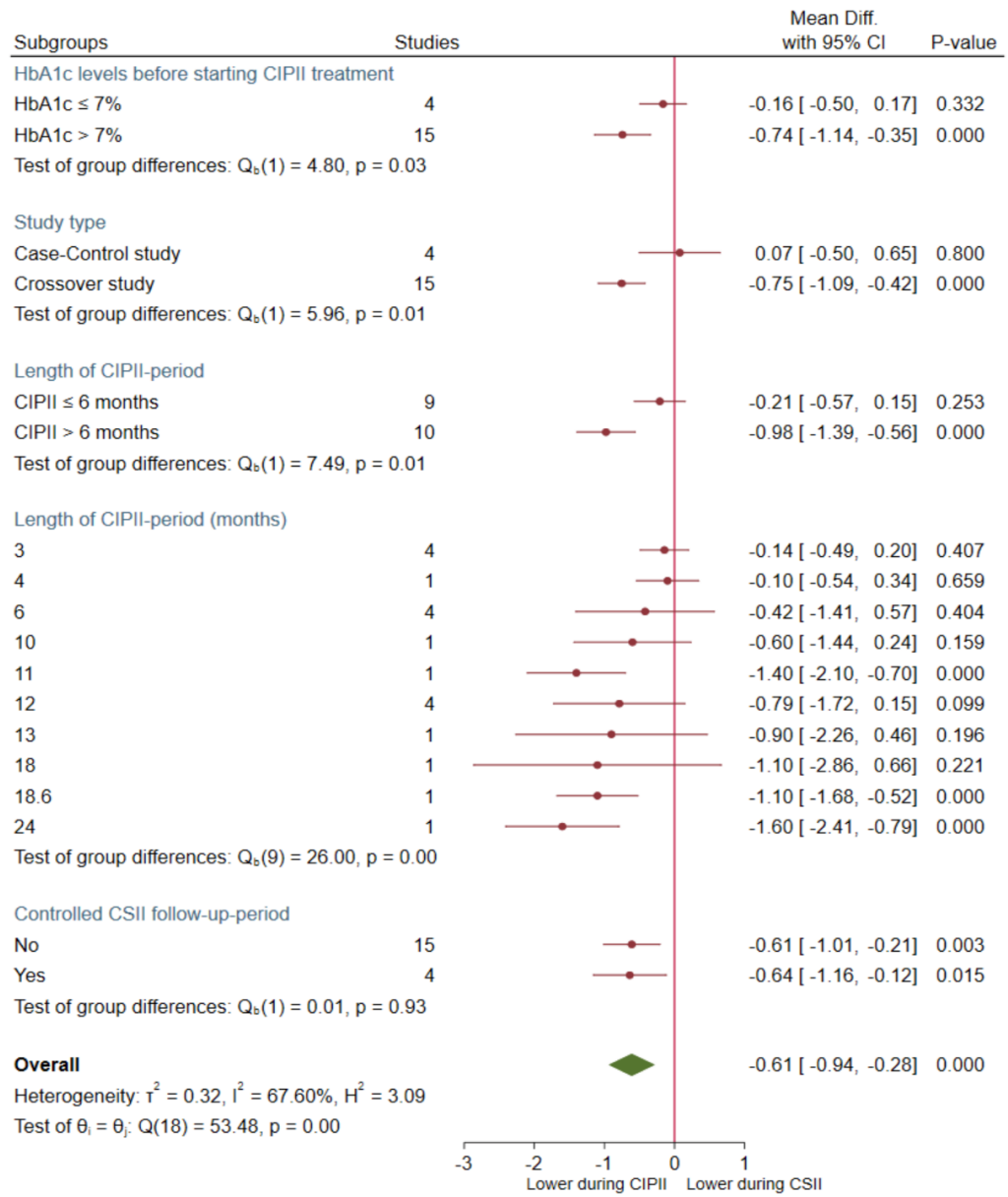


Figure 26. Subgroup meta-analysis for HbA1c (%) in DM1 patients during CIPII treatment compared to controls (CSII). All 19 studies were arranged in four subgroups: according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); according to study type (Case-Control studies and Crossover studies); according to length of the CIPII-period (≤ 6 months and > 6 months); according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period. Additional subgroup was made and sorted by length of CIPII-period.

6.3.2. IP insulin delivery (Paper II)

In **paper II,** we observed that 5 U and 10 U IP insulin boluses have a similar glucose-lowering effect. However, circulating insulin levels were higher after the 10 U insulin IP bolus. A similar observation was made in rat study after IV insulin boluses [315]. The hepatic first-pass effect was observed with distinguishable hepatic saturation of insulin and minimal absorption in kidneys, muscles, and skin [315]. The fact that despite doubling the IP insulin boluses from 5 U to 10 U, barely any further decrease in circulating BG levels was observed is compatible with the liver being saturated with insulin after a 5 U IP bolus and no additional effect on hepatic glucose disposal was achievable despite increased IP insulin doses. Similar results were observed in human studies where higher insulin doses were provided during the IP insulin treatment without any increase in hypoglycemic events than SC insulin delivery [201]. However, in this particular study, two different insulin analogues were used for CIPII and SCII treatment. It could be essential to compare the exact same insulin analogues because each insulin analogue in the same route has different pharmacokinetics (Fig 27) that affect pharmacodynamics.

By including three IP insulin doses (2 U, 5 U, and 10 U), we observed three different reactions: (1) no impact on BG levels and no impact on change in circulating insulin levels; (2) lowering effect on BG levels and no impact on change in circulating insulin levels; and (3) lowering effect on BG levels and elevation of circulating insulin levels. Depending on the site of delivering insulin, it is crucial to know beforehand the needed dose. Low-dose insulin administration primarily inhibits hepatic glucose production [68]. On the contrary, high-dose IV insulin infusion decreases hepatic glucose production and increases glucose uptake in the tissues [316].

The results from **paper II** are used in the development of an algorithm for an IP AP system by the APT research group.

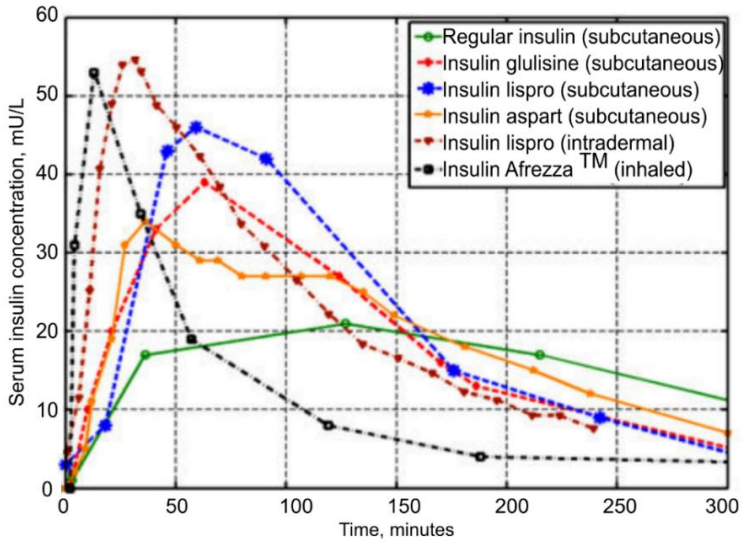


Figure 27. Pharmacokinetics of currently available insulin delivery options. Serum insulin concentration after insulin injection at time zero for various insulin formulations and delivery routes. Reused without changes from Lee et al. [317].

6.3.3. IP vs. SC vs. IV glucagon delivery (Paper III)

Paper III shows that IP, SC, and IV delivery of glucagon all induces a glucose response. Thus, our paper confirms the already known glucagon elevating effect on BG levels [318]. This paper also shows that glucose response is faster after IP glucagon boluses and with an earlier decline in BG levels compared to SC glucagon boluses. Earlier glucose response after IP glucagon administration was confirmed in experiments in pigs [255]. It is not possible to compare the present results with previous studies, as BG levels measurements were not taken frequently enough for detailed and precise descriptions of BG levels change after IP or SC glucagon boluses [251, 252, 318]. The results from **paper III** are used in the development of the algorithm for an IP AP system by the APT research group.

Compared with SC glucagon absorption, IP glucagon absorption may be faster due to a shorter distance to reach the capillaries and easier diffusion into the bloodstream. Previous studies reported that most intraperitoneally injected glucagon enters the portal vein and passes the liver, with approximately 30 % hepatic extraction, before entering the systemic circulation [319]. In our rat study, we limited our analyses to BG measurement only after IV, SC and IP glucagon boluses, as, for taking frequent blood samples, volume per one sample could not exceed 35 µl. Therefore, it was impossible to estimate how much glucagon was present in the blood after boluses. However, we observed that BG levels increased similarly after IP and after IV glucagon delivery, as reported in another study [319].

Another positive observation during the rat study was that BG increase by 2.5 – 3.0 mmol/L after a small glucagon dose of 5 μg/kg. This BG level increase was observed in all routes, i.e., IP, SC, and IV, showing that mini-boluses of glucagon could be used in an AP to reduce hypoglycaemia without producing subsequent hyperglycaemia. This was confirmed in another study from the APT research group in pigs [255]. However, this will have to be investigated further in awake pigs as anaesthetised pigs showed lower glucose elevation after IP and SC glucagon administration as compared to rat study [255].

6.3.4. Strengths and limitations

In our systematic review, we included only studies that applied continuous insulin infusion. Thus, we minimised potential confounding effects introduced by different procedures related to insulin delivery other than the site of injection. During MDI, repeated use of the same injection site increases the risk of lipoatrophy over time. These areas are pain-free; therefore, patients tend to continue to use them. However, the absorption of insulin from lipoatrophic areas is inconsistent, leading to frequent difficulties to achieve normal BG control [320]. Using MDI, incidental injections of insulin in IM tissues could happen, particularly in slim and average weight DM1 patients.

In our studies on IP insulin delivery, we included more animals than other comparable studies. Further, we performed some additional experiments with SC insulin delivery to be able to compare IP to SC insulin delivery in the same animal model. However, it should be noted that these pigs were mainly used to study IP glucagon delivery. The additional experiment did not in any way influence the results of the original study studying the effect of different IP glucagon boluses.

As rats, pigs and humans have some differences in metabolism, structure, size and DNA, **paper II** and **paper III** should be considered only as pilot studies. Our studies in rats and pigs should be confirmed in future studies in humans, preferentially in patients with DM1.

During the preparation of the study protocols, we considered the total amount of blood that could be withdrawn without affecting the animal's wellbeing during and after the experiments. By meticulously planning this and cutting down on the volume of each sample, we could draw frequent blood samples allowing for more precise results.

We protocoled and described every step and complication encountered during the analysis of studies for the systematic review and during our animal experiments; thus, providing transparency and open research. By incorporating an accurate description of our procedures, we tried to increase our studies' reproducibility; thus, increasing the value of our research and studies that would be produced based on our provided information. We provided extensive supplementary materials by including all calculations and figures that

could give more profound insight into the results and provide additional discussion. In particular, we hold that our meticulous reporting of our animal studies will be valuable for other researchers as the challenges that we encountered can be avoided. Thereby, in future studies, the number of included animals can be reduced.

The studies included in this thesis hold some limitations that were mentioned in Materials and methods (Chapter 4) and Methodological consideration (section 6.1). Briefly, some of the studies included in **paper I** had spare some methodological descriptions; thus, comparing individual studies was nearly impossible and affected the overall result description. Another limitation was that the CIPII period variated between studies (2– 48 months); thus, possibly introducing bias of CIPII treatment on metabolism compared to CSII treatment.

In **paper II,** the weights of the animals varied somewhat (36.0 – 42.6 kg) while insulin boluses were fixed. The number of included animals for IP insulin delivery was small (n = 8) and even smaller for SC insulin delivery (5 U SC insulin bolus, n = 1; 10 U SC insulin bolus, n = 2). Thus, this study should only be considered a pilot study with necessary future repeated experiments, possibly in awake pigs. Because all animals were anaesthetised during the study, obtained data may not reflect the awake animal dynamics of insulin absorption and glucose effects. The last limitation in the study could be the accumulation of different amounts of IP fluid in the peritoneum during the experimental days, therefore, possibly affecting insulin absorption. However, it should be mentioned that, overall, the observations in the review (**paper I**) and the observed effect of IP insulin delivery in pigs (**paper II**) are not contradictory but rather support each other.

A major limitation of the observations in **paper III** is the blind IP glucagon injections in the rats. We do not have 100 % certainty about the exact location of the needle during the injection. Although we believe that glucagon was injected IP, the needle tip could have been in the wall of the intestines or the lumen of the intestines. However, to minimize the error, rats were held in a position that gives better access for injection into the peritoneal cavity.

6.4. Relevance for development of IP AP

Absorption of exogenous insulin administration in patients with DM1 should be fast, effective and predictable. However, even with the most fast-acting meal insulin, present SC insulin injections show delayed insulin absorption and effect, with high variability of effectivity and low predictability of glucose-lowering. The work underlying this thesis aimed to investigate the possibilities and advantages of using the IP space as an alternative location for an AP system by investigating and comparing insulin pharmacodynamics and

pharmacokinetics after administration in IP space and SC tissues. In addition, glucagon pharmacodynamics were investigated after administration in IP space and SC tissues.

This thesis strongly indicates that improved glucose control is achieved by simply by changing from CSII to CIPII. CIPII *per se* could improve HbA1c levels in patients with DM1. During CIPII, insulin absorption is faster, maximum levels are higher, and circulating levels declines earlier than with CSII treatment. This shorter feedback loop would be an obvious advantage in an AP making improved glucose control easier to achieve.

Another noteworthy observation presented in the **Paper I,** is that no metabolic disadvantages were observed during CIPII compared to CSII treatment. During the extraction of data for the **Paper I** with a focus on complications after surgical intervention to establish IP access, not all authors reported ongoing complications and errors during the studies. Severe complications were reported in one study [321], presuming that was the reason for extensive drop-out during the study (almost 50 %). In another study, severe pain was explained as a too-long catheter inserted in the abdominal space and erythema was observed for all patients; successfully, local cleaning and disinfection removed the condition [322]. Moreover, reasons for catheter explantation or replacement were the individual reaction of a catheter which resulted in encapsulation of the tip of a catheter; reasons for pump replacement were insulin infusion system errors. These results indicate that the IP space can be used as a possible location for an AP, with minimal infection possibility.

The **Paper II** underline the importance of the liver in glucose homeostasis as a first-pass effect after a smaller IP insulin dose approached 100 %. Our observations were not supported by portal vein blood samples. However, it was observed that after a small IP insulin bolus (5 U), BG levels decreased without observed elevation of the systemic insulin levels. After the larger IP insulin boluses, the glucose-lowering effect of insulin achieved in extrahepatic tissues throughout the body was quite minor compared to the hepatic effect. This is an important observation when the algorithm for an IP AP should be established.

This thesis provides new basic knowledge related to IP delivery of insulin and glucagon. **Paper II** present results underlining that the algorithms in an AP with IP insulin delivery need to reflect the non-linear relationship between IP insulin delivered and the subsequent effect on systemic circulating glucose levels. **Paper III** indicated faster glucose response after IP glucagon injection; thus, it gives further insight into the potential benefits of IP bi-hormonal AP.

7. Concluding remarks

In this thesis, we found that CIPII can improve BG levels of DM1 patients with brittle diabetes or uncontrolled with CSII. CIPII treatment improves HbA1c levels, reduces severe hypo- and hyperglycaemic events and increases time spent in normoglycaemia. We found that IP insulin administration has a pattern more similar to endogenous insulin secretion, such as early insulin peak and early insulin level decline in circulating blood.

We found that two different IP insulin boluses (5 U and 10 U) have more or less the same effect on BG levels but very different effects on circulating insulin levels that were increased insulin levels only after the highest insulin boluses (10 U). Our pig experiments also indicate a more extensive first-pass effect and insulin absorption in the liver than previously anticipated. This indicates a non-linear association between IP insulin boluses and the impact of circulating BG levels. This is important knowledge in future work on an IP AP.

We found that IP glucagon injections gives higher BG levels elevation only after four minutes and affects BG levels for a shorter period than SC glucagon injections. This is compatible with IP glucagon reaching the primary target, the liver, earlier than after SC glucagon injection. Also, IP glucagon is subject to a first-pass effect in the liver, potentially reducing the risk of systemic adverse effects of frequent long-term use of glucagon in an IP AP compared to an AP with SC delivery of hormones.

Summarising all observations on the thesis, we hold that an IP bi-hormonal AP system has a greater potential to achieve satisfying glucose control compared to an SC approach. This thesis also gives important information on the effects of IP hormone delivery and, as such, is an essential basis for the future work on an IP AP by the APT research group.

8. Future perspectives

All patients included in the studies in the **paper I** had normal weight (on average 75 kg or BMI 25 kg/m^2). Thus, it would be of great interest to compare patients with overweight and/or underweight.

In the future, all studies that follow the inclusion criteria of the systematic review should be uploaded for the meta-analysis.

Future research with animal studies should focus on awake animals to avoid reduced intestinal motility and anaesthetics negative side effects on BG levels.

Data collected during the animal studies will be used to identify mathematical models to be used in both for simulating 'virtual patients' and in a controller for the AP system.

Data from the pig experiments should be considered when other research groups make mathematical models, focusing on the first-pass effect of insulin, specifically for IV and IP insulin administration.

Our systematic review paper and animal studies included in the thesis can alter how people look at IP insulin administration and possibly motivate more research and development in that direction.

9. References

1. Kim, C.S., S.H. Cho, H.S. Chun, S.Y. Lee, H. Endou, Y. Kanai, and D.K. Kim, *BCH, an inhibitor of system L amino acid transporters, induces apoptosis in cancer cells.* Biol Pharm Bull, 2008. **31**(6): p. 1096-100. DOI: 10.1248/bpb.31.1096
2. Gill, G.V., *The spectrum of brittle diabetes.* J R Soc Med, 1992. **85**(5): p. 259-61. PMC 1294600
3. WHO. *Diabetes Fact sheet No 312.* 2013 [cited 2020 SEptember 20]; Available from: https://web.archive.org/web/20130826174444/http://www.who.int/mediacentre/factsheets/fs312/en/.
4. Tarbell, J.M. and M.Y. Pahakis, *Mechanotransduction and the glycocalyx.* J Intern Med, 2006. **259**(4): p. 339-50. DOI: 10.1111/j.1365-2796.2006.01620.x
5. Institute, N.C. *NCI Dictionary of cancer terms: reactive oxygen species*. 2020 [cited 2020 September 16]; Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/reactive-oxygen-species.
6. Association, A.D., *Gestational diabetes mellitus.* Diabetes Care, 2003. **26 Suppl 1**: p. S103-5.
7. Fu, B.M. and J.M. Tarbell, *Mechano-sensing and transduction by endothelial surface glycocalyx: composition, structure, and function.* Wiley Interdiscip Rev Syst Biol Med, 2013. **5**(3): p. 381-90. DOI: 10.1002/wsbm.1211
8. Nitin, S., *HbA1c and factors other than diabetes mellitus affecting it.* Singapore Med J, 2010. **51**(8): p. 616-22. PMID: 20848057
9. Longmore M., W.I., Turmezei T., Cheung CK., *Endocrinology*. Oxford Handbook of Clinical medicine Seventh Edition. Vol. Seventh. 2007, Oxford University Press:: The United States by Oxford University Press Inc., New York.
10. Balijepalli, C., E. Druyts, G. Siliman, M. Joffres, K. Thorlund, and E.J. Mills, *Hypoglycemia: a review of definitions used in clinical trials evaluating antihyperglycemic drugs for diabetes.* Clin Epidemiol, 2017. **9**: p. 291-296. DOI: 10.2147/clep.s129268
11. Diabetes, N.I.o. *Prediabetes and Insulin Resistance*. [cited 2020 SEptember 16]; Available from: https://www.niddk.nih.gov/health-information/diabetes/overview/what-is-diabetes/prediabetes-insulin-resistance.
12. American diabetes association. *DKA (Ketoacidosis) & Ketones*. [cited 2020 September 16]; Available from: https://www.diabetes.org/diabetes/complications/dka-ketoacidosis-ketones.
13. community, D.c.u.t.g.d. *Multiple Dose Insulin Therapy – Multiple Daily Injections*. 2019 [cited 2020 September 16]; Available from: https://www.diabetes.co.uk/insulin/multiple-dose-insulin-injection-therapy.html.
14. Mlinar B, M.J., Pfeifer M., *Molecular Mechanisms of Insulin Resistance, Obesity and Metabolic Syndrome.* Biochem Med (Zagreb), 2006. **16**: p. 8-24. DOI: 10.11613/BM.2006.003
15. M, A., *PID control*, in *Encyclopedia of Life Support Systems (EOLSS)*, H. Unbehauen, Editor. 2002, the Auspices of the UNESCO, Eolss Publishers: Control systems, robotics and automation.
16. Amy Hess-Fischl MS, R., LDN, BC-ADM. *CDE Hyperglycemia: When Your Blood Glucose Level Goes Too High*. 2018 [cited 2020 September 16]; Available from: https://www.endocrineweb.com/conditions/hyperglycemia/hyperglycemia-when-your-blood-glucose-level-goes-too-high.

17. community, D.c.u.t.g.d. *Severe Hypoglycemia*. 2019 [cited 2020 September 16]; Available from: https://www.diabetes.co.uk/severe-hypoglycemia.html.
18. WHO. *Diabetes*. 2020 [cited 2020 September 10]; Available from: https://www.who.int/health-topics/diabetes#tab=tab_1.
19. Association, T.N.D. *Diabetes*. 2020 [cited 2020 September 10]; Available from: https://www.diabetes.no/english/.
20. Federation, I.d. *Type 1 diabetes*. 2020 [cited 2020 September 10]; Available from: https://www.idf.org/aboutdiabetes/type-1-diabetes.html.
21. Stenström, G., A. Gottsäter, E. Bakhtadze, B. Berger, and G. Sundkvist, *Latent Autoimmune Diabetes in Adults.* Definition, Prevalence, β-Cell Function, and Treatment, 2005. **54**(suppl 2): p. S68-S72. DOI: 10.2337/diabetes.54.suppl_2.S68
22. Mouri MI, B.M. *Hyperglycaemia*. 2020 [cited 2020 September 10]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK430900/.
23. Fisher, L., D. Hessler, W. Polonsky, L. Strycker, U. Masharani, and A. Peters, *Diabetes distress in adults with type 1 diabetes: Prevalence, incidence and change over time.* J Diabetes Complications, 2016. **30**(6): p. 1123-8. DOI: 10.1016/j.jdiacomp.2016.03.032
24. Martyn-Nemeth, P., J. Duffecy, C. Fritschi, and L. Quinn, *Challenges Imposed By Hypoglycemia in Adults With Type 1 Diabetes.* Clin Nurs Res, 2019. **28**(8): p. 947-967. DOI: 10.1177/1054773818774702
25. Thompson, R., D. Christie, and P.C. Hindmarsh, *The role for insulin analogues in diabetes care.* Current Paediatrics, 2006. **16**(2): p. 117-122. DOI: 10.1016/j.cupe.2005.12.011
26. de Bock, M., S.A. McAuley, M.B. Abraham, G. Smith, J. Nicholas, G.R. Ambler, F.J. Cameron, J.M. Fairchild, B.R. King, E.A. Geelhoed, E.A. Davis, D.N. O'Neal, and T.W. Jones, *Effect of 6 months hybrid closed-loop insulin delivery in young people with type 1 diabetes: a randomised controlled trial protocol.* BMJ Open, 2018. **8**(8): p. e020275. DOI: 10.1136/bmjopen-2017-020275
27. Musolino, G., J.M. Allen, S. Hartnell, M.E. Wilinska, M. Tauschmann, C. Boughton, F. Campbell, L. Denvir, N. Trevelyan, P. Wadwa, L. DiMeglio, B.A. Buckingham, S. Weinzimer, C.L. Acerini, K. Hood, S. Fox, C. Kollman, J. Sibayan, S. Borgman, P. Cheng, and R. Hovorka, *Assessing the efficacy, safety and utility of 6-month day-and-night automated closed-loop insulin delivery under free-living conditions compared with insulin pump therapy in children and adolescents with type 1 diabetes: an open-label, multicentre, multinational, single-period, randomised, parallel group study protocol.* BMJ Open, 2019. **9**(6): p. e027856. DOI: 10.1136/bmjopen-2018-027856
28. Anderson, S.M., B.A. Buckingham, M.D. Breton, J.L. Robic, C.L. Barnett, C.A. Wakeman, M.C. Oliveri, S.A. Brown, T.T. Ly, P.K. Clinton, L.J. Hsu, R.S. Kingman, L.M. Norlander, S.E. Loebner, S. Reuschel-DiVirglio, and B.P. Kovatchev, *Hybrid Closed-Loop Control Is Safe and Effective for People with Type 1 Diabetes Who Are at Moderate to High Risk for Hypoglycemia.* Diabetes Technol Ther, 2019. **21**(6): p. 356-363. DOI: 10.1089/dia.2019.0018
29. Steil, G.M., K. Rebrin, C. Darwin, F. Hariri, and M.F. Saad, *Feasibility of automating insulin delivery for the treatment of type 1 diabetes.* Diabetes, 2006. **55**(12): p. 3344-50. DOI: 10.2337/db06-0419
30. Fritze, K., U. Fischer, E.J. Freyse, and W. Besch, *Intraindividual comparison of pharmacokinetics of insulin after intravenous, portal, subcutaneous and peritoneal administration.* Exp Clin Endocrinol, 1988. **92**(3): p. 297-306. DOI: 10.1055/s-0029-1210818

31. Vecchio, I., C. Tornali, N.L. Bragazzi, and M. Martini, *The Discovery of Insulin: An Important Milestone in the History of Medicine.* Front Endocrinol (Lausanne), 2018. **9**: p. 613. DOI: 10.3389/fendo.2018.00613
32. Wojtusciszyn, A., M. Armanet, P. Morel, T. Berney, and D. Bosco, *Insulin secretion from human beta cells is heterogeneous and dependent on cell-to-cell contacts.* Diabetologia, 2008. **51**(10): p. 1843-52. DOI: 10.1007/s00125-008-1103-z
33. Pour, P.M., J. Standop, and S.K. Batra, *Are islet cells the gatekeepers of the pancreas?* Pancreatology, 2002. **2**(5): p. 440-448. DOI: 10.1159/000064718
34. O'Toole TJ, S.S. *Physiology, Somatostatin*. 2020 2020 April 27 [cited 2020 August 20]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK538327/.
35. Ionescu-Tirgoviste, C., P.A. Gagniuc, E. Gubceac, L. Mardare, I. Popescu, S. Dima, and M. Militaru, *A 3D map of the islet routes throughout the healthy human pancreas.* Sci Rep, 2015. **5**: p. 14634. DOI: 10.1038/srep14634
36. Henry, B.M., B. Skinningsrud, K. Saganiak, P.A. Pękala, J.A. Walocha, and K.A. Tomaszewski, *Development of the human pancreas and its vasculature - An integrated review covering anatomical, embryological, histological, and molecular aspects.* Ann Anat, 2019. **221**: p. 115-124. 10.1016/j.aanat.2018.09.008
37. Weiss, M.A., *Proinsulin and the genetics of diabetes mellitus.* J Biol Chem, 2009. **284**(29): p. 19159-63. DOI: 10.1074/jbc.R109.009936
38. Hou, J.C., L. Min, and J.E. Pessin, *Insulin granule biogenesis, trafficking and exocytosis.* Vitam Horm, 2009. **80**: p. 473-506. DOI: 10.1016/s0083-6729(08)00616-x
39. Oliva, A., J. Fariña, and M. Llabrés, *Development of two high-performance liquid chromatographic methods for the analysis and characterization of insulin and its degradation products in pharmaceutical preparations.* J Chromatogr B Biomed Sci Appl, 2000. **749**(1): p. 25-34. DOI: 10.1016/s0378-4347(00)00374-1
40. Kurtzhals, P., L. Schäffer, A. Sørensen, C. Kristensen, I. Jonassen, C. Schmid, and T. Trüb, *Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use.* Diabetes, 2000. **49**(6): p. 999-1005. DOI: 10.2337/diabetes.49.6.999
41. Barrett K., B.H., Boitano S., Barman S., *Ganong`s review of medical physiology 23rd edition.* Endocrine Functions of the pancreas & Regulation of carbohydrate metabolism. 2010: The McGraw-Hill companies.
42. Conlon, J.M., Y.H. Abdel-Wahab, F.P. O'Harte, P.F. Nielsen, and J. Whittaker, *Purification and characterization of insulin, glucagon, and two glucagon-like peptides with insulin-releasing activity from the pancreas of the toad, Bufo marinus.* Endocrinology, 1998. **139**(8): p. 3442-8. DOI: 10.1210/endo.139.8.6139
43. Disotuar, M.M., M.E. Petersen, J.M. Nogueira, M.S. Kay, and D.H. Chou, *Synthesis of hydrophobic insulin-based peptides using a helping hand strategy.* Org Biomol Chem, 2019. **17**(7): p. 1703-1708. DOI: 10.1039/c8ob01212a
44. Halldén, G., G. Gafvelin, V. Mutt, and H. Jörnvall, *Characterization of cat insulin.* Arch Biochem Biophys, 1986. **247**(1): p. 20-7. DOI: 10.1016/0003-9861(86)90528-x
45. Nolan, C., E. Margoliash, J.D. Peterson, and D.F. Steiner, *The structure of bovine proinsulin.* J Biol Chem, 1971. **246**(9): p. 2780-95. PMID: 4928892
46. De Meyts, P., *Insulin and its receptor: structure, function and evolution.* Bioessays, 2004. **26**(12): p. 1351-62. DOI: 10.1002/bies.20151
47. Valverde, I., P. Garcia-Morales, M. Ghiglione, and W.J. Malaisse, *The stimulus-secretion coupling of glucose-induced insulin release. LIII. Calcium-dependency of the cyclic AMP response to nutrient secretagogues.* Horm Metab Res, 1983. **15**(2): p. 62-8. DOI: 10.1055/s-2007-1018632

48. Straub, S.G. and G.W. Sharp, *Glucose-stimulated signaling pathways in biphasic insulin secretion.* Diabetes Metab Res Rev, 2002. **18**(6): p. 451-63.DOI: 10.1002/dmrr.329
49. Dingreville, F., B. Panthu, C. Thivolet, S. Ducreux, Y. Gouriou, S. Pesenti, M.A. Chauvin, K. Chikh, E. Errazuriz-Cerda, F. Van Coppenolle, J. Rieusset, and A.M. Madec, *Differential Effect of Glucose on ER-Mitochondria Ca(2+) Exchange Participates in Insulin Secretion and Glucotoxicity-Mediated Dysfunction of β-Cells.* Diabetes, 2019. **68**(9): p. 1778-1794. DOI: 10.2337/db18-1112
50. Fu, Z., E.R. Gilbert, and D. Liu, *Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes.* Curr Diabetes Rev, 2013. **9**(1): p. 25-53. PMC 3934755
51. Henquin, J.C. and H.P. Meissner, *Effects of amino acids on membrane potential and 86Rb+ fluxes in pancreatic beta-cells.* American Journal of Physiology-Endocrinology and Metabolism, 1981. **240**(3): p. E245-E252. DOI: 10.1152/ajpendo.1981.240.3.E245
52. Unger, R.H., A. Ohneda, E. Aguilar-Parada, and A.M. Eisentraut, *The role of aminogenic glucagon secretion in blood glucose homeostasis.* J Clin Invest, 1969. **48**(5): p. 810-22. DOI: 10.1172/jci106039
53. Floyd, J.C., Jr., S.S. Fajans, J.W. Conn, R.F. Knopf, and J. Rull, *Insulin secretion in response to protein ingestion.* The Journal of Clinical Investigation, 1966. **45**(9): p. 1479-1486. DOI: 10.1172/JCI105455
54. Klein, R., C.E. Myers, K.E. Lee, R. Gangnon, and B.E. Klein, *Changes in retinal vessel diameter and incidence and progression of diabetic retinopathy.* Arch Ophthalmol, 2012. **130**(6): p. 749-55. DOI: 10.1001/archophthalmol.2011.2560
55. Tsuboi, T., H.T. McMahon, and G.A. Rutter, *Mechanisms of dense core vesicle recapture following "kiss and run" ("cavicapture") exocytosis in insulin-secreting cells.* J Biol Chem, 2004. **279**(45): p. 47115-24. DOI: 10.1074/jbc.M408179200
56. Tsuboi, T. and G.A. Rutter, *Multiple forms of "kiss-and-run" exocytosis revealed by evanescent wave microscopy.* Curr Biol, 2003. **13**(7): p. 563-7. DOI: 10.1016/s0960-9822(03)00176-3
57. Renström, E., L. Eliasson, and P. Rorsman, *Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells.* J Physiol, 1997. **502 (Pt 1)**(Pt 1): p. 105-18. DOI: 10.1111/j.1469-7793.1997.105bl.x
58. Gromada, J., M. Høy, E. Renström, K. Bokvist, L. Eliasson, S. Göpel, and P. Rorsman, *CaM kinase II-dependent mobilization of secretory granules underlies acetylcholine-induced stimulation of exocytosis in mouse pancreatic B-cells.* J Physiol, 1999. **518 (Pt 3)**(Pt 3): p. 745-59. DOI: 10.1111/j.1469-7793.1999.0745p.x
59. Schumacher, K., M. Matz, D. Brüning, K. Baumann, and I. Rustenbeck, *Granule mobility, fusion frequency and insulin secretion are differentially affected by insulinotropic stimuli.* Traffic, 2015. **16**(5): p. 493-509. DOI: 10.1111/tra.12261
60. Rorsman, P. and E. Renström, *Insulin granule dynamics in pancreatic beta cells.* Diabetologia, 2003. **46**(8): p. 1029-45. DOI: 10.1007/s00125-003-1153-1
61. Brange, J. and A. Vølund, *Insulin analogs with improved pharmacokinetic profiles.* Advanced Drug Delivery Reviews, 1999. **35**(2): p. 307-335. DOI: 10.1016/S0169-409X(98)00079-9
62. Jensen, M. and P. De Meyts, *Chapter 3 Molecular Mechanisms of Differential Intracellular Signaling From the Insulin Receptor*, in *Vitamins & Hormones*. 2009, Academic Press. p. 51-75.
63. Kawamori, D., A.J. Kurpad, J. Hu, C.W. Liew, J.L. Shih, E.L. Ford, P.L. Herrera, K.S. Polonsky, O.P. McGuinness, and R.N. Kulkarni, *Insulin signaling in alpha cells modulates glucagon secretion in vivo.* Cell Metab, 2009. **9**(4): p. 350-61. DOI: 10.1016/j.cmet.2009.02.007

64. Lee, J., Miyazaki M., Romeo G.R., and Shoelson S.E., *Insulin receptor activation with transmembrane domain ligands.* J Biol Chem, 2014. **289**(28): p. 19769-77. DOI: 10.1074/jbc.M114.578641
65. Pilch, P.F. and M.P. Czech, *The subunit structure of the high affinity insulin receptor. Evidence for a disulfide-linked receptor complex in fat cell and liver plasma membranes.* J Biol Chem, 1980. **255**(4): p. 1722-31. PMID: 6986378
66. Saltiel, A.R. and C.R. Kahn, *Insulin signalling and the regulation of glucose and lipid metabolism.* Nature, 2001. **414**(6865): p. 799-806. DOI: 10.1038/414799a
67. van der Geer, P., T. Hunter, and R.A. Lindberg, *Receptor protein-tyrosine kinases and their signal transduction pathways.* Annu Rev Cell Biol, 1994. **10**: p. 251-337. DOI: 10.1146/annurev.cb.10.110194.001343
68. Edgerton, D.S., G. Kraft, M. Smith, B. Farmer, P.E. Williams, K.C. Coate, R.L. Printz, R.M. O'Brien, and A.D. Cherrington, *Insulin's direct hepatic effect explains the inhibition of glucose production caused by insulin secretion.* JCI Insight, 2017. **2**(6): p. e91863. DOI: 10.1172/jci.insight.91863
69. Bratusch-Marrain, P.R., W.K. Waldhäusl, S. Gasić, and A. Hofer, *Hepatic disposal of biosynthetic human insulin and porcine C-peptide in humans.* Metabolism, 1984. **33**(2): p. 151-157. DOI: 10.1016/0026-0495(84)90128-8
70. Lomberk, G. and R. Urrutia, *Primers on Molecular Pathways —The Insulin Pathway.* Pancreatology, 2009. **9**(3): p. 203-205. DOI: 10.1159/000200021
71. Schauer, I.E., J.K. Snell-Bergeon, B.C. Bergman, D.M. Maahs, A. Kretowski, R.H. Eckel, and M. Rewers, *Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: The CACTI study.* Diabetes, 2011. **60**(1): p. 306-14. DOI: 10.2337/db10-0328
72. Eskens, B.J., H.L. Mooij, J.P. Cleutjens, J.M. Roos, J.E. Cobelens, H. Vink, and J.W. Vanteeffelen, *Rapid insulin-mediated increase in microvascular glycocalyx accessibility in skeletal muscle may contribute to insulin-mediated glucose disposal in rats.* PLoS One, 2013. **8**(1): p. e55399. DOI: 10.1371/journal.pone.0055399
73. An, Y., Y. Kang, J. Lee, C. Ahn, K. Kwon, and C. Choi, *Blood flow characteristics of diabetic patients with complications detected by optical measurement.* Biomed Eng Online, 2018. **17**(1): p. 25. DOI: 10.1186/s12938-018-0457-9
74. Ellenberg, M., *Diabetic neuropathy: Clinical aspects.* Metabolism, 1976. **25**(12): p. 1627-1655. DOI: 10.1016/0026-0495(76)90115-3
75. Horwitz, D.L., J.I. Starr, M.E. Mako, W.G. Blackard, and A.H. Rubenstein, *Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood.* J Clin Invest, 1975. **55**(6): p. 1278-83. DOI: 10.1172/jci108047
76. Gregory, J.M., T.J. Smith, J.C. Slaughter, H.R. Mason, C.C. Hughey, M.S. Smith, B. Kandasamy, S.A.W. Greeley, L.H. Philipson, R.N. Naylor, L.R. Letourneau, N.N. Abumrad, A.D. Cherrington, and D.J. Moore, *Iatrogenic Hyperinsulinemia, Not Hyperglycemia, Drives Insulin Resistance in Type 1 Diabetes as Revealed by Comparison With GCK-MODY (MODY2).* Diabetes, 2019. **68**(8): p. 1565-1576. DOI: 10.2337/db19-0324
77. Orchard, T.J., J.C. Olson, J.R. Erbey, K. Williams, K.Y.Z. Forrest, L. Smithline Kinder, D. Ellis, and D.J. Becker, *Insulin Resistance–Related Factors, but not Glycemia, Predict Coronary Artery Disease in Type 1 Diabetes.* Diabetes Care, 2003. **26**(5): p. 1374. DOI: 10.2337/diacare.26.5.1374
78. Ibrahim, M.M., *Subcutaneous and visceral adipose tissue: structural and functional differences.* Obes Rev, 2010. **11**(1): p. 11-8. DOI: 10.1111/j.1467-789X.2009.00623.x
79. Vidot, D.C., K.L. Arheart, G. Prado, E.S. Bandstra, and S.E. Messiah, *Illicit drug use and cardiometabolic disease risk: an analysis of 2005-2008 National Health and Nutrition*

Examination Survey data. Int J Clin Pract, 2013. **67**(11): p. 1173-81. DOI: 10.1111/ijcp.12189

80. Senniappan, S., V.B. Arya, and K. Hussain, *The molecular mechanisms, diagnosis and management of congenital hyperinsulinism.* Indian J Endocrinol Metab, 2013. **17**(1): p. 19-30. DOI: 10.4103/2230-8210.107822
81. Zhang, A.M.Y., J. Magrill, T.J.J. de Winter, X. Hu, S. Skovsø, D.F. Schaeffer, J.L. Kopp, and J.D. Johnson, *Endogenous Hyperinsulinemia Contributes to Pancreatic Cancer Development.* Cell Metab, 2019. **30**(3): p. 403-404. DOI: 10.1016/j.cmet.2019.07.003
82. Higgins, V., S. Asgari, J.K. Hamilton, A. Wolska, A.T. Remaley, B. Hartmann, J.J. Holst, and K. Adeli, *Postprandial Dyslipidemia, Hyperinsulinemia, and Impaired Gut Peptides/Bile Acids in Adolescents with Obesity.* J Clin Endocrinol Metab, 2020. **105**(4): p. 1228-41. DOI: 10.1210/clinem/dgz261
83. Halama, A., M.M. Aye, S.R. Dargham, M. Kulinski, K. Suhre, and S.L. Atkin, *Metabolomics of Dynamic Changes in Insulin Resistance Before and After Exercise in PCOS.* Front Endocrinol (Lausanne), 2019. **10**: p. 116. DOI: 10.3389/fendo.2019.00116
84. Unger, R.H. and L. Orci, *Glucagon and the a Cell.* New England Journal of Medicine, 1981. **304**(25): p. 1518-1524. DOI: 10.1056/NEJM198106183042504
85. King, M.V., *A low-resolution structural model for cubic glucagon based on packing of cylinders.* Journal of Molecular Biology, 1965. **11**(3): p. 549-IN5. DOI: 10.1016/S0022-2836(65)80010-9
86. Mojsov, S., G. Heinrich, I.B. Wilson, M. Ravazzola, L. Orci, and J.F. Habener, *Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing.* J Biol Chem, 1986. **261**(25): p. 11880-9. PMID: 3528148
87. Thiessen, S.E., J. Gunst, and G. Van den Berghe, *Role of glucagon in protein catabolism.* Curr Opin Crit Care, 2018. **24**(4): p. 228-234. DOI: 10.1097/mcc.0000000000000509
88. Lee, Y., M.Y. Wang, X.Q. Du, M.J. Charron, and R.H. Unger, *Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice.* Diabetes, 2011. **60**(2): p. 391-7. DOI: 10.2337/db10-0426
89. Galsgaard, K.D., J. Pedersen, F.K. Knop, J.J. Holst, and N.J. Wewer Albrechtsen, *Glucagon Receptor Signaling and Lipid Metabolism.* Front Physiol, 2019. **10**: p. 413. DOI: 10.3389/fphys.2019.00413
90. Drucker, D.J. and S. Asa, *Glucagon gene expression in vertebrate brain.* J Biol Chem, 1988. **263**(27): p. 13475-8. PMID: 2901414
91. McGirr, R., L. Guizzetti, and S. Dhanvantari, *The sorting of proglucagon to secretory granules is mediated by carboxypeptidase E and intrinsic sorting signals.* J Endocrinol, 2013. **217**(2): p. 229-40. DOI: 10.1530/joe-12-0468
92. Reissaus, C.A. and D.W. Piston, *Reestablishment of Glucose Inhibition of Glucagon Secretion in Small Pseudoislets.* Diabetes, 2017. **66**(4): p. 960-969. DOI: 10.2337/db16-1291
93. Schwartz, N.S., W.E. Clutter, S.D. Shah, and P.E. Cryer, *Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms.* J Clin Invest, 1987. **79**(3): p. 777-81. DOI: 10.1172/jci112884
94. Sperling, M.A., P.V. DeLamater, D. Phelps, R.H. Fiser, W. Oh, and D.A. Fisher, *Spontaneous and amino acid-stimulated glucagon secretion in the immediate postnatal period. Relation to glucose and insulin.* J Clin Invest, 1974. **53**(4): p. 1159-66. DOI: 10.1172/jci107654
95. Chuang, J.C., I. Sakata, D. Kohno, M. Perello, S. Osborne-Lawrence, J.J. Repa, and J.M. Zigman, *Ghrelin directly stimulates glucagon secretion from pancreatic alpha-cells.* Mol Endocrinol, 2011. **25**(9): p. 1600-11. DOI: 10.1210/me.2011-1001

96. Scott, R., J. Minnion, T. Tan, and S.R. Bloom, *Oxyntomodulin analogue increases energy expenditure via the glucagon receptor.* Peptides, 2018. **104**: p. 70-77. DOI: 10.1016/j.peptides.2018.04.008
97. Wewer Albrechtsen, N.J., R.E. Kuhre, J. Pedersen, F.K. Knop, and J.J. Holst, *The biology of glucagon and the consequences of hyperglucagonemia.* Biomark Med, 2016. **10**(11): p. 1141-1151. DOI: 10.2217/bmm-2016-0090
98. Ahrén, B., R.C. Veith, and G.J. Taborsky, Jr., *Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1). Effects on basal release of insulin and glucagon.* Endocrinology, 1987. **121**(1): p. 323-31. DOI: 10.1210/endo-121-1-323
99. Miyachi, A., M. Kobayashi, E. Mieno, M. Goto, K. Furusawa, T. Inagaki, and T. Kitamura, *Accurate analytical method for human plasma glucagon levels using liquid chromatography-high resolution mass spectrometry: comparison with commercially available immunoassays.* Anal Bioanal Chem, 2017. **409**(25): p. 5911-5918. DOI: 10.1007/s00216-017-0534-0
100. Quesada, I., E. Tudurí, C. Ripoll, and A. Nadal, *Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes.* J Endocrinol, 2008. **199**(1): p. 5-19. DOI: 10.1677/joe-08-0290
101. Franklin, I., J. Gromada, A. Gjinovci, S. Theander, and C.B. Wollheim, *Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release.* Diabetes, 2005. **54**(6): p. 1808-15. DOI: 10.2337/diabetes.54.6.1808
102. Rix I, N.-L.C., Bergmann NC, et al. *Glucagon Physiology*. 2019 2019 Jul 16 03/12/2020]; Feingold KR, Anawalt B, Boyce A, et al., editors:[Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK279127/.
103. González-Vélez, V., G. Dupont, A. Gil, A. González, and I. Quesada, *Model for glucagon secretion by pancreatic α-cells.* PLoS One, 2012. **7**(3): p. e32282. DOI: 10.1371/journal.pone.0032282
104. Qiao, A., S. Han, X. Li, Z. Li, P. Zhao, A. Dai, R. Chang, L. Tai, Q. Tan, X. Chu, L. Ma, T.S. Thorsen, S. Reedtz-Runge, D. Yang, M.-W. Wang, P.M. Sexton, D. Wootten, F. Sun, Q. Zhao, and B. Wu, *Structural basis of G_s and G_i recognition by the human glucagon receptor.* Science, 2020. **367**(6484): p. 1346. DOI: 10.1126/science.aaz5346
105. Hayman, G.T., P. Jayaraman, V. Petri, M. Tutaj, W. Liu, J. De Pons, M.R. Dwinell, and M. Shimoyama, *The updated RGD Pathway Portal utilizes increased curation efficiency and provides expanded pathway information.* Hum Genomics, 2013. **7**(1): p. 4. DOI: 10.1186/1479-7364-7-4
106. Conarello, S.L., G. Jiang, J. Mu, Z. Li, J. Woods, E. Zycband, J. Ronan, F. Liu, R.S. Roy, L. Zhu, M.J. Charron, and B.B. Zhang, *Glucagon receptor knockout mice are resistant to diet-induced obesity and streptozotocin-mediated beta cell loss and hyperglycaemia.* Diabetologia, 2007. **50**(1): p. 142-50. DOI: 10.1007/s00125-006-0481-3
107. Mattedi, G., S. Acosta-Gutiérrez, T. Clark, and F.L. Gervasio, *A combined activation mechanism for the glucagon receptor.* Proc Natl Acad Sci U S A, 2020. **117**(27): p. 15414-15422. DOI: 10.1073/pnas.1921851117
108. Rickels, M.R., K.J. Ruedy, N.C. Foster, C.A. Piché, H. Dulude, J.L. Sherr, W.V. Tamborlane, K.E. Bethin, L.A. DiMeglio, R.P. Wadwa, A.J. Ahmann, M.J. Haller, B.M. Nathan, S.M. Marcovina, E. Rampakakis, L. Meng, and R.W. Beck, *Intranasal Glucagon for Treatment of Insulin-Induced Hypoglycemia in Adults With Type 1 Diabetes: A Randomized Crossover Noninferiority Study.* Diabetes Care, 2016. **39**(2): p. 264-70. DOI: 10.2337/dc15-1498

109. Pietrzak, I. and A. Szadkowska, *Nasal glucagon - a new way to treat severe hypoglycemia in patients with diabetes.* Pediatr Endocrinol Diabetes Metab, 2020: p. 45-57. DOI: 10.5114/pedm.2020.94390
110. Sherr, J.L., K.J. Ruedy, N.C. Foster, C.A. Piché, H. Dulude, M.R. Rickels, W.V. Tamborlane, K.E. Bethin, L.A. DiMeglio, L.A. Fox, R.P. Wadwa, D.A. Schatz, B.M. Nathan, S.M. Marcovina, E. Rampakakis, L. Meng, and R.W. Beck, *Glucagon Nasal Powder: A Promising Alternative to Intramuscular Glucagon in Youth With Type 1 Diabetes.* Diabetes Care, 2016. **39**(4): p. 555-62. DOI: 10.2337/dc15-1606
111. Beato-Víbora, P.I. and F.J. Arroyo-Díez, *New uses and formulations of glucagon for hypoglycaemia.* Drugs Context, 2019. **8**: p. 212599. DOI: 10.7573/dic.212599
112. Mulla, C.M., S. Zavitsanou, A.J. Laguna Sanz, D. Pober, L. Richardson, P. Walcott, I. Arora, B. Newswanger, M.J. Cummins, S.J. Prestrelski, F.J. Doyle, E. Dassau, and M.E. Patti, *A Randomized, Placebo-Controlled Double-Blind Trial of a Closed-Loop Glucagon System for Postbariatric Hypoglycemia.* J Clin Endocrinol Metab, 2020. **105**(4): p. e1260-71. DOI: 10.1210/clinem/dgz197
113. Wilson, L.M., P.G. Jacobs, K.L. Ramsey, N. Resalat, R. Reddy, D. Branigan, J. Leitschuh, V. Gabo, F. Guillot, B. Senf, J. El Youssef, I.I.K. Steineck, N.S. Tyler, and J.R. Castle, *Dual-Hormone Closed-Loop System Using a Liquid Stable Glucagon Formulation Versus Insulin-Only Closed-Loop System Compared With a Predictive Low Glucose Suspend System: An Open-Label, Outpatient, Single-Center, Crossover, Randomized Controlled Trial.* Diabetes Care, 2020. **43**(11): p. 2721-2729. DOI: 10.2337/dc19-2267
114. Chabenne, J.R., M.A. DiMarchi, V.M. Gelfanov, and R.D. DiMarchi, *Optimization of the native glucagon sequence for medicinal purposes.* J Diabetes Sci Technol, 2010. **4**(6): p. 1322-31. DOI: 10.1177/193229681000400605
115. Joshi, A.B., E. Rus, and L.E. Kirsch, *The degradation pathways of glucagon in acidic solutions.* Int J Pharm, 2000. **203**(1-2): p. 115-25. DOI: 10.1016/s0378-5173(00)00438-5
116. Caputo, N., J.R. Castle, C.P. Bergstrom, J.M. Carroll, P.A. Bakhtiani, M.A. Jackson, C.T. Roberts, Jr., L.L. David, and W.K. Ward, *Mechanisms of glucagon degradation at alkaline pH.* Peptides, 2013. **45**: p. 40-7. DOI: 10.1016/j.peptides.2013.04.005
117. Rickels, M.R., S.N. DuBose, E. Toschi, R.W. Beck, A.S. Verdejo, H. Wolpert, M.J. Cummins, B. Newswanger, and M.C. Riddell, *Mini-Dose Glucagon as a Novel Approach to Prevent Exercise-Induced Hypoglycemia in Type 1 Diabetes.* Diabetes Care, 2018. **41**(9): p. 1909-1916. DOI: 10.2337/dc18-0051
118. Galochkina, T., M. Ng Fuk Chong, L. Challali, S. Abbar, and C. Etchebest, *New insights into GluT1 mechanics during glucose transfer.* Sci Rep, 2019. **9**(1): p. 998. DOI: 10.1038/s41598-018-37367-z
119. Müller, T.D., B. Finan, C. Clemmensen, R.D. DiMarchi, and M.H. Tschöp, *The New Biology and Pharmacology of Glucagon.* Physiol Rev, 2017. **97**(2): p. 721-766. DOI: 10.1152/physrev.00025.2016
120. Göpel, S.O., T. Kanno, S. Barg, and P. Rorsman, *Patch-clamp characterisation of somatostatin-secreting -cells in intact mouse pancreatic islets.* J Physiol, 2000. **528**(Pt 3): p. 497-507. DOI: 10.1111/j.1469-7793.2000.00497.x
121. Patel, Y.C., *Molecular pharmacology of somatostatin receptor subtypes.* J Endocrinol Invest, 1997. **20**(6): p. 348-67. DOI: 10.1007/bf03350317
122. Francis, B.H., D.G. Baskin, D.R. Saunders, and J.W. Ensinck, *Distribution of somatostatin-14 and somatostatin-28 gastrointestinal-pancreatic cells of rats and humans.* Gastroenterology, 1990. **99**(5): p. 1283-1291. DOI: 10.1016/0016-5085(90)91151-U

123. Wikner, J., L. Wetterberg, and S. Röjdmark, *The role of somatostatin (octreotide) in the regulation of melatonin secretion in healthy volunteers and in patients with primary hypothyroidism.* J Endocrinol Invest, 1999. **22**(7): p. 527-34. DOI: 10.1007/bf03343604
124. Schubert, M.L. and J.F. Rehfeld, *Gastric Peptides—Gastrin and Somatostatin*, in *Comprehensive Physiology*. p. 197-228.
125. Elliott, A.D., Ustione A., and Piston D.W., *Somatostatin and insulin mediate glucose-inhibited glucagon secretion in the pancreatic α-cell by lowering cAMP.* American Journal of Physiology-Endocrinology and Metabolism, 2015. **308**(2): p. E130-E143. DOI: 10.1152/ajpendo.00344.2014
126. Patel, Y.C., *Somatostatin and its receptor family.* Front Neuroendocrinol, 1999. **20**(3): p. 157-98. DOI: 10.1006/frne.1999.0183
127. Mandarino, L., D. Stenner, W. Blanchard, S. Nissen, J. Gerich, N. Ling, P. Brazeau, P. Bohlen, F. Esch, and R. Guillemin, *Selective effects of somatostatin-14, -25 and -28 on in vitro insulin and glucagon secretion.* Nature, 1981. **291**(5810): p. 76-7. DOI: 10.1038/291076a0
128. Denwood, G., A. Tarasov, A. Salehi, E. Vergari, R. Ramracheya, H. Takahashi, V.O. Nikolaev, S. Seino, F. Gribble, F. Reimann, P. Rorsman, and Q. Zhang, *Glucose stimulates somatostatin secretion in pancreatic δ-cells by cAMP-dependent intracellular Ca(2+) release.* J Gen Physiol, 2019. **151**(9): p. 1094-1115. DOI: 10.1085/jgp.201912351
129. Patel, Y.C., J.-L. Liu, A. Galanopoulou, and D.N. Papachristou, *Supplement 21. Handbook of Physiology, The Endocrine System, The Endocrine Pancreas and Regulation of Metabolism*. Production, Action, and Degradation of Somatostatin. 2011. DOI: 10.1002/cphy.cp070209
130. Patel, Y.C. and S. Reichlin, *Somatostatin in hypothalamus, extrahypothalamic brain, and peripheral tissues of the rat.* Endocrinology, 1978. **102**(2): p. 523-30. DOI: 10.1210/endo-102-2-523
131. Vanetti, M., G. Vogt, and V. Höllt, *The two isoforms of the mouse somatostatin receptor (mSSTR2A and mSSTR2B) differ m coupling efficiency to adenylate cyclase and in agonist-induced receptor desensitization.* FEBS Letters, 1993. **331**(3): p. 260-266. DOI: 10.1016/0014-5793(93)80349-Y
132. Mahipal, A., D. Shibata, E. Siegel, G. Springett, K. Almhanna, W. Fulp, I. Williams-Elson, and R. Kim, *Phase I trial of combination of FOLFIRI and pasireotide, a somatostatin analogue, in advanced gastrointestinal malignancies.* Invest New Drugs, 2015. **33**(5): p. 1093-9. DOI: 10.1007/s10637-015-0277-8
133. Cescato, R., K.A. Loesch, B. Waser, H.R. Mäcke, J.E. Rivier, J.C. Reubi, and A. Schonbrunn, *Agonist-biased signaling at the sst2A receptor: the multi-somatostatin analogs KE108 and SOM230 activate and antagonize distinct signaling pathways.* Mol Endocrinol, 2010. **24**(1): p. 240-9. DOI: 10.1210/me.2009-0321
134. Ludvigsen, E., M. Stridsberg, J.E. Taylor, M.D. Culler, K. Oberg, E.T. Janson, and S. Sandler, *Regulation of insulin and glucagon secretion from rat pancreatic islets in vitro by somatostatin analogues.* Regul Pept, 2007. **138**(1): p. 1-9. DOI: 10.1016/j.regpep.2006.07.006
135. Montminy, M., P. Brindle, J. Arias, K. Ferreri, and R. Armstrong, *Regulation of Somatostatin Gene Transcription by cAMP*, in *Advances in Pharmacology*, H. Hidaka and A.C. Nairn, Editors. 1996, Academic Press. p. 1-13. DOI: 10.1016/S1054-3589(08)60573-6
136. Takemura, J., Y.N. Kwok, and J.C. Brown, *Comparison of the effect of somatostatin and an analogue, SMS 201-995, on gastrin and insulin secretion from isolated perfused rat stomach and pancreas.* The American Journal of Medicine, 1986. **81**(6, Supplement 2): p. 65-73. DOI: 10.1016/0002-9343(86)90586-3

137. Kumar, U., R. Sasi, S. Suresh, A. Patel, M. Thangaraju, P. Metrakos, S.C. Patel, and Y.C. Patel, *Subtype-selective expression of the five somatostatin receptors (hSSTR1-5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis.* Diabetes, 1999. **48**(1): p. 77-85. DOI: 10.2337/diabetes.48.1.77
138. Reubi, J.C., U. Horisberger, A. Kappeler, and J.A. Laissue, *Localization of receptors for vasoactive intestinal peptide, somatostatin, and substance P in distinct compartments of human lymphoid organs.* Blood, 1998. **92**(1): p. 191-7. PMID: 9639516
139. Krenning, E.P., P.P. Kooij, S. Pauwels, W.A. Breeman, P.T. Postema, W.W. De Herder, R. Valkema, and D.J. Kwekkeboom, *Somatostatin receptor: scintigraphy and radionuclide therapy.* Digestion, 1996. **57 Suppl 1**: p. 57-61. DOI: 10.1159/000201398
140. Caplin, M.E., M. Pavel, J.B. Ćwikła, A.T. Phan, M. Raderer, E. Sedláčková, G. Cadiot, E.M. Wolin, J. Capdevila, L. Wall, G. Rindi, A. Langley, S. Martinez, J. Blumberg, and P. Ruszniewski, *Lanreotide in metastatic enteropancreatic neuroendocrine tumors.* N Engl J Med, 2014. **371**(3): p. 224-33. DOI: 10.1056/NEJMoa1316158
141. Schulz, S., S.U. Pauli, S. Schulz, M. Händel, K. Dietzmann, R. Firsching, and V. Höllt, *Immunohistochemical determination of five somatostatin receptors in meningioma reveals frequent overexpression of somatostatin receptor subtype sst2A.* Clin Cancer Res, 2000. **6**(5): p. 1865-74. PMID: 10815909
142. Stueven, A.K., A. Kayser, C. Wetz, H. Amthauer, A. Wree, F. Tacke, B. Wiedenmann, C. Roderburg, and H. Jann, *Somatostatin Analogues in the Treatment of Neuroendocrine Tumors: Past, Present and Future.* Int J Mol Sci, 2019. **20**(12). DOI: 10.3390/ijms20123049
143. Colao, A., M.D. Bronstein, P. Freda, F. Gu, C.C. Shen, M. Gadelha, M. Fleseriu, A.J. van der Lely, A.J. Farrall, K. Hermosillo Reséndiz, M. Ruffin, Y. Chen, and M. Sheppard, *Pasireotide versus octreotide in acromegaly: a head-to-head superiority study.* J Clin Endocrinol Metab, 2014. **99**(3): p. 791-9. DOI: 10.1210/jc.2013-2480
144. Schmid, H.A. and J. Brueggen, *Effects of somatostatin analogs on glucose homeostasis in rats.* J Endocrinol, 2012. **212**(1): p. 49-60. DOI: 10.1530/joe-11-0224
145. Anık, A., G. Çatlı, A. Abacı, and E. Böber, *Maturity-onset diabetes of the young (MODY): an update.* J Pediatr Endocrinol Metab, 2015. **28**(3-4): p. 251-63. DOI: 10.1515/jpem-2014-0384
146. Davidson, J.A., *Treatment of the patient with diabetes: importance of maintaining target HbA(1c) levels.* Curr Med Res Opin, 2004. **20**(12): p. 1919-27. DOI: 10.1185/030079904x6291
147. Umpierrez, G.E., M.B. Murphy, and A.E. Kitabchi, *Diabetic Ketoacidosis and Hyperglycemic Hyperosmolar Syndrome.* Diabetes Spectrum, 2002. **15**(1): p. 28. DOI: 10.2337/diaspect.15.1.28
148. Chiasson, J.L., N. Aris-Jilwan, R. Bélanger, S. Bertrand, H. Beauregard, J.M. Ekoé, H. Fournier, and J. Havrankova, *Diagnosis and treatment of diabetic ketoacidosis and the hyperglycemic hyperosmolar state.* Cmaj, 2003. **168**(7): p. 859-66. PMCID: PMC151994
149. Cherubini, V., E. Skrami, L. Ferrito, S. Zucchini, A. Scaramuzza, R. Bonfanti, P. Buono, F. Cardella, V. Cauvin, G. Chiari, D.A. G, A.P. Frongia, D. Iafusco, I.P. Patera, S. Toni, S. Tumini, I. Rabbone, F. Lombardo, F. Carle, and R. Gesuita, *High frequency of diabetic ketoacidosis at diagnosis of type 1 diabetes in Italian children: a nationwide longitudinal study, 2004-2013.* Sci Rep, 2016. **6**: p. 38844. DOI: 10.1038/srep38844
150. Diabetes, C., I. Complications Trial/Epidemiology of Diabetes, G. Complications Research, J.M. Lachin, S. Genuth, P. Cleary, M.D. Davis, and D.M. Nathan, *Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy.* The New England journal of medicine, 2000. **342**(6): p. 381-389. DOI: 10.1056/NEJM200002103420603

151. DeMill, D.L., M. Hussain, R. Pop-Busui, and R.M. Shtein, *Ocular surface disease in patients with diabetic peripheral neuropathy.* Br J Ophthalmol, 2016. **100**(7): p. 924-928. DOI: 10.1136/bjophthalmol-2015-307369
152. Konduracka, E., G. Cieślik, M.T. Małecki, G. Gajos, A. Siniarski, K.P. Malinowski, M. Kostkiewicz, L. Mastalerz, J. Nessler, and W. Piwowarska, *Obstructive and nonobstructive coronary artery disease in long-lasting type 1 diabetes: a 7-year prospective cohort study.* Pol Arch Intern Med, 2019. **129**(2): p. 97-105. DOI: 10.20452/pamw.4440
153. Haller, M.J., J.M. Stein, J.J. Shuster, D. Theriaque, M.M. Samyn, C. Pepine, and J.H. Silverstein, *Pediatric Atorvastatin in Diabetes Trial (PADIT): a pilot study to determine the effect of atorvastatin on arterial stiffness and endothelial function in children with type 1 diabetes mellitus.* J Pediatr Endocrinol Metab, 2009. **22**(1): p. 65-8. DOI: 10.1515/jpem.2009.22.1.65
154. Iturralde, E., J.R. Rausch, J. Weissberg-Benchell, and K.K. Hood, *Diabetes-Related Emotional Distress Over Time.* Pediatrics, 2019. **143**(6). DOI: 10.1542/peds.2018-3011
155. Sumnik, Z., A. Szypowska, V. Iotova, N. Bratina, V. Cherubini, G. Forsander, S. Jali, J.F. Raposo, G. Stipančic, A. Vazeou, H. Veeze, and K. Lange, *Persistent heterogeneity in diabetes technology reimbursement for children with type 1 diabetes: The SWEET perspective.* Pediatr Diabetes, 2019. **20**(4): p. 434-443. DOI: 10.1111/pedi.12833
156. Foster, N.C., R.W. Beck, K.M. Miller, M.A. Clements, M.R. Rickels, L.A. DiMeglio, D.M. Maahs, W.V. Tamborlane, R. Bergenstal, E. Smith, B.A. Olson, and S.K. Garg, *State of Type 1 Diabetes Management and Outcomes from the T1D Exchange in 2016-2018.* Diabetes Technol Ther, 2019. **21**(2): p. 66-72. DOI: 10.1089/dia.2018.0384
157. Tyndall, V., R.H. Stimson, N.N. Zammitt, S.A. Ritchie, J.A. McKnight, A.R. Dover, and F.W. Gibb, *Marked improvement in HbA(1c) following commencement of flash glucose monitoring in people with type 1 diabetes.* Diabetologia, 2019. **62**(8): p. 1349-1356. DOI: 10.1007/s00125-019-4894-1
158. Ranawana, V. and B. Kaur, *Chapter One - Role of Proteins in Insulin Secretion and Glycemic Control*, in *Advances in Food and Nutrition Research*, J. Henry, Editor. 2013, Academic Press. p. 1-47. DOI: 10.1016/B978-0-12-416555-7.00001-1
159. Ferrannini, E., O. Bjorkman, G.A. Reichard, Jr., A. Pilo, M. Olsson, J. Wahren, and R.A. DeFronzo, *The disposal of an oral glucose load in healthy subjects. A quantitative study.* Diabetes, 1985. **34**(6): p. 580-8. DOI: 10.2337/diab.34.6.580
160. Ishida, T., Z. Chap, J. Chou, R. Lewis, C. Hartley, M. Entman, and J.B. Field, *Differential effects of oral, peripheral intravenous, and intraportal glucose on hepatic glucose uptake and insulin and glucagon extraction in conscious dogs.* J Clin Invest, 1983. **72**(2): p. 590-601. DOI: 10.1172/jci111007
161. Nathan, D.M., P.A. Cleary, J.Y. Backlund, S.M. Genuth, J.M. Lachin, T.J. Orchard, P. Raskin, and B. Zinman, *Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes.* N Engl J Med, 2005. **353**(25): p. 2643-53. DOI: 10.1056/NEJMoa052187
162. Dhatariya, K.K. and P. Vellanki, *Treatment of Diabetic Ketoacidosis (DKA)/Hyperglycemic Hyperosmolar State (HHS): Novel Advances in the Management of Hyperglycemic Crises (UK Versus USA).* Curr Diab Rep, 2017. **17**(5): p. 33. DOI: 10.1007/s11892-017-0857-4
163. Cheisson, G., S. Jacqueminet, E. Cosson, C. Ichai, A.M. Leguerrier, B. Nicolescu-Catargi, A. Ouattara, I. Tauveron, P. Valensi, and D. Benhamou, *Perioperative management of adult diabetic patients. Review of hyperglycaemia: definitions and pathophysiology.* Anaesth Crit Care Pain Med, 2018. **37 Suppl 1**: p. S5-s8. DOI: 10.1016/j.accpm.2018.02.019
164. Westerberg, D.P., *Diabetic ketoacidosis: evaluation and treatment.* Am Fam Physician, 2013. **87**(5): p. 337-46. PMID: 23547550

165. Evans, K., *Diabetic ketoacidosis: update on management.* Clin Med (Lond), 2019. **19**(5): p. 396-398. DOI: 10.7861/clinmed.2019-0284
166. Kuppermann, N., S. Ghetti, J.E. Schunk, M.J. Stoner, A. Rewers, J.K. McManemy, S.R. Myers, L.E. Nigrovic, A. Garro, K.M. Brown, K.S. Quayle, J.L. Trainor, L. Tzimenatos, J.E. Bennett, A.D. DePiero, M.Y. Kwok, C.S. Perry, 3rd, C.S. Olsen, T.C. Casper, J.M. Dean, and N.S. Glaser, *Clinical Trial of Fluid Infusion Rates for Pediatric Diabetic Ketoacidosis.* N Engl J Med, 2018. **378**(24): p. 2275-2287. DOI: 10.1056/NEJMoa1716816
167. Hoffman, W.H., J.P. Locksmith, E.M. Burton, E. Hobbs, G.G. Passmore, A.L. Pearson-Shaver, D.A. Deane, M. Beaudreau, and R.W. Bassali, *Interstitial pulmonary edema in children and adolescents with diabetic ketoacidosis.* J Diabetes Complications, 1998. **12**(6): p. 314-20. DOI: 10.1016/s1056-8727(98)00012-9
168. Oschatz, E., M. Müllner, H. Herkner, and A.N. Laggner, *Multiple organ failure and prognosis in adult patients with diabetic ketoacidosis.* Wien Klin Wochenschr, 1999. **111**(15): p. 590-5. PMID: 10483673
169. Matz, R., *Management of the hyperosmolar hyperglycemic syndrome.* Am Fam Physician, 1999. **60**(5): p. 1468-76. PMID: 10524491
170. Stoner, G.D., *Hyperosmolar hyperglycemic state.* Am Fam Physician, 2005. **71**(9): p. 1723-30. PMID: 15887451
171. Iqbal, A. and S. Heller, *Managing hypoglycaemia.* Best Pract Res Clin Endocrinol Metab, 2016. **30**(3): p. 413-30. DOI: 10.1016/j.beem.2016.06.004
172. The American Diabetes Association, *Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia.* Diabetes Care, 2005. **28**(5): p. 1245-9. DOI: 10.2337/diacare.28.5.1245
173. Morales, J. and D. Schneider, *Hypoglycemia.* The American Journal of Medicine, 2014. **127**(10): p. S17-S24. DOI: 10.1016/j.amjmed.2014.07.004
174. Henriksen, M.M., H.U. Andersen, B. Thorsteinsson, and U. Pedersen-Bjergaard, *Asymptomatic hypoglycaemia in Type 1 diabetes: incidence and risk factors.* Diabet Med, 2019. **36**(1): p. 62-69. DOI: 10.1111/dme.13848
175. Staff, M.C. *Diabetic hypoglycemia*. 2020 April 03, 2020 December 4, 2020]; Available from: https://www.mayoclinic.org/diseases-conditions/diabetic-hypoglycemia/symptoms-causes/syc-20371525.
176. Goto, A., O.A. Arah, M. Goto, Y. Terauchi, and M. Noda, *Severe hypoglycaemia and cardiovascular disease: systematic review and meta-analysis with bias analysis.* Bmj, 2013. **347**: p. f4533. DOI: 10.1136/bmj.f4533
177. Lo, S.C., Y.S. Yang, E. Kornelius, J.Y. Huang, Y.R. Lai, C.N. Huang, and J.Y. Chiou, *Early cardiovascular risk and all-cause mortality following an incident of severe hypoglycaemia: A population-based cohort study.* Diabetes Obes Metab, 2019. **21**(8): p. 1878-1885. DOI: 10.1111/dom.13746
178. Villani, M., A. Earnest, K. Smith, D. Giannopoulos, G. Soldatos, B. de Courten, and S. Zoungas, *Outcomes of people with severe hypoglycaemia requiring prehospital emergency medical services management: a prospective study.* Diabetologia, 2019. **62**(10): p. 1868-1879. DOI: 10.1007/s00125-019-4933-y
179. Naseerullah, F.S. and A. Murthy, *Hypothermia as a forgotten sign of prolonged severe hypoglycaemia.* BMJ Case Rep, 2018. **2018**. DOI: 10.1136/bcr-2018-225606
180. Rosenfeld, L., *Insulin: discovery and controversy.* Clinical chemistry, 2002. **48 12**: p. 2270-88. PMID: 12446492
181. Poulsen, J.E., *The impact of August Krogh on the insulin treatment of diabetes and our present status.* Acta Med Scand Suppl, 1975. **578**: p. 7-14. DOI: 10.1111/j.0954-6820.1975.tb06497.x

182. Hilgenfeld, R., G. Seipke, H. Berchtold, and D.R. Owens, *The evolution of insulin glargine and its continuing contribution to diabetes care.* Drugs, 2014. **74**(8): p. 911-27. DOI: 10.1007/s40265-014-0226-4
183. Slieker, L.J., G.S. Brooke, R.D. DiMarchi, D.B. Flora, L.K. Green, J.A. Hoffmann, H.B. Long, L. Fan, J.E. Shields, K.L. Sundell, P.L. Surface, and R.E. Chance, *Modifications in the B10 and B26-30 regions of the B chain of human insulin alter affinity for the human IGF-I receptor more than for the insulin receptor.* Diabetologia, 1997. **40 Suppl 2**: p. S54-61. DOI: 10.1007/s001250051402
184. Umpierrez, G.E., S. Jones, D. Smiley, P. Mulligan, T. Keyler, A. Temponi, C. Semakula, D. Umpierrez, L. Peng, M. Cerón, and G. Robalino, *Insulin analogs versus human insulin in the treatment of patients with diabetic ketoacidosis: a randomized controlled trial.* Diabetes Care, 2009. **32**(7): p. 1164-9. DOI: 10.2337/dc09-0169
185. Johnston, K.C., A. Bruno, Q. Pauls, C.E. Hall, K.M. Barrett, W. Barsan, A. Fansler, K. Van de Bruinhorst, S. Janis, and V.L. Durkalski-Mauldin, *Intensive vs Standard Treatment of Hyperglycemia and Functional Outcome in Patients With Acute Ischemic Stroke: The SHINE Randomized Clinical Trial.* Jama, 2019. **322**(4): p. 326-335. DOI: 10.1001/jama.2019.9346
186. Brossard, J.H., J. Havrankova, D. Rioux, S. Bertrand, and P. D'Amour, *Long-term use of intramuscular insulin therapy in a type I diabetic patient with subcutaneous insulin resistance.* Diabet Med, 1993. **10**(2): p. 174-6. DOI: 10.1111/j.1464-5491.1993.tb00037.x
187. Hofman, P.L., J.G. Derraik, T.E. Pinto, S. Tregurtha, A. Faherty, J.M. Peart, P.L. Drury, E. Robinson, R. Tehranchi, M. Donsmark, and W.S. Cutfield, *Defining the ideal injection techniques when using 5-mm needles in children and adults.* Diabetes Care, 2010. **33**(9): p. 1940-4. DOI: 10.2337/dc10-0871
188. Vaag, A., K.D. Pedersen, M. Lauritzen, P. Hildebrandt, and H. Beck-Nielsen, *Intramuscular versus subcutaneous injection of unmodified insulin: consequences for blood glucose control in patients with type 1 diabetes mellitus.* Diabet Med, 1990. **7**(4): p. 335-42. DOI: 10.1111/j.1464-5491.1990.tb01401.x
189. Hövelmann, U., T. Heise, L. Nosek, B. Sassenfeld, K.M.D. Thomsen, and H. Haahr, *Pharmacokinetic Properties of Fast-Acting Insulin Aspart Administered in Different Subcutaneous Injection Regions.* Clin Drug Investig, 2017. **37**(5): p. 503-509. DOI: 10.1007/s40261-017-0499-y
190. Battelino, T., M. Phillip, N. Bratina, R. Nimri, P. Oskarsson, and J. Bolinder, *Effect of continuous glucose monitoring on hypoglycemia in type 1 diabetes.* Diabetes Care, 2011. **34**(4): p. 795-800. DOI: 10.2337/dc10-1989
191. Freckmann, G., U. Kamecke, D. Waldenmaier, C. Haug, and R. Ziegler, *Accuracy of Bolus and Basal Rate Delivery of Different Insulin Pump Systems.* Diabetes Technol Ther, 2019. **21**(4): p. 201-208. DOI: 10.1089/dia.2018.0376
192. Buckingham, B., R.W. Beck, K.J. Ruedy, P. Cheng, C. Kollman, S.A. Weinzimer, L.A. DiMeglio, A.A. Bremer, R. Slover, and W.V. Tamborlane, *Effectiveness of early intensive therapy on β-cell preservation in type 1 diabetes.* Diabetes Care, 2013. **36**(12): p. 4030-5. DOI: 10.2337/dc13-1074
193. Weisman, A., J.-W. Bai, M. Cardinez, C.K. Kramer, and B.A. Perkins, *Effect of artificial pancreas systems on glycaemic control in patients with type 1 diabetes: a systematic review and meta-analysis of outpatient randomised controlled trials.* The Lancet Diabetes & Endocrinology, 2017. **5**(7): p. 501-512. DOI: 10.1016/S2213-8587(17)30167-5
194. Brown, S.A., B.P. Kovatchev, D. Raghinaru, J.W. Lum, B.A. Buckingham, Y.C. Kudva, L.M. Laffel, C.J. Levy, J.E. Pinsker, R.P. Wadwa, E. Dassau, F.J. Doyle, 3rd, S.M. Anderson, M.M. Church, V. Dadlani, L. Ekhlaspour, G.P. Forlenza, E. Isganaitis, D.W. Lam, C. Kollman, and

R.W. Beck, *Six-Month Randomized, Multicenter Trial of Closed-Loop Control in Type 1 Diabetes.* N Engl J Med, 2019. **381**(18): p. 1707-1717. DOI: 10.1056/NEJMoa1907863

195. Hauzenberger, J.R., B.R. Hipszer, C. Loeum, P.A. McCue, M. DeStefano, M.C. Torjman, M.T. Kaner, A.R. Dinesen, I. Chervoneva, T.R. Pieber, and J.I. Joseph, *Detailed Analysis of Insulin Absorption Variability and the Tissue Response to Continuous Subcutaneous Insulin Infusion Catheter Implantation in Swine.* Diabetes Technol Ther, 2017. **19**(11): p. 641-650. DOI: 10.1089/dia.2017.0175
196. Pala, L., Dicembrini I., and Mannucci E., *Continuous subcutaneous insulin infusion vs modern multiple injection regimens in type 1 diabetes: an updated meta-analysis of randomized clinical trials.* Acta Diabetol, 2019. **56**(9): p. 973-980. DOI: 10.1007/s00592-019-01326-5
197. Jockel, J.P., Roebrock P., and Shergold O.A., *Insulin depot formation in subcutaneoue tissue.* J Diabetes Sci Technol, 2013. **7**(1): p. 227-37. DOI: 10.1177/193229681300700128
198. Heise, T., Nosek L., Dellweg S., Zijlstra E., Præstmark K.A., Kildegaard J., Nielsen G., and Sparre T., *Impact of injection speed and volume on perceived pain during subcutaneous injections into the abdomen and thigh: a single-centre, randomized controlled trial.* Diabetes Obes Metab, 2014. **16**(10): p. 971-6. DOI: 10.1111/dom.12304
199. Gradel, A.K.J., Porsgaard T., Lykkesfeldt J., Seested T., Gram-Nielsen S., Kristensen N.R., and Refsgaard H.H.F., *Factors Affecting the Absorption of Subcutaneously Administered Insulin: Effect on Variability.* J Diabetes Res, 2018. **2018**: p. 1205121. DOI: 10.1155/2018/1205121
200. van Dijk, P.R., Logtenberg S.J.J., Gans R.O.B., Bilo H.J.G., and Kleefstra N., *Intraperitoneal insulin infusion: treatment option for type 1 diabetes resulting in beneficial endocrine effects beyond glycaemia.* Clinical Endocrinology, 2014. **81**(4): p. 488-497. DOI: 10.1111/cen.12546
201. Dassau, E., E. Renard, J. Place, A. Farret, M.J. Pelletier, J. Lee, L.M. Huyett, A. Chakrabarty, F.J. Doyle, 3rd, and H.C. Zisser, *Intraperitoneal insulin delivery provides superior glycaemic regulation to subcutaneous insulin delivery in model predictive control-based fully-automated artificial pancreas in patients with type 1 diabetes: a pilot study.* Diabetes Obes Metab, 2017. **19**(12): p. 1698-1705. DOI: 10.1111/dom.12999
202. Nicolucci, A., A. Ceriello, P. Di Bartolo, A. Corcos, and M. Orsini Federici, *Rapid-Acting Insulin Analogues Versus Regular Human Insulin: A Meta-Analysis of Effects on Glycemic Control in Patients with Diabetes.* Diabetes Therapy, 2020. **11**(3): p. 573-584. DOI: 10.1007/s13300-019-00732-w
203. Giacca, A., A. Caumo, G. Galimberti, G. Petrella, M.C. Librenti, M. Scavini, G. Pozza, and P. Micossi, *Peritoneal and subcutaneous absorption of insulin in type I diabetic subjects.* J Clin Endocrinol Metab, 1993. **77**(3): p. 738-42. DOI: 10.1210/jcem.77.3.8370695
204. Radziuk, J., Pye S., Seigler D.E., Skyler J.S., Offord R., and Davies G., *Splanchnic and systemic absorption of intraperitoneal insulin using a new double-tracer method.* Am J Physiol, 1994. **266**(5 Pt 1): p. E750-9. DOI: 10.1152/ajpendo.1994.266.5.E750
205. Schade, D.S., Eaton R.P., Davis T., Akiya F., Phinney E., Kubica R., Vaughn E.A., and Day P.W., *The kinetics of peritoneal insulin absorption.* Metabolism, 1981. **30**(2): p. 149-55. DOI: 10.1016/0026-0495(81)90164-5
206. Kazumi, T., M. Vranic, H. Bar-On, and G. Steiner, *Portal v peripheral hyperinsulinemia and very low density lipoprotein triglyceride kinetics.* Metabolism, 1986. **35**(11): p. 1024-1028. DOI: 10.1016/0026-0495(86)90038-7
207. Renard, E., Rostane T., Carriere C., Marchandin H., Jacques-Apostol D., Lauton D., Gibert-Boulet F., and Bringer J., *Implantable insulin pumps: infections most likely due to seeding*

from skin flora determine severe outcomes of pump-pocket seromas. Diabetes Metab, 2001. **27**(1): p. 62-5. PMID: 11240448

208. van Dijk, P.R., S.J. Logtenberg, K.H. Groenier, J.W. Haveman, N. Kleefstra, and H.J. Bilo, *Complications of continuous intraperitoneal insulin infusion with an implantable pump.* World J Diabetes, 2012. **3**(8): p. 142-8. DOI: 10.4239/wjd.v3.i8.142
209. Wasserman, R.L., *Progress in Gammaglobulin Therapy for Immunodeficiency: From Subcutaneous to Intravenous Infusions and Back Again.* Journal of Clinical Immunology, 2012. **32**(6): p. 1153-1164. DOI: 10.1007/s10875-012-9740-x
210. Dekoninck, S., E. Hannezo, A. Sifrim, Y.A. Miroshnikova, M. Aragona, M. Malfait, S. Gargouri, C. de Neunheuser, C. Dubois, T. Voet, S.A. Wickström, B.D. Simons, and C. Blanpain, *Defining the Design Principles of Skin Epidermis Postnatal Growth.* Cell, 2020. **181**(3): p. 604-620.e22. DOI: 10.1016/j.cell.2020.03.015
211. van der Kolk, B.W., M. Kalafati, M. Adriaens, M.M.J. van Greevenbroek, N. Vogelzangs, W.H.M. Saris, A. Astrup, A. Valsesia, D. Langin, C.J.H. van der Kallen, S. Eussen, C.G. Schalkwijk, C.D.A. Stehouwer, G.H. Goossens, I.C.W. Arts, J.W.E. Jocken, C.T. Evelo, and E.E. Blaak, *Subcutaneous Adipose Tissue and Systemic Inflammation Are Associated With Peripheral but Not Hepatic Insulin Resistance in Humans.* Diabetes, 2019. **68**(12): p. 2247-2258. DOI: 10.2337/db19-0560
212. Spencer, M., R. Unal, B. Zhu, N. Rasouli, R.E. McGehee, Jr., C.A. Peterson, and P.A. Kern, *Adipose tissue extracellular matrix and vascular abnormalities in obesity and insulin resistance.* J Clin Endocrinol Metab, 2011. **96**(12): p. E1990-8. DOI: 10.1210/jc.2011-1567
213. Salans, L.B., S.W. Cushman, and R.E. Weismann, *Studies of human adipose tissue. Adipose cell size and number in nonobese and obese patients.* J Clin Invest, 1973. **52**(4): p. 929-41. DOI: 10.1172/jci107258
214. Rasmussen, C.H., R.M. Røge, Z. Ma, M. Thomsen, R.L. Thorisdottir, J.-W. Chen, E. Mosekilde, and M. Colding-Jørgensen, *Insulin aspart pharmacokinetics: An assessment of its variability and underlying mechanisms.* European Journal of Pharmaceutical Sciences, 2014. **62**: p. 65-75. DOI: 10.1016/j.ejps.2014.05.010
215. Thomsen, M., M. Poulsen, M. Bech, A. Velroyen, J. Herzen, F. Beckmann, R. Feidenhans'l, and F. Pfeiffer, *Visualization of subcutaneous insulin injections by x-ray computed tomography.* Phys Med Biol, 2012. **57**(21): p. 7191-203. DOI: 10.1088/0031-9155/57/21/7191
216. Kalra A, W.C., Tuma F. *Anatomy, Abdomen and Pelvis, Peritoneum.* 2020 2020 Aug 10 2021 Feb 9]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK534788/.
217. Pannu, H.K. and M. Oliphant, *The subperitoneal space and peritoneal cavity: basic concepts.* Abdom Imaging, 2015. **40**(7): p. 2710-22. DOI: 10.1007/s00261-015-0429-5
218. Tirkes, T., K. Sandrasegaran, A.A. Patel, M.A. Hollar, J.G. Tejada, M. Tann, F.M. Akisik, and J.C. Lappas, *Peritoneal and retroperitoneal anatomy and its relevance for cross-sectional imaging.* Radiographics, 2012. **32**(2): p. 437-51. DOI: 10.1148/rg.322115032
219. van der Wal, J.B. and J. Jeekel, *Biology of the peritoneum in normal homeostasis and after surgical trauma.* Colorectal Dis, 2007. **9 Suppl 2**: p. 9-13. DOI: 10.1111/j.1463-1318.2007.01345.x
220. Albanese, A.M., E.F. Albanese, J.H. Miño, E. Gómez, M. Gómez, M. Zandomeni, and A.B. Merlo, *Peritoneal surface area: measurements of 40 structures covered by peritoneum: correlation between total peritoneal surface area and the surface calculated by formulas.* Surgical and Radiologic Anatomy, 2009. **31**(5): p. 369-377. DOI: 10.1007/s00276-008-0456-9

221. Solass, W., P. Horvath, F. Struller, I. Königsrainer, S. Beckert, A. Königsrainer, F.J. Weinreich, and M. Schenk, *Functional vascular anatomy of the peritoneum in health and disease.* Pleura Peritoneum, 2016. **1**(3): p. 145-158. DOI: 10.1515/pp-2016-0015
222. Isaza-Restrepo, A., J.S. Martin-Saavedra, J.L. Velez-Leal, F. Vargas-Barato, and R. Riveros-Dueñas, *The Peritoneum: Beyond the Tissue - A Review.* Frontiers in physiology, 2018. **9**: p. 738-738. DOI: 10.3389/fphys.2018.00738
223. Dillman, J.R., E.A. Smith, A.C. Morani, and A.T. Trout, *Imaging of the pediatric peritoneum, mesentery and omentum.* Pediatr Radiol, 2017. **47**(8): p. 987-1000. DOI: 10.1007/s00247-017-3864-3
224. Blackburn, S.C. and M.P. Stanton, *Anatomy and physiology of the peritoneum.* Semin Pediatr Surg, 2014. **23**(6): p. 326-30. DOI: 10.1053/j.sempedsurg.2014.06.002
225. Mutsaers, S.E., C.M. Prêle, S. Pengelly, and S.E. Herrick, *Mesothelial cells and peritoneal homeostasis.* Fertil Steril, 2016. **106**(5): p. 1018-1024. DOI: 10.1016/j.fertnstert.2016.09.005
226. van Baal, J.O., K.K. Van de Vijver, R. Nieuwland, C.J. van Noorden, W.J. van Driel, A. Sturk, G.G. Kenter, L.G. Rikkert, and C.A. Lok, *The histophysiology and pathophysiology of the peritoneum.* Tissue Cell, 2017. **49**(1): p. 95-105. DOI: 10.1016/j.tice.2016.11.004
227. Mutsaers, S.E., *Mesothelial cells: Their structure, function and role in serosal repair.* Respirology, 2002. **7**(3): p. 171-191. DOI: 10.1046/j.1440-1843.2002.00404.x
228. Zhou, Q., M.A. Bajo, G. Del Peso, X. Yu, and R. Selgas, *Preventing peritoneal membrane fibrosis in peritoneal dialysis patients.* Kidney Int, 2016. **90**(3): p. 515-24. DOI: 10.1016/j.kint.2016.03.040
229. Sandoval, P., J.A. Jiménez-Heffernan, Á. Rynne-Vidal, M.L. Pérez-Lozano, Á. Gilsanz, V. Ruiz-Carpio, R. Reyes, J. García-Bordas, K. Stamatakis, J. Dotor, P.L. Majano, M. Fresno, C. Cabañas, and M. López-Cabrera, *Carcinoma-associated fibroblasts derive from mesothelial cells via mesothelial-to-mesenchymal transition in peritoneal metastasis.* The Journal of Pathology, 2013. **231**(4): p. 517-531. DOI: 10.1002/path.4281
230. Kastelein, A.W., L.M.C. Vos, K.H. de Jong, J. van Baal, R. Nieuwland, C.J.F. van Noorden, J.W.R. Roovers, and C.A.R. Lok, *Embryology, anatomy, physiology and pathophysiology of the peritoneum and the peritoneal vasculature.* Semin Cell Dev Biol, 2019. **92**: p. 27-36. DOI: 10.1016/j.semcdb.2018.09.007
231. Yoo E, J. HK, M.-J. K, J.-S. Yu, J.-J. Chung, H.-S. Yoo, and K.W. Kim, *Greater and Lesser Omenta: Normal Anatomy and Pathologic Processes.* RadioGraphics, 2007. **27**(3): p. 707-720. DOI: 10.1148/rg.273065085
232. Schaefer, B., M. Bartosova, S. Macher-Goeppinger, A. Ujszaszi, M. Wallwiener, J. Nyarangi-Dix, P. Sallay, D. Burkhardt, U. Querfeld, V. Pfeifle, B. Lahrmann, V. Schwenger, E. Wühl, S. Holland-Cunz, F. Schaefer, and C.P. Schmitt, *Quantitative Histomorphometry of the Healthy Peritoneum.* Sci Rep, 2016. **6**: p. 21344. DOI: 10.1038/srep21344
233. Coffey, J.C., *Surgical anatomy and anatomic surgery – Clinical and scientific mutualism.* The Surgeon, 2013. **11**(4): p. 177-182. DOI: 10.1016/j.surge.2013.03.002
234. Brown, S.E. and P.A. Dubbins, *Detection of free intraperitoneal fluid in healthy young men.* J Ultrasound Med, 2012. **31**(10): p. 1527-30. DOI: 10.7863/jum.2012.31.10.1527
235. Struller, F., F.J. Weinreich, P. Horvath, M.K. Kokkalis, S. Beckert, A. Königsrainer, and M.A. Reymond, *Peritoneal innervation: embryology and functional anatomy.* Pleura Peritoneum, 2017. **2**(4): p. 153-161. DOI: 10.1515/pp-2017-0024
236. Yoshikawa, T., N. Hayashi, E. Maeda, I. Matsuda, H. Sasaki, H. Ohtsu, and K. Ohtomo, *Peritoneal fluid accumulation in healthy men and postmenopausal women: evaluation on pelvic MRI.* AJR Am J Roentgenol, 2013. **200**(6): p. 1181-5. DOI: 10.2214/ajr.12.9645

237. Flessner, M.F., *Peritoneal transport physiology: insights from basic research.* J Am Soc Nephrol, 1991. **2**(2): p. 122-35. DOI: 10.1681/ASN.V22122
238. Rippe, B. and G. Stelin, *Simulations of peritoneal solute transport during CAPD. Application of two-pore formalism.* Kidney Int, 1989. **35**(5): p. 1234-44. DOI: 10.1038/ki.1989.115
239. Andreoli, M.C.C. and C. Totoli, *Peritoneal Dialysis.* Rev Assoc Med Bras (1992), 2020. **66Suppl 1**(Suppl 1): p. s37-s44. DOI: 10.1590/1806-9282.66.s1.37
240. Waniewski, J., R. Paniagua, J. Stachowska-Pietka, M.D. Ventura, M. Ávila-Díaz, C. Prado-Uribe, C. Mora, E. García-López, and B. Lindholm, *Threefold peritoneal test of osmotic conductance, ultrafiltration efficiency, and fluid absorption.* Perit Dial Int, 2013. **33**(4): p. 419-25. DOI: 10.3747/pdi.2011.00329
241. Verger, C., *Peritoneal dialysis solution and nutrition.* Contrib Nephrol, 2012. **178**: p. 6-10. DOI: 10.1159/000337784
242. Van de Sande, L., S. Cosyns, W. Willaert, and W. Ceelen, *Albumin-based cancer therapeutics for intraperitoneal drug delivery: a review.* Drug Deliv, 2020. **27**(1): p. 40-53. DOI: 10.1080/10717544.2019.1704945
243. Yu, L., Z.F. Zhang, C.X. Jing, and F.L. Wu, *Intraperitoneal administration of gonadotropin-releasing hormone-PE40 induces castration in male rats.* World J Gastroenterol, 2008. **14**(13): p. 2106-9. DOI: 10.3748/wjg.14.2106
244. Zhou, Y., P.C.M. van Zijl, X. Xu, J. Xu, Y. Li, L. Chen, and N.N. Yadav, *Magnetic resonance imaging of glycogen using its magnetic coupling with water.* Proc Natl Acad Sci U S A, 2020. **117**(6): p. 3144-3149. DOI: 10.1073/pnas.1909921117
245. van Dijk, P.R., S.J. Logtenberg, S.H. Hendriks, K.H. Groenier, J. Feenstra, F. Pouwer, R.O. Gans, N. Kleefstra, and H.J. Bilo, *Intraperitoneal versus subcutaneous insulin therapy in the treatment of type 1 diabetes mellitus.* Neth J Med, 2015. **73**(9): p. 399-409. PMID: 26582805
246. Li, Y., B. Li, B. Wang, M. Liu, X. Zhang, A. Li, J. Zhang, H. Zhang, and R. Xiu, *Integrated Pancreatic Microcirculation Profiles of Streptozotocin-Induced and Insulin- Administrated Type 1 Diabetes Mellitus.* Microcirculation, 2021: p. e12691. DOI: 10.1111/micc.12691
247. Renard E, G.B., Jeandidier N, EVADIAC. , *Long-term safety and efficacy of intraperitoneal insulin infusion from implanted pumps in a large series of patients with type 1 diabetes and initial high glucose variability* in *54(th) EASD Annual Meeting of the European Association for the Study of Diabetes*. 2018, Diabetologia: Berlin, Germany, 1 - 5 October 2018. p. 31. DOI: 10.1007/s00125-018-4693-0
248. Garcia-Verdugo, R., M. Erbach, and O. Schnell, *A New Optimized Percutaneous Access System for CIPII.* J Diabetes Sci Technol, 2017. **11**(4): p. 814-821. DOI: 10.1177/1932296817694913
249. Logtenberg, S.J., N. Kleefstra, S.T. Houweling, K.H. Groenier, R.O. Gans, and H.J. Bilo, *Health-related quality of life, treatment satisfaction, and costs associated with intraperitoneal versus subcutaneous insulin administration in type 1 diabetes: a randomized controlled trial.* Diabetes Care, 2010. **33**(6): p. 1169-72. DOI: 10.2337/dc09-1758
250. Logtenberg, S.J., N. Kleefstra, S.T. Houweling, K.H. Groenier, R.O. Gans, E. van Ballegooie, and H.J. Bilo, *Improved glycemic control with intraperitoneal versus subcutaneous insulin in type 1 diabetes: a randomized controlled trial.* Diabetes Care, 2009. **32**(8): p. 1372-7. DOI: 10.2337/dc08-2340
251. Loxham, S.J.G., J. Teague, S.M. Poucher, J. De Schoolmeester, A.V. Turnbull, and F. Carey, *Glucagon challenge in the rat: A robust method for the in vivo assessment of Glycogen*

phosphorlyase inhibitor efficacy. Journal of Pharmacological and Toxicological Methods, 2007. **55**(1): p. 71-77. DOI: 10.1016/j.vascn.2006.03.005

252. Zlotnik, A., B.F. Gruenbaum, Y. Klin, S.E. Gruenbaum, S. Ohayon, E. Sheiner, R. Kuts, M. Boyko, Y. Bichovsky, Y. Shapira, and V.I. Teichberg, *The Effects of Insulin, Glucagon, Glutamate, and Glucose Infusion on Blood Glutamate and Plasma Glucose Levels in Naive Rats.* Journal of Neurosurgical Anesthesiology, 2011. **23**(4). DOI: 10.1097/ANA.0b013e3182299b15
253. Lin, C., P.W. Chen, W.Y. Chen, C.C. Sun, and F.C. Mao, *Glucagon and insulin have opposite effects on tissue chromium distribution in an obese mouse model.* J Diabetes Investig, 2013. **4**(6): p. 528-32. DOI: 10.1111/jdi.12097
254. Åm K.M., *The intraperitoneal artificial pancreas; glucose sensing and glucagon delivery.*, in *Department of Clinical and Molecular Medicine*. 2020, NTNU: Trondheim, Norway. p. 95. ISBN: 9788232648825
255. Åm, M.K., I. Dirnena-Fusini, A.L. Fougner, S.M. Carlsen, and S.C. Christiansen, *Intraperitoneal and subcutaneous glucagon delivery in anaesthetized pigs: effects on circulating glucagon and glucose levels.* Sci Rep, 2020. **10**(1): p. 13735. DOI: 10.1038/s41598-020-70813-5
256. Hauner, H., M. Wabitsch, and E.F. Pfeiffer, *Differentiation of adipocyte precursor cells from obese and nonobese adult women and from different adipose tissue sites.* Horm Metab Res Suppl, 1988. **19**: p. 35-9. PMID: 3235057
257. Freedland, E.S., *Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: a review.* Nutrition & metabolism, 2004. **1**(1): p. 12-12. DIO: 10.1186/1743-7075-1-12
258. Mårin, P., B. Andersson, M. Ottosson, L. Olbe, B. Chowdhury, H. Kvist, G. Holm, L. Sjöström, and P. Björntorp, *The morphology and metabolism of intraabdominal adipose tissue in men.* Metabolism, 1992. **41**(11): p. 1242-8. DOI: 10.1016/0026-0495(92)90016-4
259. Abate, N., A. Garg, R.M. Peshock, J. Stray-Gundersen, and S.M. Grundy, *Relationships of generalized and regional adiposity to insulin sensitivity in men.* J Clin Invest, 1995. **96**(1): p. 88-98. DOI: 10.1172/jci118083
260. Larsson, B., K. Svärdsudd, L. Welin, L. Wilhelmsen, P. Björntorp, and G. Tibblin, *Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913.* Br Med J (Clin Res Ed), 1984. **288**(6428): p. 1401-4. DOI: 10.1136/bmj.288.6428.1401
261. Renard, E., G. Costalat, H. Chevassus, and J. Bringer, *Closed loop insulin delivery using implanted insulin pumps and sensors in type 1 diabetic patients.* Diabetes Research and Clinical Practice, 2006. **74**: p. S173-S177. DOI: 10.1016/S0168-8227(06)70026-2
262. Renard, E., J. Place, M. Cantwell, H. Chevassus, and C.C. Palerm, *Closed-loop insulin delivery using a subcutaneous glucose sensor and intraperitoneal insulin delivery: feasibility study testing a new model for the artificial pancreas.* Diabetes Care, 2010. **33**(1): p. 121-7. DOI: 10.2337/dc09-1080
263. Burnett, D.R., L.M. Huyett, H.C. Zisser, F.J. Doyle, 3rd, and B.D. Mensh, *Glucose sensing in the peritoneal space offers faster kinetics than sensing in the subcutaneous space.* Diabetes, 2014. **63**(7): p. 2498-505. DOI: 10.2337/db13-1649
264. Homko, C., A. Deluzio, C. Jimenez, J.W. Kolaczynski, and G. Boden, *Comparison of insulin aspart and lispro: pharmacokinetic and metabolic effects.* Diabetes Care, 2003. **26**(7): p. 2027-31. DOI: 10.2337/diacare.26.7.2027
265. Schaepelynck Bélicar, P., P. Vague, and V. Lassmann-Vague, *Reproducibility of plasma insulin kinetics during intraperitoneal insulin treatment by programmable pumps.* Diabetes Metab, 2003. **29**(4 Pt 1): p. 344-8. DOI: 10.1016/s1262-3636(07)70045-9

266. Cunningham D.D., *In Vivo Glucose Sensing*, ed. S. JA. 2010, Hoboken, NJ: John Wiley & Sons.
267. Walsh JT., *Pumping Insulin: Everything You Need for Success with an Insulin Pump*. 3rd ed, ed. S. B. 2000, San Diego, CA: Torrey Rines Press.
268. Pitt, H.A., C.D. Saudek, and H.A. Zacur, *Long-term intraperitoneal insulin delivery.* Ann Surg, 1992. **216**(4): p. 483-91; discussion 491-2. DOI: 10.1097/00000658-199210000-00011
269. Renard, E., *Insulin delivery route for the artificial pancreas: subcutaneous, intraperitoneal, or intravenous? Pros and cons.* J Diabetes Sci Technol, 2008. **2**(4): p. 735-8. DOI: 10.1177/193229680800200429
270. Huyett, L.M., E. Dassau, H.C. Zisser, and F.J. Doyle, 3rd, *Design and Evaluation of a Robust PID Controller for a Fully Implantable Artificial Pancreas.* Ind Eng Chem Res, 2015. **54**(42): p. 10311-10321. DOI: 10.1021/acs.iecr.5b01237
271. Christiansen, S.C., A.L. Fougner, Ø. Stavdahl, K. Kölle, R. Ellingsen, and S.M. Carlsen, *A Review of the Current Challenges Associated with the Development of an Artificial Pancreas by a Double Subcutaneous Approach.* Diabetes Ther, 2017. **8**(3): p. 489-506. DOI: 10.1007/s13300-017-0263-6
272. Chakrabarty, A., J.M. Gregory, L.M. Moore, P.E. Williams, B. Farmer, A.D. Cherrington, P. Lord, B. Shelton, D. Cohen, H.C. Zisser, F.J. Doyle, 3rd, and E. Dassau, *A New Animal Model of Insulin-Glucose Dynamics in the Intraperitoneal Space Enhances Closed-Loop Control Performance.* J Process Control, 2019. **76**: p. 62-73. DOI: 10.1016/j.jprocont.2019.01.002
273. Bekiari, E., K. Kitsios, H. Thabit, M. Tauschmann, E. Athanasiadou, T. Karagiannis, A.B. Haidich, R. Hovorka, and A. Tsapas, *Artificial pancreas treatment for outpatients with type 1 diabetes: systematic review and meta-analysis.* Bmj, 2018. **361**: p. k1310. DOI: 10.1136/bmj.k1310
274. Ward, W.K., J. Engle, H.M. Duman, C.P. Bergstrom, S.F. Kim, and I.F. Federiuk, *The Benefit of Subcutaneous Glucagon During Closed-Loop Glycemic Control in Rats With Type 1 Diabetes.* IEEE Sensors Journal, 2008. **8**(1): p. 89-96. DOI: 10.1109/JSEN.2007.912912
275. Bakhtiani, P.A., N. Caputo, J.R. Castle, J. El Youssef, J.M. Carroll, L.L. David, C.T. Roberts, Jr., and W.K. Ward, *A novel, stable, aqueous glucagon formulation using ferulic acid as an excipient.* J Diabetes Sci Technol, 2015. **9**(1): p. 17-23. DOI: 10.1177/1932296814552476
276. Tannenbaum, J. and B.T. Bennett, *Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose.* J Am Assoc Lab Anim Sci, 2015. **54**(2): p. 120-32. PMCID: PMC4382615
277. Smith, A.J., R.E. Clutton, E. Lilley, K.E.A. Hansen, and T. Brattelid, *PREPARE: guidelines for planning animal research and testing.* Lab Anim, 2018. **52**(2): p. 135-141. DOI: 10.1177/0023677217724823
278. Kilkenny, C., W.J. Browne, I.C. Cuthill, M. Emerson, and D.G. Altman, *Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research.* Osteoarthritis Cartilage, 2012. **20**(4): p. 256-60. DOI: 10.1016/j.joca.2012.02.010
279. Arifin, W.N. and W.M. Zahiruddin, *Sample Size Calculation in Animal Studies Using Resource Equation Approach.* Malays J Med Sci, 2017. **24**(5): p. 101-105. DOI: 10.21315/mjms2017.24.5.11
280. Goldstein, D.E., R.R. Little, R.A. Lorenz, J.I. Malone, D.M. Nathan, and C.M. Peterson, *Tests of glycemia in diabetes.* Diabetes Care, 2003. **26 Suppl 1**: p. S106-8. DOI: 10.2337/diacare.26.2007.s106
281. Beylot, M., Y. Khalfallah, M. Laville, G. Sautot, H. Dechaud, P. Serusclat, F. Berthezene, J.P. Riou, and R. Mornex, *Insulin-mediated glucose disposal in type 1 (insulin-dependent)*

diabetic subjects treated by continuous subcutaneous or intraperitoneal insulin fusion. Diabete Metab, 1987. **13**(4): p. 450-6. PMID: 3315766

282. Micossi, P., E. Bosi, M. Cristallo, L.D. Monti, M.C. Librenti, G. Petrella, G. Galimberti, D. Spotti, G. Giudici, C. Vergani, and et al., *Chronic continuous intraperitoneal insulin infusion (CIPII) in type I diabetic patients non-satisfactorily responsive to continuous subcutaneous insulin infusion (CSII).* Acta Diabetol Lat, 1986. **23**(2): p. 155-64. DOI: 10.1007/bf02624675
283. Swindle MM, S.A., *Comparative anatomy and physiology of the pig.* Scandinavian Journal of Laboratory Animal Science, 1998. **25**(Suppl1): p. 11-21.
284. Dawson, H. *A Comparative Assessment of the Pig, Mouse and Human Genomes: Structural and Functional Analysis of Genes Involved in Immunity and Inflammation*. The minipig in biomedical research, eds. McAnulty P., Dayan A., Ganderup N.C., Hastings K. Boca Raton, FL: CRC Press 2011. 323-342. DOI: 10.1201/b11356-28
285. Diehl, K.H., R. Hull, D. Morton, R. Pfister, Y. Rabemampianina, D. Smith, J.M. Vidal, and C. van de Vorstenbosch, *A good practice guide to the administration of substances and removal of blood, including routes and volumes.* J Appl Toxicol, 2001. **21**(1): p. 15-23. DOI: 10.1002/jat.727
286. *Cochrane Handbook for Systematic Reviews of Interventions* in *Chapter 6: Choosing effect measures and computing estimates of effect*, C.J. Higgins JPT, Cumpston M, Li T, Page MJ, Welch VA Editor. 2019, Cochrane.
287. Higgins, J.P., S.G. Thompson, J.J. Deeks, and D.G. Altman, *Measuring inconsistency in meta-analyses.* Bmj, 2003. **327**(7414): p. 557-60. DOI: 10.1136/bmj.327.7414.557
288. Deeks JJ, H.J., Altman DG, *Cochrane Handbook for Systematic Reviews of Interventions* T.J. Higgins J, Chadler J, Cumpston M, li T, Page MJ, Welch VA, Editor. 2021, Cochrane.
289. Sterne, J.A. and M. Egger, *Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis.* J Clin Epidemiol, 2001. **54**(10): p. 1046-55. DOI: 10.1016/s0895-4356(01)00377-8
290. Hirsch, I.B., B.W. Bode, S. Garg, W.S. Lane, A. Sussman, P. Hu, O.M. Santiago, and J.W. Kolaczynski, *Continuous Subcutaneous Insulin Infusion (CSII) of Insulin Aspart Versus Multiple Daily Injection of Insulin Aspart/Insulin Glargine in Type 1 Diabetic Patients Previously Treated With CSII.* Diabetes Care, 2005. **28**(3): p. 533-538. DOI: 10.2337/diacare.28.3.533
291. Kónya, A., K.C. Wright, M. Gounis, and K. Kandarpa, *Animal Models for Atherosclerosis, Restenosis, and Endovascular Aneurysm Repair*, in *Sourcebook of Models for Biomedical Research*, P.M. Conn, Editor. 2008, Humana Press: Totowa, NJ. p. 369-384. DOI: 10.1007/978-1-59745-285-4_40
292. Swindle, M.M., A. Makin, A.J. Herron, F.J. Clubb, Jr., and K.S. Frazier, *Swine as models in biomedical research and toxicology testing.* Vet Pathol, 2012. **49**(2): p. 344-56. DOI: 10.1177/0300985811402846
293. Kobayashi, E., Y. Hanazono, and S. Kunita, *Swine used in the medical university: overview of 20 years of experience.* Exp Anim, 2018. **67**(1): p. 7-13. DOI: 10.1538/expanim.17-0086
294. Smith, J.R., G.T. Hayman, S.J. Wang, S.J.F. Laulederkind, M.J. Hoffman, M.L. Kaldunski, M. Tutaj, J. Thota, H.S. Nalabolu, S.L.R. Ellanki, M.A. Tutaj, J.L. De Pons, A.E. Kwitek, M.R. Dwinell, and M.E. Shimoyama, *The Year of the Rat: The Rat Genome Database at 20: a multi-species knowledgebase and analysis platform.* Nucleic Acids Res, 2020. **48**(D1): p. D731-d742. DOI: 10.1093/nar/gkz1041
295. Blais, E.M., K.D. Rawls, B.V. Dougherty, Z.I. Li, G.L. Kolling, P. Ye, A. Wallqvist, and J.A. Papin, *Reconciled rat and human metabolic networks for comparative toxicogenomics and biomarker predictions.* Nature Communications, 2017. **8**(1): p. 14250. DOI: 10.1038/ncomms14250

296. Liu, Y., J.F. Li, H. Liu, F.Y. Liu, Y.M. Peng, Y.H. Liu, M.C. Cheng, G.C. Chen, and X. Zhou, *Functional and structural alterations of peritoneum and secretion of fibrotic cytokines in rats caused by high glucose peritoneal dialysis solutions.* Ren Fail, 2014. **36**(2): p. 292-9. DOI: 10.3109/0886022x.2013.844645
297. Böhm, B., J.W. Milsom, and V.W. Fazio, *Postoperative intestinal motility following conventional and laparoscopic intestinal surgery.* Arch Surg, 1995. **130**(4): p. 415-9. DOI: 10.1001/archsurg.1995.01430040077017
298. Hill, A.G., *Recovery after abdominal surgery: the peritoneum may be a key therapeutic target.* ANZ J Surg, 2013. **83**(12): p. 898-9. DOI: 10.1111/ans.12247
299. Daş, G., A. Vernunft, S. Görs, E. Kanitz, J.M. Weitzel, K.P. Brüssow, and C.C. Metges, *Effects of general anesthesia with ketamine in combination with the neuroleptic sedatives xylazine or azaperone on plasma metabolites and hormones in pigs.* J Anim Sci, 2016. **94**(8): p. 3229-3239. DOI: 10.2527/jas.2016-0365
300. Hein, H., J. Krieglstein, and R. Stock, *The effects of increased glucose supply and thiopental anesthesia on energy metabolism of the isolated perfused rat brain.* Naunyn Schmiedebergs Arch Pharmacol, 1975. **289**(4): p. 399-407. DOI: 10.1007/bf00508413
301. Kaniaris, P., N. Katsilambros, E. Castanas, and C. Theophanidis, *Relation between glucose tolerance and serum insulin levels in man before and after thiopental intravenous administration.* Anesth Analg, 1975. **54**(6): p. 718-21. DOI: 10.1213/00000539-197511000-00010
302. Chen, H., L. Li, and H. Xia, *Diabetes alters the blood glucose response to ketamine in streptozotocin-diabetic rats.* Int J Clin Exp Med, 2015. **8**(7): p. 11347-51. PMCID: PMC4565331
303. Illera, J.C., A. González Gil, G. Silván, and M. Illera, *The effects of different anaesthetic treatments on the adreno-cortical functions and glucose levels in NZW rabbits.* J Physiol Biochem, 2000. **56**(4): p. 329-36. DOI: 10.1007/bf03179801
304. Behdad, S., A. Mortazavizadeh, V. Ayatollahi, Z. Khadiv, and S. Khalilzadeh, *The Effects of Propofol and Isoflurane on Blood Glucose during Abdominal Hysterectomy in Diabetic Patients.* Diabetes Metab J, 2014. **38**(4): p. 311-6. DOI: 10.4093/dmj.2014.38.4.311
305. Bruns, C., I. Lewis, U. Briner, G. Meno-Tetang, and G. Weckbecker, *SOM230: a novel somatostatin peptidomimetic with broad somatotropin release inhibiting factor (SRIF) receptor binding and a unique antisecretory profile.* Eur J Endocrinol, 2002. **146**(5): p. 707-16. DOI: 10.1530/eje.0.1460707
306. Bauer, W., U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T.J. Petcher, and Pless, *SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action.* Life Sci, 1982. **31**(11): p. 1133-40. DOI: 10.1016/0024-3205(82)90087-x
307. Patel, Y.C. and C.B. Srikant, *Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hsstr 1-5).* Endocrinology, 1994. **135**(6): p. 2814-7. DOI: 10.1210/endo.135.6.7988476
308. Scavini, M., A. Pincelli, G. Petrella, G. Galimberti, P.G. Zager, M. Torri, and G. Pozza, *Intraperitoneal insulin absorption after long-term intraperitoneal insulin therapy.* Diabetes Care, 1995. **18**(1): p. 56-9. DOI: 10.2337/diacare.18.1.56
309. Gromada, J., K. Bokvist, W.G. Ding, S. Barg, K. Buschard, E. Renström, and P. Rorsman, *Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca2+ current and the number of granules close to the L-type Ca2+ channels.* J Gen Physiol, 1997. **110**(3): p. 217-28. DOI: 10.1085/jgp.110.3.217
310. Dos Santos, T.J., J.M. Donado Campos, J. Argente, and F. Rodríguez-Artalejo, *Effectiveness and equity of continuous subcutaneous insulin infusions in pediatric type 1 diabetes: A*

systematic review and meta-analysis of the literature. Diabetes Res Clin Pract, 2021. **172**: p. 108643. DOI: 10.1016/j.diabres.2020.108643

311. Jeitler, K., K. Horvath, A. Berghold, T.W. Gratzer, K. Neeser, T.R. Pieber, and A. Siebenhofer, *Continuous subcutaneous insulin infusion versus multiple daily insulin injections in patients with diabetes mellitus: systematic review and meta-analysis.* Diabetologia, 2008. **51**(6): p. 941-51. DOI: 10.1007/s00125-008-0974-3
312. Almalki, M.H., M.A. Altuwaijri, M.S. Almehthel, S.M. Sirrs, and R.S. Singh, *Subcutaneous versus intraperitoneal insulin for patients with diabetes mellitus on continuous ambulatory peritoneal dialysis: meta-analysis of non-randomized clinical trials.* Clin Invest Med, 2012. **35**(3): p. E132-43. DOI: 10.25011/cim.v35i3.16589
313. Spaan, N., A. Teplova, G. Stam, J. Spaan, and C. Lucas, *Systematic review: continuous intraperitoneal insulin infusion with implantable insulin pumps for diabetes mellitus.* Acta Diabetol, 2014. **51**(3): p. 339-51. DOI: 10.1007/s00592-014-0557-3
314. Wu, J., C. Xing, L. Zhang, H. Mao, X. Chen, M. Liang, F. Wang, H. Ren, H. Cui, A. Jiang, Z. Wang, M. Zou, and Y. Ji, *Autophagy promotes fibrosis and apoptosis in the peritoneum during long-term peritoneal dialysis.* J Cell Mol Med, 2018. **22**(2): p. 1190-1201. DOI: 10.1111/jcmm.13393
315. Izzo, J.L., J.W. Bartlett, A. Roncone, M.J. Izzo, and W.F. Bale, *Physiological processes and dynamics in the disposition of small and large doses of biologically active and inactive 131-I-insulins in the rat.* J Biol Chem, 1967. **242**(10): p. 2343-55. PMID: 6026231
316. Brown, P.M., C.V. Tompkins, S. Juul, and P.H. Sönksen, *Mechanism of action of insulin in diabetic patients: a dose-related effect on glucose production and utilisation.* Br Med J, 1978. **1**(6122): p. 1239-42. DOI: 10.1136/bmj.1.6122.1239
317. Lee, J.J., E. Dassau, H. Zisser, W. Tamborlane, S. Weinzimer, and F.J. Doyle. *The impact of insulin pharmacokinetics and pharmacodynamics on the closed-loop artificial pancreas*. in *52nd IEEE Conference on Decision and Control*. 2013. DOI: 10.1109/CDC.2013.6759870
318. Blauw, H., I. Wendl, J.H. DeVries, T. Heise, and T. Jax, *Pharmacokinetics and pharmacodynamics of various glucagon dosages at different blood glucose levels.* Diabetes Obes Metab, 2016. **18**(1): p. 34-9. DOI: 10.1111/dom.12571
319. Fischer, U., E.J. Freyse, E. Salzsieder, and K. Rebrin, *Artificial connection between glucose sensing and insulin delivery: implications of peritoneal administration.* Artif Organs, 1992. **16**(2): p. 151-62. DOI: 10.1111/j.1525-1594.1992.tb00286.x
320. Richardson, T. and D. Kerr, *Skin-related complications of insulin therapy: epidemiology and emerging management strategies.* Am J Clin Dermatol, 2003. **4**(10): p. 661-7. DOI: 10.2165/00128071-200304100-00001
321. Liebl, A., R. Hoogma, E. Renard, P.H. Geelhoed-Duijvestijn, E. Klein, J. Diglas, L. Kessler, V. Melki, P. Diem, J.M. Brun, P. Schaepelynck-Bélicar, and T. Frei, *A reduction in severe hypoglycaemia in type 1 diabetes in a randomized crossover study of continuous intraperitoneal compared with subcutaneous insulin infusion.* Diabetes Obes Metab, 2009. **11**(11): p. 1001-8. DOI: 10.1111/j.1463-1326.2009.01059.x
322. Wredling, R., U. Adamson, P.E. Lins, L. Backman, and D. Lundgren, *Experience of long-term intraperitoneal insulin treatment using a new percutaneous access device.* Diabet Med, 1991. **8**(6): p. 597-600. DOI: 10.1111/j.1464-5491.1991.tb01663.x

Appendices

Paper I

PLOS ONE

RESEARCH ARTICLE

Physiological effects of intraperitoneal versus subcutaneous insulin infusion in patients with diabetes mellitus type 1: A systematic review and meta-analysis

Ilze Dirnena-Fusini[1]*, Marte Kierulf Åm[1,2], Anders Lyngvi Fougner[3], Sven Magnus Carlsen[1,2], Sverre Christian Christiansen[1,2]

1 Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway, **2** Department of Endocrinology, St. Olav's University Hospital, Trondheim, Norway, **3** Department of Engineering Cybernetics, Faculty of Information Technology and Electrical Engineering, Norwegian University of Science and Technology, Trondheim, Norway

* ilze.dirnena-fusini@ntnu.no

Citation: Dirnena-Fusini I, Åm MK, Fougner AL, Carlsen SM, Christiansen SC (2021) Physiological effects of intraperitoneal versus subcutaneous insulin infusion in patients with diabetes mellitus type 1: A systematic review and meta-analysis. PLoS ONE 16(4): e0249611. https://doi.org/10.1371/journal.pone.0249611

Editor: Clemens Fürnsinn, Medical University of Vienna, AUSTRIA

Received: September 15, 2020

Accepted: March 22, 2021

Published: April 13, 2021

Data Availability Statement: All data are available in the article and online supplementary materials.

Funding: The Norwegian Research Council (NRC) is funding the Double Intraperitoneal Artificial Pancreas project (project number 248872) (all authors) as part of Centre for Digital Life Norway (digitallifenorway.org). The study is also supported by a scholarship from the Central Norway Regional Health Authority (CNRHA) (grant no 2014/23166) (MKA). These grants support the development to

Abstract

The intraperitoneal route of administration accounts for less than 1% of insulin treatment regimes in patients with diabetes mellitus type 1 (DM1). Despite being used for decades, a systematic review of various physiological effects of this route of insulin administration is lacking. Thus, the aim of this systematic review was to identify the physiological effects of continuous intraperitoneal insulin infusion (CIPII) compared to those of continuous subcutaneous insulin infusion (CSII) in patients with DM1. Four databases (EMBASE, PubMed, Scopus and CENTRAL) were searched beginning from the inception date of each database to 10th of July 2020, using search terms related to intraperitoneal and subcutaneous insulin administration. Only studies comparing CIPII treatment ($\geq$ 1 month) with CSII treatment were included. Primary outcomes were long-term glycaemic control (after $\geq$ 3 months of CIPII inferred from glycated haemoglobin (HbA1c) levels) and short-term ($\geq$ 1 day for each intervention) measurements of insulin dynamics in the systematic circulation. Secondary outcomes included all reported parameters other than the primary outcomes. The search identified a total of 2242 records; 39 reports from 32 studies met the eligibility criteria. This meta-analysis focused on the most relevant clinical end points; the mean difference (MD) in HbA1c levels during CIPII was significantly lower than during CSII (MD = -6.7 mmol/mol, [95% CI: -10.3 —3.1]; in percentage: MD = -0.61%, [95% CI: -0.94 — 0.28], p = 0.0002), whereas fasting blood glucose levels were similar (MD = 0.20 mmol/L, [95% CI: -0.34–0.74], p = 0.47; in mg/dL: MD = 3.6 mg/dL, [95% CI: -6.1–13.3], p = 0.47). The frequencies of severe hypo- and hyper-glycaemia were reduced. The fasting insulin levels were significantly lower during CIPII than during CSII (MD = 16.70 pmol/L, [95% CI: -23.62 —9.77], p < 0.0001). Compared to CSII treatment, CIPII treatment improved overall glucose control and reduced fasting insulin levels in patients with DM1.

create a safe and robust artificial pancreas for patients with diabetes. The funding bodies are not involved in any other aspect of this systematic review, such as the design of the review's protocol and analysis plan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Patients with diabetes mellitus type 1 (DM1) lack endogenous insulin and are completely dependent on external insulin delivery. This is usually accomplished by subcutaneous (SC) delivery, either by multiple daily injections (MDI) or via continuous subcutaneous insulin infusion (CSII). Despite considerable efforts, most patients with DM1 experience frequent episodes of hyper- and hypoglycaemia, and they often fail to keep their glucose levels within the desired range. Hence, alternative treatment options to achieve better glucose control are desired. Many research groups have explored whether intraperitoneal (IP) insulin delivery can improve overall glucose control compared with SC insulin administration.

In healthy subjects, insulin is secreted from the pancreas to the liver via the portal vein. In the portal vein, the insulin concentration can be several times higher than in the systemic circulation [1, 2]. Hepatic insulin extraction from the portal vein during the first pass through the liver varies between 20% and 80% [3]. After SC insulin injections, the systemic and portal vein insulin concentrations become more or less equalised, resulting in systemic hyperinsulinemia and hepatic hypoinsulinemia as compared to the normal physiological conditions in healthy subjects [4, 5]. Furthermore, the SC route is hampered by a variable and slow insulin absorption rate and, consequently, a slow onset of its glucose lowering effects [1]. The slow modification of glucose levels after administration of SC boluses of insulin is also a challenge in the development of an artificial pancreas (AP) that relies on SC administration [6].

Animal trials have shown that IP insulin administration appears to be more physiological than SC insulin administration [7], as a substantial percentage of IP administered insulin is primarily absorbed via the portal vein [8] and at a faster rate [9]. Accordingly, IP insulin administration is a promising means of achieving improved glucose control compared to the SC route [10]. Furthermore, IP insulin delivery may also prove to be an advantage for the implementation and use of an AP [6]. However, being an invasive treatment, IP administration of insulin also has some disadvantages, although the overall risk profile as compared to SC insulin treatment remains unknown.

We hypothesised that the CIPII normalises metabolic processes in the patients with DM1 compared to the CSII. The aim of this systematic review was to identify possible differences in the physiological effects related to CIPII versus CSII insulin administration in patients with DM1. More specifically, we aimed to determine the following: (i) whether there were benefits and harms associated with CIPII versus CSII insulin administration in patients with DM1; and (ii) whether there were methodological characteristics that could explain the divergent outcomes of previous studies. There is a lot of available information about IP versus SC insulin administration; however, more explicitly, studies with comparisons with CIPII versus CSII are limited. Meanwhile, these studies present a wide range of metabolic analyses; therefore, in this systematic review, we included clinically most relevant physiological changes during CIPII versus CSII-treatment periods.

Materials and methods

The protocol followed the PRISMA and Cochrane Handbook guidelines and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on the 30th of June 2016 (registration number CRD42016040124).

Search strategy

Systematic searches were performed in PubMed, EMBASE (Medline/Ovid), The Cochrane Library's CENTRAL database (Wiley Online Library), and Scopus. A librarian assisted in developing the search strategy (S1 Table in S1 File). Searches for trial protocols registered with

ClinicalTrials.gov and the International Standard Randomized Controlled Trial Number (ISRCTN) registry were also performed. Furthermore, the International Clinical Trials Registry Platform Search Portal was used to search for ongoing or recently completed trials. Dissertation Abstracts, Electronic Thesis Online Service (EthOS) and Network Digital Library of Theses and Dissertations database were additionally searched. For all relevant material, all references were checked to identify additional material (grey literature). The last search was performed on the 10th of July 2020.

All abstracts and titles of articles from the systematic search were uploaded to Distiller SR software. Two reviewers (IDF and MKÅ) independently screened the reports and abstracts based on predefined inclusion and exclusion criteria. During the data evaluation, we decided to restrict the results to the effects of CSII and CIPII only (see the 'Changes in the systematic review compared to the Protocol' section in the S1 File). When any disagreement occurred, two consultants with expertise in endocrinology (SCC and SMC) independently evaluated the material.

Eligibility criteria

Types of studies. All reports and abstracts from studies addressing the physiological effects of CIPII versus CSII in DM1 patients were included, including controlled trials, observational studies, case series (> 1 case), case reports (single case), as well as abstracts from clinical and scientific conference presentations.

Participants and interventions. Studies were determined to be eligible if CIPII treatment was compared to CSII treatment in DM1 patients. The CIPII treatment had to exceed one month in duration (including the wound healing period after establishing the abdominal port for insulin delivery). The minimum follow-up for the evaluation of glycated haemoglobin A1c (HbA1c) levels was set to three months, as HbA1c reflects the average glucose levels of the previous 120 days (the average erythrocyte life span) [11]. Consequently, as the follow-up was less than three months in two studies, they were excluded from the HbA1c analyses [12, 13].

Outcome measures. Any outcome reported in any of the included studies was included in the systematic review.

The primary outcomes included the following: (1) glycaemic control (HbA1c levels, fasting blood glucose (fasting BG) levels, hypoglycaemia, and hyperglycaemia); and (2) insulin levels (fasting insulin levels, time to reach peak insulin concentrations, maximum insulin levels, and time until insulin levels return to the basal level) and the mean daily insulin dose.

The secondary outcomes included any reported variable other than those listed in the primary outcomes. These included the following: (1) glycaemic control (self-monitoring of blood glucose (SMBG), mean daily BG levels, time spent in normoglycaemia, and glucose variability); (2) intermediate metabolites (triglycerides, cholesterol, free fatty acids, lactate, ketone bodies, and apolipoproteins); (3) counterregulatory hormones and other hormones (glucagon, catecholamines, growth hormone, insulin-like growth hormones, and binding proteins); (4) other metabolic outcomes (levels of anti-insulin antibodies (AIA), sex hormone binding globulin (SHBG), and plasminogen activator inhibitor-1 (PAI– 1)); and (5) any technical and/or physiological complications reported during CIPII treatment.

Data extraction

IDF and SCC independently extracted the data from each eligible study, including information on trial design and experimental interventions, the type of comparator, insulin dosage, the frequency and duration of treatment, patient characteristics (age, sex, mean duration of diabetes, types of other symptoms, and the mode of insulin delivery), number of included patients, duration of follow-up, and inclusion and exclusion criteria. When we encountered missing

information, we contacted the authors for further clarification. Five out of ten authors responded to our request for information, although only two of them provided informative answers.

Statistical analysis

Web-based tools were used to convert glucose concentration from mg/dL to mmol/L, HbA1c from percentages to mmol/mol [14], insulin levels from mU/L to pmol/L [15], and lipid levels from mg/dL to mmol/L [16].

Data were extracted from text, tables, and figures in the included reports. Data that were extracted from the figures of three studies [17–19] may be inaccurate due to the low-resolution of the figures. Data presentations in which no p-values were reported were assigned to the 'p-value not calculated' category when comparing CIPII to CSII periods/treated patients (S2.1–2.5 and S2.9–2.13 Tables in S1 File). Raw data and data for individual participants were extracted from six studies [18–23], and the standard deviations (SDs) for mean HbA1c, SMBG, insulin, cholesterol, or triglyceride levels were calculated using IBM Statistical Package for the Social Sciences (SPSS) Statistics 26.

If a study reported measurements from several time-points during the CIPII and/or CSII periods, the data from the final time-point during the CIPII and/or CSII period was selected for the meta-analysis.

Continuous outcomes were measured and analysed as mean differences and 95% confidence intervals (MD, 95% CI); skewed data and non-quantitative data were presented descriptively [24]. A meta-analysis was performed on the primary outcomes including HbA1c, fasting BG, and fasting insulin levels, and daily insulin dose, and the secondary outcomes including SMBG, cholesterol, and triglyceride levels using STATA software (Stata Corp. 2019. Stata Statistical Software: Release 16. College Station, TX: Stata Corp LLC) (Figs 2–7 and S1 –S7c Figs in S1 File). A meta-analysis could not be performed for the other secondary outcomes (levels of free fatty acids, lactate, ketone bodies, apolipoproteins, glucagon, adrenaline, noradrenaline, growth hormone, insulin-like growth factor, insulin-like growth factor binding proteins, sex hormone binding globulin, anti-insulin antibodies, and plasminogen activator inhibitor 1) due to the diversity in the presentation of the results (e.g., mean values, mean difference, only p-values, or only text descriptions without exact numbers) (S2.1 –S2.14 Tables in S1 File).

When required, the SDs were derived from the available standard errors of the mean (SEM) and the number of participants (S2.9 –S2.14 Tables in S1 File) using the calculator in the Review Manager software (RevMan, version 5.3). When the outcome variables were continuous measurements, the mean difference (MD) was used as the effect size. The heterogeneity was estimated with random effects models and restricted maximum likelihood as the analysis model. The heterogeneity was estimated by the I^2 statistic and categorised as follows: a) heterogeneity that might not be important (0–40%), b) may represent moderate or substantial heterogeneity (40–75%), or c) considerable heterogeneity (75–100%). However, the importance of the observed value depends on the magnitude and direction of the effects and the strength of the evidence for heterogeneity [25]. Each study was weighted using STATA software for continuous outcome variables, based on the SD and the sample size of the study. This weighting determined how much each individual study contributed to the pooled results estimates [26].

Assessment of the risk of bias

For randomised comparisons, the Cochrane collaboration tools were used to assess the random sequence generation, allocation concealment, the blinding of participants and personnel, the blinding of the outcome assessment, the presence of incomplete outcome data, and selective reporting and 'other bias' [27].

For observational studies, the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) checklist was used to evaluate items related to the article´s title, abstract, introduction, methods, results and discussion sections and other information such as funding [28]. The Quality Assessment Tool (QAT) was used to assess the selection bias, study design, confounders, blinding, data collection methods, withdrawals and drop-outs, intervention integrity, and statistical analyses [29].

To validate the quality of case reports and case series, the Institute of Health Economics (IHE) Quality Appraisal Checklist for Case Series Studies (QACCSS) was used [30]. The evaluation was based on the study objective, study design, study population, intervention and co-intervention, outcome measures, statistical analysis, results, conclusions, competing interests, and sources of support.

An evaluation of the risk of bias was performed for all studies by IDF and MKÅ, except for the case reports. All such evaluations are presented in S2.1 –S2.5 Tables in S1 File. Disagreements were resolved first through discussions between IDF and MKÅ, and, if necessary, by consulting the clinicians SCC and SMC.

Subgroup analyses were performed for all studies included in meta-analysis. The categories for the subgroup analyses were: (1) HbA1c levels before starting CIPII treatment ($\leq$ 7% and $>$ 7%), (2) study type (case-control studies and crossover studies), (3) duration of the CIPII-period ($\leq$ 6 months and $>$ 6 months), and (4) whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period. As an additional analysis, studies were sorted by the duration of the CIPII-period (months) to provide information about changes in the effect with time. All subgroup analyses are reported in S1 –S7c Figs in S1 File.

Evaluation of heterogeneity between studies was performed for studies reporting HbA1c levels by meta-regression and bubble plot analysis with 95% CI and linear prediction of HbA1c levels with CIPII treatment, using CSII controls for comparison.

Heterogeneity of effects was also explored with funnel plots [31] when ten or more studies were included in the meta-analysis. Assessments for publication bias across studies were performed using graphical (funnel plot) and statistical (Egger's test: random-effect model, t-distribution) analyses. For quantitative testing of skewness in the funnel plot, the Egger's test was chosen to analyse the MD of continuous outcomes.

A cumulative sequential meta-analysis of the studies was performed according to the duration of the CIPII-period.

Results

Literature selection

On the 10th of July 2020, our literature searches identified 2,263 reports. After the abstract screening, 109 potentially eligible reports remained (Fig 1). After applying the additional exclusion criteria, 70 of the 109 reports were excluded. In total, 32 studies describing a total of 39 reports were included in the systematic review, including one full-text article in Italian [32] and one full-text article in German [33].

CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections; IPII, intraperitoneal insulin infusion; CIPII, continuous intraperitoneal insulin infusion; SPAD, subcutaneous peritoneal access device; BG, blood glucose; SMBG, self-monitoring blood glucose.

Systematic review

Twenty-four [12, 13, 17, 18, 20–22, 32, 34–54] out of the 32 studies were cross-over studies with patients receiving at least three months of CSII treatment prior to 1.5–34 months of CIPII treatment. Only two of the studies were randomised studies [39, 55]. In the 30 studies that

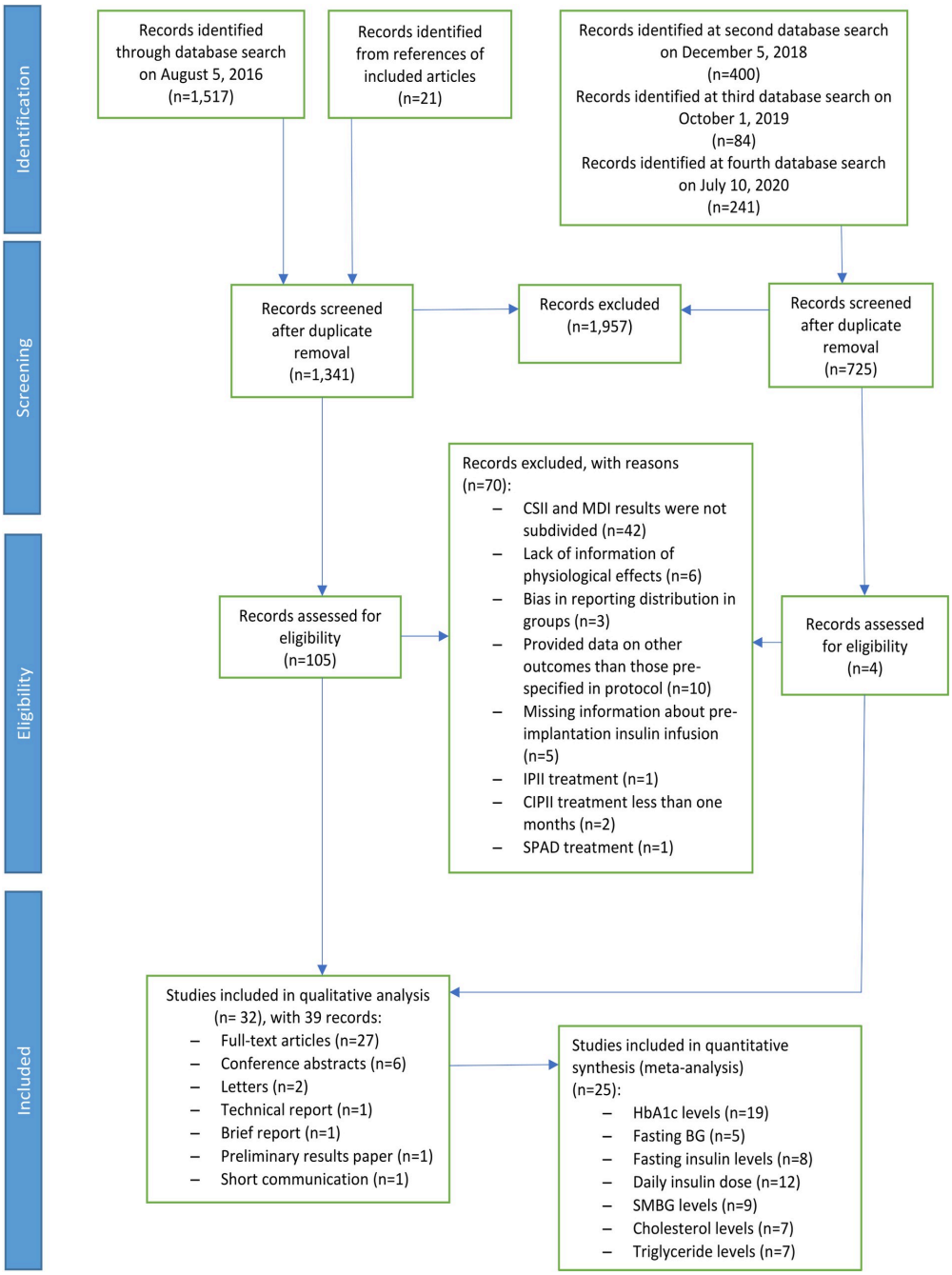


Fig 1. Flow chart of the screening and selection of included studies.

https://doi.org/10.1371/journal.pone.0249611.g001

reported the sex of the participants, more men (n = 167; 55%) than women (n = 136; 45%) were included in the CIPII period. In these 30 studies, the participants' ages ranged from 19 to 82 years (Table 1). In the ten studies [12, 13, 19, 22, 32, 33, 35, 38, 53, 56] that did report age separately for women and men, the mean age (range) was 35.1 (18–61) years in women and 38.9 (19–62) years in men. Ten out of the 32 studies were published in the 2000s; these included 122 participants during the CIPII period and 170 participants during the CSII period [23, 34–37, 45–49, 55–61].

Twenty-eight studies originated from single European countries, three from the USA [17, 18, 38], and one study was a multinational study [55] (Table 1). All overviews and procedures are summarised in the S2.1 –S2.14 Tables in S1 File.

HbA1c values were reported in 19 studies that included a total of 178 participants in the CIPII-period versus 188 participants in the CSII-period.

Glycaemic control

Meta-analysis: HbA1c. When including all 19 studies (CIPII, n = 178; CSII, n = 188) [17–19, 32–34, 36–38, 40, 41, 44–53, 58–60, 63] in the random-effect meta-analysis, the HbA1c levels were significantly lower during CIPII treatment than during CSII treatment (MD = -6.7 mmol/mol, [95% CI: -10.3 –-3.1]; in percentage: MD = -0.61%, [95% CI: -0.94 –-0.28], p = 0.0002; Fig 2). While substantial heterogeneity was present (I^2: 67.6%, p > 0.0001) and also evident in the funnel plot (Fig 3), the relative symmetry of the funnel plot was supported by a non-significant Egger's test result (p = 0.293).

Subgroup analysis. In subgroup analysis according to HbA1c levels before starting CIPII treatment, significantly lower HbA1c levels were observed during CIPII treatment than during CSII treatment in the subgroup with HbA1c levels > 53.0 mmol/mol (> 7%) and remained unchanged in the subgroup with HbA1c levels ≤ 53.0 mmol/mol (≤ 7%) (MD = -8.1 mmol/mol, [95% CI: -12.5 –-3.8], p < 0.01 and MD = -1.8 mmol/mol, [95% CI: -5.5–1.9], p = 0.33, respectively; in percentage: MD = -0.74%, [95% CI: -1.14 –-0.35], p < 0.01 and MD = -0.16%, [95% CI: -0.50–0.17], p = 0.33, respectively; S1c. A and S1d Fig in S1 File). The difference between the two subgroups was significant (p = 0.03). There was substantial heterogeneity between the studies with HbA1c levels > 53.0 mmol/mol (> 7%) (I^2: 70%, p < 0.01) and no heterogeneity in the studies with HbA1c levels ≤ 53.0 mmol/mol (≤ 7%) (I^2: 0%, p = 0.72).

In subgroup analysis according to study types, significantly lower HbA1c levels were observed during CIPII treatment than during CSII treatment in the crossover studies, while HbA1c levels remained unchanged in the case-control studies (MD -8.2 mmol/mol, [95% CI: -11.9 –-4.6], p < 0.01 and MD = 0.8 mmol/mol, [95% CI: -5.5–7.1], p = 0.8, respectively; in percentage: MD = -0.75%, [95% CI: -1.09 –-0.42], p < 0.01 and MD = 0.07%, [95% CI: -0.50–0.65], p = 0.8, respectively; S1c. B and S1d Fig in S1 File). The difference between the two subgroups was significant (p = 0.01). In both study type subgroups there was a substantial amount of heterogeneity (I^2: 62%, p < 0.01 and I^2: 34%, p = 0.19, respectively).

In subgroup analysis according to duration of the CIPII-period, significantly lower HbA1c levels were observed in the subgroup with a longer CIPII-period (> 6 months) while HbA1c levels remained unchanged in the subgroup with a shorter CIPII-period (≤ 6 months) (MD = -10.7 mmol/mol, [95% CI: -15.2 –-6.1], p < 0.01 and MD = -2.3 mmol/mol, [95% CI: -6.2–1.6], p = 0.25, respectively; in percentage: MD = -0.98%, [95% CI: -1.39 –-0.56], p < 0.01 and MD = -0.21%, [95% CI: -0.57–0.15], p = 0.25, respectively; S1c. C and S1d Fig S1 File). The difference between the two subgroups was significant (p = 0.01). In both subgroups of CIPII treatment duration there was substantial heterogeneity (I^2: 56%, p = 0.01 and I^2: 49%, p = 0.06, respectively).

Table 1. Characteristics of studies included in the systematic review.

Study	Study design	Number of Participants	Sex (Male or Female)	Age (mean±SD or range) (years)	HbA1c at inclusion (% or range)	CSII minimum period (month)	CIPII minimum period (month)
Giacca et al. 1993 (France) [39]	RCs	5	1/4	31–50	7.4	96 hours	3
Liebl et al. 2009 (Multinational) [55]	RFUs	CIPII: 15	CIPII: M:11/4	CIPII: 50.5	CIPII: 8.2	6	12
		CSII: 21	CSII: M: 9/12	CSII: 45.3	CSII:8.3		
Micossi et al. 1986 (Italy) [13]	NRCs	6	3/3	22–50	7.25	12	1 ½
Beylot et al. 1987 (France) [12]	NRCs	4	3/1	36–51	7.6 (5.0–9.2)	2	2
Wredling, Adamson et al. 1991 (technical report) (Sweden) [53]	NRCs	6	4/2	31–49	8.7 (7.0–9.5)	12	15
Wredling, Liu et al. 1991 (Sweden) [54]	NRCs	6	4/2	31–49	7.7–10.2	24	6.9
Georgopoulos et al. 1992 (USA) [38]	NRCs	7	5/2	19–40	9.83 (7.4–12.0)	ND	12
Pitt et al. 1992 (USA) [18]	NRCs	10	8/2	19–56	9.1	3	34[a]
Renard et al. 1993 (France) [22]	NRCs	8	6/2	31–53	ND	2.4	12
Georgopoulos et al 1994 (USA) [17]	NRCs	8	5/3	37±7	9.4	ND	6
Lassmann-Vague et al. 1994 (short communication) (France) [44]	NRCs	11	5/6	21–48	7.0	6	3
Raccah et al. 1994 (letter) (France) [51]	NRCs	11	6/5	21–48	6.9	3	10
Schnell et al. 1994 (Germany) [52]	NRCs	5	1/4	25–62	9.8	39	12
Lassmann-Vague et al. 1995/1998 (article/letter) (France) [20, 21]	NRCs	15	8/9	ND	ND	1	24
Guerci et al. 1996 (France) [40]	NRCs	14	9/5	40±6.2	6.1	14.2	4
Hanaire-Broutin et al. 1996 (France) [41]	NRCs	18	11/7	25–65	7.6	3	12
Lassmann-Vague et al. 1996 (France) [43]	NRCs	11	6/5	36.9±9	7.7	ND	2
Pacifico et al. 1997 (Italy) [32]	NRCs	8	5/4	18–50	6.5	3	12
Oskarsson et al. 1999 (Sweden) [50]	NRCs	7	5/2	36–50	8.5	6	11
Oskarsson et al. 2000 (Sweden) [49]	NRCs	7	5/2	36–50	8.6	12	11
Duvillard et al. 2005/2007 (brief report/article) (France) [36, 37]	NRCs	7	6/1	48±6.5	7.34	ND	3
Liebl et al. 2013/2014 (c.p) (Germany) [45–48]	NRCs	12	2/10	28–82	9.0	ND	12
Dassau et al. 2017 (France) [35]	NRCs	10	7/3	18–65	7.7	102	1
Jeandidier et al. 1992 (preliminary results) (France) [42]	Retro.Cs	8	ND	33.5±2.9	6.64	ND	10
Catargi et al. 2002 (France) [34]	Retro.Cs	14	5/9	50.6±12.8	7.8	1.5	3
Jeandidier et al. 2002 (France) [57]	NRFUs	CIPII: 13	CIPII: 6/7	CIPII: 36.8±1.7	CIPII: ND	6	6
		CSII: 11	CSII: 6/5	CSII: 43.1±3.4	CSII: ND		
Van Dijk et al. 2016	NRFUs	CIPII: 39	CIPII: 14/25	CIPII: 18–70	CIPII: 8.3	48	48
Van Dijk et al. 2020 (The Netherlands) [23, 62]		CSII: 74	CSII: 30/44	CSII: 48±12	CSII: 7.9		
Colette et al. 1989 (France) [63]	C-Cs	CIPII: 13	CIPII: ND	CIPII: 30±3	CIPII: 8.0	7	10
		CSII: 11	CSII: ND	CSII: 32±3	CSII: 8.9		
Selam et al. 1989 (UK) [19]	C-Cs	CIPII: 6	CIPII: 4/2	CIPII: 25–43	CIPII: 8.3	12	6
		CSII: 8	CSII: 5/3	CSII: 26–67	CSII: 8.7		
Walter et al. 1989 (Germany) [33]	C-Cs	CIPII: 6	CIPII: 6/0	CIPII:21–39	CIPII:8.0	6	3
		CSII: 6	CSII: 6/0	CSII:23–31	CSII:7.9		

(*Continued*)

Table 1. (Continued)

Study	Study design	Number of Participants	Sex (Male or Female)	Age (mean±SD or range) (years)	HbA1c at inclusion (% or range)	CSII minimum period (month)	CIPII minimum period (month)
Hedman et al. 2009/2014; Arnqvist et al. 2010 (c.p/article; c.p) (Sweden) [58–60]	C-Cs	CIPII: 10	CIPII: 5/5	CIPII: 53.1±9.1	CIPII: 8.6	6	6
		CSII:20	CSII:10/10	CSII:52.8±9.0	CSII:7.9		
Catargi et al 2000 (case report) (France) [56]	CR	1	1/0	32	ND	6	1.5

RCs, randomised crossover study; RFUs, randomised follow-up study; NRCs, non-randomised crossover study; Retro.Cs, retrospective crossover study; C-Cs, case-control study; NRFUs, non-randomised follow-up study; CR, case report; CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion; ND, no data available; c.p, conference poster; [a], available glycaemic control data for the first 18 months.

https://doi.org/10.1371/journal.pone.0249611.t001

In subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period, significantly lower HbA1c levels were observed in both subgroups (MD = -6.7 mmol/mol, [95% CI: -11 –-2.3], p = 0.003 and MD = -7.0 mmol/mol, [95% CI: -12.7 –-1.3], p = 0.015, respectively; in percentage: MD = -0.61%, [95% CI: -1.01 –-0.21], p = 0.003 and MD = -0.64%, [95% CI: -1.16 –-0.12], p = 0.015, respectively; S1c. D and S1d Fig in S1 File), with no significant difference between the subgroups (p = 0.93). There was substantial heterogeneity in both subgroups (I^2: 72%, $p < 0.01$ and I^2: 45%, p = 0.17, respectively).

Meta-regression. The regression coefficient for duration of the CIPII treatment was - 0.068 ($p > 0.002$). Thus, with every month of the CIPII treatment HbA1c decreased 0.7 mmol/mol (in percentage: 0.068%). The proportion of between-study variance explained by the *duration of CIPII-period* (R^2) was 52%. The residual variation was due to substantial heterogeneity (I^2: 49%, p = 0.014). In addition, a bubble plot of the observed effect size against the duration of CIPII-period overlaid with the predicted regression and confidence-interval lines, shows a similar pattern (S1e Fig in S1 File). This pattern was also observed in the subgroup meta-analysis with *duration of CIPII-period in months* (S1d Fig S1 File).

Cumulative meta-analysis. To evaluate the change in HbA1c levels with time during CIPII treatment compared to that during CSII treatment, a cumulative meta-analysis was performed (S1f Fig in S1 File). The results indicated that the HbA1c levels were progressively lower during CIPII treatment than during CSII treatment. The total difference became statistically significant ($p < 0.05$) after the inclusion of the study by Georgopoulos et al. [38] (MD = -5.3 mmol/mol, [95% CI: -9.7 –-0.8], p = 0.023; in percentage: MD = -0.48%, [95% CI: -0.89 –-0.07], p = 0.023), and the tendency of decrease in the MD during CIPII treatment, compared to that during CSII treatment, remained significant throughout the analysis (-5.1 to -5.7 mmol/mol, in percentage: -0.47 to -0.61%).

Neither of the two randomised studies [39, 55] was included in the meta-analysis to assess the effect of treatment on HbA1c levels, as the mean and SD or SEM values were not reported.

Detailed information about all studies and reported results pertaining to glycaemic control is available in S2.1 Table in S1 File.

Meta-analysis: Fasting blood glucose. When including all five studies that reported fasting BG (CIPII, n = 39; CSII, n = 41) [12, 19, 34, 42, 49], the fasting BG levels remained unchanged during CIPII treatment compared to those during CSII treatment (MD = 0.20 mmol/L, [95% CI: -0.34–0.74], p = 0.47; in mg/dL: MD = 3.6 mg/dL, [95% CI: -6.1–13.3], p = 0.47; Fig 4). The heterogeneity between the studies was low (I^2: 32%, p = 0.14).

Subgroup analysis. The mean difference in blood glucose levels was not different in any of the subgroups analysed (S2b Fig in S1 File).

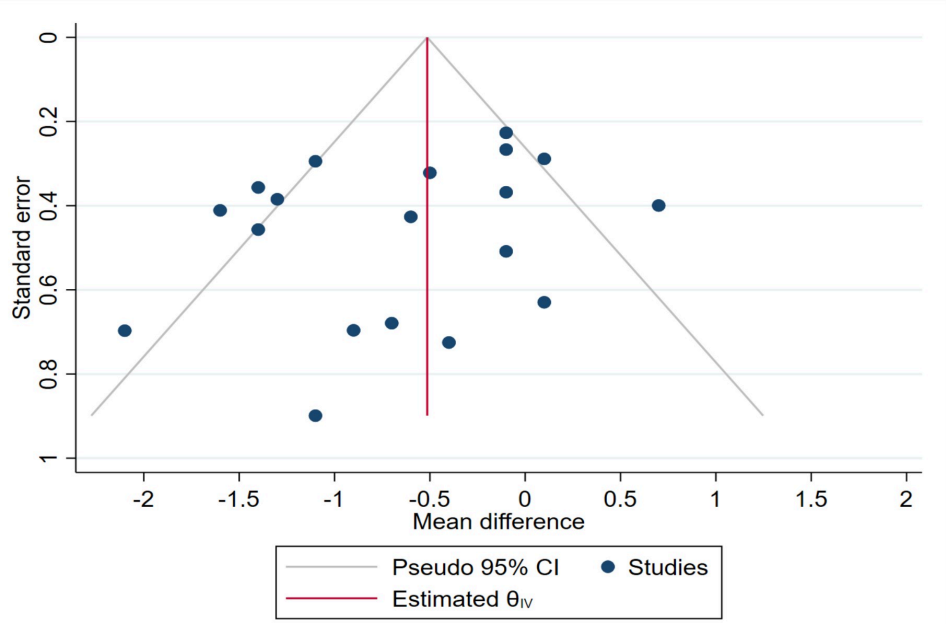


Fig 3. Funnel plot of HbA1c (%) during CIPII treatment compared to that during control treatment (CSII). The funnel plot includes diagonal lines representing expected distribution of studies in the absence of heterogeneity (95% of the studies should lie within these diagonal lines). The lines are not strict 95% confidence interval, therefore, referred as 'pseudo 95% CI'.

https://doi.org/10.1371/journal.pone.0249611.g003

The group difference in fasting BG levels remained unchanged during CIPII treatment compared to that during CSII treatment whether the HbA1c levels before starting CIPII were ≤ 53.0 mmol/mol (≤ 7%) or > 53.0 mmol/mol (> 7%) (p = 0.34); according to study type (case-control studies vs crossover studies) (p = 0.07); and whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period (p = 0.74) (S2a and S2b Fig in S1 File). Subgroup analysis according to the duration of the CIPII-period could not be performed because all the included studies had a short CIPII-period (≤ 6 months).

Other primary outcomes: Hypoglycaemia. In total, ten studies reported outcomes related to hypoglycaemia. Out of these studies, seven reported on mild hypoglycaemia [13, 18, 35, 42, 49, 50, 55] and five reported on severe hypoglycaemia [18, 22, 32, 45–48, 55], most of which defined severe hypoglycaemia as cases requiring assistance (requiring hospitalisation or IV glucose administration, or events accompanied by unconsciousness or seizure). One randomised study observed a significantly reduced frequency of severe hypoglycaemia during the CIPII-period compared to that during the CSII-period (0.35 vs 0.86 events per patient-year, p = 0.013) [55]. The frequency of severe hypoglycaemic events was unchanged for the first three months of CIPII treatment, whereas it was reduced in the subsequent nine months (0.72 vs 0.15 events per patient-year, respectively, p-value not calculated) [55].

Three studies reported, respectively, zero [22], 0.43 [18], and 1.5 [45–48] severe hypoglycaemic events per patient-year during the CIPII-period versus 0.54 [22] and 12 [45–48] events per patient-year during the CSII-period. One study did not provide data for the CSII-period [18]. Among the two studies that reported on hypoglycaemic coma, no such events occurred during the CIPII-period [18, 22] compared to 0.54 events per patient-year during the CSII-period

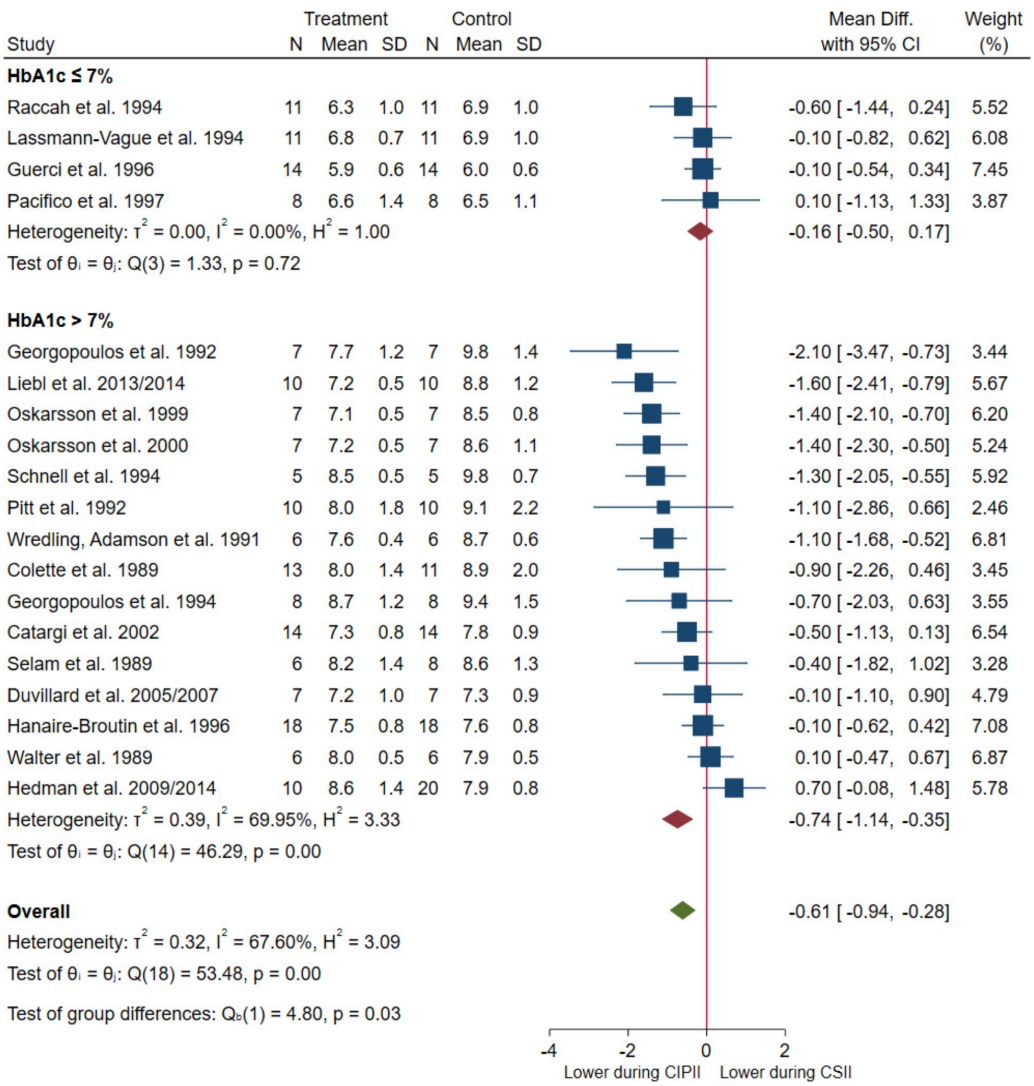


Fig 2. Meta-analysis of HbA1C (%) in patients during CIPII treatment compared to that during control treatment (CSII). Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Studies ordered by effect size (mean difference) and divided into subgroups: HBA1c levels ≤ 53.0 mmol/mol (≤ 7%) and HbA1c levels > 53.0 mmol/mol (> 7%) during control treatment (CSII).

https://doi.org/10.1371/journal.pone.0249611.g002

[22]. No other studies reported on hypoglycaemic coma during periods of CIPII or CSII. One study reported no difference in the occurrence of severe hypoglycaemia [32].

One prospective study that evaluated SMBG reported a reduced time spent in hypoglycaemia during the CIPII-period (SMBG < 3.9 mmol/L, $p < 0.05$), whereas the time spent in more pronounced hypoglycaemia (SMBG < 2.8 mmol/L) was similar between the two treatment

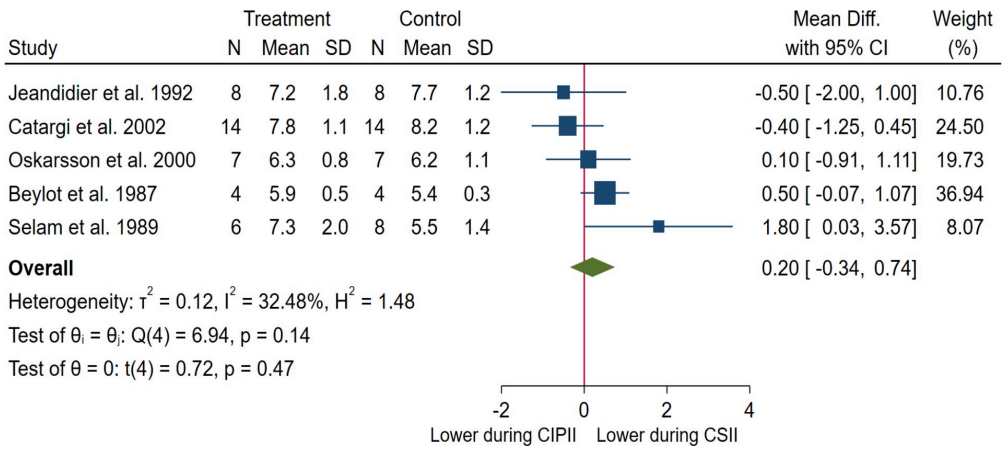


Fig 4. Meta-analysis of fasting blood glucose (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII). Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Studies are ordered by effect size (mean difference).

https://doi.org/10.1371/journal.pone.0249611.g004

periods [13]. However, four other studies observed no differences in the occurrence of hypoglycaemic events (SMBG < 3.0 mmol/L) in the last four weeks of the treatment periods [49, 50], in the frequencies of events per patient-year during those periods [55], or in the occurrence of BG levels < 3.8 mmol/L during a 24-hour period (based on a continuous glucose monitoring (CGM) profile) [35] (S2.1 and S2.8 Tables in S1 File).

Other primary outcomes: Hyperglycaemia. One prospective study that collected CGM data reported less time spent in hyperglycaemia (BG > 10.0 mmol/L, $p < 0.05$) during a 24-hour CIPII treatment compared to that during the CSII treatment [35], whereas another study that assessed SMBG observed no difference in hyperglycaemia (BG > 10.0 mmol/L) during a six-week period [13]. However, both studies reported a reduced amount of time spent in severe hyperglycaemia (BG > 14.0 mmol/L, $p < 0.05$) during the CIPII treatment compared to that during the CSII treatment (S2.1, S2.8 Tables in S1 File) [13, 35].

Insulin levels

Meta-analysis: Fasting insulin levels. When including all eight studies that reported fasting insulin levels (CIPII, n = 69; CSII, n = 67) [12, 39, 43, 44, 49–51, 63], the fasting insulin levels were significantly lower during CIPII treatment than during CSII treatment (MD = -16.70 pmol/L, [95% CI: -23.62 –-9.77], $p < 0.0001$; Fig 5). There was no heterogeneity between the studies (I^2: 0%, p = 0.99).

Subgroup analysis. In subgroup analysis according to HbA1c levels before starting CIPII treatment, significantly lower fasting insulin levels were observed during CIPII treatment than during CSII treatment in the subgroup with HbA1c levels > 53.0 mmol/mol (> 7%), while fasting insulin levels remained unchanged in the subgroup with HbA1c levels ≤ 53.0 mmol/mol (≤ 7%) (MD = -16.86 pmol/L, [95% CI: -23.87 –-9.85], $p < 0.001$ and MD = -9.94 pmol/L, [95% CI: -54.99–35.11], p = 0.66, respectively; S3a. A and S3b Fig in S1 File). However, there was no difference between the subgroups (p = 0.77) and there was no heterogeneity in the subgroups (I^2: 0%, p = 0.95 and I^2: 0%, p = 0.75, respectively).

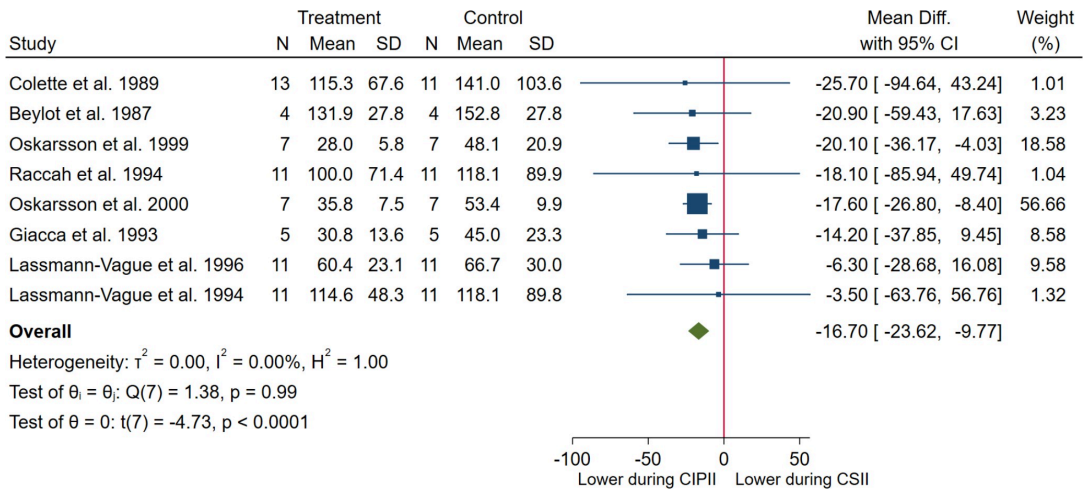


Fig 5. Meta-analysis of fasting insulin (pmol/L) in patients during CIPII treatment compared to that during control treatment (CSII). Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Studies ordered by effect size (mean difference).

https://doi.org/10.1371/journal.pone.0249611.g005

In subgroup analysis according to study types, significantly lower fasting insulin levels were observed during CIPII treatment than during CSII treatment in the crossover studies while levels remained unchanged in the case-control studies (MD = -16.61 pmol/L, [95% CI: -23.57 –-9.64], $p < 0.001$ and MD = -25.70 pmol/L, [95% CI: -94.64–43.24], p = 0.465, respectively; S3a. B and S3b Fig in S1 File). However, there was no statistical difference between the two groups (p = 0.80). There was no heterogeneity in both subgroups (I^2: 0%, p = 0.97 and I^2: 0%, p = not possible to calculate (n = 1), respectively).

In subgroup analysis according to duration of the CIPII-period, significantly lower fasting insulin levels were observed during CIPII treatment than during CSII treatment in both subgroups (MD = -15.20 pmol/L, [95% CI: -25.98 –-4.43], p = 0.006 and MD = -17.75 pmol/L, [95% CI: -26.79 –-8.71], $p < 0.001$, for CIPII-period $\leq$ 6 months and CIPII-period > 6 months, respectively; S3a. C and S3b Fig in S1 File) with no difference between the subgroups (p = 0.72). There was no heterogeneity in the subgroups (I^2: 0%, p = 0.88 and I^2: 0%, p = 0.97, respectively).

In subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period, significantly lower fasting insulin levels were observed during CIPII treatment than during CSII treatment in the subgroup without controlled CSII follow-up-period while levels remained unchanged in the subgroup with controlled CSII follow-up-period (MD = -16.99 pmol/L, [95% CI: -24.42 –-9.56], $p < 0.001$ and MD = -14.77 pmol/L, [95% CI: -33.89–4.34], p = 0.13, respectively; S3a. D and S3b Fig in S1 File), with no difference between the subgroups (p = 0.83). There was no heterogeneity in the subgroups (I^2: 0%, p = 0.89 and I^2: 0%, p = 0.89, respectively).

Meta-analysis: Daily insulin dose. When including all 12 studies that reported daily insulin dose (CIPII, n = 131; CSII, n = 141) [13, 17, 32, 36, 37, 41, 42, 44, 49–51, 55, 58–60], the daily insulin dose remained unchanged during CIPII treatment compared to that during CSII treatment (MD = 1.30 U/24 hours, [95% CI: -1.60–4.20], p = 0.38; Fig 6), with no heterogeneity between the studies (I^2: 0%, p = 0.96). Homogeneity was also evident in the funnel plot (Fig 7).

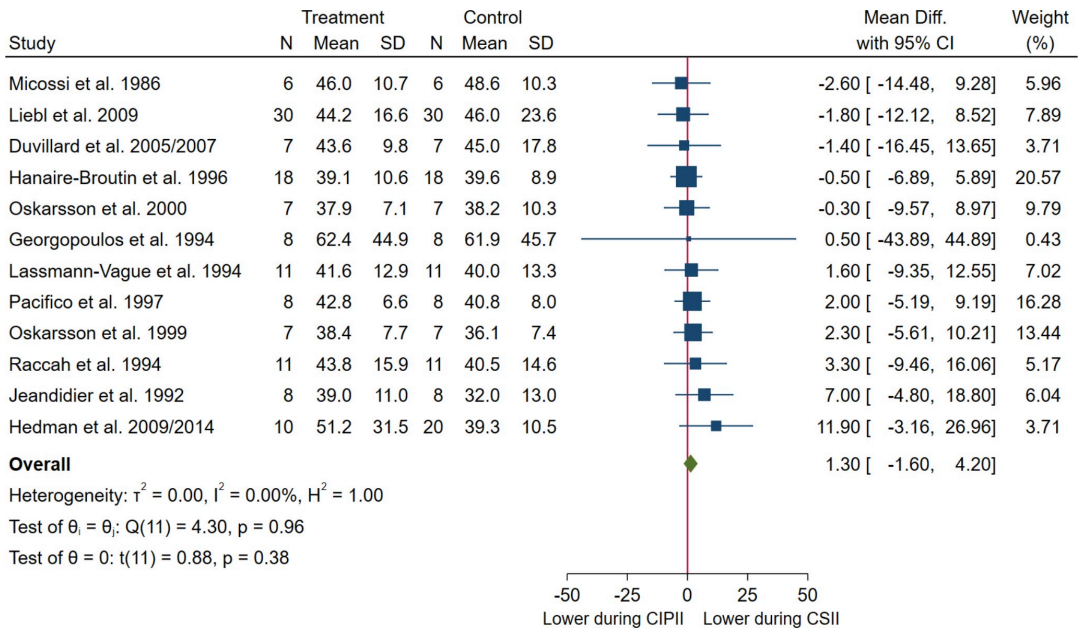


Fig 6. Meta-analysis of mean daily insulin (U/24 hours) in patients during CIPII treatment compared to that during control treatment (CSII). Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Studies ordered by effect size (mean difference).

https://doi.org/10.1371/journal.pone.0249611.g006

The relative symmetry of the funnel plot is supported by the non-significant Egger's test result (p = 0.621).

Subgroup analysis. The MD in daily insulin dose was not different in any of the subgroups analysed (S4b Fig in S1 File).

The group difference in daily insulin dose remained unchanged during CIPII treatment compared to that during CSII treatment, irrespective of whether the HbA1c levels before starting CIPII treatment were ≤ 53.0 mmol/mol (≤ 7%) or > 53.0 mmol/mol (> 7%) (p = 0.41); according to study type (case-control studies vs crossover studies) (p = 0.69); according to the duration of the CIPII-period (p = 0.71); and irrespective of whether or not there was an additional controlled CSII follow-up-period with a subsequent CIPII-period (p = 0.96) (S4a and S4b Fig in S1 File).

Other primary outcomes: Time to reach peak insulin concentrations. Three studies, all using regular human insulin, reported post-bolus systemic insulin levels at specific time-points (after 30 and 60 minutes for IP administration). All three studies observed earlier maximum insulin levels during the CIPII treatment compared to the CSII treatment (60 vs 133.6 minutes ($p < 0.006$) [54]; 60 vs 180 minutes ($p < 0.05$) [43]; and 30 minutes vs 60 minutes (p-value not reported) [19]).

Other primary outcomes: Maximum insulin levels. Two studies reported higher maximum insulin levels during the CIPII treatment than during the CSII treatment (179.18 vs 125.01 pmol/L, respectively ($p < 0.05$) [43] and 263.91 vs 145.84 pmol/L, respectively (30 minutes after bolus administration, $p < 0.05$) [19]. Another study reported no difference between the treatments 30 minutes after administering an insulin bolus [49].

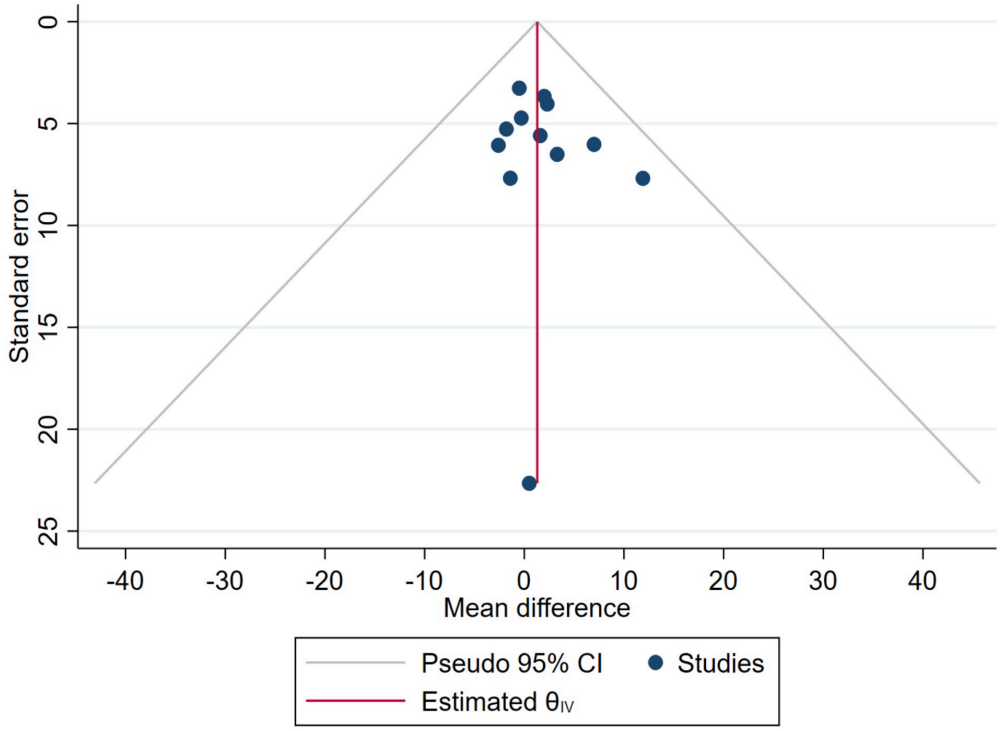


Fig 7. Funnel plot of daily insulin dose (U/24 hours) during CIPII treatment compared to that during control treatment (CSII). The funnel plot includes diagonal lines representing expected distribution of studies in the absence of heterogeneity (95% of the studies should lie within these diagonal lines). The lines are not strict 95% confidence interval, therefore, referred as 'pseudo 95% CI'.

https://doi.org/10.1371/journal.pone.0249611.g007

Other primary outcomes: Time until insulin levels returned to basal levels. After the administration of a pre-breakfast insulin bolus, two studies observed that the insulin levels returned to baseline values after three hours during the CIPII treatment [19, 43] whereas during the CSII treatment, insulin levels either returned to baseline values after four hours [19] or remained elevated after five-and-a-half hours [43].

Secondary outcomes

Meta-analysis: SMBG. When including all nine studies that reported SMBG levels (CIPII, n = 85; CSII, n = 85) [12, 13, 17, 18, 34, 38, 40, 44, 51], the SMBG levels were significantly lower during CIPII treatment than during CSII treatment (MD = -0.62 mmol/L, [95% CI: -1.01 –-0.23], p = 0.002; in mg/dL: MD = -11.2 mg/dL, [95% CI: -18.2 –-4.1], p = 0.002; S5a Fig in S1 File). However, there was moderate heterogeneity (I^2: 42%, p = 0.05).

Subgroup analysis. In subgroup analysis according to HbA1c levels before starting CIPII treatment, significantly lower SMBG levels were observed during CIPII treatment than during CSII treatment in the subgroup with HbA1c levels > 53.0 mmol/mol (> 7%) while SMBG levels remained unchanged in the subgroup with HbA1c levels ≤ 53.0 mmol/mol (≤ 7%) (MD =

-0.88 mmol/L, [95% CI: -1.34 –-0.42], p < 0.001 and MD = -0.19 mmol/L, [95% CI: -0.59–0.21], p = 0.345, respectively; in mg/dL: MD = -15.8 mg/dL, [95% CI: -24.1 –-7.6], p < 0.001 and MD = -3.4 mg/dL [95% CI: -10.6–3.8], p = 0.345, respectively; S5b. A and S5c Fig in S1 File). There was a significant difference between the subgroups (p = 0.03). There was a low heterogeneity between the studies with HbA1c levels > 53.0 mmol/mol (> 7%) (I^2: 29%, p = 0.14) and no heterogeneity in the studies with HbA1c levels ≤ 53.0 mmol/mol (≤ 7%) (0%, p = 0.94).

A subgroup analysis according to study types was not possible to calculate as all the studies were crossover studies (S5b. B and S5c Fig in S1 File).

In subgroup analysis according to duration of the CIPII-period, significantly lower SMBG levels were observed during CIPII treatment than during CSII treatment in the subgroup with longer CIPII-period (> 6 months) while levels remained unchanged in the subgroup with shorter CIPII-period (≤ 6 months) (MD = -1.24 mmol/L, [95% CI: -2.40 –-0.07], p = 0.037 and MD = -0.30 mmol/L, [95% CI: -0.63–0.03], p = 0.074, respectively; in mg/dL: MD = -22.3 mg/dL, [95% CI: -43.2 –-1.3], p = 0.037 and MD = -5.4 mg/dL, [95% CI: -11.3–0.5], p = 0.074, respectively; S5b. C and S5c Fig in S1 File). The difference between the subgroups was not significant (p = 0.13). There was a substantial heterogeneity in both subgroups (I^2: 56%, p = 0.01 and 49%, p = 0.06, respectively).

In subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period, significantly lower SMBG levels were observed during CIPII treatment than during CSII treatment in the subgroup with controlled CSII follow-up-period, while levels remained unchanged in the subgroup without controlled CSII follow-up-period (MD = -0.72 mmol/L, [95% CI: -1.20 –-0.23], p = 0.004 and MD = -0.70 mmol/L, [95% CI: -1.62–0.22], p = 0.138, respectively; in mg/dL: MD = -13.0 mg/dL, [95% CI: -21.6 –-4.1], p = 0.004 and MD = -22.6 mg/dL, [95% CI: -29.2–4.0], p = 0.138, respectively; S5b. D and S5c Fig in S1 File). There was no significant difference between the subgroups (p = 0.97). There was non-essential heterogeneity in the subgroup with controlled CSII follow-up-period (I^2: 29%, p = 0.34) and a substantial heterogeneity in the subgroup without controlled CSII follow-up-period (I^2: 70%, p = 0.04).

Meta-analysis: Cholesterol. When including all seven studies that reported cholesterol levels (CIPII, n = 61; CSII, n = 61) [13, 17, 32, 36–38, 40, 51], the cholesterol levels remained unchanged during CIPII treatment compared to those during CSII treatment (MD = -0.06 mmol/L, [95% CI: -0.35–0.22], p = 0.67; S6a Fig in S1 File). There was no heterogeneity between the studies (I^2: 0%, p = 0.81).

Subgroup analysis. The MD in cholesterol levels was not different in any of the subgroups analysed (S6c Fig in S1 File).

The group difference in cholesterol levels remained unchanged during CIPII treatment compared to that during CSII treatment whether the HbA1c levels before starting the CIPII treatment were ≤ 53.0 mmol/mol (≤ 7%) or > 53.0 mmol/mol (> 7%) (p = 0.52); according to length of the CIPII-period (p = 0.89); and whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period (p = 0.20) (S6b and S6c Fig in S1 File). Subgroup analysis according to study type could not be performed because all the included studies were crossover studies.

Meta-analysis: Triglycerides. When including all seven studies that reported triglyceride levels (CIPII, n = 61; CSII, n = 61) [13, 17, 32, 36–38, 40, 51], the triglyceride levels remained unchanged during CIPII treatment compared to those during CSII treatment (MD = 0.09 mmol/L, [95% CI: -0.03–0.22], p = 0.15; S7a Fig in S1 File). There was non-essential heterogeneity between the studies (I^2: 17%, p = 0.17).

Subgroup analysis. The MD in triglyceride levels was significantly different only in the subgroups according to whether or not there was an additional controlled CSII follow-up-period.

Significantly higher triglyceride levels were observed in the subgroup with controlled CSII follow-up-period while levels remained unchanged in the subgroup without controlled CSII follow-up-period (MD = 0.60 mmol/L, [95% CI: 0.20–1.00], p = 0.003 and MD = 0.04 mmol/L, [95% CI: -0.07–0.16], p = 0.455, respectively; S7b. D and S7c Fig in S1 File). There was a significant difference between the subgroups (p = 0.01). There was no heterogeneity in the subgroups without a controlled CSII follow-up-period (I^2: 0%, p = 0.80), while in the other subgroup, heterogeneity could not be calculated as only one study was included.

The group difference in triglyceride levels remained unchanged during CIPII treatment compared to that during CSII treatment, irrespective of whether the HbA1c levels before starting the CIPII treatment were ≤ 53.0 mmol/mol (≤ 7%) or > 53.0 mmol/mol (> 7%) (p = 0.44; S7b. A and S7c Fig in S1 File). There was a substantial heterogeneity in the subgroup with HbA1c levels > 53.0 mmol/mol (> 7%) (I^2: 66%, p = 0.04) and no heterogeneity in the subgroup with HbA1c levels ≤ 53.0 mmol/mol (≤ 7%) (I^2: 0%, p = 0.86; S7b. A Fig in S1 File).

Subgroup analysis according to the study type could not be performed since all the included studies were crossover studies (S7b. B and S7c Fig in S1 File).

The group difference in triglyceride levels remained unchanged during CIPII treatment compared to that during CSII treatment in the subgroup according to duration of the CIPII-period (p = 0.27). There was a substantial heterogeneity between the studies in the subgroup with CIPII-period ≤ 6 months (I^2: 58%, p = 0.07), and no heterogeneity between the studies in the subgroup with CIPII-period > 6 months (I^2: 0%, p = 0.70; S7b. C Fig in S1 File).

Other secondary outcomes. Analyses for the other secondary outcomes (levels of free fatty acids, lactate, ketone bodies, apolipoproteins, glucagon, adrenaline, noradrenaline, growth hormone, insulin-like growth factor, insulin-like growth factor binding proteins, sex hormone binding globulin, anti-insulin antibodies, and plasminogen activator inhibitor 1) are available in S2.1 –S2.14 Tables in S1 File.

Other secondary outcomes: Technical and medical complications. Technical and medical complications during the CIPII treatment (including inflammation, severe abdominal pain, severe insulin underdelivery, erythema, pump re-implantation, change of catheter, and insulin pump technical problems) are summarised in S2.6 Table in S1 File. Due to missing comparisons with the CSII treatment, these data were not evaluated in the main article.

Discussion

This qualitative analysis and meta-analysis strongly indicated that improved glucose control can be achieved by switching from CSII treatment to CIPII treatment in patients with DM1. This included improved overall glucose control, as evaluated by HbA1c levels, as well as a reduced frequency of severe hyperglycaemia and severe hypoglycaemia. However, despite highly significant differences, the effect of the CIPII treatment was not overwhelmingly large, as it resulted in a reduction of HbA1c levels by only 6.7 mmol/mol (0.61%). Meta-regression analysis showed that the linear prediction of HbA1c levels decreased over time during CIPII treatment. This trend, which was also observed in the cumulative meta-analysis, increases the evidence that CIPII treatment lowers HbA1c levels during the treatment period and is not an 'inclusion effect' or 'study effect' mentioned previously [13, 18]. Subgroup analysis did not find a source of substantial heterogeneity. Furthermore, the funnel plot symmetry and non-significant Egger's test (Fig 3) supported the conclusion that publication bias did not influence the results.

In the current systematic review and meta-analysis, subgroup analyses according to HbA1c levels before starting CIPII treatment, study type, duration of the CIPII-period, and whether or not there was a controlled CSII follow-up-period with a subsequent CIPII-period, were also performed.

These subgroup analyses revealed that a larger decrease in HbA1c levels was observed in the subgroup with HbA1c levels > 53.0 mmol/mol (> 7%) before starting CIPII treatment and in the crossover studies with CIPII treatment longer than six months compared to that in the meta-analysis for HbA1c levels with all studies. Additionally, in subgroups according to whether or not there was a controlled CSII follow-up period, the MD of the HbA1c levels was unchanged. Heterogeneity between studies was higher in the first three aforementioned subgroups (according to HbA1c levels before starting CIPII treatment, study type, duration of the CIPII period) with a higher MD.

The effect of decreased fasting insulin levels appeared to be related to the HbA1c levels before CIPII treatment and study type, as a significant difference appeared in the subgroup with HbA1c levels ≤ 53 mmol/mol (≤ 7%) in the crossover studies. However, fasting insulin levels decreased in the first six months and continued to decrease throughout CIPII treatment.

A similar pattern was observed in the SMBG levels in the subgroup with HbA1c levels ≤ 53 mmol/mol (≤ 7%) in the crossover studies. However, compared to that in the previous subgroup, significant difference appeared only in the subgroup with a CIPII treatment duration > 6 months.

Other metabolic variables included in the meta-analysis did not change by switching from CSII treatment to CIPII treatment.

Implications for clinical practice

Although the reduction in HbA1c levels by CIPII treatment is limited, the fact that such an improvement can be achieved simply by switching the site of insulin delivery is noteworthy, especially as the total daily insulin dose remains unchanged, combined with a reduction in the frequency of severe hypoglycaemia.

The latter observation seems even more robust, as when the frequency of severe hypoglycaemia decreased during optimized treatment with CSII, a further decrease was observed during treatment with CIPII [55]. However, in four studies that reported outcome related to severe hypoglycaemia [18, 22, 32, 45–48], the raw data were presented without any statistical comparisons. Therefore, it remains uncertain whether CIPII improves the rate of overall hypoglycaemia, as only one study reported an overall decrease [13].

The fasting insulin levels were reduced by 16.70 pmol/L during CIPII treatment. More importantly, the post-bolus circulating insulin levels peaked earlier and returned to baseline levels faster during CIPII treatment than during CSII treatment. The reduced frequency of severe hyper- or hypoglycaemias is probably due to insulin concentrations peaking faster and returning to baseline levels more quickly after the administration of insulin boluses during CIPII treatment. Concurrently, glucose control inferred from HbA1c levels improved during CIPII treatment [13, 40, 42, 49, 50]. This probably reflects the fact that CIPII mimics the physiological, endogenous insulin profile more closely than does CSII treatment.

The difference we observed in the study's main outcome measures could translate into long-term health benefits in those treated with CIPII compared to those receiving CSII treatment, even in the absence of clinically and significantly improved glucose control, and with the same overall insulin requirements. For instance, it should be noted that the incidence of myocardial infarction is increased 10-fold in young and middle-aged patients with DM1 compared to that in those in the general population without DM1 [64], and that circulating insulin levels have been linked to the pathogenesis of cardiovascular diseases [65]. Thus, reducing systemic hyperinsulinemia by switching to IP insulin delivery may, in the long-term, translate into a reduced prevalence of cardiovascular diseases in patients with DM1.

Development of an artificial pancreas

Another possible benefit of IP insulin delivery could be the potential to improve the performance of an AP. At present, only SC insulin-hybrid APs are available; with these set-ups, patients have to inform the system of the amount of carbohydrates ingested, from which the system calculates the SC bolus of insulin to be administered. The development of a fully closed-loop AP requiring no regular daily intervention by the patient and at the same time maintaining glucose levels in the normal or close-to-normal range remains a distant dream; however, a switch from SC to IP insulin delivery could help such a dream to come true [6].

Comparison with previous systematic reviews

As our focus was on the potential metabolic effects of IP versus. SC insulin delivery *per se*, we limited our systematic review only to studies of patients with DM1 that compared CIPII to CSII. Thus, we excluded MDI, as it implies the use of medium- and long-acting insulin formulations, which could influence the metabolic effects to a different extent. By focusing only on a comparison of CIPII and CSII, we limited the investigation exclusively to the use of continuously infused short-acting insulins.

In the past, two systematic reviews have been published comparing IP and SC insulin administration [66, 67]. In one of these, Almalki et al. compared IP to SC insulin delivery in patients with peritoneal dialysis, whereas Spaan et al. included a mixed group of patients with diabetes mellitus type 2 and DM1, in addition to trying to compare the effects of CIPII to MDI or CSII. Thus, neither of these existing reviews could provide useful information about the effects of IP versus SC insulin delivery *per se* in patients with DM1, specifically. Interestingly, and probably as a consequence of the study objectives, none of the studies included in our own systematic review were included in those two previous systematic reviews [66, 67]. Although nine out of the 13 studies that were included by Spaan et al. were identified in our first screening, they were subsequently excluded after applying the additional exclusion criteria.

In the current meta-analysis, additional subgroup analyses were performed, including those according to fasting BG levels, fasting insulin levels, daily insulin dose, SMBG levels, cholesterol levels, and triglyceride levels. However, in the main article we present data that show significant differences between CIPII treatment and CSII treatment. All meta-analyses and extracted qualitative data and analysis are available in the online supporting material.

Strengths and limitations

As we included only studies that applied continuous insulin infusion, we minimised the potential confounding effects of other factors, increasing the likelihood that the difference in the site of insulin delivery was the main reason for the different effects we observed. This strict approach adds to the quality of systematic reviews and meta-analyses.

It should be noted that the majority of the articles included were published during the 1990s, and only ten studies were published after the year 2000. This limitation results in a relative lack of reports of patients treated with current pump technologies, CGM, and newer and faster-acting insulin formulations. Another limitation is that the lengths of the treatment periods differed between studies (ranging from 2–48 months for the CIPII periods), probably contributing to the high heterogeneity observed between studies. In addition, the majority of the reports were not purely prospective, but were rather small cohorts assembled during consecutive periods of clinical use of CIPII, with the data from the control period (CSII) often being less well-described. Sadly, this, combined with the limited number of studies reporting on many of the outcomes, limits the scientific robustness of many of our observations. This is particularly true for the secondary outcomes reported in the S1 File.

In general, the poor descriptions of the CSII treatment periods and the open design of all the studies increases the possibility of substantial 'inclusion benefit' or 'study effect' [18]. In all of the studies that reported HbA1c levels, only one avoided this effect by maintaining the same follow-up procedure during both treatment periods [13]. However, no improvement in glycaemic control was observed during one year of intense medical surveillance in the CSII-period. Unfortunately, the follow-up period in this study was only six weeks for each of the treatments. Thus, the HbA1c results in this study are likely to be less trustworthy than those of the other outcome measures.

Conclusions

This meta-analysis suggests that CIPII treatment is superior to CSII treatment in improving glucose control in patients with DM1 who have poor glycaemic control. The effect is observed as a reduction in HbA1c levels and in reduced frequencies of severe hyperglycaemic and severe hypoglycaemic events. CIPII decreases circulating insulin levels and results in a more physiological insulin profile post bolus administration.

Thus, we hold that CIPII could be beneficial in terms of improved glucose control and more dynamic insulin effect on glucose levels. In the future, further work is needed to strengthen the evidence of the benefits of the use of CIPII.

Supporting information

S1 Checklist.
(DOC)

S1 File.
(PDF)

Acknowledgments

We sincerely thank Ingrid Ingeborg Riphagen (IIR), for providing helpful advice during the protocol drafting and for advice on the development of the search strategy.

Prior presentation

Preliminary data from this study were presented as an abstract and poster at the 10th International conference on 'Advanced Technology and Treatment for Diabetes' on the 15th– 18th of February 2017 in Paris, France. The data was also presented as an abstract and poster at the 13th International conference on 'Advanced Technology and Treatment for Diabetes' on the 19th– 22th of February 2020 in Madrid, Spain.

Author Contributions

Conceptualization: Ilze Dirnena-Fusini, Marte Kierulf Åm, Sven Magnus Carlsen, Sverre Christian Christiansen.

Data curation: Ilze Dirnena-Fusini, Marte Kierulf Åm, Sven Magnus Carlsen, Sverre Christian Christiansen.

Formal analysis: Ilze Dirnena-Fusini.

Funding acquisition: Sven Magnus Carlsen, Sverre Christian Christiansen.

Investigation: Ilze Dirnena-Fusini, Marte Kierulf Åm, Sverre Christian Christiansen.

Methodology: Ilze Dirnena-Fusini, Sverre Christian Christiansen.

Project administration: Ilze Dirnena-Fusini, Sven Magnus Carlsen, Sverre Christian Christiansen.

Resources: Ilze Dirnena-Fusini, Marte Kierulf Åm.

Software: Ilze Dirnena-Fusini.

Supervision: Anders Lyngvi Fougner, Sven Magnus Carlsen, Sverre Christian Christiansen.

Validation: Ilze Dirnena-Fusini, Marte Kierulf Åm, Anders Lyngvi Fougner, Sven Magnus Carlsen, Sverre Christian Christiansen.

Visualization: Ilze Dirnena-Fusini.

Writing – original draft: Ilze Dirnena-Fusini, Sven Magnus Carlsen, Sverre Christian Christiansen.

Writing – review & editing: Ilze Dirnena-Fusini, Marte Kierulf Åm, Anders Lyngvi Fougner, Sven Magnus Carlsen, Sverre Christian Christiansen.

References

1. Greco A.V., et al., Insulin and glucagon concentrations in portal and peripheral veins in patients with hepatic cirrhosis. Diabetologia, 1979. 17(1): p. 23–28. https://doi.org/10.1007/BF01222973 PMID: 467851
2. Felig P., et al., Concentrations of glucagon and the insulin:glucagon ratio in the portal and peripheral circulation. Proc Soc Exp Biol Med, 1974. 147(1): p. 88–90. https://doi.org/10.3181/00379727-147-38286 PMID: 4438348
3. Selam J.L., et al., Determination of portal insulin absorption from peritoneum via novel nonisotopic method. Diabetes, 1990. 39(11): p. 1361–1365. https://doi.org/10.2337/diab.39.11.1361 PMID: 2227110
4. Edgerton D.S., et al., Targeting insulin to the liver corrects defects in glucose metabolism caused by peripheral insulin delivery. JCI Insight, 2019. 5. https://doi.org/10.1172/jci.insight.126974 PMID: 30830873
5. Home P.D., Plasma insulin profiles after subcutaneous injection: how close can we get to physiology in people with diabetes? Diabetes Obes Metab, 2015. 17(11): p. 1011–20. https://doi.org/10.1111/dom.12501 PMID: 26041603
6. Christiansen S.C., et al., A Review of the Current Challenges Associated with the Development of an Artificial Pancreas by a Double Subcutaneous Approach. Diabetes Ther, 2017. 8(3): p. 489–506. https://doi.org/10.1007/s13300-017-0263-6 PMID: 28503717
7. Wan C.K.N., et al., Increased responses of glucagon and glucose production to hypoglycemia with intraperitoneal versus subcutaneous insulin treatment. Metabolism: Clinical and Experimental, 2000. 49(8): p. 984–989. https://doi.org/10.1053/meta.2000.7727 PMID: 10954014
8. Schade D.S., et al., The kinetics of peritoneal insulin absorption. Metabolism, 1981. 30(2): p. 149–55. https://doi.org/10.1016/0026-0495(81)90164-5 PMID: 7007802
9. Schade D.S., et al., The intravenous, intraperitoneal, and subcutaneous routes of insulin delivery in diabetic man. Diabetes, 1979. 28(12): p. 1069–72. https://doi.org/10.2337/diab.28.12.1069 PMID: 510804
10. Husband D.J., et al., Continuous intraperitoneal insulin infusion in the management of severely brittle diabetes-a metabolic and clinical comparison with intravenous infusion. Diabet Med, 1984. 1(2): p. 99–104. https://doi.org/10.1111/j.1464-5491.1984.tb01937.x PMID: 6242795
11. Goldstein D.E., et al., Tests of glycemia in diabetes. Diabetes Care, 2003. 26 Suppl 1: p. S106–8. https://doi.org/10.2337/diacare.26.2007.s106 PMID: 12502632
12. Beylot M., et al., Insulin-mediated glucose disposal in type 1 (insulin-dependent) diabetic subjects treated by continous subcutaneous or intraperitoneal insulin fusion. Diabete et Metabolisme, 1987. 13 (4): p. 450–456. PMID: 3315766
13. Micossi P., Bosi E., and Cristallo M., Chronic continuous intraperitoneal insulin infusion (CIPII) in type I diabetic patients non-satisfactorily responsive to continuous subcutaneous insulin infusion (CSII). Acta Diabetologica Latina, 1986. 23(2): p. 155–164. https://doi.org/10.1007/BF02624675 PMID: 3751449

14. Diabetes.co.uk. *Blood sugar convertor*. 2019 [cited 2019 18/11/2019]; Blood sugar converter].
15. endmemo.com. *Insulin Unit Conversion*. 2016 [cited 2019 11/11/2019]; Insulin Unit Conversion].
16. Calculator O. *Cholesterol Units Converter*. 2019 [cited 2019 14/12].
17. Georgopoulos A. and Saudek C.D., Intraperitoneal insulin delivery decreases the levels of chylomicron remnants in patients with IDDM. Diabetes Care, 1994. 17(11): p. 1295–1299. https://doi.org/10.2337/diacare.17.11.1295 PMID: 7821170
18. Pitt H.A., Saudek C.D., and Zacur H.A., Long-term intraperitoneal insulin delivery. Ann Surg, 1992. 216 (4): p. 483–91; discussion 491–2. https://doi.org/10.1097/00000658-199210000-00011 PMID: 1417197
19. Selam J.L., et al., Comparison of intraperitoneal and subcutaneous insulin administration on lipids, apolipoproteins, fuel metabolites, and hormones in type I diabetes mellitus. Metabolism, 1989. 38(9): p. 908–12. https://doi.org/10.1016/0026-0495(89)90240-0 PMID: 2671601
20. Lassmann-Vague V., et al., Immunogenicity of long-term intraperitoneal insulin administration with implantable programmable pumps: Metabolic consequences. Diabetes Care, 1995. 18(4): p. 498–503. https://doi.org/10.2337/diacare.18.4.498 PMID: 7497859
21. Lassmann-Vague V., et al., Autoimmunity and intraperitoneal insulin treatment by programmable pumps [14] (multiple letters). Diabetes Care, 1998. 21(11): p. 2041–2044. https://doi.org/10.2337/diacare.21.11.2041 PMID: 9802775
22. Renard E., et al., Experience with intraperitoneal insulin infusion from implantable programmable systems in Type 1 (insulin-dependent) diabetes mellitus previously treated by external pumps. Diabete et Metabolisme, 1993. 19(4): p. 364–371. PMID: 8293863
23. Van Dijk P.R., et al., Different effects of intraperitoneal and subcutaneous insulin administration on the GH-IGF-1 axis in type 1 diabetes. Journal of Clinical Endocrinology and Metabolism, 2016. 101(6): p. 2493–2501. https://doi.org/10.1210/jc.2016-1473 PMID: 27115061
24. Higgins JPT, T.J., Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Chapter 6: Choosing effect measures and computing estimates of effect*. Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019., 2019.
25. Higgins J.P., et al., Measuring inconsistency in meta-analyses. Bmj, 2003. 327(7414): p. 557–60. https://doi.org/10.1136/bmj.327.7414.557 PMID: 12958120
26. Deeks JJ H.J., Altman DG (editors), *Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020)*, T.J. In: Higgins JPT, Chandler J, Cumpston M, Li T, Page MJ, Welch VA(editors). Editor. 2020, Cochrane.
27. Higgins JPT, A.D., *Sterne JAC Chapter 8: Assessing risk of bias in included studies. In*: Higgins JPT, Green S *(editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*. The Cochrane Collaboration, 2011.
28. von Elm E., et al., The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol, 2008. 61(4): p. 344–9. https://doi.org/10.1016/j.jclinepi.2007.11.008 PMID: 18313558
29. Project E.P.H.P., *National Collaborating Centre for Methods and Tools (*2008*). Quality Assessment Tool for Quantitative Studies*. Hamilton, ON: McMaster University. (Updated 13 April, 2010) Retrieved from http://www.nccmt.ca/resources/search/14. 2010.
30. Guo B., et al., A principal component analysis is conducted for a case series quality appraisal checklist. J Clin Epidemiol, 2016. 69: p. 199–207.e2. https://doi.org/10.1016/j.jclinepi.2015.07.010 PMID: 26307459
31. Sterne J.A.C. and Egger M., Funnel plots for detecting bias in meta-analysis: Guidelines on choice of axis. Journal of Clinical Epidemiology, 2001. 54(10): p. 1046–1055. https://doi.org/10.1016/s0895-4356(01)00377-8 PMID: 11576817
32. Pacifico A., et al., Our experience with programmable implantable pump for intraperitoneal insulin infusion. [Italian]. Giornale Italiano di Diabetologia, 1997. 17(1): p. 21–27.
33. Walter H., et al., Peripheral hyperinsulinemia in type I diabetics: Reduction via continuous infusion. [German]. Aktuelle Endokrinologie und Stoffwechsel, 1989. 10(4): p. 224–228.
34. Catargi B., et al., Comparison of blood glucose stability and HbA1c between implantable insulin pumps using U400 hoe 21pH insulin and external pumps using lispro in type 1 diabetic patients: A pilot study. Diabetes and Metabolism, 2002. 28(2): p. 133–137. PMID: 11976565
35. Dassau E., et al., Intraperitoneal insulin delivery provides superior glycaemic regulation to subcutaneous insulin delivery in model predictive control-based fully-automated artificial pancreas in patients with type 1 diabetes: a pilot study. Diabetes Obes Metab, 2017. 19(12): p. 1698–1705. https://doi.org/10.1111/dom.12999 PMID: 28474383

36. Duvillard L., et al., Comparison of apolipoprotein B100 metabolism between continuous subcutaneous and intraperitoneal insulin therapy in type 1 diabetes. Journal of Clinical Endocrinology and Metabolism, 2005. 90(10): p. 5761–5764. https://doi.org/10.1210/jc.2005-0989 PMID: 16091481

37. Duvillard L., et al., No change in apolipoprotein AI metabolism when subcutaneous insulin infusion is replaced by intraperitoneal insulin infusion in type 1 diabetic patients. Atherosclerosis, 2007. 194(2): p. 342–347. https://doi.org/10.1016/j.atherosclerosis.2006.10.034 PMID: 17141785

38. Georgopoulos A. and Saudek C.D., Normalization of composition of triglyceride-rich lipoprotein subfractions in diabetic subjects during insulin infusion with programmable implantable medication system. Diabetes Care, 1992. 15(1): p. 19–26. https://doi.org/10.2337/diacare.15.1.19 PMID: 1737538

39. Giacca A., et al., Peritoneal and subcutaneous absorption of insulin in type I diabetic subjects. J Clin Endocrinol Metab, 1993. 77(3): p. 738–42. https://doi.org/10.1210/jcem.77.3.8370695 PMID: 8370695

40. Guerci B., et al., Intraperitoneal insulin infusion improves the depletion in choline- containing phospholipids of lipoprotein B particles in type I diabetic patients. Metabolism: Clinical and Experimental, 1996. 45(4): p. 430–434. https://doi.org/10.1016/s0026-0495(96)90215-2 PMID: 8609827

41. Hanaire-Broutin H., et al., Effect of intraperitoneal insulin delivery on growth hormone binding protein, insulin-like growth factor (IGF)-I, and IGF-binding protein-3 in IDDM. Diabetologia, 1996. 39(12): p. 1498–1504. https://doi.org/10.1007/s001250050604 PMID: 8960832

42. Jeandidier N., et al., Comparison of intraperitoneal insulin infusion (using implantable pump) and subcutaneous insulin administration: Preliminary results of a crossover study. Transplantation Proceedings, 1992. 24(3): p. 948–949. PMID: 1604678

43. Lassmann-Vague V., et al., Insulin kinetics in type I diabetic patients treated by continuous intraperitoneal insulin infusion: Influence of anti-insulin antibodies. Diabetic Medicine, 1996. 13(12): p. 1051–1055. https://doi.org/10.1002/(SICI)1096-9136(199612)13:12<1051::AID-DIA286>3.0.CO;2-Y PMID: 8973887

44. Lassmann-Vague V., et al., SHBG (sex hormone binding globulin) levels in insulin dependent diabetic patients according to the route of insulin administration. Hormone and Metabolic Research, 1994. 26 (9): p. 436–437. https://doi.org/10.1055/s-2007-1001725 PMID: 7835829

45. Liebl A., et al., Evaluation of the new Accu-Chek diaport, a port system for continuous intraperitoneal insulin infusion, in patients with type 1 diabetes: First 3-month results. Diabetes Technology and Therapeutics, 2013. 15: p. A13.

46. Liebl A., et al., Evaluation of the New ACCU-CHEK (R) DIAPORT, a Port System for Continuous Intraperitoneal Insulin Infusion, in Patients With Type 1 Diabetes: First 6-Month Results. Diabetes 2013. 62: p. pp.A247–A248.

47. Liebl A., et al., Evalution of the new ACCU-CHEK DIAPORT system, a port system for continous intraperitoneal insulin infusion, in patients with type 1 diabetes: final 12-month results, in International diabetes federation (IDF) World diabetes congress 2013, 2–6 December. 2013: Melbourne, Australia.

48. Liebl A., et al., Long-term clinical evaluation of the new Accu-Chek diaport, a port system for continuous intraperitoneal insulin infusion: 24-month results. Diabetes, 2014. 63: p. A241.

49. Oskarsson P.R., et al., Continuous intraperitoneal insulin infusion partly restores the glucagon response to hypoglycaemia in type 1 diabetic patients. Diabetes Metab, 2000. 26(2): p. 118–24. PMID: 10804326

50. Oskarsson P.R., et al., Metabolic and hormonal responses to exercise in type 1 diabetic patients during continuous subcutaneous, as compared to continuous intraperitoneal, insulin infusion. Diabetes and Metabolism, 1999. 25(6): p. 491–497. PMID: 10633873

51. Raccah D., et al., Intraperitoneal insulin administration does not modify plasminogen activator inhibitor 1 levels in IDDM patients. Diabetes Care, 1994. 17(8): p. 941–2. https://doi.org/10.1055/s-2007-1001725 PMID: 7835829

52. Schnell O., et al., Continuous intraperitoneal insulin therapy via port-system in type 1 diabetes with delayed absorption of subcutaneously applied insulin [Kontinuierliche intraperitoneale insulintherapie mit portsystem bei typ 1 diabetikern mit subkutaner insulinaufnahmestorung]. Diabetes und Stoffwechsel, 1994. 3: p. 51–55.

53. Wredling R., et al., Experience of long-term intraperitoneal insulin treatment using a new percutaneous access device. Diabetic Medicine, 1991. 8(6): p. 597–600. https://doi.org/10.1111/j.1464-5491.1991.tb01663.x PMID: 1832367

54. Wredling R., et al., Variation of insulin absorption during subcutaneous and peritoneal infusion in insulin-dependent diabetic patients with unsatisfactory long-term glycaemic response to continuous subcutaneous insulin infusion. Diabete Metab, 1991. 17(5): p. 456–9. PMID: 1752346

55. Liebl A., et al., A reduction in severe hypoglycaemia in type 1 diabetes in a randomized crossover study of continuous intraperitoneal compared with subcutaneous insulin infusion. Diabetes, Obesity and

Metabolism, 2009. 11(11): p. 1001–1008. https://doi.org/10.1111/j.1463-1326.2009.01059.x PMID: 19740082

56. Catargi B., et al., Glucose profiles in a type 1 diabetic patient successively treated with CSII using regular insulin, lispro and an implantable insulin pump. Diabetes Metab, 2000. 26(3): p. 210–4. PMID: 10880895

57. Jeandidier N., et al., Comparison of antigenicity of Hoechst 21PH insulin using either implantable intraperitoneal pump or subcutaneous external pump infusion in type 1 diabetic patients. Diabetes Care, 2002. 25(1): p. 84–8. https://doi.org/10.2337/diacare.25.1.84 PMID: 11772906

58. Hedman C.A., et al., Intraperitoneal insulin delivery gives higher circulating IGF-I activity than CSII in type 1 diabetes. Diabetologia, 2009. 52 (S1): p. S376–S377.

59. Arnqvist H., et al., Higher circulating IGF-I bioactivity and total IGF-I with intraperitoneal insulin delivery than with CSII in type 1 diabetes. Growth Hormone and IGF Research, 2010. 20: p. S24–S25.

60. Hedman C.A., et al., Intraperitoneal insulin delivery to patients with type 1 diabetes results in higher serum IGF-I bioactivity than continuous subcutaneous insulin infusion. Clin Endocrinol (Oxf), 2014. 81 (1): p. 58–62. https://doi.org/10.1111/cen.12296 PMID: 23865977

61. van Dijk P.R., et al., Favourable serum calcification propensity with intraperitoneal as compared with subcutaneous insulin administration in type 1 diabetes. Therapeutic Advances in Endocrinology and Metabolism, 2020. 11: p. 2042018820908456. https://doi.org/10.1177/2042018820908456 PMID: 32166012

62. van Dijk P.R., et al., Favourable serum calcification propensity with intraperitoneal as compared with subcutaneous insulin administration in type 1 diabetes. Ther Adv Endocrinol Metab, 2020. 11: p. 2042018820908456. https://doi.org/10.1177/2042018820908456 PMID: 32166012

63. Colette C., et al., Effect of different insulin administration modalities on vitamin D metabolism of insulin-dependent diabetic patients. Hormone and Metabolic Research, 1989. 21(1): p. 37–41. https://doi.org/10.1055/s-2007-1009144 PMID: 2647606

64. Laing S.P., et al., Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia, 2003. 46(6): p. 760–5. https://doi.org/10.1007/s00125-003-1116-6 PMID: 12774166

65. Conway B., Costacou T., and Orchard T., Is glycaemia or insulin dose the stronger risk factor for coronary artery disease in type 1 diabetes? Diabetes and Vascular Disease Research, 2009. 6(4): p. 223–230. https://doi.org/10.1177/1479164109336041 PMID: 20368215

66. Almalki M.H., et al., Subcutaneous versus intraperitoneal insulin for patients with diabetes mellitus on continuous ambulatory peritoneal dialysis: meta-analysis of non-randomized clinical trials. Clin Invest Med, 2012. 35(3): p. E132–43. https://doi.org/10.25011/cim.v35i3.16589 PMID: 22673316

67. Spaan N., et al., Systematic review: continuous intraperitoneal insulin infusion with implantable insulin pumps for diabetes mellitus. Acta Diabetol, 2014. 51(3): p. 339–51. https://doi.org/10.1007/s00592-014-0557-3 PMID: 24595518

Supporting information

Physiologic effects of intraperitoneal versus subcutaneous insulin delivery in patients with diabetes mellitus type 1:

A systematic review

Ilze Dirnena-Fusini et al. 2020

Contents

Literature search strategy

Table S1. Literature search strategy.

Embase		PubMed		Scopus		Central	
1	exp diabetes mellitus/	1	Diabetes mellitus[mh]	1	TITLE-ABS-KEY (diabet*)	1	Diabet*:ti,ab,kw
2	diabet*.ti,ab,kw.	2	diabet*[tiab] OR diabet*[ot]	2	TITLE-ABS-KEY (insulin resistan*)	2	insulin resistan*:ti,ab,kw
3	insulin resistan*.ti,ab,kw.	3	insulin resistan*[tiab] OR insulin resistan*[ot]	3	TITLE-ABS-KEY (impaired glucose tolerance)	3	impaired glucose tolerance:ti,ab,kw
4	impaired glucose tolerance.ti,ab,kw.	4	impaired glucose tolerance [tiab] OR impaired glucose tolerance [ot]	4	TITLE-ABS-KEY (Wolfram syndrome)	4	Wolfram syndrome:ti,ab,kw
5	Wolfram syndrome.ti,ab,kw.	5	Wolfram syndrome [tiab] OR Wolfram syndrome [ot]	5	#1 OR #2 OR #3 OR #4	5	#1 or #2 or #3 or #4
6	1 or 2 or 3 or 4 or 5	6	#1 OR #2 OR #3 OR #4 OR #5	6	TITLE-ABS-KEY (peritoneum)	6	intraperitone*:ti,ab,kw
7	exp peritoneum/	7	Peritoneum [mh]	7	TITLE-ABS-KEY (intraperitoneal)	7	peritone*:ti,ab,kw
8	exp intraperitoneal drug administration/	8	peritoneum[tiab] OR peritoneum[ot]	8	TITLE-ABS-KEY (peritoneal cavity)	8	#6 or #7
9	exp peritoneal cavity/	9	intraperitoneal [tiab] OR intraperitoneal [ot]	9	#6 OR #7 OR #8	9	subcutaneous*:ti,ab,kw
10	(peritone* or intraperitone*).ti,ab,kw.	10	#7 OR #8 OR #9	10	TITLE-ABS-KEY (subcutaneous*)	10	insulin:ti,ab,kw
11	7 or 8 or 9 or 10	11	Subcutaneous*[tw]	11	TITLE-ABS-KEY (insulin)	11	inject*:ti,ab,kw
12	exp subcutaneous drug administration/	12	Insulin [mh]	12	TITLE-ABS-KEY (inject*)	12	infus*:ti,ab,kw
13	subcutaneous.ti,ab,kw.	13	Insulin [tiab] OR Insulin [ot]	13	TITLE-ABS-KEY (infus*)	13	admin*:ti,ab,kw
14	12 or 13	14	#12 OR #13	14	TITLE-ABS-KEY (admin*)	14	absorption:ti,ab,kw
15	exp insulin derivative/	15	Drug administration routes[mh]	15	TITLE-ABS-KEY (absorption*)	15	therap*:ti,ab,kw
16	insulin.ti,ab,kw.	16	injection[tiab] OR injection[ot]	16	TITLE-ABS-KEY (therap*)	16	treatment:ti,ab,kw
17	15 or 16	17	infusion[tiab] OR infusion[ot]	17	TITLE-ABS-KEY (insulin treatment)	17	insulin infusion system*:ti,ab,kw
18	exp injection/	18	administration[tiab] OR administration[ot]	18	TITLE-ABS-KEY (pump)	18	pump:ti,ab,kw
19	infus*.ti,ab,kw.	19	absorption[tiab] OR absorption[ot]	19	#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18	19	#11 or #12 or #13 or #14 or #15 or #16 or #17 or #18
20	admin*.ti,ab,kw.	20	therap*[tiab] OR therap*[ot]	20	#5 AND #9 AND #10 AND #11 AND #19	20	#5 and #8 and #9 and #10 and #19
21	absorption.ti,ab,kw.	21	treatment[tiab] OR treatment[ot]				
22	inject*.ti,ab,kw.	22	Infusion pump[mh]				
23	exp therapy/	23	pump[tiab] OR pump [ot]				
24	therap*.ti,ab,kw.	24	#15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23				
25	exp insulin treatment/	25	#6 AND #10 AND #11 AND #14 AND #24				
26	exp pump/						
27	insulin pump.ti,ab,kw.						
28	18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27						
29	6 and 11 and 14 and 17 and 28						

Changes in the systematic review compared to the Protocol

During the data evaluation, we decided to restrict the results to a comparison of the effects of continuous subcutaneous insulin infusion (CSII) and continuous intraperitoneal insulin infusion (CIPII) only, as the pharmacokinetics (and possibly the pharmacodynamics) of multiple daily injections (MDI) differ between the two routes of administration. In general, we observed improved glycaemic control when continuous insulin delivery systems (either intravenous, subcutaneous, or intraperitoneal) were compared to MDI of insulin [1-4] and we concluded that reporting a comparison between CIPII and MDI or mixed MDI/CSII treatment would introduce unnecessary bias. The inability to compare MDI and CSII is also reflected by the differences in pharmacokinetics of the various insulin regimes used with MDI (short-, medium-, or long-lasting) versus the exclusive use of continuous short-lasting insulin infusions during CSII. Therefore, bias could be introduced based on differences in the daily profile of insulin delivery or the type of insulin used, and not just the route of administration *per se*. Furthermore, studies with missing or insufficient information pertaining to the methods of insulin delivery were also excluded.

In the Protocol, one of the outcomes was identified as 'Different locations of IP and SC delivered insulin'. After the data extraction, however, we observed that in some included studies [5, 6], patients had been given the choice about where the intraperitoneal (IP) catheter was inserted; in addition, the location could also be changed during the study (e.g., after the replacement of an implanted pump). For instance, in one study, the pumps were placed on the left side of the abdomen in the IP space because all the participants were right-handed [6]. Therefore, the main outcome described as 'Insulin absorption and parameters that can affect it: Different location of IP and subcutaneous (SC) delivered insulin; Different types of insulin used in the same location' could not be evaluated.

Regarding the case-control studies, we revised the inclusion criteria, from "we need at least one before CIPII-period and one after CIPII-period measurement point", to 'the study is included if measurements from CSII and CIPII patients/periods are reported separately'.

During the data collection, we demoted some of the primary outcomes (Stated in the Protocol) to secondary outcomes. Consequently, we made a decision based on the clinical relevance of the results. The original primary and secondary outcomes were described as follows:

Primary outcomes

The main outcomes in the included studies were: (1) Glycaemic control (glycated haemoglobin A1c (HbA1c) levels, self-monitoring of blood glucose (SMBG), fasting blood glucose (BG) and mean BG levels, hypoglycaemic and hyperglycaemic events, time spent in normoglycaemia, and glucose variability), (2) Insulin

levels (fasting insulin level, time until maximum insulin level, maximum insulin level, and elevation of insulin level after administration of a pre-meal insulin bolus), (3) Mean daily insulin requirement.

Secondary outcomes

Secondary outcomes were physiological variables other than the primary outcomes, including the following: (1) Intermediate metabolites (levels of triglycerides, cholesterol, free fatty acids, lactate, ketone bodies, and apolipoproteins), (2) Counterregulatory hormones (levels of glucagon, catecholamines, growth hormone, insulin-like growth hormones, and binding proteins), (3) Other metabolic outcomes (levels of anti-insulin antibodies (AIA), sex hormone binding globulin (SHBG), and plasminogen activator inhibitor-1 (PAI–1)), (4) Any technical and/or physiological complications reported during the CIPII treatment.

Extended information not described in the results

Excluded articles and reasons for exclusion

The search strategy identified 1,517 records. After the removal of duplicates and irrelevant articles, 108 potentially eligible articles remained for consideration (Fig 1).

After full-text and manual reference screening of potential articles and the evaluation of the quality of evidence, 105 articles were included. After additional searches, four more articles were considered for inclusion. After the introduction of additional exclusion criteria (See section above titled: 'Changes in the Systematic review compared to the Protocol'), 70 of the 109 articles were excluded for the following reasons:

- Forty-one articles did not report CSII and MDI patients/periods separately [7-47];
- two articles reported on only MDI and CIPII, but not CSII [48, 49];
- four technical reports lacked information on physiological effects [50-54];
- two reports were review articles [55, 56];
- three articles compared intravenous (IV) versus IP insulin administration [57-59];
- two articles exhibited biased reporting of the distribution of patients per group [60, 61];
- one article did not provide information about the distribution of patients per groups [62];
- five articles were missing information about pre-implantation SC insulin infusion/injection [63-67];
- one article was an epidemiological study [68];
- two articles assessed patients with a mixture of diabetes mellitus type 1 (DM1) and diabetes mellitus type 2 (DM2) [69, 70];
- two articles did not provide any relevant information [71, 72];

- one article assessed patients treated with IP insulin injections (IPII) delivered as separate boluses, not as a continuous infusion as was used for CIPII [73];
- two articles assessed a CIPII treatment period lasting less than one month [74, 75];
- one article investigated an SC peritoneal access device (SPAD). SPAD allows for absorption of insulin at the tissue close to the peritoneal lining, not from the inside of the peritoneal cavity [76];
- one article did not mention the length of the CSII and CIPII-periods [77].

In the second literature search (follow-up), which screened for studies published in 2016 to 2018, 209 additional records were identified. After the exclusion of irrelevant articles, only one additional article was included in the systematic review [78]. In the third literature search (follow-up) in which we screened studies from the year 2019, 84 additional records were identified. After the removal of all irrelevant articles, no additional articles were included in the systematic review. In the fourth literature search (follow-up) in which we screened for the studies published from 2017 to 2020, 241 records were identified. After the exclusion of irrelevant articles, four records were considered for inclusion; ultimately, only one was included in the systematic review.

In total, 32 studies from 39 articles were included in the systematic review.

Risk of biases

Some studies [79-81] included participants who received MDI therapy, however, the data were also separately available for the CSII and CIPII treatment groups.

One study that provided data for the CSII-period vs. the CIPII-period used a programmable implantable medication system (PIMS). Afterwards, the PIMS was changed to the MiniMed Implantable Pump (MIP). Because two different CIPII pumps were used, the data from the period in which patients were treated with a PIMS insulin pump were compared with the data from the CSII-period. Data pertaining to the complications experienced during the CIPII-period were extracted from both the PIMS and MIP periods [6]. One study included two different experiments with overlapping patient groups; however, data from the study's second experiment fulfilled our inclusion criteria, and the data for the CIPII and CSII treated patients were extracted [82].

One study did not report essential unit information regarding the daily insulin expenditure [83]. However, we assumed that the insulin expenditure in Table 2 was reported as U/24 hours.

One study did not provide unit information for the mean amplitude of glycaemic excursion (MAGE) [84]. To try to obtain the missing information, we used the reference for the MAGE from the article provided by the authors [85], where, the reported unit was listed as 'mg/100 mL'.

One study did not state whether the error of the reported data was listed as the SD or the standard error (SE) [86]. Another study did not describe the statistical analysis method [87]. A third study did not state the mean values of the patients' HbA1c levels [5]. Consequently, these studies were excluded from the HbA1c meta-analyses.

In one study, the units for BG were defined differently in Table 2 (mg/mL) and in the main text (mg/dL); we assumed the correct units to be mg/dL, and those values were used in the analysis. The percentage of blood glucose levels that were high, low or in the normal range were not available due to missing information about the definition of the normal range in that study [88].

Two independent studies provided very similar base line data, with similar methodological description and with identical study periods. However, the authors did not state whether the data in these reports were derived from the same study, from two separate studies, or whether they contained partially overlapping patient populations [89, 90]. E-mails, sent to the authors by IDF to verify the uniqueness of these two studies were not answered.

Another two studies provided similar base line data, with the same year of publication [91, 92]. Those two studies had identical male: female sex ratios, and age ranges (Table 1); however, they differed in the lengths of the follow-up periods, and the baseline HbA1c levels. Therefore, we assumed that the follow-up periods in these two reports were from different time periods, although we cannot discount the possibility of an overlap in the follow-up for these two studies. One of these articles [91] reported HbA1c levels (Fig 2) in the addition to the insulin expenditure, the anti-insulin antibody levels, and complications that occurred during the CIPII-period (Table S2.6). From the other article [92] the data were derived from a figure showing changes in insulin levels, and it was not possible to determine the SD. Therefore, these data were not included in the meta-analysis.

In one study, the data reported in the text were given as the geometric mean values, whereas we used the estimated mean value (Table 2) [93].

One study was a multinational, open, randomised, controlled, crossover study [5]. Due to a high dropout rate (15 out of 30 patients in the CIPII group and 9 out of 30 in the CSII group), the results were analysed as a randomised follow-up study between two parallel treatment groups (i.e., before the crossover).

One study did not provide a definition of severe hypoglycaemia. During the extended periods of the study's reporting (including conference posters presentations for data at 3, 6, 12, 24 months), the number of severe hypoglycaemic events reportedly increased during the CSII-period [94-97].

Results of the search

The primary search strategy identified 1,517 reports, and 21 more were added after screening of the reference lists. After abstract screening, 105 potentially eligible reports remained (Fig 1). After additional searches, four more articles were considered for inclusion in the analysis.

When applying the additional exclusion criteria (which are described above in the "Changes in the Systematic review compared to the Protocol), 70 of the 109 reports were excluded; these are described in the 'Excluded reports and reasons for exclusion' section above.

In total, 38 reports from 32 studies, including one report in Italian [98] and one in German [99], were included (Fig 1).

Data extraction and quality assessment

There was considerable heterogeneity among the studies (Tables S2.1 – S2.6), although most were crossover studies (23 of 32 studies), with at least three months of CSII treatment, followed by 1.5 to 14 months of CIPII treatment. More men (n = 167; 55 %) than women (n = 136; 45 %) were included in the CIPII-period. Thirty out of 32 studies reported the sex of participants, and the ages ranged from 19 to 82 years (Table 1). In the nine studies that reported age separately for each sex, the mean age range (min – max) was 37.1 years (19 – 67) in men and 32.6 years (18 – 50) in women.

Twenty-four studies originated from single European countries (Table 1), four originated from a French multicentre study (EVADIAC: EVAluation dans le Diabète des Implants ACtifs Group) [86, 88, 100, 101], three studies were from the USA [6, 83, 102], and one was a multinational study [5] (Table 1).

All results of these studies are summarised in Tables S2.1 – S2.13.

Qualitative data analysis

Primary outcome: Glycaemic control

In addition to including patients who were already being treated with CSII, one randomised [5] and six nonrandomised studies [6, 84, 88, 91, 103, 104] provided participants with an additional CSII follow-up before transitioning them to the CIPII treatment. In three of these studies, the HbA1c levels decreased during this additional CSII follow-up period [5, 103, 104].

Randomised follow-up studies

One prospective, randomised, follow-up study (for details see the section titled, 'Risk of biases') observed equivalent reduction in HbA1c levels in the two treatment groups (CIPII: - 0.5 %; CSII: - 0.6 %, p = 0.374) and no difference in SMBG values during the twelve months of CIPII treatment and the six months of CSII treatment [5].

Non-randomised and retrospective crossover studies

Glycated haemoglobin A1c

Significantly lower ($p < 0.05$) mean HbA1c levels were reported during the CIPII treatment period in eight prospective studies and one retrospective study. HbA1c level decreased from 83.6 – 56.3 mmol/mol (9.8 – 7.3 %) to 60.7 – 44.3 mmol/mol (7.7 – 6.2 %) (Fig 2) [6, 83, 87-90, 94-97, 105].

No differences in mean HbA1c levels were reported in five studies [98, 101, 102, 106-108]. In one study the HbA1c levels decreased after three months of CIPII treatment (54.1 mmol/mol (7.1 %)), whereas no statistical difference was observed after 12 months of CIPII treatment compared to the previous CSII treatment (58.5 vs. 59.6 mmol/mol (7.5 % vs. 7.6 %)) [101]. Five studies did not report statistical analyses comparing the two treatments (Table S2.1) [86, 91, 103, 104, 109]. The lack of SD/SE data resulted in the exclusion of three of these studies from the meta-analysis (Fig 2) [5, 86, 87].

Self-monitored blood glucose

Three studies that reported on SMBG concentrations showed a decrease in BG levels from 7.8 – 10.5 mmol/L to 7.4 – 8.0 mmol/L ($p < 0.05$) [83, 88, 96, 102], whereas four studies reported no difference in SMBG levels (Fig S1, Table S2.1) [6, 84, 86, 108]. However, in one of these studies, SMBG levels decreased during the first 16 months of CIPII treatment, but was equal to those following CSII after 18 months [6]. Three studies did not conduct statistical testing to compare the two treatments [103, 104, 109].

Glucose variability

One study reported a lower MAGE value during the CIPII treatment period compared to the CSII treatment period (6.9 vs. 9.5 mmol/L, $p < 0.005$) [84]. Another five studies reported a decrease in SD of BG levels during CIPII-period compared to the CSII-period (3.0 – 3.8 mmol/L vs. 3.4 – 5.1 mmol/L, $p < 0.04$) (Table S2.1) [86, 88-90, 108].

Continuous glucose monitoring

One study reported decreased mean BG levels (measured by continuous glucose monitoring (CGM)) (8.3 vs. 10.5 mmol/L, $p = 0.004$), increased time spent in normoglycaemia (3.9 –10.0 mmol/L, $p = 0.001$), and a narrower BG range (4.4 – 7.8 mmol/L, $p = 0.03$) in the CIPII-period than in the CSII-period [78]. Another study with CGM reported an increase in the time spent in normoglycaemia (3.9 – 10.0 mmol/L, $p = 0.027$) during the CIPII-period [94-97].

One study reported decreased pre-prandial BG levels ($p < 0.05$) [88], whereas another observed decreased post-prandial BG levels ($p < 0.01$) [87]. Two studies reported no difference in pre-prandial BG levels [86, 88]

and two studies reported no difference in post-prandial BG levels during the CIPII-period [86, 88]. One study did not conduct statistical comparison of the two treatments [103].

Case-control studies

Among the four included case-control studies that reported HbA1c levels, no difference was observed between the treatment groups (Fig 2) [82, 88, 99, 110-112]. One of these studies also reported no difference in pre-prandial and post-prandial BG levels [82].

Case studies

Only one case study was included, which reported no difference in glycaemic control between the CIPII and CSII treatments (Table S2.1) [113]. Due to large SD values, these results could not be included in the meta-analysis.

Primary outcome: Hypo-/ hyperglycaemia

Randomised follow-up studies

In one study, the frequency of severe hypoglycaemia (requiring hospitalization or IV glucose administration, or events accompanied by unconsciousness or seizure) was significantly reduced during the CIPII compared to the CSII follow-up periods (0.35 vs. 0.86 events/patient-years, p = 0.013). During the first three months after the initiation of CIPII treatment, the frequency of severe hypoglycaemic events was unchanged, whereas it was reduced in the subsequent nine months (0.72 vs. 0.15 events/patient-years). During CSII treatment the frequency of severe hypoglycaemia was 1.6 events per one patient-year at baseline which was reduced to 0.86 events per one patient-years during the CSII follow-up period [5]. No difference in the frequency of hypoglycaemic episodes (SMBG level < 3 mmol/L) was observed during the CIPII treatment period. Furthermore, no difference was observed between the first three months and the subsequent nine months of CIPII treatment (Tables S2.1 and S2.8) [5]. Statistical analyses were only reported for comparison between the CIPII and CSII treatment groups; no within-group analyses were performed.

Non-randomised crossover studies

Severe hypoglycaemia and hypoglycaemic coma

Four studies recorded severe hypoglycaemia, but none conducted any statistical analyses [6, 81, 94-98]. One study reported no difference in the frequency of hypoglycaemic coma events (CIPII: 0 vs. CSII: 0.54 events/patient-year) [81]. Another study reported that the frequency of severe hypoglycaemia (requiring assistance) was 0.43 events per one patient-year during the CIPII-period while no episodes of hypoglycaemic coma were observed [6].

One study reported 1.5 severe hypoglycaemic (requiring assistance) events per one patient-year during the CIPII compared to the 12 events per one patient-year during CSII-period [94-97]. Another study reported no severe hypoglycaemic (requiring assistance) events during the CIPII-period [81], and one study reported no difference in the occurrence of severe hypoglycaemia [98].

Hypoglycaemia

One study reported a reduction in the time spent in hypoglycaemia during CIPII-period (SMBG level < 3.9 mmol/L, $p < 0.05$), whereas the duration of time spent with SMBG levels < 2.8 mmol/L was similar between the treatment periods [84]. On the contrary, one 24-hour BG profile study reported no difference in the time spent in hypoglycaemia (BG < 3.8 mmol/L, measured by CGM) [78]. Similarly, two other studies reported no difference in hypoglycaemic events (SMBG level < 3.0 mmol/L) [89, 90].

One study reported at least one hypoglycaemic event (SMBG level < 3.3 mmol/L) per patient during CIPII-period [6].

Hyperglycaemia

One study using CGM [78] reported less time spent in hyperglycaemia (BG > 10 mmol/L, $p < 0.05$), whereas another study using SMBG reported no difference [84]. However, both reported a reduction in the time spent in severe hyperglycaemia (BG > 14 mmol/L, $p < 0.05$, measured by SMBG and CGM) during CIPII-period. (Tables S2.1 and S2.8) [78, 84].

Primary outcome: Insulin levels

Randomised crossover and follow-up studies

In one study, five patients being treated during the CIPII-period were crossed over to receive 96-hour CSII treatment temporarily. Insulin was infused for 12 hours at a fixed basal rate. Fasting serum free insulin levels were decreased during the CIPII-period compared to the CSII-period (30.8 vs. 45.0 pmol/L, $p < 0.001$) [100]. Subsequently, insulin was infused a rate of 15 nmol/h for 150 minutes, then 42 nmol/h for the following 150 minutes. During these two short-term periods with increased infusion rates, the rate of appearance (Ra) of insulin in the systemic circulation was greater during CIPII treatment ($p < 0.05$ and $p < 0.01$, respectively) [100].

No difference in the mean daily insulin requirement was observed in a prospective study with 36 patients, although no statistical analyses were performed [5].

Non-randomised crossover studies and follow-up studies

Two studies reported lower fasting insulin levels ($p < 0.05$ and $p < 0.01$) [89, 90], despite a higher basal insulin infusion rate during CIPII ($p = 0.02$) [89]. Two studies reported no difference in fasting insulin levels between

the two periods [87, 109]. Another two studies did not perform statistical comparisons between treatments [103, 104]. Two studies (with 20-hour and 16-hour insulin profiles) reported decreased night-time insulin levels during CIPII (127.8 vs. 163.2 pmol/L, $p < 0.05$; and 70.1 vs. 128.5 pmol/L, $p < 0.01$, respectively) [87, 103].

Two studies reported earlier post-bolus maximum insulin levels, peripherally, during the CIPII-period (60 vs. 133.6 minutes, $p < 0.006$ [92]; and 60 vs. 180 minutes, $p < 0.05$ [87]). The latter study reported increased maximum insulin levels during the CIPII-period (179.18 vs. 125.01 pmol/L, $p < 0.05$) [87].

Furthermore, during the CIPII-period, insulin levels returned to baseline values three hours after administration of a pre-breakfast bolus, whereas during the CSII-period, the post-bolus insulin level remained elevated five-and-half hours later [87].

One study that performed insulin clamp testing reported no difference in the maximum insulin levels between the periods; however, the first measurement was recorded 30 minutes after the administration of insulin boluses [89]. One study reported increased insulin levels ($p < 0.05$) during exercise in those receiving CSII, although, insulin levels did not change during exercise in the CIPII group [90].

One study reported a lower total area under curve (AUC) (16 hours) (72 vs. 100 mU/L/h, $p < 0.01$) and a lower night-time AUC (12 vs 36 mU/L/h, $p < 0.01$) during the CIPII period. The AUC following administration of an insulin bolus did not differ between the periods; however, the duration of the period for which the AUC was calculated was not specified [87].

In two studies, day-time mean insulin requirements were increased ($p < 0.05$) during CIPII-period [86, 108]. However, in one of these studies, the insulin requirement was increased only during the first two months of CIPII treatment before decreasing to levels that were similar to those in the previous CSII-period [108].

Other studies reported no change in insulin requirements between the periods, 12 of which performed statistical analyses [83, 84, 89, 90, 94-98, 101, 102, 105-109] (Table S2.2.).

On the contrary, one 24-hour closed-loop artificial pancreas study reported increased insulin delivery during closed-loop CIPII than during closed-loop CSII (43.7 U vs. 32.3 U, $p < 0.001$) [78].

Case-control studies

One study reported decreased mean night-time insulin levels in the CIPII-treated patients (65.56 vs. 86.53 pmol/L, $p < 0.005$) [99], whereas two studies reported no difference in fasting insulin levels between the two groups [82, 114].

One study reported earlier peaking of post-bolus (0.15 U/kg) insulin levels in CIPII-treated patients (30 minutes vs. 60 minutes, p-value not reported), increased maximum insulin levels (263.91 vs. 145.84 pmol/L

(significance between groups starting 30 minutes after bolus administration, $p < 0.05$)), and a decreased duration of elevated insulin levels (180 minutes vs. 240 minutes, p-value not reported) [82].

No differences in the mean daily insulin requirement were reported in three studies that performed statistical analyses [99, 110-112, 114] (Table S2.2).

Case reports

One case report showed no difference in daily insulin requirements [113].

Secondary outcomes: Intermediate metabolites

All reports that analysed intermediate metabolites are summarised in Table S2.3.

Non-randomised crossover studies

One study reported decreased total cholesterol levels after six months of the CIPII-period compared to those in the CSII-period (4.56 mmol/L vs. 4.85 mmol/L, $p = 0.044$) [102]. In the remaining six studies, no differences in total cholesterol levels were observed after six weeks to one year of CIPII treatment (Fig S2) [83, 84, 98, 106-109].

In one study, high-density lipoprotein (HDL)-cholesterol levels were lower during CIPII-periods compared to the CSII-periods (1.2 mmol/L vs. 1.4 mmol/L, $p < 0.05$) [84]. In five studies, no difference in HDL-cholesterol levels was observed between the periods [83, 98, 102, 106-108]. No difference in low-density lipoprotein (LDL)-cholesterol levels was observed in four studies [98, 102, 106-108].

One study reported an increase in fasting serum triglyceride levels after the CIPII-period (1.5 mmol/L vs. 0.9 mmol/L, $p < 0.005$) [84]. In six studies, no difference in triglyceride levels was observed between the two periods (Fig S3) [83, 98, 102, 106-109].

The chylomicron remnant levels, the ratio of retinyl ester: apoB lipoproteins, and the HDL compositions reported in the studies are provided in Table S2.3.

Case-control studies

One study reported decreased fasting free fatty acid (FFA) levels during the CIPII-period compared to the CSII-period ($p = 0.05$), whereas during the 60 minutes after the administration of a pre-meal insulin bolus, no changes in FFA levels were observed within the groups. However, decreased FFA levels were observed in the CIPII-period after administration of a pre-meal insulin bolus ($p = 0.05$) [82].

The measurements of lactate, vitamin D metabolites, creatinine, calcium, magnesium, phosphorus, parathyroid hormone, osteocalcin, and alanine reported in the studies are summarised in Table S2.3.

Secondary outcomes: counterregulatory hormones

All reported counterregulatory hormone analyses are summarised in Table S2.4.

Non-randomised crossover studies and follow-up studies

During a hypoglycaemic clamp, one study reported a significant incremental glucagon response during CIPII ($p = 0.003$), whereas the glucagon response was non-significant during CSII. Consequently, the maximal glucagon response was higher during CIPII (17.0 pg/mL vs.7.5 pg/mL, $p = 0.048$) [89]. One study reported increased glucagon levels post-exercise during CIPII-periods ($p = 0.01$); however, no difference in glucagon levels was observed between the CIPII and CSII-periods [90]. Significantly larger AUC was observed for the incremental glucagon response in the CIPII-period during hypoglycaemic insulin clamp testing and after intense exercise compared to pre-clamp testing and pre-exercise testing (44.4 pg/mL/h vs. 5.1 pg/mL/h, $p = 0.027$; and 23.4 pg/mL/h vs. 10.3 pg/mL/h, $p = 0.04$, respectively) [89, 90]. A significantly larger incremental post-exercise AUC compared to post-exercise (23.4 pg/mL/h vs. 10.3 pg/mL/h, $p = 0.04$) was also observed [90].

Two studies reported no change in epinephrine and norepinephrine incremental responses between the two periods during respective hypoglycaemic insulin clamp testing [89] or intensive exercise [90].

The results of measured changes in growth hormone (GH), insulin like growth factor 1 (IGF-1) and 2 (IGF-2), growth hormone binding protein (GHBP), insulin-like growth factor binding protein 2 (IGFBP-2) and 3 (IGFBP-3), and cortisol are summarised in Table S2.4.

Case-control studies

One study reported no difference in fasting and postprandial glucagon levels between the treatment groups [82].

Secondary outcome: Other metabolic outcomes

All other reported analyses are summarised in Table S2.5.

Non-randomised crossover and follow-up studies

Increased levels of anti-insulin antibodies (AIA) measured by enzyme-linked immunosorbent assay (ELISA), were observed after three and twelve months of the CIPII-period (39.3 % and 42.5 % vs. 23.7 %, respectively, $p < 0.01$), but not after 24 months [79, 80], and at three months of the CIPII-period in another study (11.0 % vs. 3.6 %, $p < 0.05$) [86]. No difference was observed in one study [91], and another reported no changes in the AIA levels (p-value not reported) [78].

One follow-up study observed increased AIA levels after six months of the CIPII-period vs. six months of the CSII-period (41.8 % vs. 24.9 %, $p = 0.009$), as measured by radioimmunoassay (RIA), although they observed no difference when AIA levels were measured by ELISA [115].

Studies reporting sex hormone binding globulin (SHBG) levels are summarised in Table S2.5.

Secondary outcome: Complications

All reported technical and physical complications are summarised in Table S2.6.

How to read the tables

The source column lists the main author and the year of publication. In cases where the authors and year of publication are the same for two studies, some additional information is provided in differentiation. Alternatively, when there is no information given in other columns, information is provided that could explain the missing data. For example, if there is no information provided under the 'Reported study objectives' and/or 'methodological quality' columns, it could be because information was extracted from a letter to the editor.

The 'Participant characteristics' column supplies information about the number of participants and some characteristics we believe are important for describing the actual patients. More detailed information can be found in the original publications.

In the 'Length of' column, we provide information about the duration of the CIPII and/or CSII-periods, and, if available, some information about patient follow-up. Most data are given as the means.

In the 'Reported study objectives' column we present the precise information as stated in the articles.

We extracted data from text, tables, and graphics, all of which is included it in the 'Outcomes' column. In cases, where information was missing, possible biases are indicated in the systematic review's Results section.

Some articles included figures showing measurements of continuous variables (for example, 16-hour measurements). From such figures, we extracted data from fasting periods and noted data that was significantly different between the two periods. If data for continuous variables measurements were not significantly different, it was mentioned in the Results without providing any additional data.

Units of the measurement are indicated after the CSII data (for example, HbA1C measurements, CIPII: 8.7; CSII: 8.8 %).

Definition of words used:

Increases means that in the CIPII-period, levels were statistically significantly higher ($p < 0.05$) than those in the CSII-period.

Decreases means that in the CIPII-period, levels are statistically significantly lower ($p < 0.05$) than those in the CSII-period.

Decreases/increases in both means that the values followed the same pattern when compared at different time-points.

No change means a statistically non-significant difference ($p > 0.05$) or the p-value not provided (ND). If possible, data are shown in parentheses.

M3, M6, and M12, for example, should be read as 'three months', 'six months', and 'twelve months'.

The 'Methodological quality' column contains quality assessment tools that are appropriate for that particular study.

Table S2.1. Intervention studies: Participant characteristics, description, outcomes: glycaemic control

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, CIPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Glycaemic control	**Randomised follow-up studies**					**Cochrane risk of bias tool (CRB):**
	Liebl et al. 2009 [5]	N = 60[#] (CIPII: 30 /CSII: 30) Age: 50.5/45.3 Diabetes duration: 26.3/25.1 Sex: (male) 73 %/43 % HbA1c: 8.2/8.3 C-peptide: ND Reasons: Poor metabolic control	CSII use: ND CSII f-u: 26 CIPII f-u: 52	Comparison of frequency of hypoglycaemia, severe hypoglycaemia, metabolic control, diabetic QoL and safety between CSII and CIPII in type 1 diabetic patients.	**HbA1c:** Decreases in both groups (CIPII: - 0.5; CSII: - 0.6 %, p=0.374) **SMBG:** No change (CIPII: + 0.1; CSII: ± 0.0 mmol/L, p=NS) **BG < 3 mmol/L:** No change (All CIPII-period: 118.2; M1-3: 138.1; M4-12: 108.9; CSII: 115.8 events/patient-years, p=NS) **Severe hypoglycaemia:** Decreases (Before CIPII: 0.7; All CIPII-period: 0.35, M1-3: 0.72; M4-12: 0.15, p=ND; Before CSII: 1.6; CSII-period: 0.86 events/patient-years, p=ND; CIPII vs CSII-period: p=0.013)	CRB: Unclear risk of bias: Random sequence generation, allocation concealment, blinding Low risk of bias: Incomplete outcome data, selective reporting, treatment procedure
	Non-randomised crossover studies					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Micossi et al. 1986 [84]	N = 6 Age: 38.8 Diabetes duration: 12.6 Sex: 3/3 HbA1c: 7.25 C-peptide: ≤ 0.02 pmol/mL Reasons: Pmc	CSII use: 12 CSII f-u: 6 CIPII f-u: 6	To investigate the hormonal and metabolic patterns produced by CIPII in group of severely unstable DM1 who has previously responded poorly to CSII. To compare clinical and metabolic effects of CSII and CIPII.	**HbA1c:** Decreases (CIPII: 6.2; CSII: 7.25 % (CIPII: 44; CSII: 56 mmol/mol), p<0.05) **SMBG:** No change (CIPII: 8.8; CSII: 9.7 mmol/l, p=NS) **BG > 14 mmol/l**: Decreases (CIPII: 8.9; CSII: 16.1 %, p<0.05) **BG > 10mmol/l:** No change (CIPII: 31.8; CSII: 44.7 %, p=NS) **BG < 3.9 mmol/l:** Decreases (CIPII: 4.5; CSII: 6.2 %, p<0.05) **BG < 2.8 mmol/l:** No change (CIPII: 1.2; CSII: 1.6 %, p=NS) **MAGE:** Decreases (CIPII: 6.9; CSII: 9.5 mmol/L, p<0.005)	STROBE: 15/22 QAT: Strong: Data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design Weak: Confounders
	Beylot et al. 1987 [103]	N = 4 Age: 42 Diabetes duration: 21.5 Sex: 3/1 HbA1c: 7.6 (9.2 – 5) C-peptide: ND Reasons: Volunteers	CSII use: ND CSII f-u: 8 CIPII f-u: 8 Washout: 1 day	To determine if IP insulin administration could, in addition to decreasing peripheral insulin levels, improve the insulin resistance of DM1.	**HbA1c [DT]:** No change (CIPII: 6.2; CSII: 6.5 % (CIPII: 44; CSII:48 mmol/mol), p=ND)) **SMBG [DT]:** No change (CIPII: 8.20; CSII: 8.77 mmol/l, p=ND) **Pre-prandial BG**: No change (CIPII: 5.9; CSII: 5.4 mmol/L, p=ND) **Endogenous glucose production in basal period:** No change (CIPII: 2.92; CSII: 2.93mg/kg/min, p=ND) **Glucose utilization in basal period:** No change (CIPII: 3.30; CSII: 3.62 mg/kg/min, p=ND)	STROBE: 15/22 QAT: Strong: Data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design, confounders
	Wredling, Adamson et al. 1991 (technical report) [91]	N = 6 Age: 41.3 Diabetes duration: 23.2 Sex: 4/2 HbA1c: 8.7 C-peptide: Neg Reasons: Pmc	CSII use: 52+ CSII f-u: 8 (n=3) CIPII f-u: median 72	To determine the efficacy of a new percutaneous device.	**HbA1c*:** No change (CIPII: 7.6; CSII: 8.7 % (CIPII: 60; CSII: 72 mmol/mol), p=ND)	STROBE:15/22 QAT: Moderate: Selection bias, study design, data collection method Weak: Withdrawals and drop-outs Unclear: Confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; Pmc, Poor metabolic control; NS, Not significant; BG, blood glucose; MPG, mean plasma glucose; SMBG, self-monitored BG; MAGE, mean amplitude of glycaemic excursion; [#] dropouts in this study (at the end of the periods N= 36 (CIPII: 15 /CSII: 21); *, HbA1c calculated as mean of all determinations (every 4 weeks); [DT]: data calculated from table.

Table S2.1. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
	Non-randomised crossover studies					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
Glycaemic control	Georgopoulos et al. 1992 [83]	N = 7 Age: 27 Diabetes duration: 12 Sex: 5/2 HbA1c: 9.8 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 52-60	To investigate whether long-term improved glycaemic control by intraperitoneal insulin infusion normalizes the compositional abnormalities of triglyceride (TG)-rich lipoproteins in DM1.	**HbA1c:** Decreases (CIPII: 7.7; CSII: 9.8 % (CIPII: 61; CSII: 84 mmol/mol), p<0.001) **SMBG:** Decreases (CIPII: 7.7; CSII: 10.5 mmol/L, p<0.02)	STROBE: 11/22 QAT: Strong: Data collection methods, withdrawals and drop-puts Moderate: Selection bias, study design, confounders
	Pitt et al. 1992 [6]	N = 10 Age: 33.2 Diabetes duration: 23.2 Sex: 8/2 HbA1c: 9.1 C-peptide: Neg Reasons: Volunteers	CSII use: 12+ CSII f-u: 8 CIPII f-u: 240	Document nearly 70 patient-years of experience with IP insulin delivery, with longest over 5 years, in 21 patients with type I diabetes.	**HbA1c [FF]:** Decreases (CIPII: M18: 8.0, p<0.05; M16: 8.6, p=NS; M12: 8.0, p<0.05; M6: 7.5, p<0.05; CSII: 9.1 % (CIPII: M18: 64; M16: 70; M12: 64; M6: 58; CSII: 76 mmol/mol)) **SMBG [FF]:** No change (CIPII: M18: 7.8, p=NS; M16: 7.7, p<0.05; M12: 7.8, p<0.05; M6: CIPII: 7.2, p<0.05; CSII: 8.9 mmol/L, p<0.05) **BG < 3.3 mmol/L:** No change (ND) **Severe hypoglycaemia**: 3 episodes during 7 years in CIPII-period **Hypoglycaemic coma:** No events occurred during CIPII-period	STROBE: 18/22 QAT: Strong: Confounders, withdrawals and dropouts Moderate: Selection bias, study design, data collection methods
	Renard et al. 1993 [81]	N = 8 Age: 41.6 Diabetes duration: 14.0 Sex: 6/2 HbA1c: ND C-peptide: Neg Reasons: Volunteers	CSII use: 52 CIPII f-u: 52	To gain experience in assessing the feasibility of therapeutical mode in DM1 patients, who had previous long-term experience of ambulatory SC insulin delivery portable devices.	**SMBG:** Based on mixed results (MDI and CSII) data is not included in the review **Severe hypoglycaemia**: Decreases (CIPII: 0; CSII: 0.54 events/patient-year, p=ND) **Hypoglycaemic coma:** Decreases (CIPII: 0; CSII: 0.54 events/patient-years, p=ND) **Ketoacidosis:** Decreases (CIPII: 0; CSII: 0.14 events/patient-years, p=ND)	STROBE: 19/22 QAT: Strong: Confounders, data collection methods Moderate: Selection bias, study design Weak: Withdrawals and drop-outs
	Georgopoulos et al. 1994 [102]	N = 8 Age: 37 Diabetes duration: 21.6 Sex: 5/3 HbA1c: 9.4 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 26	Test hypothesis that CIPII will decrease the level of circulating chylomicron remnants in patients with DM1.	**HbA1c:** No change (CIPII: 8.7; CSII: 9.4 %, p=NS) **SMBG:** Decreases (CIPII: 7.4; CSII: 7.82 mmol/l, p=0.027)	STROBE: 14/22 QAT: Strong: Data collection method, withdrawals and dropouts Moderate: Study design, confounders Unclear: Selection bias
	Lassmann-Vague et al. 1994 (short communication) [104]	N = 11 Age: 34.4 Diabetes duration: 22.4 Sex: 5/6 HbA1c: 7.0 C-peptide: Neg Reasons: ND	CSII use: 26+ CSII f-u: 4 CIPII f-u: 12	ND	**HbA1c**: No change (CIPII: 6.8; CSII: 6.9 %, p=ND) **SMBG**: No change (CIPII: M1: 7.9; M3: 8.3; CSII: 8.3 mmol/L, p=ND)	NP

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; BG, blood glucose; SMBG, self-monitored BG; Severe hypoglycaemia, requiring assistance; Ketoacidosis, vomiting and/or nausea in the presence of hyperglycaemia (BG>13 mmol/L), more detains in the main article; [FF], data extracted from figure.

Table S2.1. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Glycaemic control	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Raccah et al. 1994 (letter) [109]	N = 11 Age: 34.4 Diabetes duration: 22.3 Sex: 6/5 HbA1c: 6.9 C-peptide: ND Reasons: ND	CSII use: 12 CIPII f-u: 40	ND	**HbA1c:** No change (CIPII: M10: 6.3; M3: 6.8; CSII: 6.9 %, p=ND) **SMBG:** No change (CIPII: M3: 8.3; M10: 8; CSII: 8.3 mmol/L, p=ND)	NP
	Schnell et al. 1994 [105]	N = 5 Age: 35.8 Diabetes duration: 20.2 Sex: 1/4 HbA1c: 9.8 C-peptide: ND Reasons: ND	CSII use: 156-364 CIPII f-u: 52	To compare insulin demands during 24 h in IPII and CSII patients. To compare HbA1c levels in CIPII and CSII patients.	**HbA1c:** Decreases (CIPII: M12: 8.5, p<0.05; M3: 8.6, p<0.05; CSII: 9.8 %)	STROBE: 17/22 QAT: Strong: Withdrawals and drop-outs Moderate: Selection bias, study design, confounders, data collection method
	Guerci et al. 1996 [108]	N = 14 Age: 40.0 Diabetes duration: 16.4 Sex: 9/5 HbA1c: 6.1 C-peptide: Neg Reasons: Volunteers	CSII use: 52+ CIPII f-u: 16	To determine the effects of IPII on qualitative lipoprotein abnormality.	**HbA1c:** No change (CIPII: 5.9; CSII: 6.1 %, p=NS) **SMBG:** No change (CIPII: 7.55; CSII: 7.78 mmol/L, p=NS) **SD of BG:** Decreases (CIPII: 3.0; CSII: 3.4 mmol/L, p<0.01)	STROBE: 16/22 QAT: Strong: Selection bias, confounders, data collection method, withdrawals and drop-outs Moderate: Study design
	Hanaire-Broutin et al 1996 [101]	N = 18 Age: 43.0 Diabetes duration: 20.0 Sex: 11/7 HbA1c: 7.6 C-peptide: Neg Reasons: Volunteers	CSII use:128 CIPII f-u: 52	To evaluate the impact of IP insulin therapy, which results in preferential insulin absorption by the portal system, on the hepatic growth hormone-resistant state of DM1.	**HbA1c:** No change (M12: 7.5, p=NS; M3: 7.1, p<0.02; CSII: 7.6 %)	STROBE:16/22 QAT: Strong: Study design, data collection methods Moderate: Selection bias, confounders, withdrawals and drop-outs
	Lassmann-Vague et al. 1996 [87]	N = 11 Age: 36.3 Diabetes duration: 17.8 Sex: 6/5 HbA1c: ND C-peptide: ND Reasons: ND	CSII use: ND CSII f-u: ND CIPII f-u: 8	To compare plasma free insulin levels achieved in patients with DM1 chronically treated with CSII and CIPII.	**HbA1c:** Decreases (CIPII: 6.9; CSII: 7.7 %, p<0.001) **16-hour blood glucose profile:** **BG during night (12:00 am):** No change (CIPII: 9.1; CSII: 9.3 mmol/L, p=ND) **4:00 am:** No change (CIPII: 7.7; CSII: 7.9 mol/L, p=ND) **Post-prandial BG (9:30 am):** Decreases (CIPII: 7.8; CSII: 12.7 mmol/L, p<0.01) **3:00 pm:** Decreases (CIPII: 7.5; CSII: 12.8 mmol/L, p<0.01)	STROBE: 14/22 QAT: Strong: Data collection method, withdrawals and drop-outs Moderate: Selection bias, study design Weak: Confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; BG, blood glucose; SMBG, self-monitored BG; SD of BG, standard deviation of BG.

Table S2.1. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Glycaemic control	**Non-randomised crossover studies**			**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**		
	Pacifico et al. 1997 [98]	N = 8 Age: 35.1 Diabetes duration: 19 Sex: 5/4 HbA1c: 6.5 C-peptide: Neg Reasons: Volunteers	CSII use: 12+ CIPII f-u: 52+	To evaluate the safety, the efficacy and the results after 3 years of CIPII	**HbA1c:** No change (M12: 6.6 CSII: 6.5 %, p=NS) **Severe hypoglycaemia:** No change (CIPII: 0.11 events/patients/year CSII: ND)	STROBE:19/22 QAT: Strong: Study design, data collection methods, selection bias Moderate: Confounders, withdrawals and drop-outs
	Oskarsson et al. 1999 [90]	N = 7 Age: 42 Diabetes duration: 15 Sex: 5/2 HbA1c: 8.5 C-peptide: < 0.2 nM Reasons: Pmc	CSII use: 26+ CIPII f-u: 47-82	To assess the clinical relevance of the blood glucose, hypoglycaemia, glucagon secretion during exercise by comparing glycaemic and hormonal responses to a 40-min bicycle exercise test at 60% of $VO_{2\ max}$ during CSII and CIPII in type 1 diabetic patients.	**HbA1c:** Decreases (CIPII: 7.1; CSII: 8.5 %, p<0.01) **SD of BG (stability index):** Decreases (CIPII: 3.5; CSII: 5.1 mmol/L, p=0.02) **BG < 3.0 mmol/L**: No change (CIPII: 0.7; CSII: 3.8 events/months, p=0.07)	STROBE:16/22 QAT: Strong: Confounders, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design
	Oskarsson et al. 2000 [89]	N = 7 Age: 42 Diabetes duration: 17 Sex: 5/2 HbA1c: 8.6 C-peptide: Neg Reasons: Pmc	CSII use: 52+ CIPII f-u: 47-86	To expose the patients to an identical hyperinsulinemic clamp with special emphasis on the glucagon response in the same patients during continuous treatment with CSII and CIPII.	**HbA1c:** Decreases (CIPII: 7.2 CSII: 8.6 %, p<0.01) **SD of BG**: Decreases (CIPII: 3.5; CSII: 5.1 to mmol/L, p=0.02) **Pre-prandial BG**: No change (CIPII: 6.3; CSII: 6.2 mmol/L p=NS) **BG < 3.0 mmol/l**: No change (CIPII: 0.7; CSII: 3.8 event/month, p=0.07)	STROBE: 16/22 QAT: Strong: Confounders, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design
	Duvillard et al. 2005 (Brief report) [106] Duvillard et al 2007 [107]	N = 7 Age: 48 Diabetes duration: 17 Sex: 6/1 HbA1c: 7.34 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 12	Compare if replacement of SCII with IPII restores the normal physiological gradient between the portal vein and peripheral circulation, which is likely to modify lipoprotein metabolism. To compare HDL apolipoprotein (apo) AI metabolism in patients treated with CSII and CIPII.	**HbA1c:** No change (CIPII: 7.24; CSII: 7.34 %, p=NS)	Strobe: 19/22 QAT: Moderate: Data collection methods, study design, withdrawals and drop-outs Poor: Selection bias, confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; NS, Not significant; BG, blood glucose; SMBG, self-monitored BG.

Table S2.1. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Glycaemic control	**Non-randomised crossover studies**				**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**	
	Liebl et al. 2013(conf. Abstracts/ Poster) [94-96] Liebl et al. 2014 (c.poster) [97]	N = 12 (n = 10)* Age: 49 Diabetes duration: 30 Sex: 2/10 HbA1c: 9.0 (8.8)* C-peptide: ND Reasons: Pmc	CSII use: ND CIPII f-u: 104	To investigate the clinical long-term performance and safety of the new Accu-Chek DiaPort system.	**HbA1c:** Decreases (CIPII: M24*: 7.2, p=0.003; M12: 7.6, p=0.002; M6: 7.57, p<0.001; CSII: 9.0 %) **BG (by CGM) > 10.0 mmol/l**: Decreases (CIPII: M6: 38: CSII: 53 %, p=0.036) **BG (by CGM) in range 3.9 - 10.0 mmol/l:** Increases (CIPII: M6: 58; CSII: 45 %, p=0.027) **Severe hypoglycaemia:** No change (CIPII: 3 events/24 months; CSII: 12 events/12 months, p=ND)	NP
	Dassau et al. 2017 [78]	N = 10 Age: 49 Diabetes duration: 29 Sex: 7/3 HbA1c: 7.7 C-peptide: ND Reasons: Pmc	CSII use: 443 CSII f-u: 24h CIPII f-u: 4 to 20 Washout: 4 to 20	To compare closed-loop zone MPC using the DiaPort IP insulin delivery system with the traditional SC insulin delivery method during a 24-hour in-clinic protocol.	**BG (by CGM)**: Decreases (CIPII: 8.3; CSII: 10.5 mmol/L, p=0.004) **BG > 14 mmol/L:** Decreases (CIPII: 5.9; CSII: 23.0 %, p=0.0004) **BG > 10mmol/L:** Decreases (CIPII: 32.4; CSII: 53.5 %, p=0.0014) **BG in range 3.9 to 10 mmol/L:** Increases (CIPII: 65.7; CSII: 43.9 %, p=0.001) **BG in range 4.4 to 7.8 mmol/L:** Increases (CIPII: 39.8; CSII: 25.6 %, p=0.03) **BG < 3.8mmol/L:** No change (CIPII: 2.5; CSII: 4.1 %, p=0.42)	STROBE: 20/22 QAT: Strong: Data collection methods, withdrawals and drop-outs, study design Moderate: Selection bias, confounders
	Retrospective crossover studies					**STROBE and QAT:**
	Jeandidier et al. 1992 (Preliminary results) [86]	N = 8 Age: 33.5 Diabetes duration: 14.5 Sex: ND HbA1c: 6.64 C-peptide: Neg Reasons: ND	CSII use: 1 CIPII use: 12	To assess the potential benefits of CIPII vs SCII.	**HbA1c:** No change (CIPII: 6.7; CSII: 6.64 %, p=ND) **SD of BG:** Decreases (CIPII: 3.3; CSII: 3.6 mmol/L/24h, p=0.038) **Pre-prandial BG**: No change (CIPII: 7.2; CSII: 7.8 mmol/L, p=0.051) **Post-prandial BG**: No change (CIPII: 8.7; CSII: 10.1 mmol/L, p=0.051) **BG < 3.6 mmol/L**: No change (CIPII: 3.6; CSII: 4.0 events/week, p=ND)	STROBE: 12/22 QAT: Weak: Study design Unclear: Selection bias, confounders, data collection methods
	Catargi et al. 2002 [88]	N = 14 Age: 50.6 Diabetes duration: 28.0 Sex: 5/9 HbA1c: 7.8 C-peptide: Neg Reasons: ND	CSII use: ND CSII f-u: 6.4 Healing period: 6.4 CIPII f-u: 6.4[a]	To compare the efficacy of IPII and CSII of therapy in terms of glycaemic control, glycaemic stability and hypoglycaemia frequency.	**HbA1c:** Decreases (CIPII: 7.3; CSII: 7.8 %, p<0.05) **Pre-prandial BG:** Decreases (CIPII: 7.8; CSII: 8.1 mmol/L, p<0.05) **SMBG:** Decreases (CIPII: 8.0; CSII: 8.5 mmol/L, p<0.01) **SD of BG:** Decreases (CIPII: 3.8; CSII: 4.4 mmol/L, p<0.01) **Post-prandial BG:** No change (CIPII: 8.2; CSII: 8.5 mmol/L, p=0.07)	STROBE: 15/22 QAT: Moderate: Study design, data collection method; withdrawals and drop-outs Unclear: Selection bias, confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; NS, Not significant; BG, blood glucose; SMBG, self-monitored BG; CGM, continuous glucose monitoring; SD of BG, standard deviation of BG. Note, *, dropout in the study at 24months; [a], three patients first were treated with CIPII, and then with CSII.

Table S2.1. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Glycaemic control	**Case-control studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Colette et al. 1989 [114]	N = 24 (CIPII: 13 /CSII: 11) Age: 30/32 Diabetes duration: 17/20 Sex: ND HbA1c: 8.0/8.9 C-peptide: ND Reasons: ND	CSII use: 40 CIPII use: 60	Study the effects of prolonged tight diabetic control and insulin delivery through portal route on vitamin D metabolism in DM1.	**HbA1c:** No change (CIPII: 8.0; CSII: 8.9 %, p=NS)	STROBE: 18/22 QAT: Strong: Data collection method Moderate: Selection bias, study design, confounders
	Selam et al. 1989 [82]	N = 14 (CIPII: 6 /CSII: 8) Age: 32/44.3 Diabetes duration: 16/23.1 Sex: 4/2 / 5/3 HbA1c: 8.3/8.7 C-peptide: ND Reasons: ND	CSII use: 52+ CIPII use: 26	Compare the effects of intensive SC vs. implantable pump IP insulin delivery on intermediary metabolites in DM1 patients.	**HbA1c:** No change (CIPII: 8.2; CSII: 8.6 %, p=NS) **Pre-prandial BG[FF]:** No change (CIPII: 7.3; CSII: 5.5 mmol/L, p=NS) **Post-prandial BG**: No change (p=NS)	STROBE: 14/22 QAT: Strong: Data collection methods Moderate: Study design, confounders Weak: Confounders Unclear: Selection bias, blinding
	Walter et al. 1989 [99]	N = 12 (CIPII: 6 /CSII: 6) Age: 28.3/26.6 Diabetes duration: 10.8/10.5 Sex: 6/0 / 6/0 HbA1c: 8.0/7.9 C-peptide: ND Reasons: ND	CSII use: 26+ CIPII use: 12+	To compare metabolism control at night time in the patients with MDI and continuous insulin administration.	**HbA1c:** No change (CIPII: 8.0; CSII: 7.9 %, p=NS)	STROBE: 15/22 QAT: Strong: Data collection methods Moderate: Selection bias, study design, confounders Unclear: Blinding Not applicable: Withdrawals and drop-outs
	Hedman et al. 2009 (c.a) [111] Arnqvist et al. 2010 (c.a.) [116] Hedman et al. 2014 [112]	N = 30 (CIPII: 10 /CSII: 20) Age: 53.1/52.8 Diabetes duration: 124.2/30.8 Sex: 5/5 / 10/10 HbA1c: 8.6/7.9 C-peptide: ND Reasons: Pmc	CSII use: 26+ CIPII use: 26+	Investigate in cross-sectional study if the different modes of insulin administration, CIPII or CSII were associated with a change in the circulating IGF system.	**HbA1c:** No change (CIPII: 8.6; CSII: 7.9 %, p=NS)	STROBE: 21/22 QAT: Strong: Selection bias, confounders, data collection method, withdrawals and drop-outs Moderate: Study design

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; NS, Not significant; BG, blood glucose; SMBG, self-monitored BG; SPAD, SC peritoneal access device; c.a., conference abstract; [FF], data extracted from figure.

Table S2.1. (Continued)

	Source	**Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate**	**Length of: CSII use, CSII follow-up, IPII follow-up (weeks)**	**Reported study objectives**	**Outcomes (mean, p-value)**	**Methodological quality**
Glycaemic control	**Case report**					**Critical appraisal tool of Center for Evidence-based management:**
	Catargi et al. 2000 [113]	N = 1 Age: 32 Diabetes duration: 6 Sex: 1/0 HbA1c: ND C-peptide: Neg Reasons: Pmc	CSII f-u (rapid -acting) (1): 12 CSII f-u (Lispro) (2): 3 CIPII use: 1.5+	To evaluate a new catheter design.	**HbA1c:** No change (CIPII: 5.9; CSII (1): 6.2; CSII (2): 6.1 %, p=ND) **SMBG:** No change (CIPII: 6.3; CSII (1): 7.8; CSII (2): 7.3 mmol/L, p=ND) **Pre-prandial BG:** No change (CIPII: 5.9; CSII (1): 6.4; CSII (2): 6.6 mmol/L, p=ND) **Post-prandial BG:** No change (CIPII: 6.6; CSII (1): 9.6; CSII (2): 8.8 mmol/L, p=ND) **LBGI^:** No change (CIPII: 4.3; CSII (1): 5.5; CSII (2): 4.0, p=ND) **AUC (mean of 7 times/day SMBG):** No change (CIPII: 43.9; CSII (1): 49.5; CSII (2): 44.3 h.mmol/L, p=ND)	8/10 (2 cannot tell)

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; BG, blood glucose; LBGI, low blood glucose index. Note, LBGI^ < 5, low or moderate risk of future severe hypoglycaemia; LBGI > 5, a high-risk; AUC, area under curve.

Table S2.2. Intervention studies, Participant characteristics, description, outcomes: Insulin levels

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Insulin levels	**Randomised crossover studies with wash-out period**					**Cochrane risk of bias tool (CRB):**
	Giacca et al. 1993 [100]	N = 5 Age: 31 - 50 Diabetes duration: 8 - 39 Sex: 1/4 HbA1c: 7.4 C-peptide: Neg Reasons: Volunteers	CSII use: ND CSII f-u: 96+ hours CIPII f-u: 12+ Washout: serum free insulin level measurements after IV insulin bolus	To compare the rate of appearance of insulin in the peripheral circulation during IP and SC insulin administration in T1D, in steady and non-steady state.	**Fasting insulin levels**: Decreases (CIPII: 30.8; CSII: 45.0 pmol/L, p<0.001) **Plasma clearance rate of insulin:** No change (CIPII: 14.7; CSII: 13.1 mL/kg*min, p=ND) **Fasting recovery rate of insulin:** Decreases (CIPII: 27; CSII: 40 %, p<0.001) **Insulin infusion 15 nmol/L for 150 min + 42nmol/L for another 150 min:** Increases recovery rate (with first increase (15nmol/h), p<0.05; with second increase (42nmol/h), p<0.01) **Basal insulin requirement**: No change (CIPII: 5.4; CSII: 5.6 nmol/h, p=ND)	CRB: Unclear risk of bias: Random sequence generation, allocation concealment, blinding Low risk of bias: Incomplete outcome data, selective reporting, treatment procedure
	Randomised follow-up studies					**Cochrane risk of bias tool (CRB):**
	Liebl et al. 2009 [5]	N = 60[a] (CIPII: 30 /CSII: 30) Age: 50.5/45.3 Diabetes duration: 26.3/25.1 Sex: (male) 73 %/43 % HbA1c: 8.2/8.3 C-peptide: ND Reasons: Pmc	CSII use: ND CSII f-u: 26 CIPII f-u: 52	Comparison of frequency of hypoglycaemia, severe hypoglycaemia, metabolic control, diabetic QoL and safety between CSII and CIPII in type 1 diabetic patients.	**Mean daily insulin requirement**: No change (CIPII: 44.2; CSII: 46.0 U/24h, p=ND)	CRB: Unclear risk of bias: Random sequence generation, allocation concealment, blinding Low risk of bias: Incomplete outcome data, selective reporting, treatment procedure
	Non-randomised crossover studies					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Micossi et al. 1986 [84]	N = 6 Age: 38.8 Diabetes duration: 12.6 Sex: 3/3 HbA1c: 7.25 C-peptide: ≤ 0.02 ρmol/mL Reasons: Pmc	CSII use: 12 CSII f-u: 6 CIPII f-u: 6	To investigate the hormonal and metabolic patterns produced by CIPII in group of severely unstable DM1 who has previously responded poorly to CSII. To compare clinical and metabolic effects of CSII and CIPII.	**Mean daily insulin requirement**: No change (CIPII: 46.02; CSII: 48.67 U/24h, p=NS)	STROBE: 15/22 QAT: Strong: Data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design Weak: Confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; NS, Not significant; [a], dropouts in this study (at the end of the periods N = 36 (CIPII: 15 /CSII: 21).

Table S2.2. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Insulin levels	**Non-randomised crossover studies**				**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**	
	Beylot et al. 1987 [103]	N = 4 Age: 42 Diabetes duration: 21.5 Sex: 3/1 HbA1c: 7.6 (9.2 – 5) C-peptide: ND Reasons: Volunteers	CSII use: ND CSII f-u: 8 CIPII f-u: 8 Washout: 1 day	To determine if IP insulin administration could, in addition to decreasing peripheral insulin levels, improve the insulin resistance of DM1.	**Fasting insulin levels**: No change (CIPII: 131.95; CSII: 152.79 pmol/L, p=ND) **Plasma free insulin (night-time):** Decreases (CIPII: 127.78; CSII: 163.2 pmol/L, p<0.05), **Mean daily insulin requirement** [DT]: No change (CIPII: 0.0.57; CSII: 0.0.59 U/kg/day, p=ND)	STROBE: 15/22 QAT: Strong: Blinding, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design, confounders
	Wredling, Lui et al. 1991 [92]	N = 6 Age: 42.8 Diabetes duration: 24.0 Sex: 4/2 HbA1c: 7.7 – 10.2 C-peptide: Neg Reasons: Pmc	CSII use: ND CSII f-u: 208 CIPII f-u: 38	To compare the reproducibility of the plasma-insulin profile of IP and SC administered insulin in a group of C-peptide-negative, diabetic patients.	**Pre-meal insulin bolus (time till max. conc.):** Decreases (CIPII: 60; CSII: 133 minutes, p=0.006) **Total insulin AUC (0-240 minutes)**: No change (CIPII (bolus 0.05 U/kg/BW): 56.1 mU; CSII (bolus 0.1 U/kg/BW): 94.6 mU, p=0.0023) **Insulin AUC 0-60 min**: No change (CIPII: 16.3; CSII: 20.6 mU, p=NS) **Intra-patient CV (AUC 0-60 min):** No change (CIPII: 19.8; CSII: 38.6 %, p=NS) **Intra-patient CV (AUC 0-240 min):** No change (CIPII: 11.5; CSII: 20.2 %, p=NS) **Inter-patient peak time:** No change (CIPII: 22.4; CSII: 28.3 %, p=NS) **Inter-patient CV (AUC 0-60 min):** No change (CIPII: 43.6; CSII: 27.9 %, p=NS) **Inter-patient CV (AUC 0-240 min):** No change (CIPII: 30.9; CSII: 29.7 %, p=NS) **Inter-patient peak time:** No change (CIPII: 44.0; CSII: 28.0 %, p=NS)	STROBE: 15/22 QAT: Strong: Data collection method Moderate: Study design Weak: Selection bias Unclear: Confounders Not applicable: Withdrawals and drop-outs
	Wredling, Adamson et al. 1991 (Technical report) [91]	N = 6 Age: 41.3 Diabetes duration: 23.2 Sex: 4/2 HbA1c: 8.7 C-peptide: Neg Reasons: Pmc	CSII use: 52+ CSII f-u: 8 (n=3) CIPII f-u: median 72	To determine the efficacy of a new percutaneous device.	**Mean daily insulin requirement**: No change (CIPII: 44.8 U/24h; CSII: ND)	STROBE:15/22 QAT: Moderate: Selection bias, study design, data collection method Weak: Withdrawals and drop-outs Unclear: Confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; NS, Not significant; CV, coefficient of variation; AUC, area under curve; [DT], data calculated from table.

Table S2.2. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
	Non-randomised crossover studies					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
Insulin levels	Georgopoulos et al. 1992 [83]	N = 7 Age: 27 Diabetes duration: 12 Sex: 5/2 HbA1c: 9.8 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 52-60	To investigate whether long-term improved glycaemic control by intraperitoneal insulin infusion normalizes the compositional abnormalities of triglyceride (TG)-rich lipoproteins in DM1.	**Mean daily insulin requirement**: No change (CIPII: 57.2; CSII: 52 (units of measurements are not provided, p=NS)	STROBE: 11/22 QAT: Strong: Data collection methods, withdrawals and drop-puts Moderate: Selection bias, study design, confounders
	Georgopoulos et al. 1994 [102]	N = 8 Age: 37 Diabetes duration: 21.6 Sex: 5/3 HbA1c: 9.4 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 26	Test hypothesis that IPII will decrease the level of circulating chylomicron remnants in patients with DM1.	**Mean daily insulin requirement**: No change (CIPII: 62.4; CSII: 61.9 U/24h, p=NS)	STROBE: 14/22 QAT: Strong: Data collection method, withdrawals and dropouts Moderate: Study design, confounders Unclear: Selection bias
	Lassmann-Vague et al. 1994 (short communication) [104]	N = 11 Age: 34.4 Diabetes duration: 22.4 Sex: 5/6 HbA1c: 6.9 C-peptide: Neg Reasons: ND	CSII use: 26+ CSII f-u: 4 CIPII f-u: 12	ND	**Fasting insulin levels:** No change (CIPII: M1: 111.12; M3: 114.59; CSII: 118.06 pmol/L, p=ND) **Mean daily insulin requirement**: No change (CIPII: 41.6; CSII: 40.5 U/24h, p=ND)	NP
	Raccah et al. 1994 (letter) [109]	N = 11 Age: 34.4 Diabetes duration: 22.3 Sex: 6/5 HbA1c: 6.9 C-peptide: ND Reasons: ND	CSII use: 12 CIPII f-u: 40	ND	**Fasting insulin levels:** No change (CIPII: M3: 114.59; M10: 100; CSII: 118.06 pmol/L, p=NS) **Mean daily insulin requirement**: No change (CIPII: 62.4; CSII: 40.5 U/24h, p=NS)	NP
	Schnell et al. 1994 [105]	N = 5 Age: 25-62 Diabetes duration: 20.2 Sex: 1/4 HbA1c: 9.8 C-peptide: ND Reasons: ND	CSII use: 156-364 CIPII f-u: 52	To compare insulin demands during 24 h in CIPII and CSII patients. To compare HbA1c levels in CIPII and CSII patients.	**Mean daily insulin requirement:** No change (CIPII: 46; CSII: 48 U/24h, p=NS)	STROBE: 17/22 QAT: Strong: Withdrawals and drop-outs Moderate: Selection bias, study design, confounders, data collection method

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; NP, not possible to evaluate.

Table S2.2. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Insulin levels	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Guerci et al. 1996 [108]	N = 14 Age: 40.0 Diabetes duration: 16.4 Sex: 9/5 HbA1c: 6.1 C-peptide: Neg Reasons: Volunteers	CSII use: 52+ CIPII f-u: 16	To determine the effects of IPII on qualitative lipoprotein abnormality.	**Mean daily insulin requirement:** No change (CIPII: M2: 0.69, p<0.01; M4: 0.64; CSII: 0.60 U/kg/24h, p=NS)	STROBE: 16/22 QAT: Strong: Selection bias, confounders, data collection method, withdrawals and drop-outs Moderate: Study design
	Hanaire-Broutin et al. 1996 [101]	N = 18 Age: 43.0 Diabetes duration: 20.0 Sex: 11/7 HbA1c: 7.6 C-peptide: Neg Reasons: Volunteers	CSII use: 128 CIPII f-u: 52	To evaluate the impact of intraperitoneal insulin therapy, which results in preferential insulin absorption by the portal system, on the hepatic growth hormone-resistant state of DM1.	**Mean daily insulin requirement:** No change (CIPII: 39.4; CSII: 39.1 U/24h, p=NS)	STROBE: 16/22 QAT: Strong: Study design, data collection methods, withdrawals and drop-outs Moderate: Selection bias, confounders
	Lassmann-Vague et al. 1996 [101]	N = 11 Age: 36.3 Diabetes duration: 17.8 Sex: 6/5 HbA1c: ND C-peptide: ND Reasons: ND	CSII use: ND CSII f-u: ND CIPII f-u: 8	To compare plasma free insulin levels achieved in patients with DM1 chronically treated with CSII and CIPII.	**Fasting insulin levels** (7:00 am): No change (CIPII: 60.42; CSII: 66.67 pmol/L, p=NS) **Plasma free insulin (night-time (12:00 am)):** Decreases (CIPII: 70.15; CSII: 128.48 pmol/L, p<0.01) **Pre-meal insulin bolus (time till max conc.):** Decreases (CIPII: 1 h; CSII: 3 h, p<0.05) **(max. insulin conc.):** Increases (CIPII: 179.18; CSII: 125.01 pmol/L, p<0.05) **elevation (return to basal concentration)**: Decreases (CIPII: 3 h; CSII: did not return till next bolus) **Total insulin AUC**: Decreases (CIPII: 72; CSII: 100 mU/h/L, p<0.01) **Night-time AUC:** Decreases (CIPII: 12; CSII: 36 mU/L/h, p<0.01) **AUC after insulin bolus:** No change (CIPII: 32; CSII: 30 mU/L/h, p=NS) **Mean daily insulin requirement:** No change (1.3 U/h)	STROBE: 14/22 QAT: Strong: Data collection method, withdrawals and drop-outs Moderate: Selection bias, study design Weak: Confounders
	Pacifico et al. 1997 [98]	N = 8 Age: 35.1 Diabetes duration: 19 Sex: 5/4 HbA1c: 6.5 C-peptide: Neg Reasons: Volunteers	CSII use: 12+ CIPII f-u: 52+	To evaluate the safety, the efficacy and the results after 3 years of CIPII.	**Mean daily insulin requirement**: No change (CIPII: 42.8; CSII: 40.8 U/24h, p=NS)	STROBE:19/22 QAT: Strong: Study design, data collection methods, Selection bias Moderate: Confounders, withdrawals and drop-outs

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; NP, not possible to evaluate; AUC, area under curve.

Table S2.2. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Insulin levels	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Oskarsson et al. 1999 [90]	N = 7 Age: 42 Diabetes duration: 15 Sex: 5/2 HbA1c: 8.5 C-peptide: < 0.2nM Reasons: Pmc	CSII use: 26+ CIPII f-u: 47-82	To assess the clinical relevance of the BG, hypoglycaemia, glucagon secretion during exercise by comparing glycaemic and hormonal responses to a 40-min bicycle exercise test at 60 % of $VO_{2\,max}$ during CSII and CIPII in type 1 diabetic patients.	**Fasting insulin levels:** decreases (CIPII: 28.0; CSII: 48.1 pmol/L, p=0.043) **Change in insulin levels during the time of exercises[FF]:** No change (in the groups); increases (between groups, through the study, p<0.05) **Mean daily insulin requirement:** No change (CIPII: 38.4; CSII: 36.1 U/24h, p=0.06)	STROBE:16/22 QAT: Strong: Confounders, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design
	Oskarsson et al. 2000 [89]	N = 6 Age: 42 Diabetes duration: 17 Sex: 5/2 HbA1c: 8.6 C-peptide: Neg Reasons: Unsatisfactory on CSII	CSII use: 52+ CIPII f-u: 69	To expose the patients to an identical hyperinsulinemic challenge with special emphasis on the glucagon response in the same patients during continuous treatment with CSII and CIPII.	**Fasting insulin levels:** Decreases (CIPII: 35.8; CSII: 53.4 pmol/L, p<0.01) **Change in plasma hormone levels from basal level to peak level in time of insulin clamp; and change between CIPII and CSII:** **Insulin(+30 min):** Increases in both (CIPII: 66.9, p=0.01; CSII: 42.4 pmol/L, p=0.03); No change (p=0.32) **Basal rate:** Increases (CIPII: 1.34; CSII: 1.14 U/h, p=0.02) **Bolus doses:** Decreases (CIPII: 7.1; CSII: 11.6 U/24h, p=0.04) **Mean daily insulin requirement:** No change (CIPII: 37.9; CSII: 38.2 U/24h, p=0.95)	STROBE: 16/22 QAT: Strong: Confounders, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design
	Duvillard et al. 2005 (Brief report) [106] Duvillard et al 2007 [107]	N = 7 Age: 48 Diabetes duration: 17 Sex: 6/1 HbA1c: 7.34 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 12	Compare if replacement of SCII with IPII restores the normal physiological gradient between the portal vein and peripheral circulation, which is likely to modify lipoprotein metabolism. To compare HDL apolipoprotein (apo) AI metabolism in patients treated with CSII and IPII.	**Mean daily insulin requirement:** No change (CIPII: 43.6; CSII: 45.0 U/24h, p=0.69)	Strobe: 19/22 QAT: Moderate: Data collection methods, study design, withdrawals and drop-outs Poor: Selection bias, confounders
	Liebl et al. 2013 (c.a) [94-96] Liebl et al 2014 (c.a) [97]	N = 12 (n = 10)* Age: 49 Diabetes duration: 30 Sex: 2/10 HbA1c: 9.0 (8.8)* C-peptide: ND Reasons: Pmc	CSII use: ND CIPII f-u: 104	To investigate the clinical long-term performance and safety of the new Accu-Chek DiaPort system.	**Mean daily insulin requirement:** No change (CIPII: M6: 45; CSII: 49 U, p=NS)	NP

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; [FF], data extracted from figure; *, dropouts in the study; Pmc, Poor metabolic control.

Table S2.2. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Insulin levels	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Dassau et al. 2017 [78]	N = 10 Age: 49 Diabetes duration: 29 Sex: 7/3 HbA1c: 7.7 C-peptide: ND Reasons: Pmc	CSII use: 443 CSII f-u: 24h CIPII f-u: 4 to 20	To compare closed-loop zone MPC using the DiaPort IP insulin delivery system with the traditional SC insulin delivery method during a 24-hour in-clinic protocol.	**In-clinical measurements: 24-hour total insulin delivery:** Increases (CIPII: 43.66; CSII: 32.29 U, p<0.001) **Mean daily insulin requirement**: No change (CIPII: ND; CSII: 43 U/24h)	STROBE: 20/22 QAT: Strong: Data collection methods, withdrawals and drop-outs, study design Moderate: Selection bias, confounders
	Retrospective crossover studies					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Jeandidier et al. 1992 (Preliminary results) [86]	N = 8 Age: 33.5 Diabetes duration: 14.5 Sex: ND HbA1c: 6.64 C-peptide: Neg Reasons: ND	CSII use: 1 CIPII use: 12	To assess the potential benefits of CIPII vs SCII.	**Mean daily insulin requirement**: Increase (CIPII: 39; CSII: 32 U/24h, p<0.05)	STROBE: 12/22 QAT: Weak: Study design Unclear: Selection bias, confounders, data collection methods
	Non-randomised follow-up studies					**STROBE and QAT:**
	Van Dijk et al. 2016 [93]	N = 101 (CIPII: 32 /CSII: 69)[b] Age: 50/48 Diabetes duration: 29/27 Sex: 14/25 / 30/44 HbA1c: 8.3/7.9 C-peptide: ND Reasons: Pmc	CSII/MDI use: 208+ CIPII use: 208+ CSII f-u: 27 CIPII f-u: 27	To compare the effects of CIPII to SC insulin therapy, on the GH-IGF-1 axis in a large prospective, observational matched case-control study in T1DM patients.	**Mean daily insulin requirement**: No change (CIPII: 0.7; CSII: 0.6 U/24h/kg, p=NS)	STROBE: 16/22 QAT: Strong: Selection bias, study design, data collection method Moderate: Study design, withdrawals and drop-outs
	Case-control studies					**STROBE and QAT:**
	Colette et al. 1989 [114]	N = 24 (CIPII: 13 /CSII: 11) Age: 30/32 Diabetes duration: 17/20 Sex: ND HbA1c: 8.0/8.9 C-peptide: ND Reasons: ND	CSII use: 40 CIPII use: 60	Study the effects of prolonged tight diabetic control and insulin delivery through portal route on vitamin D metabolism in insulin dependent diabetic patients.	**Fasting insulin levels:** No change (CIPII: 115.28; CSII: 140.98 pmol/L, p=NS)	STROBE: 18/22 QAT: Strong: Data collection method, withdrawals and drop-outs Moderate: Selection bias, study design, confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; Pmc, Poor metabolic control; c.a, conference abstract. Note: [b], for analysis participant nr. changed (dropouts).

Table S2.2. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Insulin levels	**Case-control studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Selam et al.1989 [82]	N = 14 (CIPII: 6 /CSII: 8) Age: 32/44.3 Diabetes duration: 16/23.1 Sex: 4/2 / 5/3 HbA1c: 8.3/8.7 C-peptide: ND Reasons: ND	CSII use: 52+ CIPII use: 26	Compare the effects of intensive SC vs. implantable pump IP insulin delivery on intermediary metabolites in DM1 patients.	**Fasting insulin levels** [FF]: No change (NS) **Pre-meal insulin bolus (bolus + 4 h basal rate = 0.15 U/kg) (time till max conc.):** No change (CIPII: 30 min; CSII: 60 min, p=ND) **(max. insulin conc.):** Increases (CIPII: 263.91; CSII: 145.84 pmol/L) (at +30 min, p<0.05); **elevation (return to basal concentration:** Decreases (CIPII: 180; CSII: 240 minutes, p=ND).	STROBE: 14/22 QAT: Strong: Data collection methods Moderate: Study design, confounders Weak: Confounders Unclear: Selection bias, blinding Not applicable: Withdrawals and drop-outs
	Walter et al. 1989 [99]	N = 12 (CIPII: 6 /CSII: 6) Age: 28.3/26.6 Diabetes duration: 10.8/10.5 Sex: 6/0 / 6/0 HbA1c: 8.0/7.9 C-peptide: ND Reasons: ND	CSII use: 26+ CIPII use: 12+	To compare metabolism control at night time in the patients with ICT and continuous insulin administration.	**Mean night insulin values (At night (23:00–7:00)):** Decreases (CIPII: 65.56; CSII: 86.53 pmol/L, p<0.005). **Mean daily insulin requirement**: No change (CIPII: 0.56; CSII: 0.55 U/kg/24h, p=NS)	STROBE: 15/22 QAT: Strong: Data collection methods Moderate: Selection bias, study design, confounders Unclear: Blinding Not applicable: Withdrawals and drop-outs
	Hedman et al. 2009 (poster) [111] Arnqvist et al. 2010 (poster) [116] Hedman et al. 2014 [112]	N = 30 (CIPII: 10 /CSII: 20) Age: 53.1/52.8 Diabetes duration: 124.2/30.8 Sex: 5/5 / 10/10 HbA1c: 8.6/7.9 C-peptide: ND Reasons: Pmc	CSII use: 26+ CIPII use: 26+	Investigate in cross-sectional study if the different modes of insulin administration, CIPII or CSII were associated with a change in the circulating IGF system.	**Mean daily insulin requirement**: No change (CIPII: 51.2; CSII: 39.3 U/24h, p=0.260)	STROBE: 21/22 QAT: Strong: Selection bias, confounders, data collection method, withdrawals and drop-outs Moderate: Study design
	Case report					**Critical appraisal tool of Centre for Evidence-based management:**
	Catargi et al. 2000 [113]	N = 1 Age: 32 Diabetes duration: 6 Sex: 1/0 HbA1c: ND C-peptide: Neg Reasons: Pmc	CSII f-u: (rapid-acting insulin) (1): 12 CSII f-u (Lispro): 12 CIPII: 1.5+	To evaluate a new catheter design	**Mean daily insulin requirement**: No change (CIPII: 52; CSII (1): 51.2; CSII (2): 50.9, p=ND)	8/10 (2 cannot tell)

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, no data available; NS, Not significant; [FF], data extracted from figure.

Table S2.3. Intervention studies, Participant characteristics, description, outcomes: Intermediate metabolites

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Secondary outcomes: Intermediate metabolites	**Non-randomised crossover studies**				**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**	
	Micossi et al. 1986 [84]	N = 6 Age: 38.8 Diabetes duration: 12.6 Sex: 3/3 HbA1c: 7.25 C-peptide: ≤ 0.02 pmol/mL Reasons: Poor glucose control	CSII use: 12 CSII f-u: 6 CIPII f-u: 6	To investigate the hormonal and metabolic patterns produced by CIPII in group of severely unstable DM1 who has previously responded poorly to CSII. To compare clinical and metabolic effects of CSII and CIPII.	**Total cholesterol**: No change (CIPII: 5.1; CSII: 4.4 mmol/L, p=NS) **HDL-cholesterol:** Decreases (CIPII: 1.2; CSII: 1.4 mmol/L, p<0.05) **HDL_2 cholesterol**: Decreases (CIPII: 0.3; CSII: 0.6 mmol/L, p<0.01) **HDL_3 cholesterol**: No change (CIPII: 0.95; CSII: 0.9 mmol/L, p=NS) **Fasting serum triglycerides**: Increases (CIPII: 1.5; CSII: 0.9 mmol/L, p<0.005) **Mean daily glycerol**: No change (CIPII: 61.7; CSII: 35.4 µmol/L, p=NS)	STROBE: 15/22 QAT: Strong: Data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design Weak: Confounders
	Georgopoulos et al. 1992 [83]	N = 7 Age: 27 Diabetes duration: 12 Sex: 5/2 HbA1c: 9.8 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 52-60	To investigate whether long-term improved glycaemic control by intraperitoneal insulin infusion normalizes the compositional abnormalities of triglyceride (TG)-rich lipoproteins in DM1.	**Total cholesterol:** No change (CIPII: 4.6; CSII: 4.9 mmol/L, p=NS) **HDL cholesterol:** No change (CIPII: 1.30; CSII: 1.33 mmol/L, p=NS) **Fasting plasma triglyceride:** No change (CIPII: 1.23; CSII: 1.35 mmol/L, p=NS) **Differences after fat ingestion:** Plasma TG increased in both groups (no statistically significant changes in any time point). **Mean ratios of constituents in fasting lipoprotein mass:** **Total cholesterol-triglyceride:** — **Sf 100-400:** CIPII: 0.20; CSII: 0.29, p<0.008 — **Sf 20-100:** CIPII: 0.375; CSII: 0.483, p<0.01 **Total cholesterol-phospholipid:** — CIPII: 0.594; CSII: 0.975, p<0.001 — CIPII: 0.73; CSII: 1.295, p<0.004 **Lipid-protein:** — CIPII: 14.07; CSII: 13.93, p=NS — CIPII: 10.16; CSII: 10.92, p=NS	STROBE: 11/22 QAT: Strong: Data collection methods, withdrawals and drop-puts Moderate: Selection bias, study design, confounders
	Raccah et al. 1994 (letter) [109]	N = 11 Age: 34.4 Diabetes duration: 22.3 Sex: 6/5 HbA1c: 6.9 C-peptide: ND Reasons: ND	CSII use: 12 CIPII f-u: 40	ND	**Total cholesterol:** No change (CIPII: M3: 4.74; M10: 4.92; CSII: 5.03 mmol/L, p=NS) **Fasting plasma triglycerides:** No change (CIPII: M3: 0.88; M10: 0.83; CSII: 0.83 mmol/L, p=NS)	NP

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; NP, not possible to evaluate; TG, triglycerides; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table S2.3. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Secondary outcomes: Intermediate metabolites	**Non-randomised crossover studies**				**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**	
	Georgopoulos et al. 1994 [102]	N = 8 Age: 37 Diabetes duration: 21.6 Sex: 5/3 HbA1c: 9.4 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 26	Test hypothesis that IPII will decrease the level of circulating chylomicron remnants in patients with DM1.	**Fasting:** **Total cholesterol:** Decreases (CIPII: 4.56; CSII: 4.85 mmol/L, p=0.044) **HDL cholesterol:** No change (CIPII: 1.26; CSII: 1.30 mmol/L, p=NS) **LDL cholesterol:** No change (CIPII: 2.87; CSII: 3.10 mmol/L, p=NS) **Plasma triglycerides:** No change (CIPII: 0.93; CSII: 0.93 mmol/L, p=NS) **Differences after fat ingestion [FF]:** **Max. conc. TG: Sf. > 100:** No change (follows similar pattern) (CIPII: 0.6; CSII: 0.7 mmol/L, p=NS) **Time till TG Sf > 100 max conc.:** No change (follows similar pattern) (CIPII: 4; CSII: 4 hours, p=NS) **Plasma TG Sf. 20-100:** No change (follows similar pattern) (p=NS) **ApoB: Sf. > 100:** No change (follows similar pattern) (p=NS) **ApoB Sf. 20-100:** No change (p=NS) **Retinyl esters Sf > 100:** Decreases (+4 hours: CIPII: 2500; CSII: 6000 µg/L, p=0.05) **Retinyl esters Sf 20-100:** No change (follows similar pattern) decreases (+ 8 hours; CIPII: 450; CSII: 700 µg/L, p=0.075) **Retinyl ester: apoB ratio: (S_f > 100)**: Decreases (p=0.0002) **S_f 60-100:** No change (p=0.06)	STROBE: 14/22 QAT: Strong: Data collection method, withdrawals and dropouts Moderate: Study design, confounders Unclear: Selection bias
	Guerci et al. 1996 [108]	N = 14 Age: 40.0 Diabetes duration: 16.4 Sex: 9/5 HbA1c: 6.1 C-peptide: Neg Reasons: Volunteers	CSII use: 52+ CIPII f-u: 16	To determine the effects of IPII on qualitative lipoprotein abnormality.	**Fasting:** **Total cholesterol**: No change (CIPII: 5.01; CSII: 4.97 mmol/L, p=NS) **HDL cholesterol:** No change (CIPII: 1.49; CSII: 1.57 mmol/L, p=NS) **LDL cholesterol:** No change (CIPII: 1.49; CSII: 1.57 mmol/L, p=NS) **Plasma triglyceride:** No change (CIPII: 1.13; CSII: 1.1 mmol/L, p=NS) **Total plasma lipids:** No change (CIPII: 3.02; CSII: 2.95 mmol/L, p=NS) **Apo A-I:** No change (CIPII: 3.96; CSII: 4.06 mmol/L, p=NS) **Apo B:** No change (CIPII: 2.56; CSII: 2.46 mmol/L, p=NS) **Lp B-PL:** Increases (CIPII: 1.36; CSII: 1.09 mmol/L, p<0.01) **Lp B-PL/apo B:** Increases (CIPII: 1.39; CSII: 1.17 mmol/L, p<0.05) **Lp B-TC:** No change (CIPII: 3.51; CSII: 3.35 mmol/L, p=NS) **Lp no B-PL:** No change (CIPII: 1.75; CSII: 1.88 mmol/L, p=NS) **Lp no B-TC:** No change (CIPII: 1.50; CSII: 1.62 mmol/L, p=NS)	STROBE: 16/22 QAT: Strong: Selection bias, confounders, data collection method, withdrawals and drop-outs Moderate: Study design

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, not significant; HDL, high density lipoprotein; LDL, low density lipoprotein; LpB, Apo B-containing lipoprotein particles; LP no B, no-apo-B containing particles; Sf, lipoprotein size; TC, total cholesterol; PL, plasma lipids; VLDL, very-low-density lipoproteins; [FF], data extracted from figure. Note: Retinyl esters – a marker of intestinal lipoproteins.

Table S2.3. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Secondary outcomes: Intermediate metabolites	**Non-randomised crossover studies**				**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**	
	Pacifico et al. 1997 [98]	N = 8 Age: 35.1 Diabetes duration: 19 Sex: 5/4 HbA1c: 6.5 C-peptide: Neg Reasons: Volunteers	CSII use: 12+ CIPII f-u: 52+	To evaluate the safety, the efficacy and the results after 3 years of CIPII.	**Total cholesterol:** No change (CIPII: 4.81; CSII: 4.72 mmol/L, p=NS) **HDL cholesterol:** No change (CIPII: 1.14; CSII: 1.17 mmol/L, p=NS) **LDL (chol.):** No change (CIPII: 3.05; CSII: 2.96 mmol/L, p=NS) **LDL (trigl.):** No change (CIPII: 0.36; CSII: 0.35 mmol/L, p=NS) **VLDL (chol.):** No change (CIPII: 0.29; CSII: 0.23 mmol/L, p=NS) **VLDL (trigl.):** No change (CIPII: 0.43; CSII: 0.27 mmol/L, p=NS) **HDL_2(chol.):** No change (CIPII: 0.26; CSII: 0.27 mmol/L, p=NS) **HDL_2(trigl.):** No change (CIPII: 0.07; CSII: 0.07 mmol/L, p=NS) **HDL_3(chol.):** No change (CIPII: 0.89; CSII: 0.84 mmol/L, p=NS) **HDL_3(trigl.):** No change (CIPII: 0.12; CSII: 0.09 mmol/L, p=NS) **Triglyceride:** No change (CIPII: 0.88; CSII: 0.81 mmol/L, p=NS)	STROBE:19/22 QAT: Strong: Study design, data collection methods, selection bias Moderate: Confounders, withdrawals and drop-outs
	Duvillard et al. 2005 (Brief report) [106] Duvillard et al. 2007 [107]	N = 7 Age: 48 Diabetes duration: 17 Sex: 6/1 HbA1c: 7.34 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 12	Compare if replacement of SCII with IPII restores the normal physiological gradient between the portal vein and peripheral circulation, which is likely to modify lipoprotein metabolism.	**Total cholesterol:** No change (CIPII: 5.04; CSII: 5.33 mmol/L, p=0.45) **HDL cholesterol:** No change (CIPII: 1.47; CSII: 1.47 mmol/L, p=0.99) **LDL cholesterol**: No change (CIPII: 3.1; CSII: 3.2 mmol/L, p=0.45) **Fasting plasma triglyceride:** No change (CIPII:1.28; CSII: 1.08 mmol/L, p=0.22) **Apo B100-containing lipoprotein production and fractional catabolic rates**: No change (ND, p=NS) **ApoA1**: No change (CIPII: 1.28; CSII: 1.34 g/L, p=0.45) **HDL composition:** **Esterified cholesterol:** No change (CIPII: 24.0; CSII: 20.1 %, p=0.45) **Free cholesterol:** No change (CIPII: 3.3; CSII: 3.4 %, p=0.99) **Triglycerides:** No change (CIPII: 2.1; CSII: 2.4 %, p=0.99) **Phospholipids:** No change (CIPII: 25.2; CSII: 22.7 %, p=0.99) **Proteins:** No change (CIPII: 45.5; CSII: 51.2 %, p=0.13)	Strobe: 19/22 QAT: Moderate: Data collection methods, study design, withdrawals and drop-outs Poor: Selection bias, confounders

Legends: CSII, continuous subcutaneous insulin infusion; CIPII, continuous intraperitoneal insulin infusion; ND, no data available; NS, Not significant; HDL, high density lipoprotein; LDL, low density lipoprotein; Apo, apolipoprotein; trigl., triglycerides; chol., cholesterol.

Table S2.3. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Secondary outcomes: Intermediate metabolites	**Case-control studies**				**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**	
	Colette et al. 1989 [114]	N = 24 (CIPII: 13 / CSII: 11) Age: 30/32 Diabetes duration: 17/20 Sex: ND HbA1c: 8.0/8.9 C-peptide: ND Reasons: ND	CSII use: 40 CIPII use: 60	Study the effects of prolonged tight diabetic control and insulin delivery through portal route on vitamin D metabolism in IDDP.	**Plasma creatinine:** No change (CIPII: 1.08; CSII: 1.11 mg/dl, p=NS) **Plasma calcium:** No change (CIPII: 9.3; CSII: 9.1 mg/dl, p=NS) **Plasma magnesium:** No change (CIPII: 1.81; CSII: 1.85 mg/dL, p=NS) **Plasma phosphorus:** No change (CIPII: 3.5; CSII: 3.3 mg/dL, p=NS) **Plasma iPTH:** No change (CIPII: 2.6; CSII: 2.7 mU/mL, p=NS) **Osteocalcin:** No change (CIPII: 5.7; CSII: 6.4 ng/mL, p=NS) **Mean vitamin D intake:** No change (CIPII: 89; CSII: 99 U/day, p=NS) **Vitamin D metabolites:** **25 OH D:** Increases (CIPII: 22.1; CSII: 12.5 ng/mL, p<0.02) **24,25-$(OH)_2$D**: Increases (CIPII: 2.3; CSII: 1.4 ng/mL, p<0.05) **1,25-$(OH)_2$D:** No change (CIPII: 45; CSII: 35 pg/mL, p=NS)	STROBE: 18/22 QAT: Strong: Data collection method, withdrawals and drop-outs Moderate: Selection bias, study design, confounders
	Selam et al.1989 [82]	N = 14 (CIPII: 6 / CSII: 8) Age: 32/44.3 Diabetes duration: 16/23.1 Sex: 4/2 / 5/3 HbA1c: 8.3/8.7 C-peptide: ND Reasons: ND	CSII use: 52+ CIPII use: 26	Compare the effects of intensive SC vs. implantable pump IP insulin delivery on intermediary metabolites in DM1 patients.	**Pre-meal insulin bolus (bolus + 4 h basal rate = 0.15 U/kg): Time point 0: FFA [FF]:** Decreases (CIPII: 0.20; CSII: 0.47 mmol/L, p<0.05) **Postprandial FFA [FF]:** Decreases (at +30min: CIPII: 0.2; CSII: 0.45 mmol/L, p<0.05); decreases (+60 min; CIPII: 0.2; CSII: 0.47 nmol/L, p=0.05) **Time point 0: lactate [FF]:** No change (CIPII: 0.5; CSII: 0.45 mmol/L, p=NS) **Postprandial lactate [FF]:** Increases (at +30 minutes: CIPII: 0.7; CSII: 0.4 mmol/L, p=NS. At +60 min.: CIPII: 1.0; CSII: 0.5 mmol/L, p<0.05) **Alanine [FF]**: No change (p=NS) **3 OH butyrate [FF]:** No change (p=NS)	STROBE: 14/22 QAT: Strong: Data collection methods Moderate: Study design, confounders Weak: Confounders Unclear: Selection bias
	Van Dijk et al. 2016 [93] Van Dijk et al. 2020 [117]	N = 181 (CIPII: 39 / CSII: 74 Age: 49.6/47.9 Diabetes duration: 28.5/24.7 Sex: 14/25 30/44 HbA1c: 66.9/63.4 C-peptide: neg Reasons: Poor glucose control*	CSII use: 208 CSII follow-up: 26 CIPII use: 208 CIPII follow-up: 26	To test the hypothesis that among persons with T1DM treated with IP insulin therapy there is a decreased calcification propensity (expressed as a higher T50) as compared with treatment with SC insulin therapy.	**Calcium:** no change (CIPII: 2.3; CSII: 2.3 mmol/L, p=ND) **T_{50} within groups**: no change (CIPII baseline: 372; CIPII end: 362 minutes, difference within group: (median [with interquartile range (IQR)]) -10[-29,9] no change (CSII baseline: 360; CSII end: 359 minutes, difference within group: (median [with interquartile range (IQR)]) -0.2[-19,9] **T_{50} after follow-up**: no change after (CIPII: 362; CSII: 359 minutes, difference CIPII vs. CSII: (median [with interquartile range (IQR)]) -8 [-22,7]	STROBE: 21/22 QAT: Strong: Data collection method, study design Moderate: Confounders Unclear: Selection bias

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; ND, No data available; Neg, negative; NS, Not significant; FFA, Free fatty acids; iPTH, Immunoreactive parathyroid hormone; 25 OH D, Calcifediol; 24,25-$(OH)_2$D, (inactive) hydroxycalcidiol; 1,25-$(OH)_2$D, active form of vitamin D_3; 3 OH butyrate, beta-hydroxybutyrate (by-product of ketosis); [FF], data extracted from figure; *, HbA1 c⩾ 58 mmol/mol (7.5 %) or at least five incidents of hypoglycaemia (defined as glucose < 4.0 mmol/L).

Table S2.4. Intervention studies, Participant characteristics, description, outcomes: Counterregulatory hormones

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Secondary outcomes: Counterregulatory hormones	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Hanaire-Broutin et al. 1996 [101]	N = 18 Age: 43.0 Diabetes duration: 20.0 Sex: 11/7 HbA1c: 7.6 C-peptide: Neg Reasons: Volunteers	CSII use: 128 CIPII f-u: 52	To evaluate the impact of intraperitoneal insulin therapy, which results in preferential insulin absorption by the portal system, on the hepatic growth hormone-resistant state of DM1.	**Fasting growth hormone:** No change (CIPII: M3: 3.46; M12: 1.47; CSII: 2.23 ng/mL) **GHBP activity** [DT]: Increases (CIPII: M3: 14.5; M12: 15.5; CSII: 10.2 %, p<0.0001)	STROBE: 16/22 QAT: Strong: Study design, data collection methods, withdrawals and drop-outs Moderate: Selection bias, confounders
	Oskarsson et al. 1999 [90]	N = 7 Age: 42 Diabetes duration: 15 Sex: 5/2 HbA1c: 8.5 C-peptide: < 0.2nM Reasons: Unsatisfactory on CSII	CSII use: 26+ CIPII f-u: 61	To assess the clinical relevance of the blood glucose, hypoglycaemia, glucagon secretion during exercise by comparing glycaemic and hormonal responses to a 40-min bicycle exercise test at 60% of $VO_{2\,max}$ during CSII and CIPII in type 1 diabetic patients.	**Change in hormone levels from pre- to post-exercises; and change between CIPII and CSII:** **Glucagon:** Increases (CIPII: 15.1, p=0.01; CSII: 7.4 pg/mL, p=0.08); no change (CIPII vs CSII: p=0.07) **Epinephrine:** Increases in both groups (CIPII: 0.81, p=0.03; CSII: 0.43 nmol/L, p=0.009); no change (CIPII vs CSII: p=0.49) **Norepinephrine**: Increases in both groups (CIPII: 3.75, p=0.006; CSII: 4.02 nmol/L, p=0.006); no change (CIPII vs CSII: p=0.09) **Growth hormone:** Increases in both groups (CIPII: 9.4, p=0.03; CSII: 11.9 mg/mL, p=0.01); no change (CIPII vs CSII: p=0.34) **Cortisol**: Increases in both groups (CIPII: 135.1, p=0.02; CSII: 92.9 nmol/L, p=0.03); no change (CIPII vs CSII: p=0.47) **C-peptide:** No change (CIPII: -0.02, p=0.19; CSII: -0.01 nmol/L, p=0.59); no change (CIPII vs CSII: p=0.91)	STROBE:16/22 QAT: Strong: Confounders, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design
	Oskarsson et al. 2000 [89]	N = 7 Age: 42 Diabetes duration: 17 Sex: 5/2 HbA1c: 8.6 C-peptide: Neg Reasons: Unsatisfactory on CSII	CSII use: 52+ CIPII f-u: 69	To expose the patients to an identical hyperinsulinemic challenge with special emphasis on the glucagon response in the same patients during continuous treatment with CSII and CIPII.	**Change in plasma hormone levels from basal level to peak level in time of hyperinsulinemia; and change between CIPII and CSII:** **Glucagon**: Increases (CIPII: 17.0, p=0.003; CSII: 7.5 pg/mL, p=0.06); increases (CIPII vs CSII: p=0.048) **Epinephrine:** Increases in both groups (CIPII: 2.05, p=0.004; CSII: 2.92 nmol/L, p=0.04); no change (CIPII vs CSII: p=0.50) **Norepinephrine**: Increases (CIPII: 0.91, p=0.003; CSII: 0.74 nmol/L, p=0.11); no change (CIPII vs CSII: p=0.68) **Growth hormone**: Increases in both groups (CIPII: 13.4, p=0.02; CSII: 19.3 mg/mL, p=0.03); no change (CIPII vs CSII: p=0.34) **Cortisol:** Increases in both groups (CIPII: 286, p=0.0003; CSII: 277 nmol/L, p=0.0003); no change (CIPII vs CSII: p=0.77) **C-peptide:** No change (CIPII: 0.02, p=0.30; CSII: 0.05 nmol/L, p=0.74); no change (CIPII vs CSII: p=0.44)	STROBE: 16/22 QAT: Strong: Confounders, data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; ND, No data available; NS, Not significant; FFA GHBP, Growth hormone binding proteins; [DT], data calculated from table.

Table S2.4. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Secondary outcomes: Counterregulatory hormones	**Non-randomised follow-up studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Van Dijk et al. 2016 [93]	N = 113 (CIPII: 39/CSII: 74) Age: 50/48 Diabetes duration: 29/27 Sex: 14/25 / 30/44 HbA1c: 8.3/7.9 C-peptide: ND Reasons: Pmc	CSII/MDI use: 208+ CIPII use: 208+ CSII f-u: 27 CIPII f-u: 27	To compare the effects of CIPII to SC insulin therapy, on the GH-IGF-1 axis in a large prospective, observational matched case-control study in T1DM patients.	**Growth hormone:** Decreases (CIPII: 0.63; CSII: 1.39 µg/L, p=0.039)	STROBE: 16/22 QAT: Strong: Selection bias, study design, data collection method Moderate: Study design, withdrawals and drop-outs
	Case-control studies					**STROBE and QAT:**
	Selam et al. 1989 [82]	N = 14 (CIPII: 6 /CSII: 8) Age: 32/44.3 Diabetes duration: 16/23.1 Sex: 4/2 / 5/3 HbA1c: 8.3/8.7 C-peptide: ND Reasons: ND	CSII use: 52+ CIPII use: 26	Compare the effects of intensive SC vs. implantable pump IP insulin delivery on intermediary metabolites in DM1 patients.	**Fasting glucagon** [FF]**:** No change (CIPII: 25; CSII: 25 pg/mL, p=NS) **Postprandial glucagon** [FF] (+30 minutes): No change (CIPII: 30; CSII: 20 pg/mL, p=NS)	STROBE: 14/22 QAT: Strong: Data collection methods Moderate: Study design, confounders Weak: Confounders Unclear: Selection bias, blinding Not applicable: Withdrawals and drop-outs

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; c.a., Conference abstract; ND, No data available; NS, Not significant; NP, Not possible to evaluate; [FF], data extracted from figure.

Table S2.5. Intervention studies, Participant characteristics, description, outcomes: Other outcomes

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Other outcomes	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Wredling, Adamson et al. 1991 (Technical report) [91]	N = 6 Age: 41.3 Diabetes duration: 23.2 Sex: 4/2 HbA1c: 8.7 C-peptide: Neg Reasons: Pmc	CSII use: 52+ CSII f-u: 8 (n=3) CIPII f-u: median 18 (15 – 24 months)	To determine the efficacy of a new percutaneous device.	**Anti-insulin antibodies**: No change (CIPII: 34.8; CSII: 21.7 %, p=NS)	STROBE:15/22 QAT: Moderate: Selection bias, study design, data collection method Weak: Withdrawals and drop-outs Unclear: Confounders
	Lassmann-Vague et al. 1994 (short communication) [104]	N = 11 Age: 34.4 Diabetes duration: 22.4 Sex: 5/6 HbA1c: 6.9 C-peptide: Neg Reasons: ND	CSII use: 26+ CIPII f-u: 12	ND	**SHBG levels in men**: Decreases (CIPII: M1: 31; M3: 33; CSII: 39 nM/L, p<0.05) **SHBG levels in women**: Decreases (CIPII: M1: 67; M3: 63; CSII: 80 nM/L, p<0.01)	NP
	Raccah et al. 1994 (letter) [109]	N = 11 Age: 34.4 Diabetes duration: 22.3 Sex: 6/5 HbA1c: 6.9 C-peptide: ND Reasons: ND	CSII use: 12 CIPII f-u: 40	ND	**Plasminogen activator inhibitor (PAI) 1** levels: No change (CIPII: M3: 4; M10: 6.6; CSII: 5.1 U/mL, p=NS)	NP
	Hanaire-Broutin et al. 1996 [101]	N = 18 Age: 43.0 Diabetes duration: 20.0 Sex: 11/7 HbA1c: 7.6 C-peptide: Neg Reasons: Volunteers	CSII use: 128 CIPII f-u: 52	To evaluate the impact of intraperitoneal insulin therapy, which results in preferential insulin absorption by the portal system, on the hepatic growth hormone-resistant state of DM1.	**Plasma IGF I** [DT]: Increases (CIPII: M3: 114.0; M12: 146.9; CSII: 89.4 ng/mL, p<0.002) **IGFBP-3** [DT]: Increases (CIPII: M3: 2275; M12: 3534; CSII: 1974 ng/mL, p<0.0001)	STROBE: 16/22 QAT: Strong: Study design, data collection methods, withdrawals and drop-outs Moderate: Selection bias, confounders
	Lassmann-Vague et al. 1995 [79] Lassmann-Vague et al. 1998 (letter) [80]	N = 15 Age: 36 Diabetes duration: 20.9 Sex: 8/9 HbA1c: 7.1 C-peptide: Neg Reasons: ND	CSII use: ND CSII f-u: 4 CIPII f-u: 104	To assess immunogenicity of intraperitoneal insulin infusion via implanted pumps by two methods. To evaluate the possible influence of an increased antibody level on metabolic and clinical parameters.	**Anti-insulin antibodies**[IE] (measured by using RIA) [DT]: Increases (CIPII: M3: 39.9, p<0.01; M12: 42.5, p<0.01; M24: 48, p=0.964; CSII: 23.7 %)	STROBE: 12/22 QAT: Moderate: Selection bias, study design, data collection method Weak: Withdrawals and dropouts Unclear: Confounders

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, No data available; NS, Not significant; NP, Not possible to evaluate; SHBG, Sex hormone binding globulin; IGF 1, Insulin-like growth factor – 1; BP, Binding proteins; [IE], 100 % is optical density between 1.5 and 2 U of AI IgG in solution; RIA, radioimmunoassay; [DT], data calculated from table.

Table S2.5. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Other outcomes	**Non-randomised crossover studies**					**Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Thomas quality assessment toll (QAT):**
	Duvillard et al. 2005 (Brief report) [106] Duvillard et al. 2007 [107]	N = 7 Age: 48 Diabetes duration: 17 Sex: 6/1 HbA1c: 7.34 C-peptide: ND Reasons: ND	CSII use: ND CIPII f-u: 12	Compare if replacement of SCII with IPII restores the normal physiological gradient between the portal vein and peripheral circulation, which is likely to modify lipoprotein metabolism.	**Fructosamine:** No change (CIPII: 352; CSII: 348 µmol/L, p=0.69)	Strobe: 19/22 QAT: Moderate: Data collection methods, study design, withdrawals and drop-outs Poor: Selection bias, confounders
	Dassau et al. 2017 [78]	N = 10 Age: 49 Diabetes duration: 29 Sex: 7/3 HbA1c: 7.7 C-peptide: ND Reasons: Poor metabolic control	CSII use: 443 CSII f-u: 24h CIPII f-u: 4 to 20 Washout: 4 to 20	To compare closed-loop zone MPC using the DiaPort IP insulin delivery system with the traditional SC insulin delivery method during a 24-hour in-clinic protocol.	**Anti-insulin antibodies:** No change (ND)	STROBE: 20/22 QAT: Strong: Data collection methods, withdrawals and drop-outs, study design Moderate: Selection bias, confounders
	Non-randomised follow-up studies					**STROBE and QAT:**
	Jeandidier et al. 2002 [115]	N = 24 (CIPII: 13/CSII: 11) Age: 36.8/43.1 Diabetes duration: 19.2/24.4 Sex: 6/7 / 6/5 HbA1c: ND C-peptide: Neg Reasons: ND	CSII/MDI use: ND CSII f-u: 26 CIPII f-u: 26	To assess the antigenicity of the insulin Hoechst 21PH using CSII and to compare the antigenicity of this insulin when administered IP or SC.	**Anti-insulin antibodies:** (measured by using RIA): Increases (CIPII: M6: 41.8; CSII: M6: 24.9 %, p=0.009) **ELISA:** No change (CIPII: M6: 10.1; CSII: 4.4 %, p=0.07)	STROBE: 16/22 QAT: Strong: Data collection methods, withdrawals and drop-outs Moderate: Selection bias, study design, confounders
	Van Dijk et al. 2016 [93]	N = 113 (CIPII: 39/CSII: 74) Age: 50/48 Diabetes duration: 29/27 Sex: 14/25 / 30/44 HbA1c: 8.3/7.9 C-peptide: ND Reasons: Pmc	CSII/MDI use: 208+ CIPII use: 208+ CSII f-u: 27 CIPII f-u: 27	To compare the effects of CIPII to SC insulin therapy, on the GH-IGF-1 axis in a large prospective, observational matched case-control study in T1DM patients.	**IGF-1:** Increases (CIPII: 123; CSII: 107 µg/L, P=NS) **IGFBP-1:** Decreases (CIPII: 40.2; CSII: 85.4 µg/L, p=0.004) **IGFBP-3:** Increases (CIPII: 3.75; CSII: 3.22 mg/L, p=0.015)	STROBE: 16/22 QAT: Strong: Selection bias, study design, data collection method Moderate: Study design, withdrawals and drop-outs

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; ND, No data available; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay.

Table S2.5. (Continued)

	Source	Participant characteristics (Number, age (mean years), diabetes duration (mean years), sex (Male/Female), HbA1c (%), C-peptide, reasons to participate	Length of: CSII use, CSII follow-up, IPII follow-up (weeks)	Reported study objectives	Outcomes (mean, p-value)	Methodological quality
Other outcomes	**Retrospective crossover studies**					**STROBE and QAT:**
	Jeandidier et al. 1992 (Preliminary results) [86]	N = 8 Age: 33.5 Diabetes duration: 14.5 Sex: ND HbA1c: 6.64 C-peptide: Neg Reasons: ND	CSII use: 1 CIPII use: 12	To assess the potential benefits of CIPII vs SCII.	**Anti-insulin antibodies:** Increases (CIPII: 11.0; CSII: 3.6 %, p<0.05)	STROBE: 12/22 QAT: Weak: Study design Unclear: Selection bias, confounders, data collection methods
	Case-control studies					**STROBE and QAT:**
	Hedman et al. 2009 (c.a.) [111] Arnqvist et al. 2010 (c.a.) [110] Hedman et al. 2014 [112]	N = 30 (CIPII: 10 /CSII: 20) Age: 53.1/52.8 Diabetes duration: 124.2/30.8 Sex: 5/5 / 10/10 HbA1c: 8.6/7.9 C-peptide: ND Reasons: Pmc	CSII use: 26+ CIPII use: 26+	Investigate in cross-sectional study if the different modes of insulin administration, CIPII or CSII were associated with a change in the circulating IGF system.	**Fasting levels of bioactive IGF-I:** Increases (CIPII: 1.83; CSII: 1.16 µg/L, p=0.024). **Total IGF-I:** Increases (CIPII: 120; CSII: 81 µg/L, p=0.007) **IGF-II:** Increases (CIPII: 1050; CSII: 879 µg/L, p=0.015) **IGFBP-1**: Decreases (p=0.013) **IGFBP-2:** No change (p=NS)	STROBE: 21/22 QAT: Strong: Selection bias, confounders, data collection method, withdrawals and drop-outs Moderate: Study design

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; Pmc, Poor metabolic control; ND, No data available; NS, Not significant; NP, Not possible to evaluate; IGF 1, Insulin-like growth factor – 1; BP, Binding proteins.

Table S2.6. Technical and physiological complications with intraperitoneal insulin pump and its attached system

Study ID	Study design	Nr. of partici-pants	Min. CIPII-period (months)	Min. CIPII-period (patient - years)™	Complications (events/study) during CIPII-period										Removal of implanted system because of compli-cations	Insulin pumps technical problems
					Local inf./ inflam-mation	Severe abdo-minal pain	Severe insulin under-delivery (catheter obstruction/ encapsulation)	Eryt-hema	Pump change/ reimplan-tation	Catheter change	Necrosis in abdominal skin «pocket"	Exhaustion of batteries of pump	Peritoneal abscess	Loss of catheter		
Liebl et al. 2009 [5]	RFUs[#]	CIPII: 30	12	30	20	9	6	-	-	-	-	-	-	-	8	-
Wredling, Adamson et al. 1991 [91]	NRCs	6	15	9.4[a]	1	3	4	6	-	-	-	-	-	-	5	-
Pitt et al. 1992 [6]	NRCs	10	34	28.3	-	-	6	?	12	1	-	-	-	-	1	2
Renard et al. 1993 [81]	NRCs	8	12[⊕]	-EP: 12[a] -CSII: 9[a]	-	-	-EP: 13 -CSII: 0	-	0	-	-	-	-	-	0	26
Schnell et al. 1994 [105]	NRCs	5	12	5	-	-	1	-	1	-	-	-	1	1	-	-
Hanaire-Broutin et al. 1996 [101]	NRCs	18	12	18	-	-	-	-	-	-	-	-	-	-	-	0
Pacifico et al. 1997 [98]	NRCs	8	12	8	-	-	6	-	-	-	1	2	-	-	9	1
Liebl et al. 2013/2014 [94-97]	NRCs	12	24	24	5	-	-	-	1	8	-	-	-	-	-	-
Dassau et al. 2017 [78]	NRCs	10	1	0.8	-	-	0	-	-	-	-	-	-	-	-	0
Jeandidier et al. 1992 [86]	Retro. Cs	8	10	6.7	-	-	8	-	-	-	-	-	-	-	8	-
TOTAL		**115**	**144**	**130.2[+]**	**26**	**12**	**44**	**6**	**14**	**9**	**1**	**2**	**1**	**1**	**31**	**29**

Legends: CIPII, Continuous intraperitoneal insulin infusion; RCs, Randomised crossover study; RFUs, Randomised follow-up study; NRCs, Non-randomised crossover study; Retro.Cs, Retrospective crossover study; C-Cs, Case-control study; NRFUs, Non-randomised follow-up study; (–), no data available; [a], authors provided data; [#], dropouts in this study (at the end of the periods N = 36 (CIPII: 15 /CSII: 21); [⊕], included patients with previous use of external CIPII (-EP) and with previous CSII (-CSII); [+], Renard et al. study is not included; ™, multiplication of the number of patients and min. CIPII-period

Table S2.7. Methodological aspects of the included studies.

Study ID	Study design	Min. CSII period (month)	Min. CIPII period (month)	CSII-period insulin	CIPII-period insulin	CIPII implantation system	Insulin pump (CSII/CIPII)	CIPII catheter position (quadrant)	SMBG tests (times/day)	SMBG parameter	Nr. of laboratory visits during the study (CSII/CIPII)
Micossi et al. 1986 [84]	NRCs	12	1 ½	-	-	Siemens	Microjet syringe/Promedos E1[E]	4 cm below umbilicus	6: Fasting, before and 2-h after lunch and dinner, at bedtime	-	1/1
Beylot et al. 1987 [103]	NRCs	2	2	Porcine	-	Siemens AG	Betatron IICPJ 9200/Promedos	Umbilical area	3-6	Mean of all BG data from second months of treatment	1/1
Colette et al. 1989 [114]	C-Cs	7	10	Actrapid (regular) or CS21 Hoechst U40	CS21 Hoechst U40 (regular)	-	Microjet Infuser or Promedos/ Promedos[P]	Through umbilicus	-	-	1/1
Selam et al. 1989 [82]	C-Cs	12	6	-	Hoechst U400 (surfactant stabilized)	PIMS (telemetry using a battery-operated programmer)	ND/MiniMed[I]	Lower portion of the IP cavity	-	-	1/1
Walter et al. 1989 [99]	C-Cs	6	3	Semisynthetic human insulin U100	Semisynthetic human insulin U40	-	Betatron II; AS8MP/Promedos E1	-	-	-	1/1
Wredling, Adamson et al. 1991 [91]	NRCs	12	15	-	Velosulin Human (2 mo, n=2), afterwards H-Tronin	Percuseal	-/-[E]	Upper right (n=1), upper left (n=2), lower left (n=3)	-	-	1/ every 4 weeks
Wredling, Liu et al. 1991 [92]	NRCs	24	6.9	Velosulin Human U100	H-Tronin U100	Percuseal	MiniMed 504-S /MiniMed 504-S[E]	-	4: before each meal + before evening snack	-	2/2
Georgopoulos et al. 1992 [83]	NRCs	ND	12	-	-	PIMS	-/-	-	4-6	Mean blood glucose over 4 weeks before end of the period	1/1
Jeandidier et al. 1992 [86]	Retro. Cs	ND	10	-	Hoechst 21 PH U100	Telemetry using a battery-operated programmer.	-/Infusaid 1000[I]	-	-	-	1/1

Pitt et al. 1992 [6]	NRCs	3	34	-	Hoechst U400	PIMS	-[E]/-[I]	Left from umbilicus above or below the waistline	2-4	Mean of all BG values for the 2 mo before and each 2 mo after implantation	2/9
Giacca et al. 1993 [100]	RCs	96 hours	3	HOE21gh U100 (human)	HOE21gh U100 (human)	-	Microjet MC-20/Promedos ID 1[I]	-	-	-	1/1
Renard et al. 1993 [81]	NRCs	2.4	12	Porcine (Velosulin) U100	Hoechst 21 PH U400 (for MiniMed pump) U100 (for Insufaid pump)	-	Portable pump/ MiniMed 2001[I] (n=6) or Insufaid 1000[I] (n=2)	-	-	-	1/4 (3,6,9,12 mo)
Georgopoulos et al. 1994 [102]	NRCs	ND	6	-	-	-	-/-	-	4-6	Mean blood glucose over 4 weeks before end of the period	1/1
Lassmann-Vague et al. 1994 [104]	NRCs	6	3	-	Hoechst 21 PH U100 (for Infusaid) or U400 (for MIP)	-	ND/Infusaid 1000[I] or MiniMed MIP 2001[I]	-	-	Mean of monthly blood glucose	2/2 (-1,0/1,3 mo)
Raccah et al. 1994 [109]	NRCs	3	10	-	-	-	ND/Infusaid 1000[I] (n=6) or MIP 2001[I] (MiniMed) (n=5)	-	4-5	Mean of monthly blood glucose	1/3 (1,3,10 mo)
Schnell et al. 1994 [105]	NRCs	36	12	-	-	Percuseal	-	Left of right above navel	-	-	1/2 (3,12 mo)
Lassmann-Vague et al.1995/1998 [79, 80]	NRCs	1	24	Actrapid U100 (n=3), Velosulin U100 (n=10), Ultratardu m U40 (n=2)	Hoechst 21 PH U100 (for Infusaid) or U400 (for MIP)	-	ND/ Infusaid 1000[I] (n=4) or MIP 2001[I] (n=11)	-	4	-	1/3 (3,12,24 mo)
Guerci et al. 1996 [108]	NRCs	14.2	4	-	Hoechst 21 PH U400	Battery-operated telemetry systems	ND[E]/MiniMed 2001[I]	Lower left	-	Mean of monthly blood glucose	1/2 (2,4 mo)
Hanaire-Broutin et al. 1996 [101]	NRCs	3	12	-	-	-	ND[E]/MIP 2001 (MiniMed)[I]	-	>4	-	1/2 (3,12 mo)
Lassmann-Vague et al. 1996 [87]	NRCs	ND	2	Actrapid Novo (n=6) or Velosulin	Hoechst 21 PH U100 (n=4) U400 (n=7)	-	ND/ND[I]	-	-	-	1/1

				Nordisk (n=5)							
Pacifico et al. 1997 [98]	NRCs	3	12	-	Hoechst 21 PH U400	-	ND/MIP 2001[I] (MiniMed)	Lower left	-	-	1/2 (6,12 mo)
Oskarsson et al. 1999 [90]	NRCs	6	11	-	-	-	MiniMed 506/ MiniMed 2001[I]	-	-	-	1/1
Oskarsson et al. 2000 [89]	NRCs	12	11	-	-	-	MiniMed 506/ MiniMed 2001[I]	-	5: morning, before lunch and dinner, 2 h after dinner, before bed	Mean of monthly blood glucose	1/1
Catargi et al. 2002 [88]	Retro. Cs	1.5	3*	Lispro U100	Hoechst 21 PH U400	Telemetry using a battery-operated programmer.	MiniMed 506 or 507/MIP 2001[I] or 2007[I] (MiniMed)	Lower left	>4	Mean of all BG values for the periods (45 days/ last 45 days)	1/1
Jeandidier et al. 2002 [115]	NRFUs	6	6	Regular or Lente or Humalog	Insuman Infusat U100	-	H-Tron/ MIP 2001[I] (MiniMed)	-	-	-	3/3 (0,3,6 mo)
Duvilard et al. 2005/2007 [106, 107]	NRCs	ND	3	-	-	-	MiniMed 506 or 507/Minimed 2007C[I] or 2007A[I]	-	-	-	1/1
Liebl et al. 2009 [5]	RFUs	6	12	Lispro U100	Insuman Infusat U100 or H-Tronin U100	Diaport	H-TRONplus/ H-TRONplus	Lower left or right	4: prior each meal+ before bedtime	-	1/1
Hedman et al. 2009/2014 [111, 112] Arnqvist et al. 2010 [110]	C-Cs	6	6	Aspart U100(Novo rapid) or lispro U100 (Humalog)	Semisynthetic human insulin of porcine origin (Sanofi) U400	-	ND/MIP 2007C[I] (Medtronic/Mini med)	-	-	-	1/1
Liebl et al. 2013/2014 [94-97]	NRCs	-	24	-	-	DiaPort	ND/Accu-Chek[E]	-	-	-	1/4 (3,6,12,24 mo)
van Dijk et al. 2016 [93] van Dijk et al 2020 [117]	NRFUs	48	48	Fast acting	Human U400 (of E. coli origin)	-	ND/MIP 2007D[I]	-	-	-	2/2 (0,6 mo)
Dassau et al. 2017 [78]	NRCs	102	1	Fast acting	Insuman Infusat U100 (regular)	DiaPort	Accu-Check Spirit Combo[Φ,E]/ Accu-Check Spirit Combo[Φ,E]	-	CGM (every 5 min)	-	1/1

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; RCs, Randomised crossover study; RFUs, Randomised follow-up study; NRCs, Non-randomised crossover study; Retro.Cs, Retrospective crossover study; C-Cs: Case-control study; NRFUs, Non-randomised follow-up study; ND, No data available; Asterix (*), three patients first were treated with CIPII, and then with CSII; ❖, pump provided only for 24-hour glucose profile; PIMS, The programmable implantable medication system; MIP, MiniMed Implantable Pump; [E], external insulin pump; [I], implantable insulin pump; [P], peristaltic pump; (–), no data available; mo: months. Note: Studies are sorted by year of publication.

Table S2.8. Glycaemic control during the CIPII-period: Hypoglycaemia, normoglycaemia and hyperglycaemia events and/or time spent in

Study ID	Study design	Nr. of partici-pants	Minimal CIPII period (month)	Hypo-glycaemic coma	Severe hypo-glycaemic events/ patient-year (requiring assistance)	Hypo-glycaemic events/ patient year (BG < 3.0 mmol/L)	Time spent in hypo-glycaemia (BG < 2.8 mmol/L), % ± SD	Time spent in hypo-glycaemia (BG < 3.9 mmol/L), % ± SD	Time spent in normo-glycaemia (3.9 – 10.0 mmol/l)[AP], %	Time spent in normo-glycaemia (4.4 – 7.8 mmol/L), %	Time spent in hyper-glycaemia (BG > 10 mmol/L), % ± SD	Time spent in hyper-glycaemia (BG > 14 mmol/L), % ± SD
Micossi et al. 1986 [84]	NRCs	6	1 ½	-	-	-	1.65±0.51	4.51±2.42	-	-	31.84±19.66	8.9±8.69
Pitt et al. 1992 [6]	NRCs	10	84	0	0.43	>1 /patient	-	8.8-6.0 (MIP)	-	-	M2-16:15±5 M18:20±5 (MIP)	-
Renard et al. 1993 [81]	NRCs	8	12	0	0	-	M3: 10.0±7.2 M6: 7.6±7.7 M9: 6.1±5.5 M12: 6.1±6.1	-	-	-	M3: 11.9±6.8 M6: 14.3±8.5 M9: 13.6±6.4 M12: 13.1±4.5	-
Pacifico et al. 1997 [98]	NRCs	8	12									
Oskarsson et al. 1999 [90]	NRCs	7	11	-	-	8.4	-	-	-	-	-	-
Oskarsson et al. 2000 [89]	NRCs	7	11	-	-	8.4	-	-	-	-	-	-
Liebl et al. 2009 [5]	RFUs	(CIPII: 30 /CSII: 30)	12	-	Total: 0.35: M1-3: 0.72; M4-12: 0.15	Total:118.2: M1-3: 138.1; M4-12: 108.9	-	-	-	-	-	-
Liebl et al. 2013/2014 [94-97]	NRCs	12 (n=10)*	24	-	1.5	-	-	-	M6: 58	-	M6: 38	-
Dassau et al. 2017 [78]	NRCs	10	1	-	-	-	-	2.5±2.9	65.7±9.2	39.8±7.6	32.4±8.9	5.9±5.6

Legends: RCs, Randomised crossover study; RFUs, Randomised follow-up study; NRCs, Non-randomised crossover study; Retro.Cs, Retrospective crossover study; C-Cs, Case-control study; NRFUs, Non-randomised follow-up study; ND, No data available; [AP], suggested BG range for artificial pancreas systems; (–), no data available; Asterix (*), dropouts in the study; M, month.

Table S2.9. Data modification for STATA: HbA1c.

Study ID	Data in forest plot, HbA1c (%)						Original data								
	CIPII			CSII			CIPII				CSII				Unit
	Mean	SD	Total	Mean	SD	Total	Mean	SD	SEM	Total	Mean	SD	SEM	Total	
Georgopoulos et al. 1992 [83]	7.7	1.2	7	9.8	1.4	7	7.7	1.2	-	7	9.8	1.4	-	7	%, SD
Liebl et al. 2013/2014 [94-97]	7.2	0.5	10	8.8	1.2	10	7.2	0.54	-	10	8.8	1.15	-	10	%, SD
Oskarsson et al. 1999 [90]	7.1	0.5	7	8.5	0.8	7	7.1	-	0.2	7	8.5	-	0.3	7	%, SEM
Oskarsson et al. 2000 [89]	7.2	0.5	7	8.6	1.1	7	7.2	-	0.2	7	8.6	-	0.4	7	%, SEM
Schnell et al. 1994 [105]	8.5	0.5	5	9.8	0.7	5	8.5	0.5[x]	-	5	9.8	0.7[x]	-	5	%, SD
Wredling, Adamson et al. 1991 [91]	7.6	0.4	6	8.7	0.6	6	7.6*	-	-	6	8.7*	-	-	6	%, (min-max)
Pitt et al. 1992 (data extracted from figure by IDF) [6]	8	1.8	10	9.1	2.2	10	-	-	-	10	-	-	-	10	%, SEM
Colette et al. 1989 [114]	8	1.4	13	8.9	2	11	8	-	0.4	13	8.9	-	0.6	11	%, SEM
Georgopoulos et al. 1994 [102]	8.7	1.2	8	9.4	1.5	8	8.7	1.2	-	8	9.4	1.5	-	8	%, SD
Raccah et al. 1994 [109]	6.3	1	11	6.9	1	11	6.3	-	0.3	11	6.9	-	0.3	11	%, SEM
Catargi et al. 2002 [88]	7.3	0.8	14	7.8	0.9	14	7.3	0.8	-	14	7.8	0.9	-	14	%, SD
Selam et al. 1989 (SD calculated in SPSS by IDF) [82]	8.2	1.4	6	8.6	1.3	8	-	-	-	6	-	-	-	8	%
Lassmann-Vague et al. 1994 [104]	6.8	0.7	11	6.9	1	11	6.8	-	0.2	11	6.9	-	0.3	11	%, SEM
Guerci et al. 1996 [108]	5.9	0.6	14	6	0.6	14	5.9	0.63	-	14	6	0.6	-	14	%, SD
Hanaire-Boutin et al. 1996 [101]	7.5	0.8	18	7.6	0.8	18	7.5	-	0.2	18	7.6	-	0.2	18	%, SEM
Duvillard et al. 2005/2007 [106, 107]	7.2	1	7	7.3	0.9	7	7.24	1	-	7	7.34	0.94	-	7	%, SD
Pacifico et al. 1997 [98]	6.6	1.4	8	6.5	1.1	8	6.6	1.4	-	8	6.5	1.1	-	8	%, SD
Walter et al. 1989 [99]	8	0.5	6	7.9	0.5	6	8	0.5	-	6	7.9	0.5	-	6	%, SD
Hedman et al. 2009/2014, Arnqvist et al. 2010 [110-112]	8.6	1.4	10	7.9	0.8	20	8.6	1.4	-	10	7.9	0.8	-	20	%, SD

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; (–), no data; SD, standard deviation; SEM, standard error of means; SPSS, statistical software program; IDF, Ilze Dirnena-Fusini; *, data given as mean (min-max) (CIPII 7.6 (7.0 – 8.6); CSII 8.7 (7.0 – 9.5)); [x], Authors of the study did not provide statistical term for difference (SD or SEM), decision to use SD or SEM was made by reproducing statistical test by using raw data from article.

Table S2.10. Data modification for STATA: SMBG.

Study ID	Data in forest plot, SMBG (mmol/L)						Original data								
	CIPII			CSII			CIPII				CSII				Unit
	Mean	SD	Total	Mean	SD	Total	Mean	SD	SEM	Total	Mean	SD	SEM	Total	
Pitt et al. 1992 (data extracted from figure) [6]	7.8	0.4	10	8.9	0.6	10	-	-	-	10	-	-	-	10	mg/dL, SEM
Georgopoulos et al. 1992 [83]	7.7	1.2	7	10.5	2	7	7.7	1.2	-	7	10.5	2	-	7	mM, SD
Micossi et al. 1986 [84]	8.8	1.3	6	9.7	1.4	6	8.8	-	0.55	6	9.68	-	0.58	6	mmol/L, SEM
Beylot et al. 1987 (SD calculated in SPSS by IDF) [103]	8.2	0.9	4	8.8	1.3	4	-	-	-	4	-	-	-	4	mmol/L
Catargi et al. 2002 [88]	8.1	1	14	8.5	0.9	14	145.4	18.3	-	14	153.3	17.3	-	14	mg/dL, SD
Georgopoulos et al. 1994 [102]	7.4	1.1	8	7.8	1.1	8	7.4	1.1	-	8	7.8	1.1	-	8	mmol/L, SD
Guerci et al. 1996 [108]	7.6	0.5	14	7.8	0.7	14	7.55	0.47	-	14	7.78	0.7	-	14	mmol/L, SD
Raccah et al. 1994 [109]	8	1.8	11	8.3	0.8	11	151	-	9.3	11	146	-	5.5	11	mg/dL, SEM
Lassmann-Vague et al. 1994 [104]	8.3	1.8	11	8.3	1.2	11	151	-	8	11	151	-	9	11	mg/dL, SEM

Legends: SMBG, self-monitoring of blood glucose; CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; (–), no data; SD, standard deviation; SEM, standard error of means; SPSS, statistical software program; IDF, Ilze Dirnena-Fusini.

Table S2.11. Data modification for STATA: Insulin levels.

Study ID	Data in forest plot, insulin levels (pmol/L)						Original data								
	CIPII			CSII			CIPII				CSII				Unit
	Mean	SD	Total	Mean	SD	Total	Mean	SD	SEM	Total	Mean	SD	SEM	Total	
Oskarsson et al. 1999 [90]	28	5.8	7	48.1	20.9	7	28	-	2.2	7	48.1	-	7.9	7	pmol/L, SEM
Oskarsson et al. 2000 [89]	35.8	7.5	7	53.4	9.9	7	35.8	-	2.9	7	53.4	-	3.8	7	pmol/L, SEM
Giacca et al. 1993 [100]	30.8	13.6	5	45	23.3	5	30.8	-	6.1	5	45	-	10.4	5	pmol/L, SEM
Beylot et al. 1987 [103]	131.9	27.8	4	152.8	27.8	4	19	-	2	4	22	-	2	4	mU/L, SEM
Colette et al. 1989 [114]	115.3	67.6	13	141	103.6	11	16.6	-	2.7	13	20.3	-	4.5	11	µU/mL, SEM
Lassmann-Vague et al. 1996 [87]	60.4	23.1	11	66.7	30	11	8.7	-	1	11	9.6	-	1.3	11	mU/L, SEM
Raccah et al. 1994 [109]	100	71.4	11	118.1	89.9	11	14.4	-	3.1	11	17	-	3.9	11	mU/L, SEM
Lassmann-Vague et al. 1994 [104]	114.6	48.3	11	118.1	89.8	11	16.5	-	2.1	11	17	-	3.9	11	µU/mL, SEM

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; (–), no data; SD, standard deviation; SEM, standard error of means.

Table S2.12. Data modification for STATA: cholesterol levels.

Study ID	Data in forest plot, cholesterol levels (mmol/L)						Original data								
	CIPII			CSII			CIPII				CSII				Unit
	Mean	SD	Total	Mean	SD	Total	Mean	SD	SE	Total	Mean	SD	SE	Total	
Duvillard et al. 2005/2007 [106, 107]	5	0.6	7	5.4	0.7	7	5.04	0.58	-	7	5.36	0.72	-	7	mmol/L, SD
Georgopoulos et al. 1994 [102]	4.6	0.8	8	4.8	0.8	8	4.56	0.83	-	8	4.85	0.8	-	8	mmol/L, SD
Georgopoulos et al. 1992 [83]	4.6	1.1	7	4.9	1.3	7	4.6	1.1	-	7	4.9	1.3	-	7	mM, SD
Raccah et al. 1994 [109]	4.9	2.3	11	5	1.3	11	4.92	-	0.69	11	5.03	-	0.38	11	mM, SEM
Guerci et al. 1996 [108]	5	0.6	14	5	0.6	14	5.01	0.59	-	14	4.97	0.65	-	14	mmol/L, SD
Pacifico et al. 1997 [98]	4.8	0.8	8	4.7	0.8	8	185.8	31	-	8	182.5	33	-	8	mg/dL, SD
Micossi et al. 1986 [84]	5.1	1.2	6	4.4	0.9	6	5.1	-	0.5	6	4.4	-	0.38	6	mmol/L, SEM

Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; (–), no data; SD, standard deviation; SEM, standard error of means.

Table S2.13. Data modification for STATA: triglyceride levels.

Study ID	Data in forest plot, triglyceride levels (mmol/L)						Original data								
	CIPII			CSII			CIPII				CSII				Unit
	Mean	SD	Total	Mean	SD	Total	Mean	SD	SE	Total	Mean	SD	SE	Total	
Georgopoulos et al. 1992 [83]	1.2	0.3	7	1.3	0.4	7	1.23	0.27	-	7	1.35	0.27	-	7	mM, SD
Georgopoulos et al. 1994 [102]	0.9	0.2	8	0.9	0.3	8	0.93	0.2	-	8	0.93	0.3	-	8	mmol/L, SD
Raccah et al. 1994 [109]	0.8	0.3	11	0.8	0.3	11	0.83	-	0.1	11	0.83	-	0.1	11	mM, SEM
Guerci et al. 1996 [108]	1.1	0.6	14	1.1	0.4	14	1.13	0.56	-	14	1.1	0.4	-	14	mmol/L, SD
Pacifico et al. 1997 [98]	0.9	0.3	8	0.8	0.3	8	77.6	25.6	-	8	71.6	27.6	-	8	mg/dL, SD
Duvillard et al. 2005/2007 [106, 107]	1.3	0.3	7	1.1	0.2	7	1.29	0.29	-	7	1.1	0.24	-	7	mmol/L, SD
Micossi et al. 1986 [84]	1.5	0.4	6	0.9	0.3	6	1.5	-	0.17	6	0.9	-	0.12	6	mmol/L, SEM

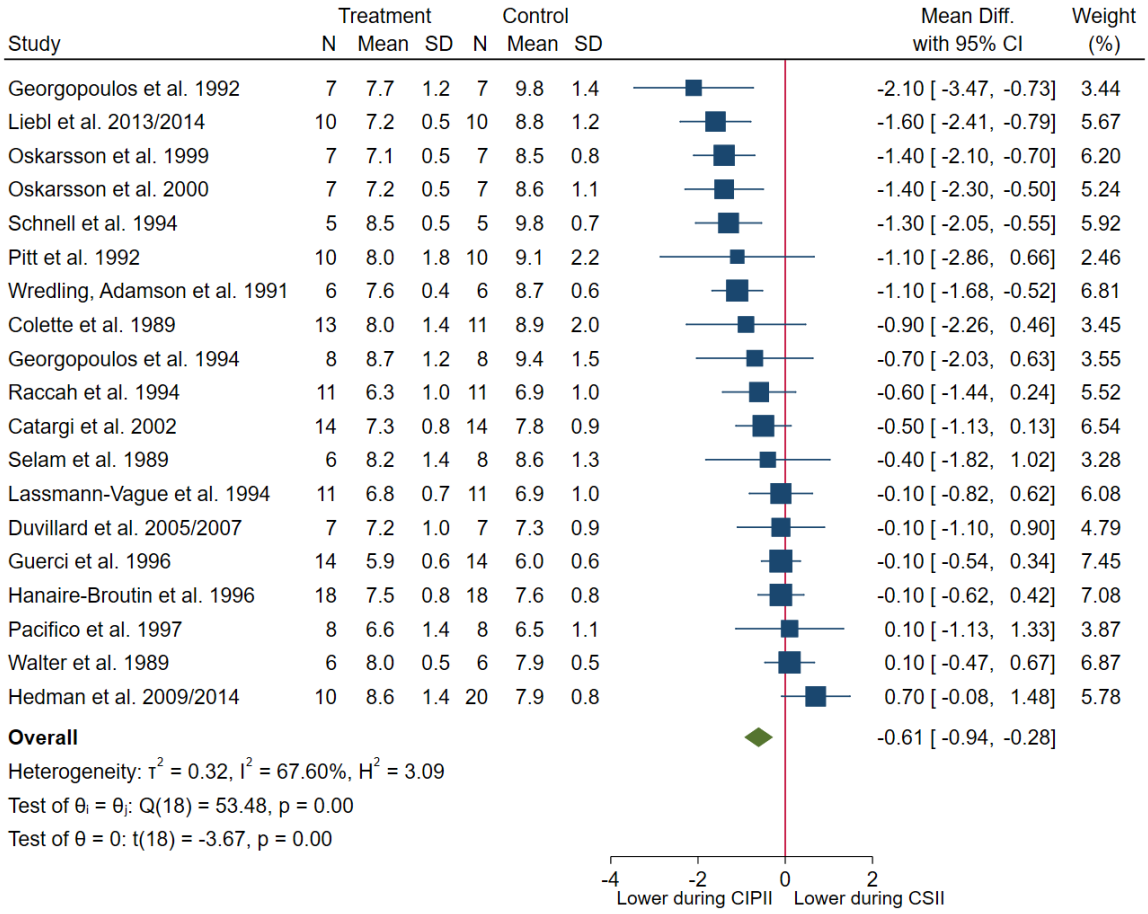
Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; (–), no data; SD, standard deviation; SEM, standard error of means.

Table S2.14. Data modification for STATA: insulin requirement

Study ID	Data in forest plot, insulin requirement (U/24 hours)						Original data								
	CIPII			CSII			CIPII				CSII				Unit
	Mean	SD	Total	Mean	SD	Total	Mean	SD	SE	Total	Mean	SD	SE	Total	
Micossi et al. 1986 [84]	46.0	10.7	6	48.6	10.3	6	46.0	-	4.37	6	48.6	-	4.22	6	SEM, U/24h
Liebl et al. 2009 [5]	44.2	16.6	30	46	23.6	30	44.2	16.6	-	30	46	44.2	-	30	SD, U/24h
Duvillard et al. 2005/2007 [106, 107]	43.6	9.8	7	45	17.8	7	43.6	9.8	-	7	45	17.8	-	7	SD, U/24h
Hanaire-Broutin et al. 1996 [101]	39.1	10.6	18	39.6	8.9	18	39.1	-	2.5	18	39.6	-	2.1	18	SEM, U/24h
Oskarsson et al. 2000 [89]	37.9	7.1	7	38.2	10.3	7	37.9	-	2.7	7	38.2	-	3.9	7	SEM, U/24h
Georgopoulos et al. 1994 [102]	62.4	44.9	8	61.9	45.7	8	62.4	44.9	-	8	61.9	45.7	-	8	SD, U/24h
Lassmann-Vague et al. 1994 [104]	41.6	12.9	11	40	13.3	11	41.6	-	3.9	11	40	-	4	11	SEM, U/24h
Pacifico et al. 1997 [98]	42.8	6.6	8	40.8	8	8	42.8	6.6	-	8	40.8	8		8	SD, U/24h
Oskarsson et al. 1999 [90]	38.4	7.7	7	36.1	7.4	7	38.4	-	2.9	7	36.1	-	2.8	7	SEM, U/24h
Raccah et al. 1994 [109]	43.8	15.9	11	40.5	14.6	11	43.8	-	4.8	11	40.5	-	4.4	11	SEM, U/24h
Jeandidier et al. 1992 [86]	39	11	8	32	13	8	39	11	-	8	32	13	-	8	SD, U/24h
Dassau et al. 2017*	43.7	0.1	10	32.3	0.1	10	43.7	0.08	-	10	32.3	0.05	-	10	SD, U/24h
Hedman et al. 2009/2014, Arnqvist et al. 2010 [110-112]	51.2	31.5	10	39.3	10.5	20	51.2	31.5	-	10	39.3	10.5	-	20	SD, U/24h

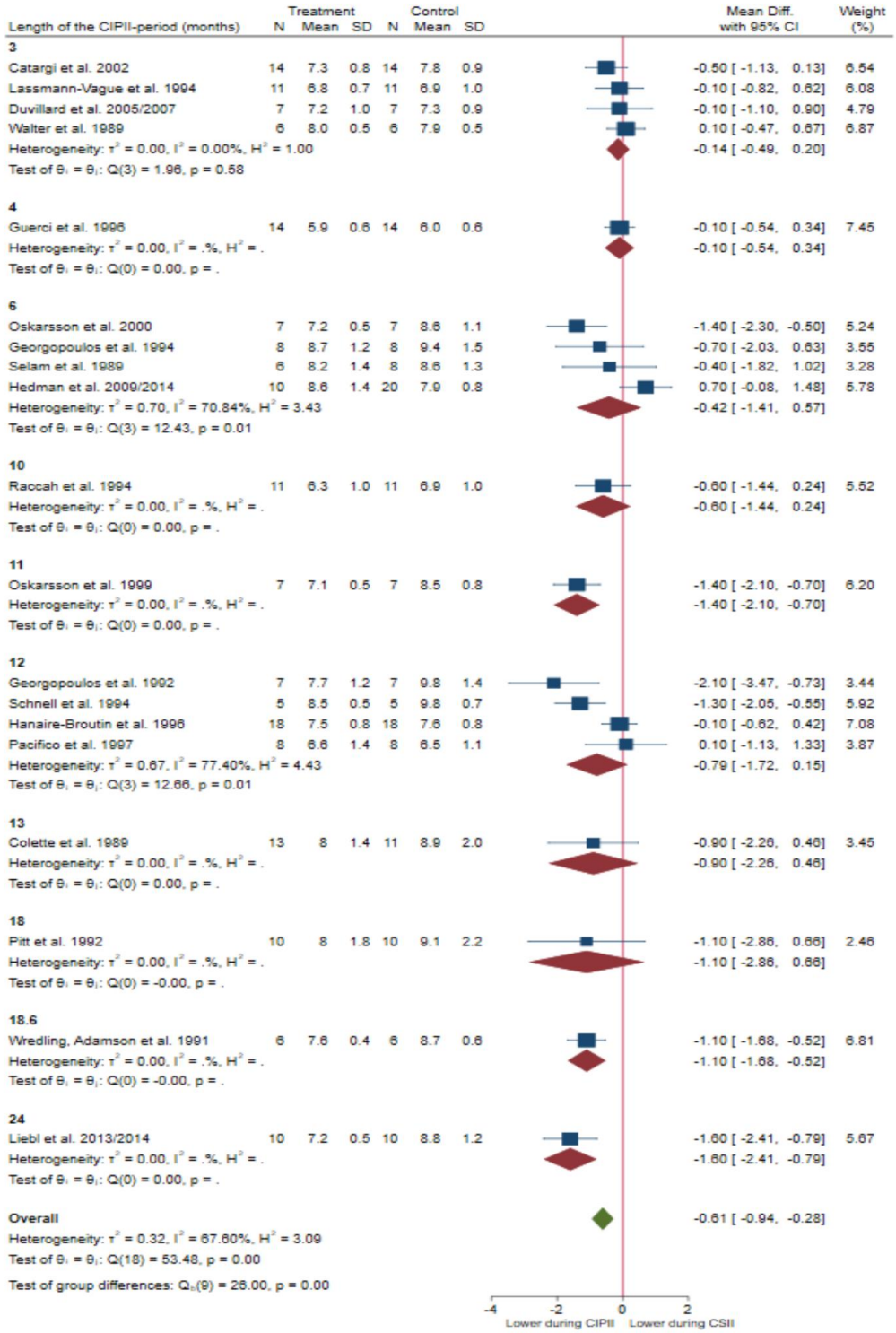
Legends: CSII, Continuous subcutaneous insulin infusion; CIPII, Continuous intraperitoneal insulin infusion; (–), no data; SD, standard deviation; SEM, standard error of means, Asterix (*), 24-hour measurements

Figure S1a. Meta-analysis of HbA1c (%) in patients during CIPII treatment compared to that during control treatment (CSII).



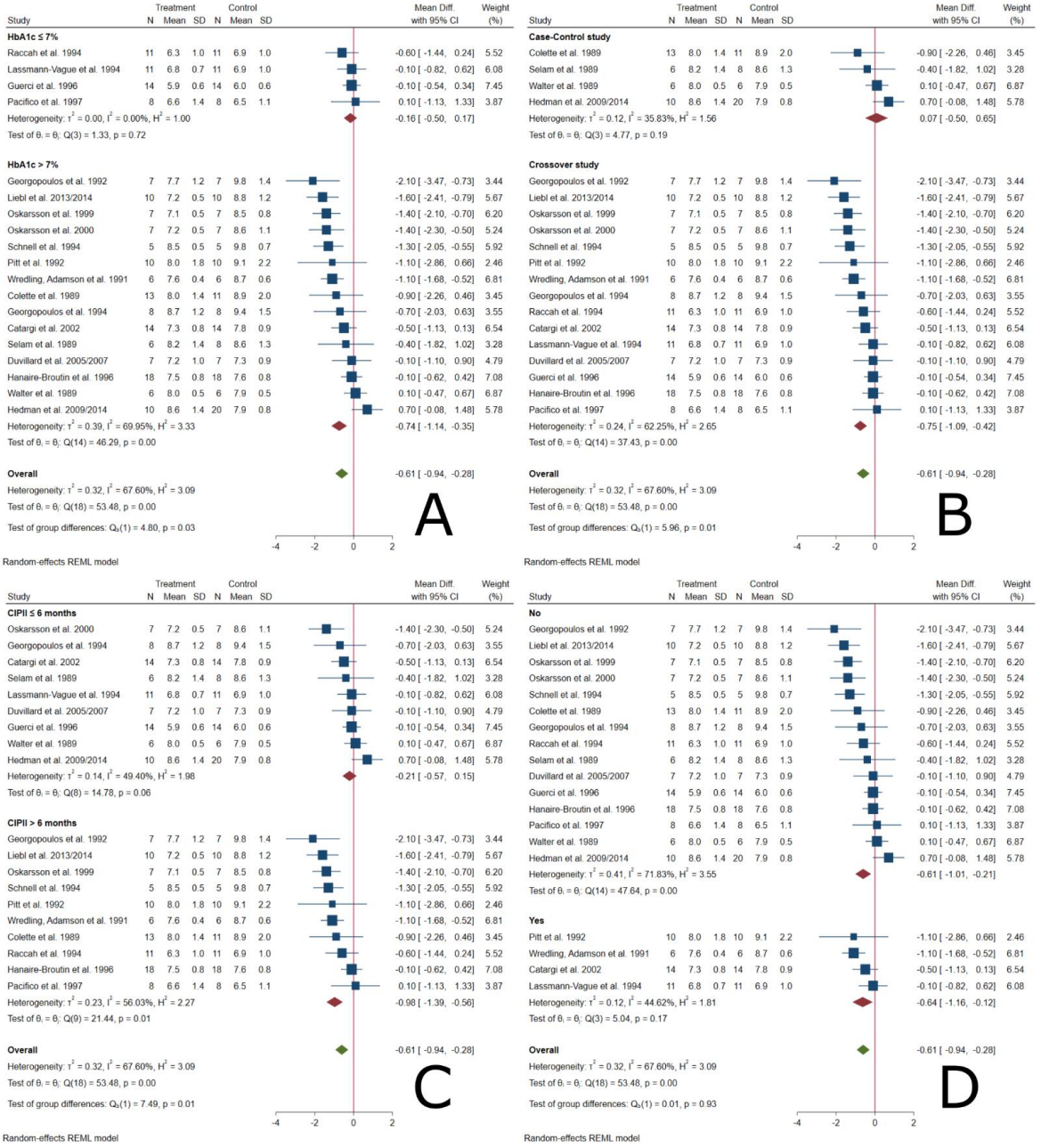
Legends: Treatment, continuous intraperitoneal insulin infusion; Control, continuous subcutaneous insulin infusion.

Figure S1b. Subgroup meta-analysis of HbA1c (%) according to duration in patients during CIPII treatment compared to that during control treatment (CSII).



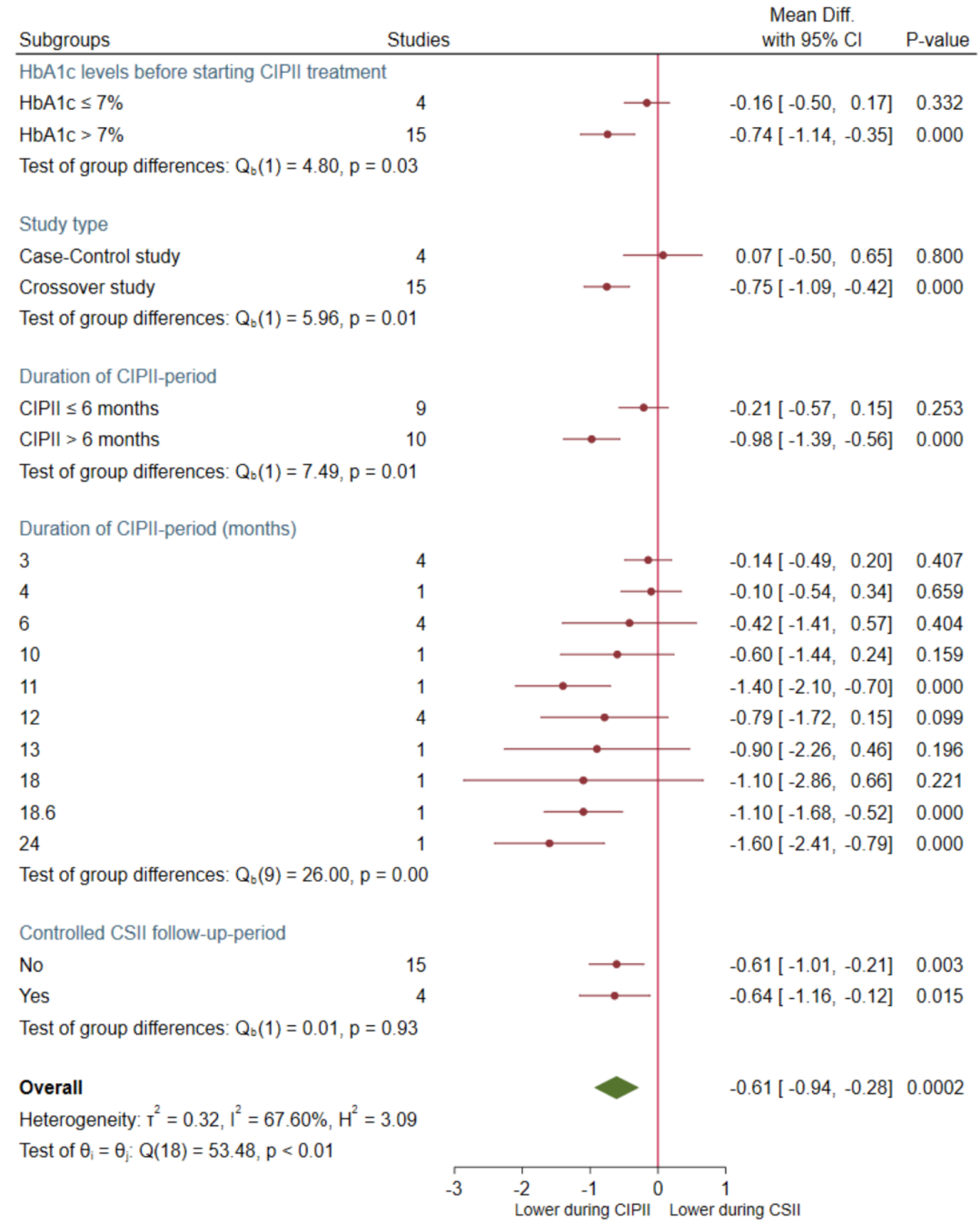
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII).

Figure S1c. Subgroup meta-analysis of HbA1c (%) in patients during CIPII treatment compared to that during control treatment (CSII).



Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S1d. Overall subgroup meta-analysis of HbA1c (%) in patients during CIPII treatment compared to that during control treatment (CSII).



Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

Figure S1e. Meta-regression analysis bubble-plot of HbA1c (%) in patients during CIPII treatment compared to that during control treatment (CSII).

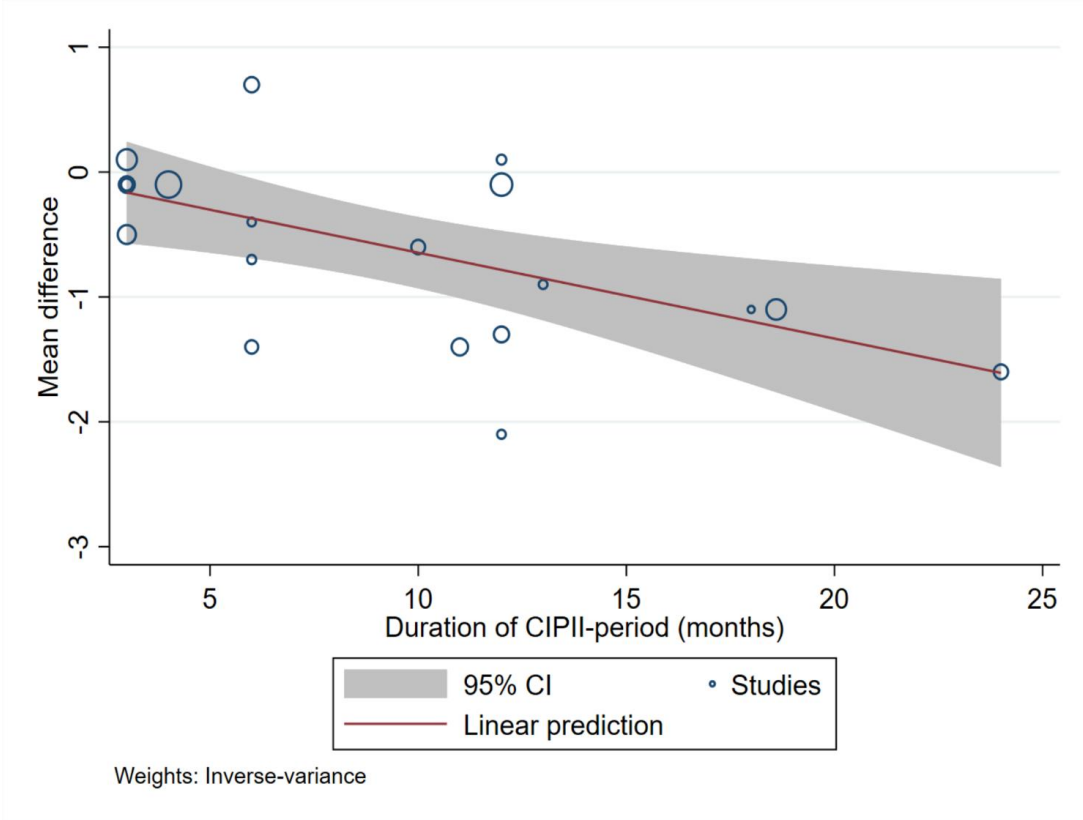
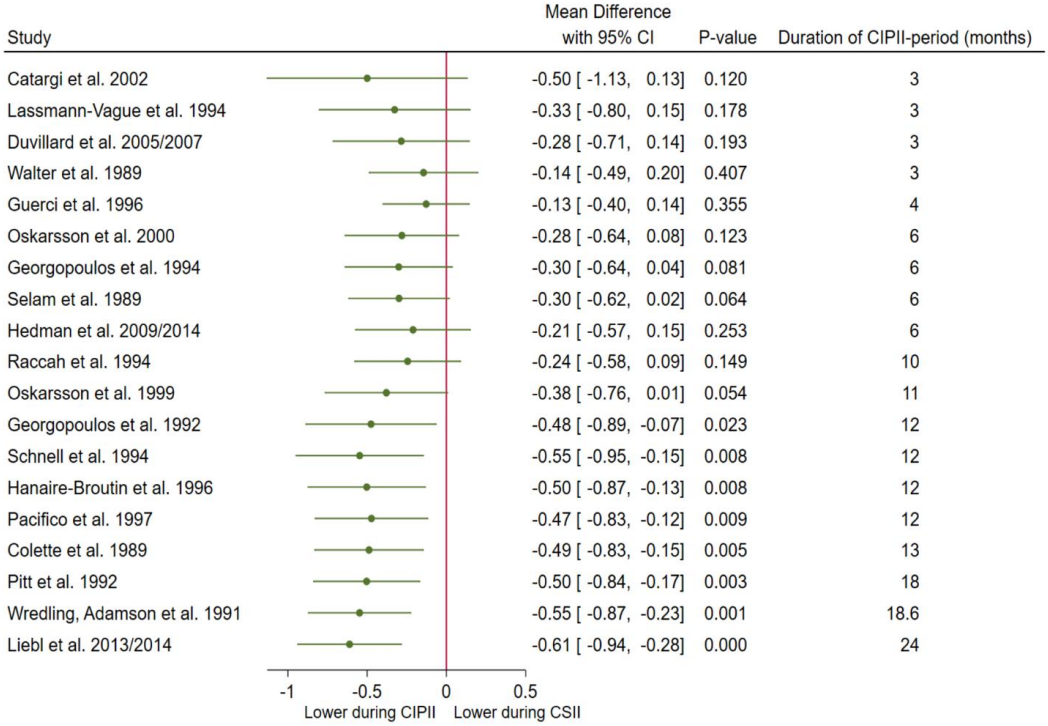
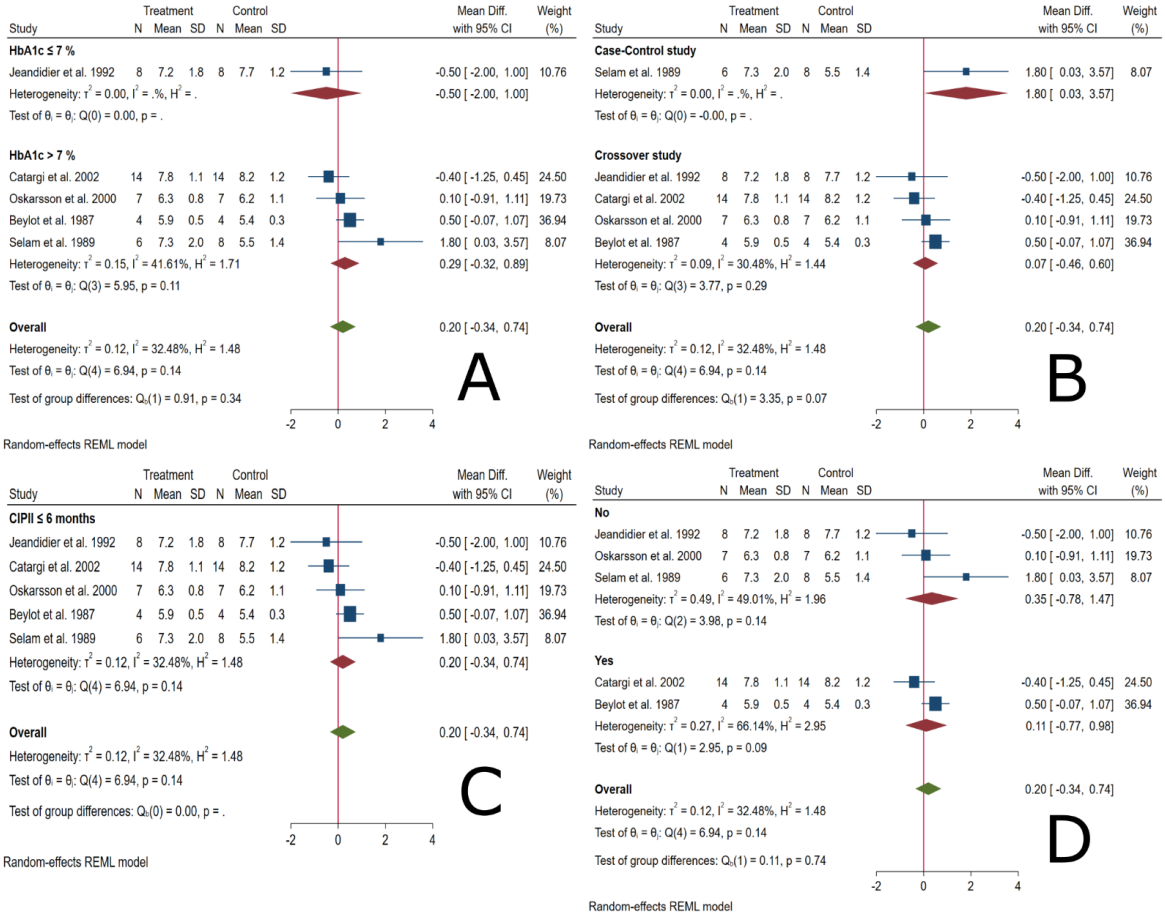


Figure S1f. Cumulative meta-analysis of HbA1c (%) in patients during CIPII treatment compared to that during control treatment (CSII) according to duration of CIPII treatment.



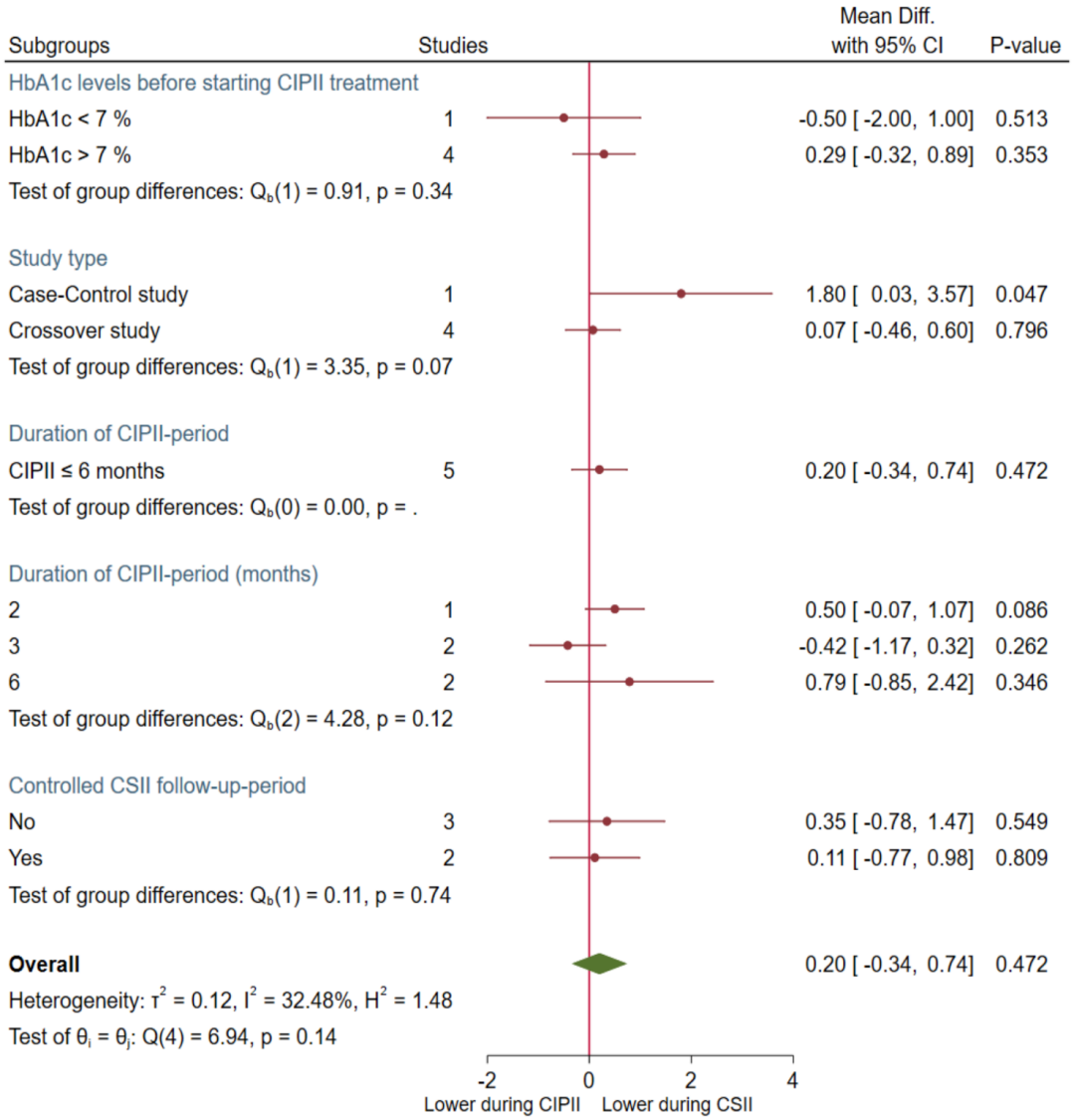
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII).

Figure S2a. Subgroup meta-analysis of fasting blood glucose (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



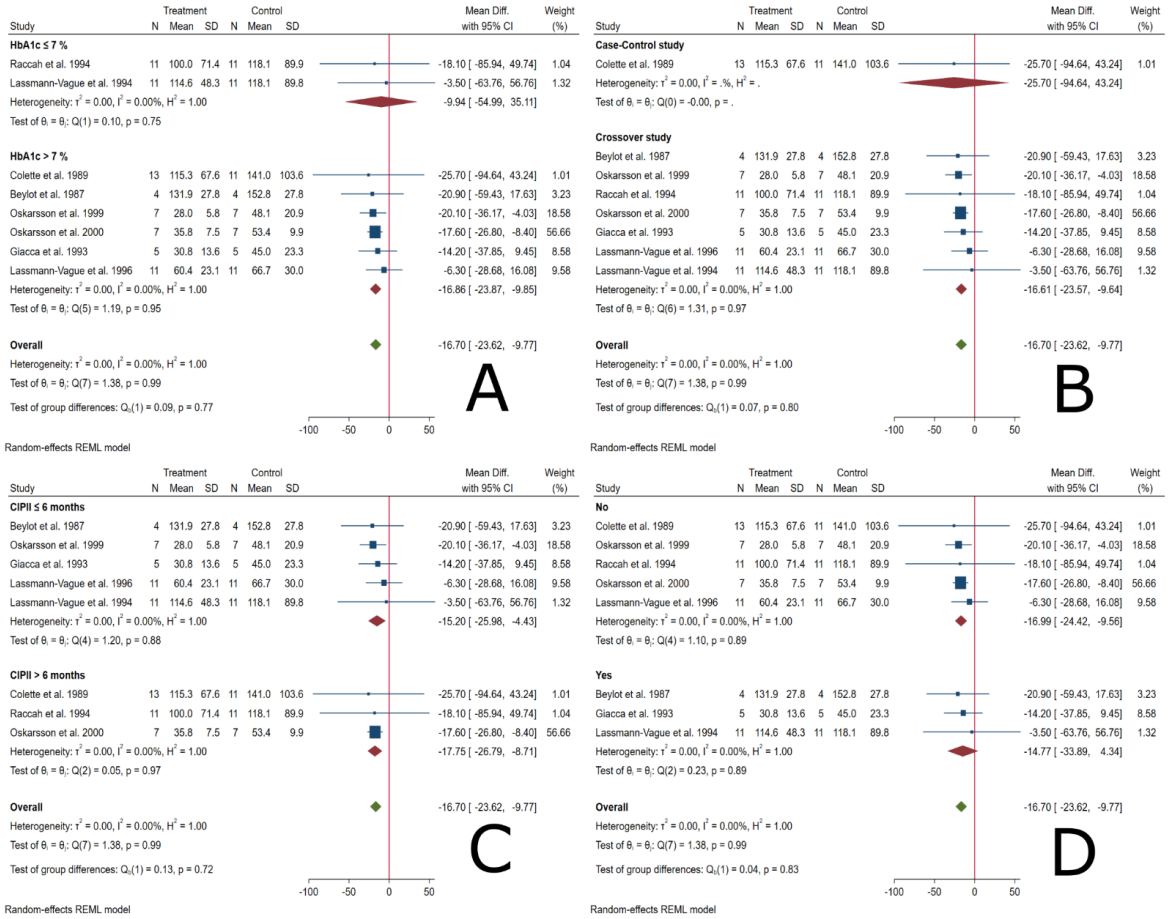
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S2b. Summarised subgroup meta-analysis of fasting blood glucose (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



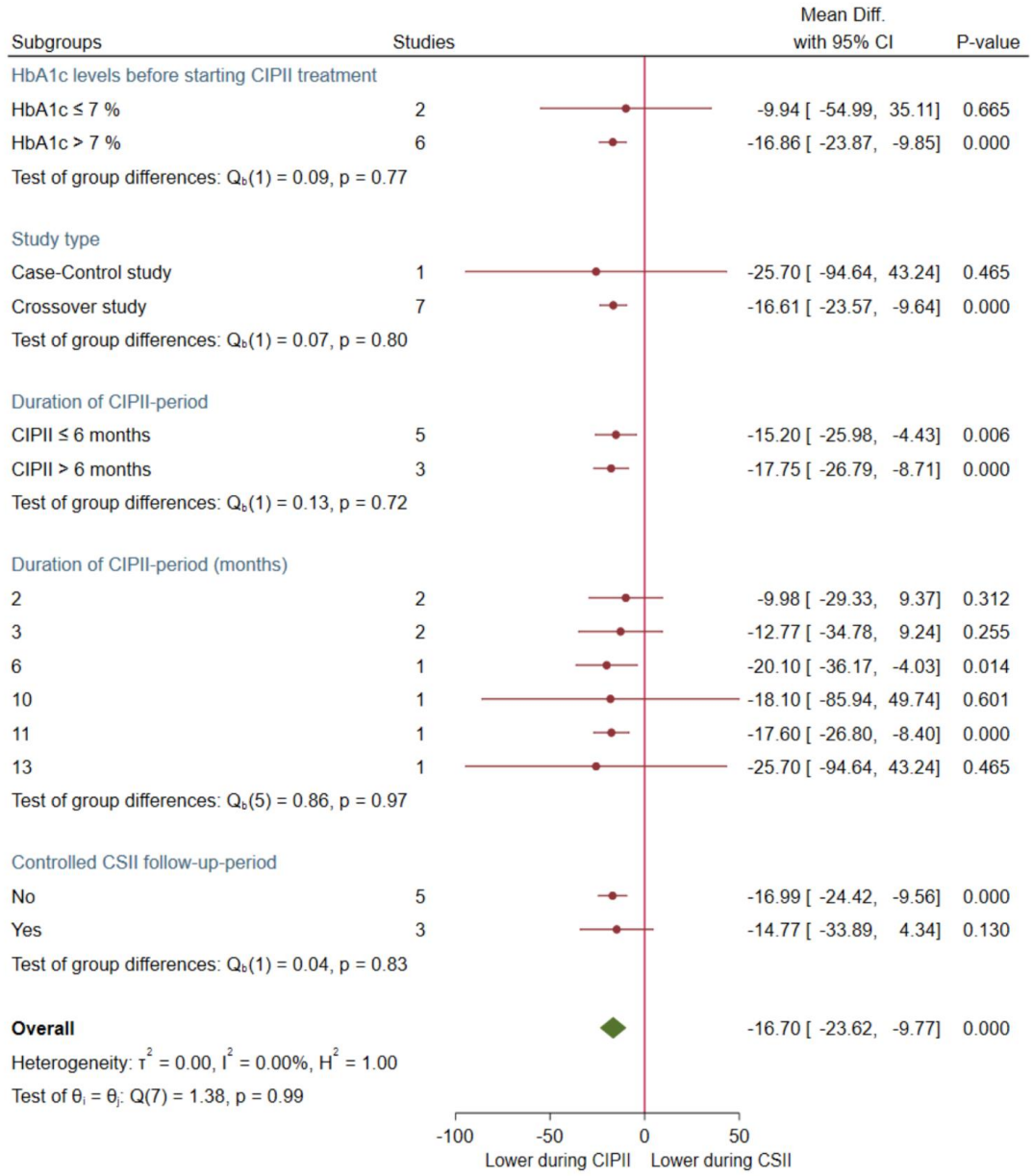
Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

Figure S3a. Subgroup meta-analysis of fasting insulin (pmol/L in patients during CIPII treatment compared to that during control treatment (CSII).



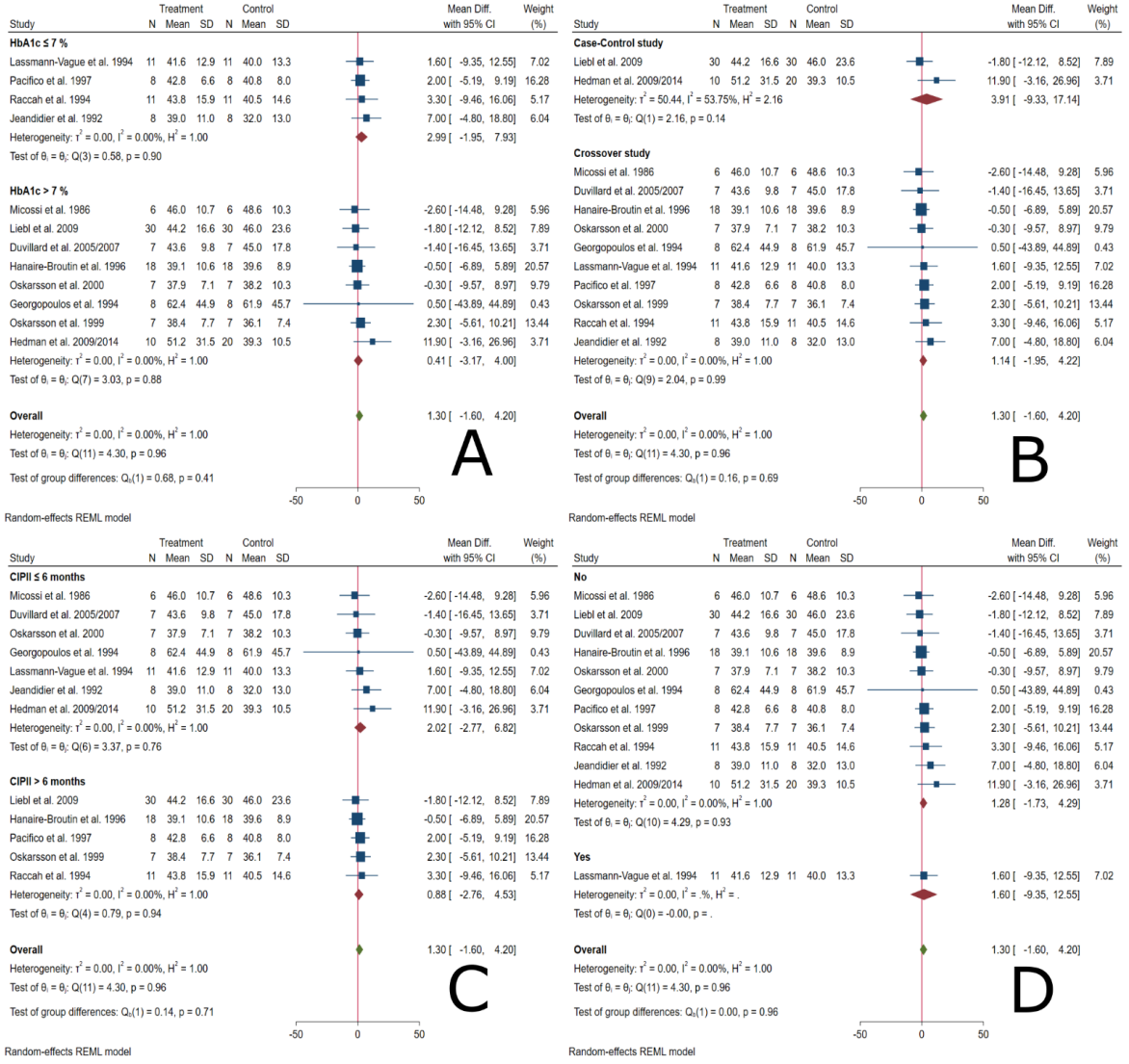
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S3b. Summarised subgroup meta-analysis of fasting insulin (pmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



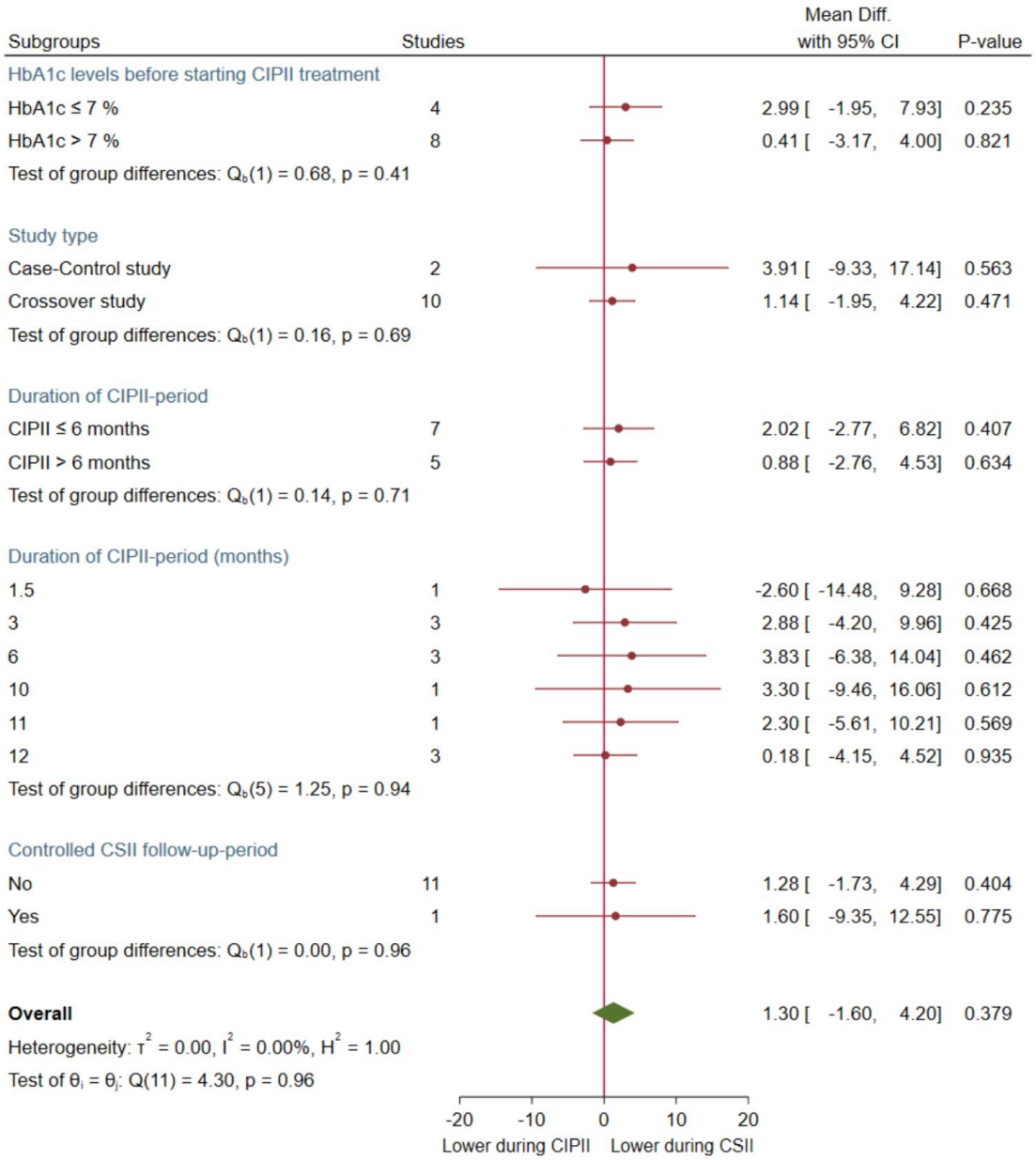
Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

Figure S4a. Subgroup meta-analysis of daily insulin dose (U/24 hours) in patients during CIPII treatment compared to that during control treatment (CSII).



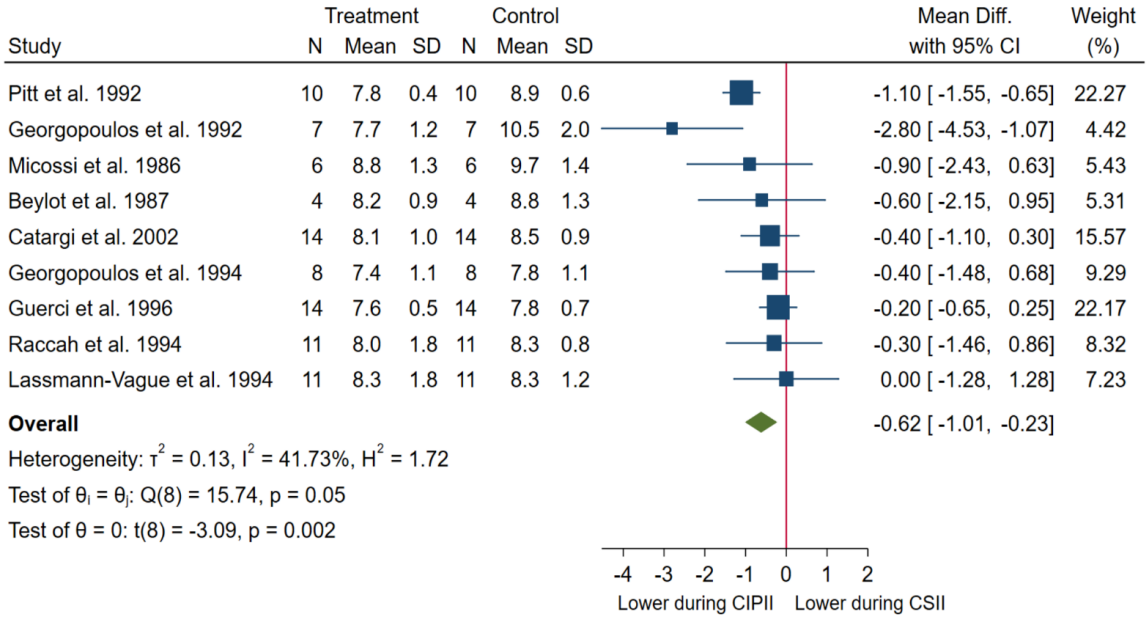
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S4b. Summarised subgroup meta-analysis of daily insulin dose (U/24 hours) in patients during CIPII treatment compared to that during control treatment (CSII).



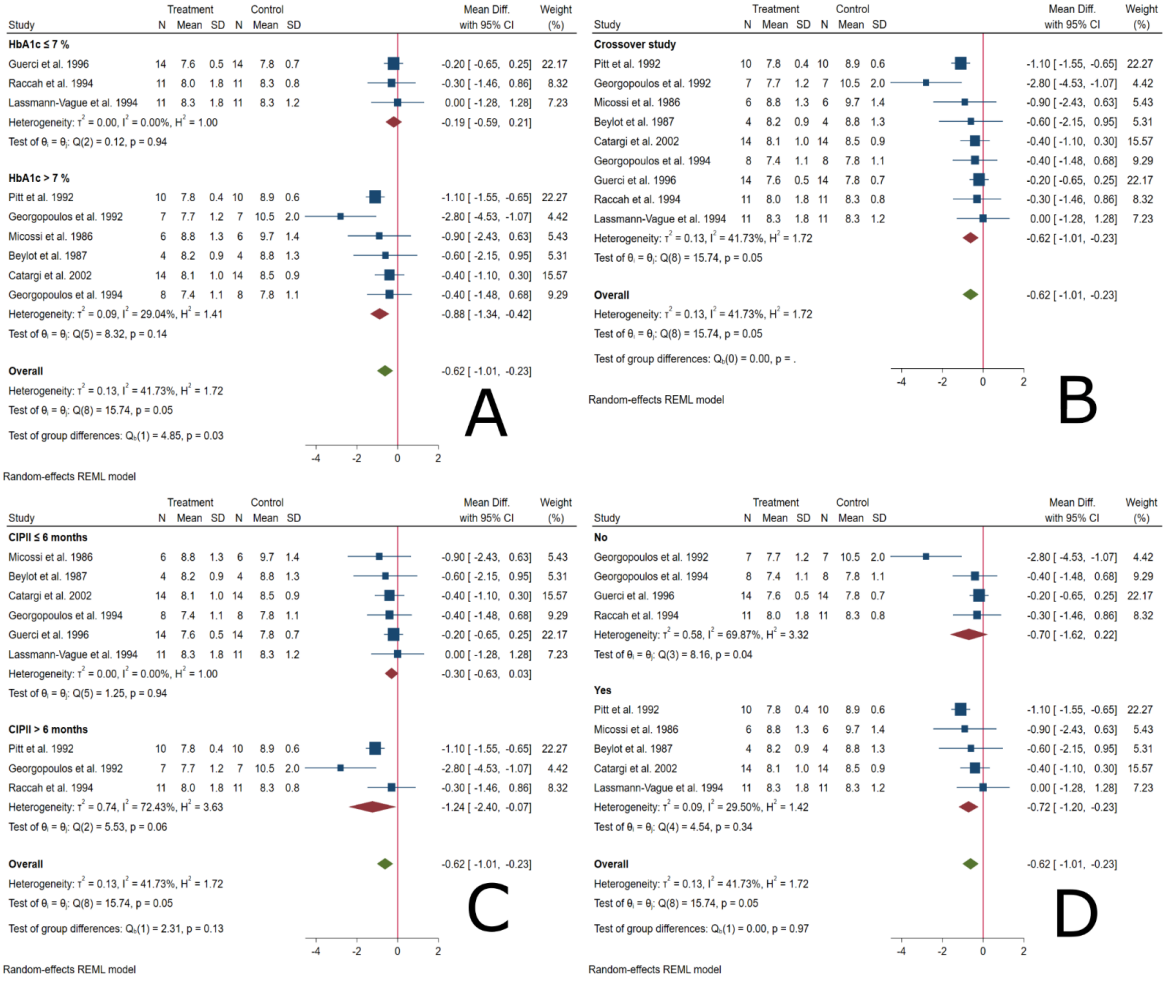
Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

Figure S5a. Meta-analysis of SMBG (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



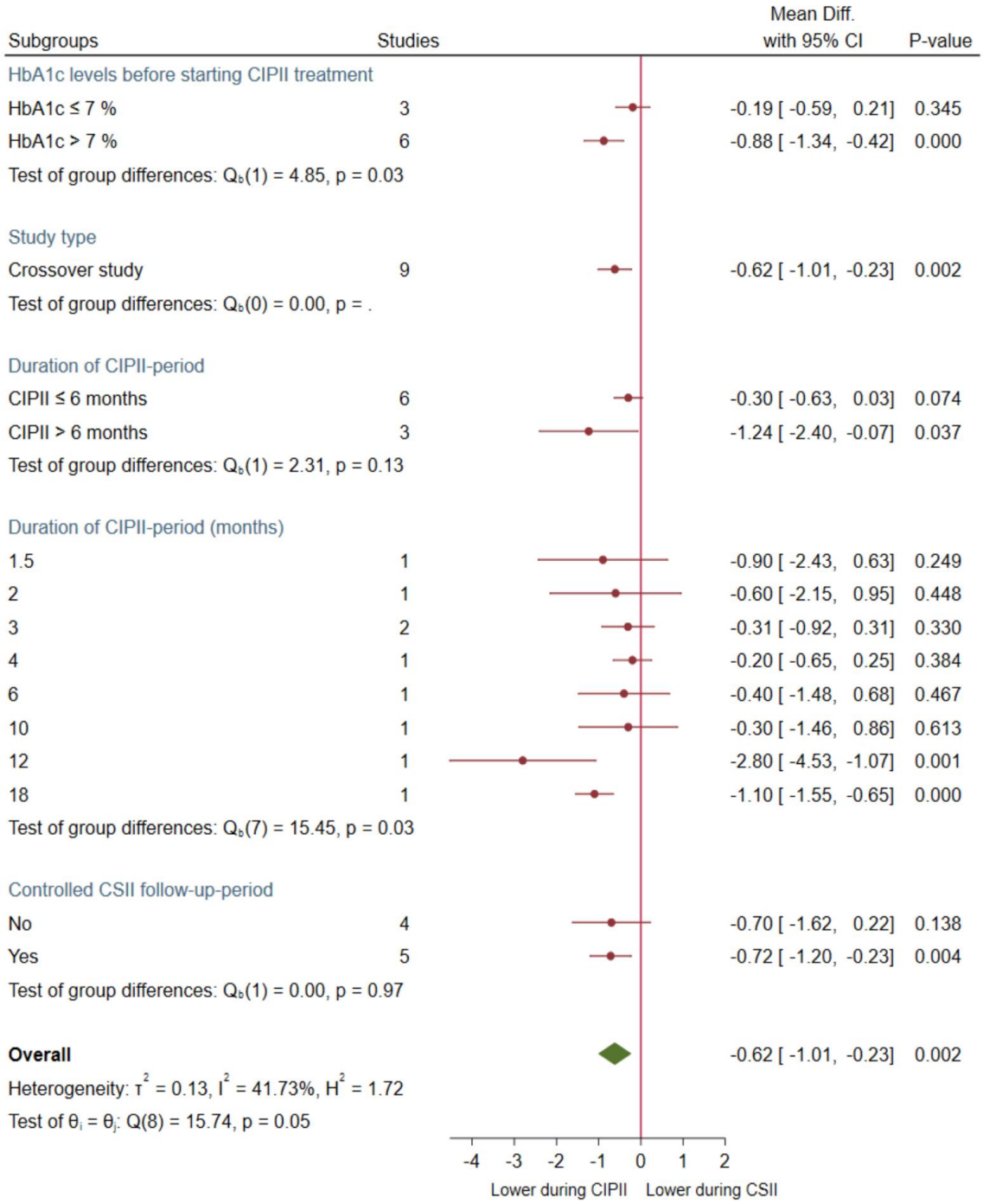
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII); SMBG, self-monitoring of blood glucose.

Figure S5b. Subgroup meta-analysis of SMBG (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



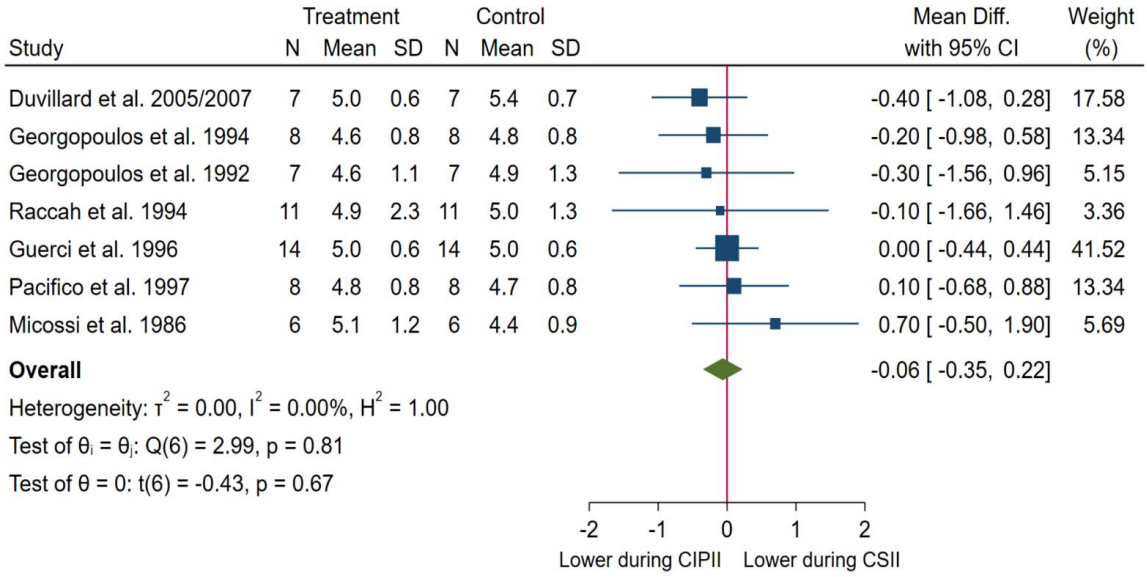
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S5c. Summarised subgroup meta-analysis of SMBG (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



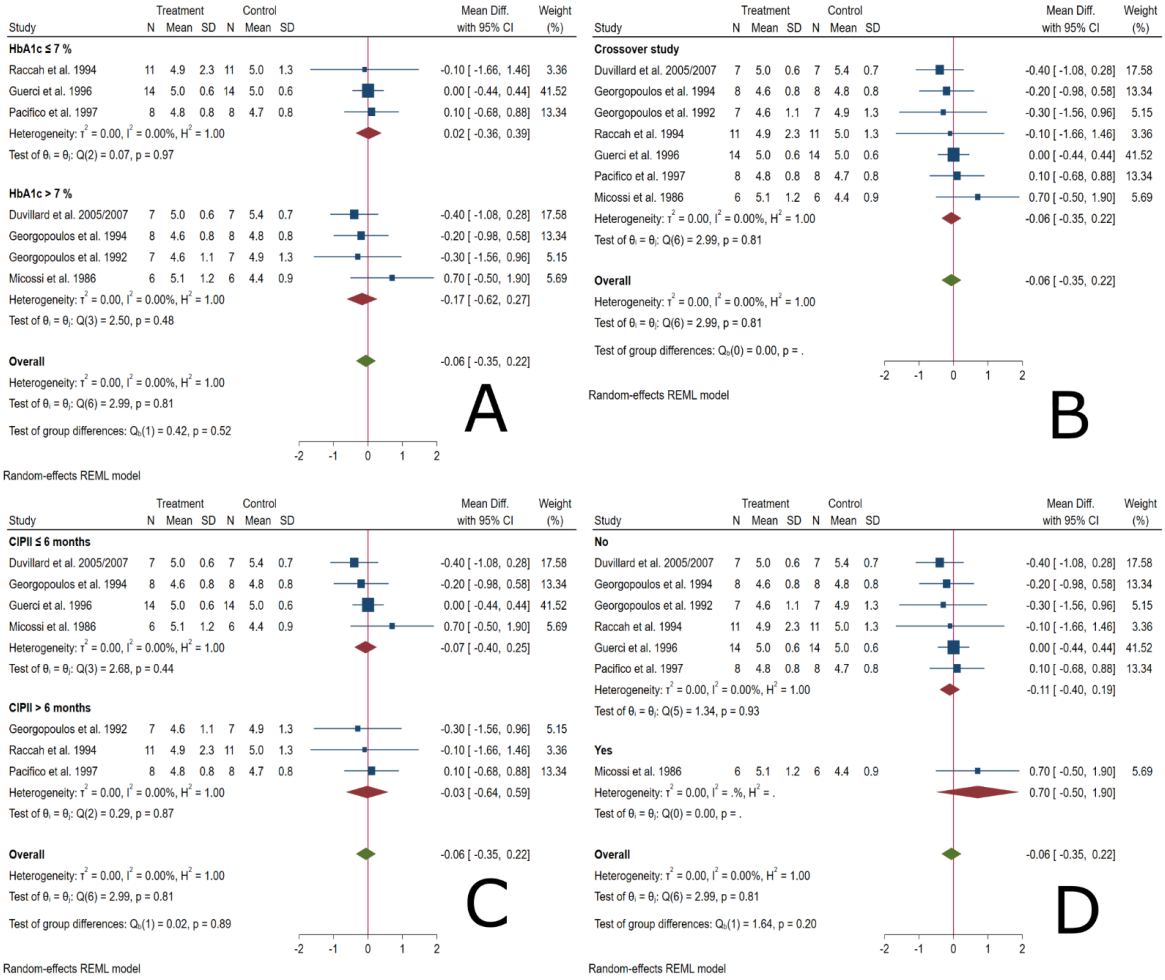
Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

Figure S6a. Meta-analysis of cholesterol (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



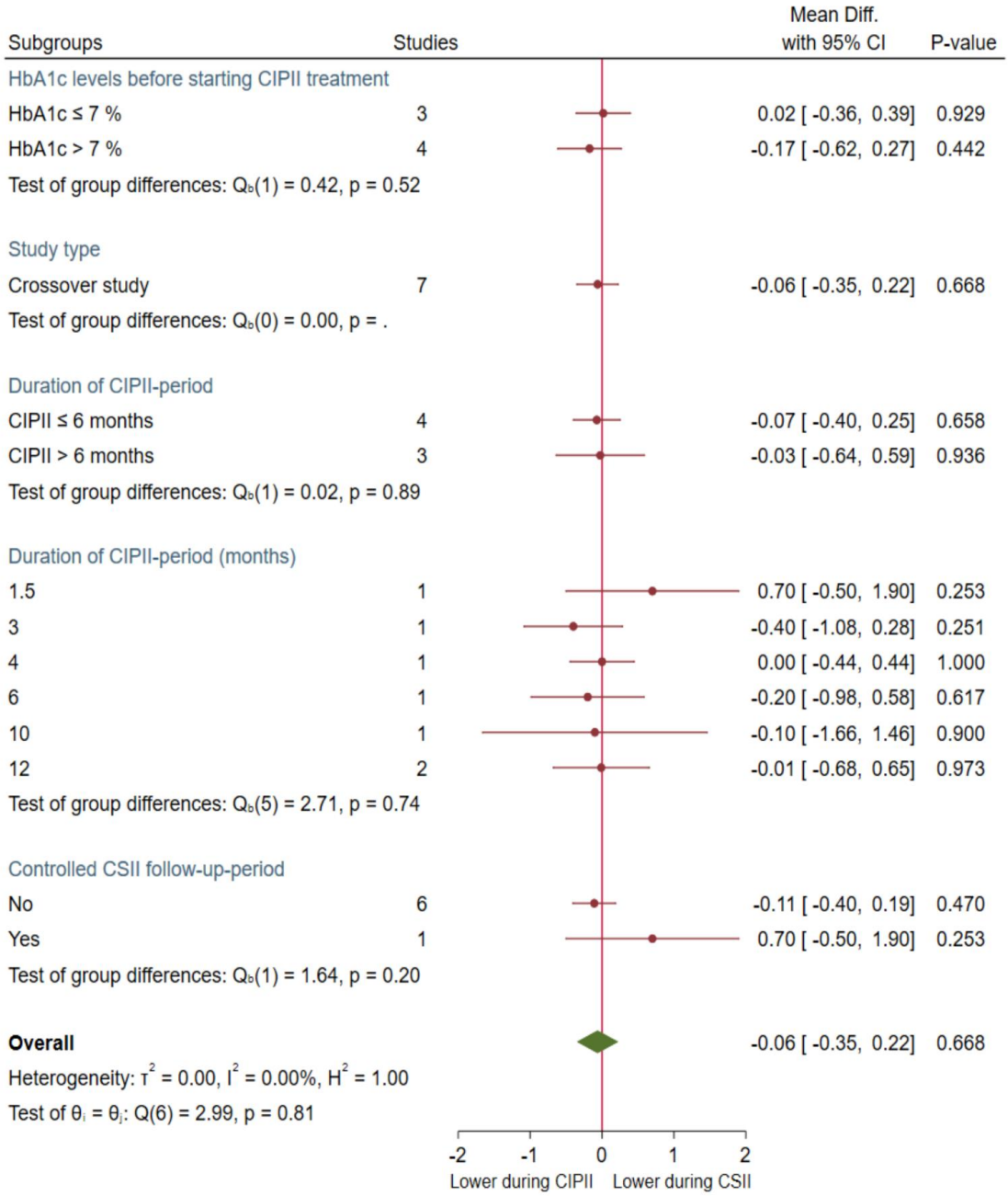
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII).

Figure S6b. Subgroup meta-analysis of cholesterol (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



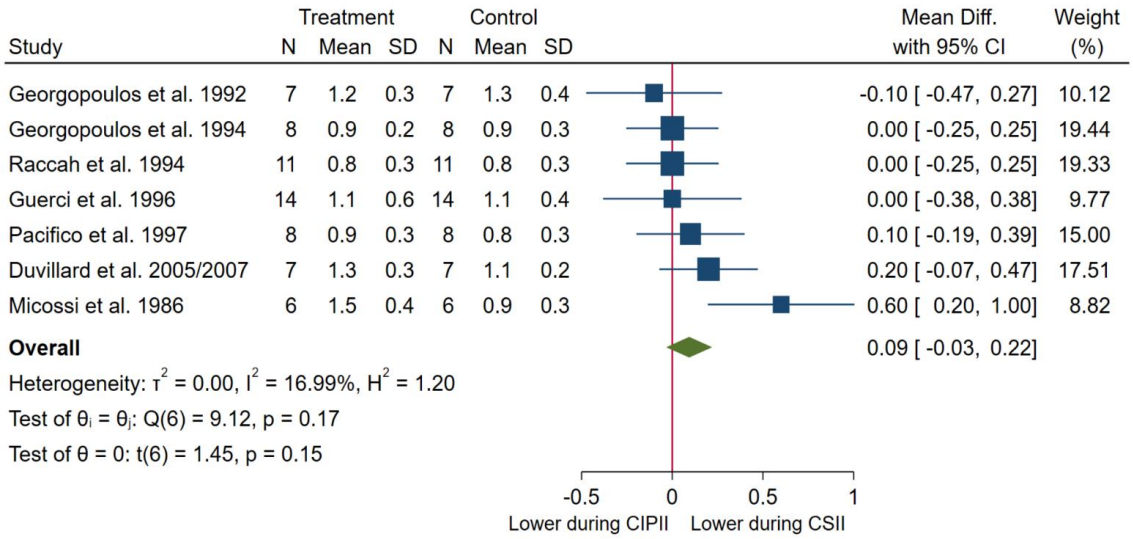
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S6c. Summarised subgroup meta-analysis of cholesterol (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



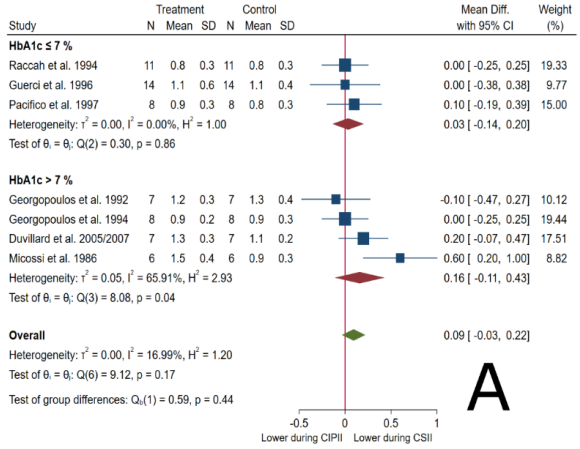
Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

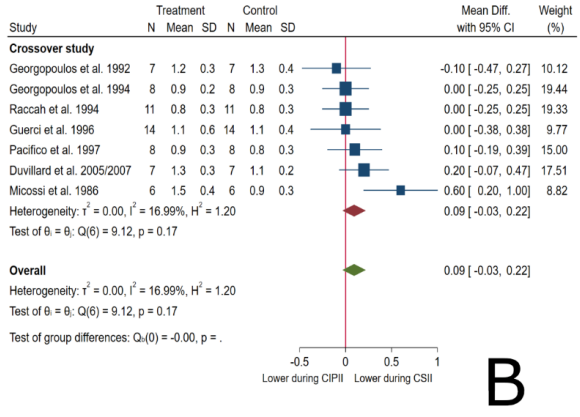
Figure S7a. Meta-analysis of triglycerides (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).

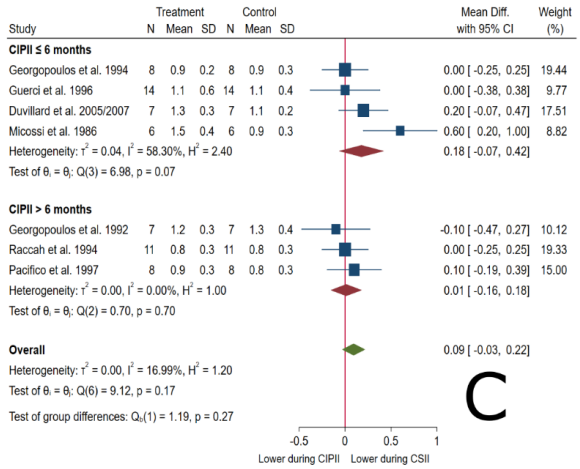


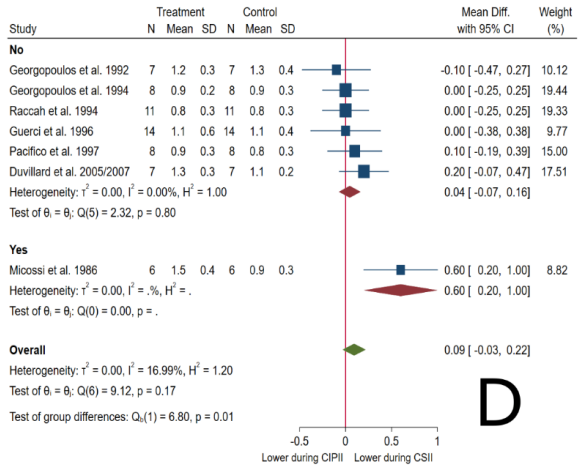
Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII).

Figure S7b. Subgroup meta-analysis of triglycerides (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



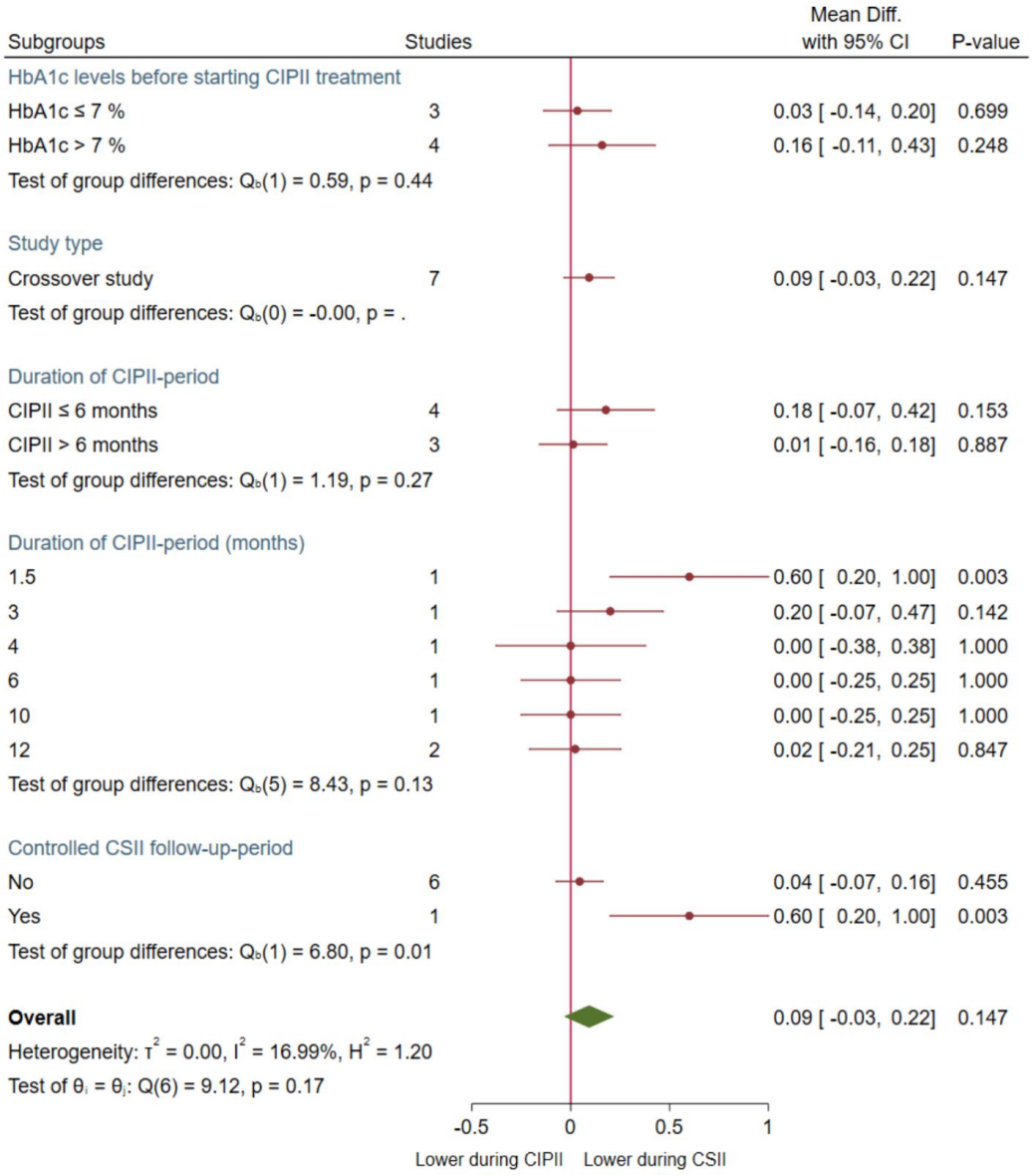






Legends: Treatment, continuous intraperitoneal insulin infusion (CIPII); Control, continuous subcutaneous insulin infusion (CSII). Figure A: Subgroup analysis according to HbA1c levels before starting CIPII treatment (≤ 7 % and > 7 %); Figure B: Subgroup analysis according to study type (Case-Control studies and Crossover studies); Figure C: Subgroup analysis according to length of the CIPII-period (≤ 6 months and > 6 months); Figure D: Subgroup analysis according to whether or not there was an additional controlled CSII follow-up-period with subsequent CIPII-period.

Figure S7c. Summarised subgroup meta-analysis of triglycerides (mmol/L) in patients during CIPII treatment compared to that during control treatment (CSII).



Legends: CIPII, continuous intraperitoneal insulin infusion; CSII, continuous subcutaneous insulin infusion.

Data for Egger`s test from STATA

HbA1c

meta bias, egger random(reml) tdistribution
Effect-size label: Mean Diff.
Effect size: _meta_es
Std. Err.: _meta_se
Regression-based Egger test for small-study effects
Random-effects model
Method: REML
H0: beta1 = 0; no small-study effects
beta1 = -1.10
SE of beta1 = 1.017
t = -1.08
Prob > t = 0.2932

Daily insulin dose

Model and method
Model: Random-effects
Method: REML
. meta bias, egger random(reml) tdistribution
Effect-size label: Mean Diff.
Effect size: _meta_es
Std. Err.: _meta_se
Regression-based Egger test for small-study effects
Random-effects model
Method: REML
H0: beta1 = 0; no small-study effects
beta1 = 0.43
SE of beta1 = 0.834
t = 0.51
Prob > t = 0.6212

References

1. Hofmann, H.M.H., P.A.M. Weiss, and J.G. Haas, *Continuous insulin delivery systems for the pregnant diabetic patient.* Acta Diabetologica Latina, 1986. **23**(3): p. 201-214.
2. Rautiainen, P., H. Tirkkonen, and T. Laatikainen, *Glycemic Control in Adult Type 1 Diabetes Patients with Insulin Glargine, Insulin Detemir, or Continuous Subcutaneous Insulin Infusion in Daily Practice.* Diabetes Technol Ther, 2018. **20**(5): p. 363-369.
3. Ruiz-de-Adana, M.S., et al., *Comparison between a multiple daily insulin injection regimen (basal once-daily glargine plus mealtime lispro) and continuous subcutaneous insulin infusion (lispro) using continuous glucose monitoring in metabolically optimized type 1 diabetes patients: A randomized open-labelled parallel study.* Med Clin (Barc), 2016. **146**(6): p. 239-46.
4. Rys, P.M., et al., *Continuous subcutaneous insulin infusion vs multiple daily injections in pregnant women with type 1 diabetes mellitus: a systematic review and meta-analysis of randomised controlled trials and observational studies.* Eur J Endocrinol, 2018. **178**(5): p. 545-563.
5. Liebl, A., et al., *A reduction in severe hypoglycaemia in type 1 diabetes in a randomized crossover study of continuous intraperitoneal compared with subcutaneous insulin infusion.* Diabetes, Obesity and Metabolism, 2009. **11**(11): p. 1001-1008.
6. Pitt, H.A., C.D. Saudek, and H.A. Zacur, *Long-term intraperitoneal insulin delivery.* Ann Surg, 1992. **216**(4): p. 483-91; discussion 491-2.
7. Schade, D.S., et al., *The peritoneal absorption of insulin in diabetic man: a potential site for a mechanical insulin delivery system.* Metabolism, 1979. **28**(3): p. 195-7.
8. *Advances in peritoneal dialysis : proceedings of the Second International Symposium on Peritoneal Dialysis : Berlin (-West), June 16-19, 1981 / editors, G.M. Gahl, M. Kessel, K.D. Nolph.* International congress series ; no. 567, ed. G.G. Gahl, 1938-, M.M. Kessel, 1926-, and K.D. Nolph. 1981, Amsterdam ; Princeton, NJ : Excerpta Medica ; New York, N.Y. : Sole distributors for the USA and Canada, Elsevier North-Holland, 1981.
9. Schade, D.S., et al., *Prolonged peritoneal insulin infusion in a diabetic man.* Diabetes Care, 1980. **3**(2): p. 314-317.
10. Schade, D.S., et al., *Normalization of plasma insulin profiles with intraperitoneal insulin infusion in diabetic man.* Diabetologia, 1980. **19**(1): p. 35-9.
11. Schade, D.S., et al., *Intraperitoneal delivery of insulin by a portable microinfusion pump.* Metabolism, 1980. **29**(8): p. 699-702.
12. Schade, D.S., R.P. Eaton, and N.M. Friedman, *Five-day programmed intraperitoneal insulin delivery in insulin-dependent diabetic man.* Journal of Clinical Endocrinology and Metabolism, 1981. **52**(6): p. 1165-1170.
13. Irsigler, K., et al., *Long-term continuous intraperitoneal insulin infusion with an implanted remote-controlled insulin infusion device.* Diabetes, 1981. **30**(12): p. 1072-5.
14. Selam, J.L., et al., *Total implantation of a remotely controlled insulin minipump in a human insulin-dependent diabetic.* Artif Organs, 1982. **6**(3): p. 315-9.
15. Selam, J.L., A. Slingeneyer, and B. Hedon, *Long-term ambulatory peritoneal insulin infusion of brittle diabetes with portable pumps: Comparison with intravenous and subcutaneous routes.* Diabetes Care, 1983. **6**(2): p. 105-111.
16. Fonseca, V.A., R.K. Menon, and P.M.S. O'Brien, *Diabetic pregnancy managed with intraperitoneal insulin.* Diabetic Medicine, 1987. **4**(1): p. 74-76.
17. Saudek, C.D., et al., *A preliminary trial of the programmable implantable medication system for insulin delivery.* N Engl J Med, 1989. **321**(9): p. 574-9.
18. Walter, H., et al., *Implantation of programmable infusion pumps for insulin delivery in type I diabetic patients.* Klinische Wochenschrift, 1989. **67**(11): p. 583-587.
19. Mirouze, J., et al., *Experience with external insulin pumps using the intraperitoneal route in 31 type I diabetic patients continuously followed for at least four years.* Diabetes, Nutrition and Metabolism - Clinical and Experimental, 1990. **3**(3): p. 185-189.
20. Selam, J.L., et al., *Clinical trial of programmable implantable insulin pump for type I diabetes.* Diabetes Care, 1992. **15**(7): p. 877-885.
21. Hopkins, K.D., et al., *Intraperitoneal insulin affects insulin-like growth factor binding protein-1 in a well-controlled type I diabetic patient.* Diabetes Care, 1993. **16**(10): p. 1404-5.
22. Bauersachs, R., et al., *Hormone and substrate levels after long-term continuous intraperitoneal insulin infusion in insulin-dependent diabetes mellitus.* Diab.Nurt.Metab, 1993. **6**: p. 25-32.

23. Olsen, C.L., et al., *Long-term safety and efficacy of programmable implantable insulin delivery systems.* International Journal of Artificial Organs, 1993. **16**(12): p. 847-854.
24. Haardt, M.J., et al., *A cost-benefit comparison of intensive diabetes management with implantable pumps versus multiple subcutaneous injections in patients with type I diabetes.* Diabetes Care, 1994. **17**(8): p. 847-857.
25. Ruotolo, G., et al., *Normalization of lipoprotein composition by intraperitoneal insulin in IDDM: Role of increased hepatic lipase activity.* Diabetes Care, 1994. **17**(1): p. 6-12.
26. Selam, J.L., et al., *Alterations in reverse cholesterol transport associated with programmable implantable intraperitoneal insulin delivery.* Metabolism: Clinical and Experimental, 1994. **43**(6): p. 665-669.
27. Bagdade, J.D., et al., *Intraperitoneal insulin therapy corrects abnormalities in cholesteryl ester transfer and lipoprotein lipase activities in insulin-dependent diabetes mellitus.* Arteriosclerosis and Thrombosis, 1994. **14**(12): p. 1933-1939.
28. Pinget, M., et al., *Multicentre trial of a programmable implantable insulin pump in type I diabetes.* International Journal of Artificial Organs, 1995. **18**(6): p. 322-325.
29. Hanaire-Broutin, H., et al., *Feasibility of intraperitoneal insulin therapy with programmable implantable pumps in IDDM. A multicenter study. The EVADIAC Study Group. Evaluation dans le Diabete du Traitement par Implants Actifs.* Diabetes Care, 1995. **18**(3): p. 388-92.
30. Jeandidier, N., et al., *Intraperitoneal insulin pump therapy during pregnancy: Two cases.* Practical Diabetes International, 1995. **12**(6): p. 280-280.
31. Jeandidier, N., et al., *Five cases of hyperthyroidism in type I diabetic patients treated with intraperitoneal insulin infusion.* Diabetes Care, 1995. **18**(6): p. 888-9.
32. Jeandidier, N., et al., *Immunogenicity of intraperitoneal insulin infusion using programmable implantable devices.* Diabetologia, 1995. **38**(5): p. 577-84.
33. Bagdade, J.D. and F.L. Dunn, *Improved lipoprotein surface and core lipid composition following intraperitoneal insulin delivery in insulin-dependent diabetes mellitus.* Diabetes and Metabolism, 1996. **22**(6): p. 420-426.
34. Logtenberg, S.J., et al., *Improved glycemic control with intraperitoneal versus subcutaneous insulin in type 1 diabetes: A randomized controlled trial.* Diabetes Care, 2009. **32**(8): p. 1372-1377.
35. Logtenberg, S.J., et al., *Health-related quality of life, treatment satisfaction, and costs associated with intraperitoneal versus subcutaneous insulin administration in type 1 diabetes: A randomized controlled trial.* Diabetes Care, 2010. **33**(6): p. 1169-1172.
36. Logtenberg, S.J.J., et al., *30 month post trial follow up of HbA1c with continuous intraperitoneal insulin infusion in type 1 diabetes.* Diabetologia, 2010. **53**: p. S8.
37. Schaepelynck, P., et al., *A recent survey confirms the efficacy and the safety of implanted insulin pumps during long-term use in poorly controlled type 1 diabetes patients.* Diabetes Technol Ther, 2011. **13**(6): p. 657-60.
38. Van Dijk, P.R., et al., *Effect of intraperitoneal insulin administration on IGF-1 concentrations in type 1 diabetes.* Diabetologia, 2012. **55**: p. S382.
39. van Dijk, P.R., et al., *Effect of i.p. insulin administration on IGF1 and IGFBP1 in type 1 diabetes.* Endocrine Connections, 2014. **3**(1): p. 17-23.
40. Van Dijk, P., et al., *Continuous intraperitoneal insulin infusion in type 1 diabetes: A 6 year post trial follow-up.* Diabetes Technology and Therapeutics, 2014. **16**: p. A20-A21.
41. Bilo, H.J.G., et al., *Continuous intraperitoneal insulin infusion versus subcutaneous insulin for type 1 diabetes: A prospective, case-control trial proving noninferiority.* Diabetologia, 2014. **1)**: p. S89.
42. van Dijk, P.R., et al., *Report of a 7 year case-control study of continuous intraperitoneal insulin infusion and subcutaneous insulin therapy among patients with poorly controlled type 1 diabetes mellitus: favourable effects on hypoglycaemic episodes.* Diabetes Res Clin Pract, 2014. **106**(2): p. 256-63.
43. van Dijk, P.R., et al., *After 6 years of intraperitoneal insulin administration IGF-I concentrations in T1DM patients are at low-normal level.* Growth Hormone and IGF Research, 2015. **25**(6): p. 316-319.
44. van Dijk, P.R., et al., *Intraperitoneal versus subcutaneous insulin therapy in the treatment of type I diabetes mellitus.* Netherlands Journal of Medicine, 2015. **73**(9): p. 399-409.
45. Van Dijk, P.R., et al., *Continuous intraperitoneal insulin infusion versus subcutaneous insulin therapy in the treatment of type 1 diabetes: Effects on glycemic variability.* Diabetes Technology and Therapeutics, 2015. **17**(6): p. 379-384.

46. van Dijk, P.R., et al., *Different routes of insulin administration do not influence serum free thiols in type 1 diabetes mellitus.* Endocrinol Diabetes Metab, 2019. **2**(4): p. e00088.
47. Boering, M., et al., *Effects of intraperitoneal insulin versus subcutaneous insulin administration on sex hormonebinding globulin concentrations in patients with type 1 diabetes mellitus.* Endocrine Connections, 2016. **5**(3): p. 136-142.
48. Mirouze, J., et al., *One year continuous run with a totally implantable insulin infusion pump in a human diabetic.* Transactions - American Society for Artificial Internal Organs, 1983. **29**: p. 709-713.
49. Rouaud, R., et al., *Long term ambulatory peritoneal insulin infusion operating life of the chronic catheters and the portable pumps* Pacing and Clinical Electrophysiology, 1988. **11**(6): p. 790-976.
50. van Dijk, P.R., et al., *Complications of continuous intraperitoneal insulin infusion with an implantable pump.* World journal of diabetes, 2012. **3**(8): p. 142-148.
51. Gin, H., et al., *Clinical evaluation of a newly designed compliant side port catheter for an insulin implantable pump: the EVADIAC experience. Evaluation dans le Diabete du Traitement par Implants Actifs.* Diabetes Care, 2001. **24**(1): p. 175.
52. Belicar, P. and V. Lassmann-Vague, *Local adverse events associated with long-term treatment by implantable insulin pumps. The French EVADIAC Study Group experience. Evaluation dans le Diabete du Traitement par Implants Actifs.* Diabetes Care, 1998. **21**(2): p. 325-6.
53. Renard, E., et al., *Insulin underdelivery from implanted pumps using peritoneal route. Determinant role of insulin pump compatibility.* Diabetes Care, 1996. **19**(8): p. 812-7.
54. Renard, E., et al., *Catheter Complications Associated With Implantable Systems for Peritoneal Insulin Delivery: An analysis of frequency, predisposing factors, and obstructing materials.* Diabetes Care, 1995. **18**(3): p. 300.
55. Mirouze, J., et al., *Clinical experience in human diabetics with portable and implantable insulin minipumps.* Life Support Syst, 1983. **1**(1): p. 39-49.
56. Irsigler, K. and H. Kritz, *On the clinical application of the insulin infusion in the open-loop-system. [German].* Zeitschrift fur die Gesamte Innere Medizin und Ihre Grenzgebiete, 1981. **36**(1): p. 8-19.
57. Liebl, A., et al., *Successful treatment of type 1 diabetes with intraperitoneal insulin infusion when subcutaneous insulin application is not possible: Two case reports.* Diabetes, 2012. **61**: p. A230-A231.
58. Schade, D.S., R.P. Eaton, and R.M. Warhol, *Subcutaneous peritoneal access device for type I diabetic patients nonresponsive to subcutaneous insulin.* Diabetes, 1982. **31**(5 I): p. 470-473.
59. Irsigler, K., et al., *Preprogrammed insulin infusion with a portable pump system.* Horm Metab Res Suppl, 1979(8): p. 193-7.
60. Selam, J.L., et al., *Randomized comparison of metabolic control achieved by intraperitoneal insulin infusion with implantable pumps versus intensive subcutaneous insulin therapy in type I diabetic patients.* Diabetes Care, 1992. **15**(1): p. 53-8.
61. Jeandidier, N., et al., *Decreased severe hypoglycemia frequency during intraperitoneal insulin infusion using programmable implantable pumps [1].* Diabetes Care, 1996. **19**(7): p. 780.
62. Renard, E., B. Guerci, and N. Jeandidier, *Long-term safety and efficacy of intraperitoneal insulin infusion from implanted pumps in a large series of patients with type 1 diabetes and initial high glucose variability.* Diabetologia, 2018. **61 (Supplement 1)**: p. S31.
63. Stephen, R.L., J.G. Maxwell, and J.J. Harrow, *Intervention in nephropathy due to insulin-dependent diabetes mellitus (IDDM).* Kidney International, 1985. **28**(SUPPL. 17): p. S-60-S-65.
64. Broussolle, C., N. Jeandidier, and H. Hanaire-Broutin, *French multicentre experience of implantable insulin pumps.* Lancet, 1994. **343**(8896): p. 514-515.
65. Olsen, C.L., et al., *Insulin antibody responses after long-term intraperitoneal insulin administration via implantable programmable insulin delivery systems.* Diabetes Care, 1994. **17**(3): p. 169-176.
66. Udelsman, R., et al., *Implanted programmable insulin pumps: one hundred fifty-three patient years of surgical experience.* Surgery, 1997. **122**(6): p. 1005-11.
67. Udelsman, R., et al., *Intraperitoneal delivery of insulin via mechanical pump: surgical implications.* Langenbecks Arch Surg, 2000. **385**(6): p. 367-72.
68. Dufaitre-Patouraux, L., et al., *Continuous intraperitoneal insulin infusion does not increase the risk of organ-specific autoimmune disease in type 1 diabetic patients: results of a multicentric, comparative study.* Diabetes Metab, 2006. **32**(5 Pt 1): p. 427-32.

69. DeVries, J.H., et al., *Continuous intraperitoneal insulin infusion in patients with 'brittle' diabetes: favourable effects on glycaemic control and hospital stay.* Diabet Med, 2002. **19**(6): p. 496-501.
70. Stephen, R., et al., *Long-term intraperitoneal insulin treatment: Preliminary Studies in 12 diabetic patients.".* Diabetic Renal-Retinal Syndrome, 1982. **2**: p. 447.
71. Campbell, I.W., et al., *Treatment of type I diabetic with subcutaneous insulin resistance by a totally implantable insulin infusion device ("Infusaid").* Diabetes Res, 1984. **1**(2): p. 83-8.
72. Othonos, N., et al., *Continuous intra-peritoneal insulin infusion: An alternative option for insulin administration.* Diabetic Medicine, 2017. **34**(Supplement 1): p. 16.
73. Schade, D.S., et al., *The intravenous, intraperitoneal, and subcutaneous routes of insulin delivery in diabetic man.* Diabetes, 1979. **28**(12): p. 1069-72.
74. Gooch, B.R., N.N. Abumrad, and R.P. Robinson, *Exercise in insulin-dependent diabetes mellitus: The effect of continuous insulin infusion using the subcutaneous, intravenous, and intraperitoneal sites.* Diabetes Care, 1983. **6**(2): p. 122-128.
75. Gooch, B.R., et al., *Near normalization of metabolism of IDDM: Comparison of continuous subcutaneous (CSII) versus intraperitoneal (CIPII) insulin delivery.* Hormone and Metabolic Research, 1984. **16**(SUPPL. 1): p. 190-194.
76. Dandona, P., V. Fonseca, and O. Fernando, *Control of diabetes through a subcutaneous peritoneal access device (SPAD) in patients with resistance to subcutaneous injected insulin.* Diabetes Research, 1987. **5**(1): p. 47-50.
77. Hermans, M.P., et al., *Fasting and postprandial plasma glucose and peripheral insulin levels in insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus subjects during continuous intraperitoneal versus subcutaneous insulin delivery.* Transplantation Proceedings, 1995. **27**(6): p. 3329-3330.
78. Dassau, E., et al., *Intraperitoneal insulin delivery provides superior glycaemic regulation to subcutaneous insulin delivery in model predictive control-based fully-automated artificial pancreas in patients with type 1 diabetes: a pilot study.* Diabetes Obes Metab, 2017. **19**(12): p. 1698-1705.
79. Lassmann-Vague, V., et al., *Immunogenicity of long-term intraperitoneal insulin administration with implantable programmable pumps: Metabolic consequences.* Diabetes Care, 1995. **18**(4): p. 498-503.
80. Lassmann-Vague, V., et al., *Autoimmunity and intraperitoneal insulin treatment by programmable pumps [14] (multiple letters).* Diabetes Care, 1998. **21**(11): p. 2041-2044.
81. Renard, E., et al., *Experience with intraperitoneal insulin infusion from implantable programmable systems in Type 1 (insulin-dependent) diabetes mellitus previously treated by external pumps.* Diabete et Metabolisme, 1993. **19**(4): p. 364-371.
82. Selam, J.L., et al., *Comparison of intraperitoneal and subcutaneous insulin administration on lipids, apolipoproteins, fuel metabolites, and hormones in type I diabetes mellitus.* Metabolism, 1989. **38**(9): p. 908-12.
83. Georgopoulos, A. and C.D. Saudek, *Normalization of composition of triglyceride-rich lipoprotein subfractions in diabetic subjects during insulin infusion with programmable implantable medication system.* Diabetes Care, 1992. **15**(1): p. 19-26.
84. Micossi, P., E. Bosi, and M. Cristallo, *Chronic continuous intraperitoneal insulin infusion (CIPII) in type I diabetic patients non-satisfactorily responsive to continuous subcutaneous insulin infusion (CSII).* Acta Diabetologica Latina, 1986. **23**(2): p. 155-164.
85. Service, F.J., et al., *Mean Amplitude of Glycemic Excursions, a Measure of Diabetic Instability.* Diabetes, 1970. **19**(9): p. 644-655.
86. Jeandidier, N., et al., *Comparison of intraperitoneal insulin infusion (using implantable pump) and subcutaneous insulin administration: Preliminary results of a crossover study.* Transplantation Proceedings, 1992. **24**(3): p. 948-949.
87. Lassmann-Vague, V., et al., *Insulin kinetics in type I diabetic patients treated by continuous intraperitoneal insulin infusion: Influence of anti-insulin antibodies.* Diabetic Medicine, 1996. **13**(12): p. 1051-1055.
88. Catargi, B., et al., *Comparison of blood glucose stability and HbA1c between implantable insulin pumps using U400 hoe 21pH insulin and external pumps using lispro in type 1 diabetic patients: A pilot study.* Diabetes and Metabolism, 2002. **28**(2): p. 133-137.
89. Oskarsson, P.R., et al., *Continuous intraperitoneal insulin infusion partly restores the glucagon response to hypoglycaemia in type 1 diabetic patients.* Diabetes Metab, 2000. **26**(2): p. 118-24.

90. Oskarsson, P.R., et al., *Metabolic and hormonal responses to exercise in type 1 diabetic patients during continuous subcutaneous, as compared to continuous intraperitoneal, insulin infusion.* Diabetes and Metabolism, 1999. **25**(6): p. 491-497.
91. Wredling, R., et al., *Experience of long-term intraperitoneal insulin treatment using a new percutaneous access device.* Diabetic Medicine, 1991. **8**(6): p. 597-600.
92. Wredling, R., et al., *Variation of insulin absorption during subcutaneous and peritoneal infusion in insulin-dependent diabetic patients with unsatisfactory long-term glycaemic response to continuous subcutaneous insulin infusion.* Diabete Metab, 1991. **17**(5): p. 456-9.
93. Van Dijk, P.R., et al., *Different effects of intraperitoneal and subcutaneous insulin administration on the GH-IGF-1 axis in type 1 diabetes.* Journal of Clinical Endocrinology and Metabolism, 2016. **101**(6): p. 2493-2501.
94. Liebl, A., et al., *Evaluation of the new Accu-Chek diaport, a port system for continuous intraperitoneal insulin infusion, in patients with type 1 diabetes: First 3-month results.* Diabetes Technology and Therapeutics, 2013. **15**: p. A13.
95. Liebl, A., et al., *Evaluation of the New ACCU-CHEK (R) DIAPORT, a Port System for Continuous Intraperitoneal Insulin Infusion, in Patients With Type 1 Diabetes: First 6-Month Results.* Diabetes 2013. **62**: p. pp.A247-A248.
96. Liebl , A., et al., *Evalution of the new ACCU-CHEK DIAPORT system, a port system for continous intraperitoneal insulin infusion, in patients with type 1 diabetes: final 12-month results*, in *International diabetes federation (IDF) World diabetes congress 2013, 2-6 December*. 2013: Melbourne, Australia.
97. Liebl, A., et al., *Long-term clinical evaluation of the new Accu-Chek diaport, a port system for continuous intraperitoneal insulin infusion: 24-month results.* Diabetes, 2014. **63**: p. A241.
98. Pacifico, A., et al., *Our experience with programmable implantable pump for intraperitoneal insulin infusion. [Italian].* Giornale Italiano di Diabetologia, 1997. **17**(1): p. 21-27.
99. Walter, H., et al., *Peripheral hyperinsulinemia in type I diabetics: Reduction via continuous infusion. [German].* Aktuelle Endokrinologie und Stoffwechsel, 1989. **10**(4): p. 224-228.
100. Giacca, A., et al., *Peritoneal and subcutaneous absorption of insulin in type I diabetic subjects.* J Clin Endocrinol Metab, 1993. **77**(3): p. 738-42.
101. Hanaire-Broutin, H., et al., *Effect of intraperitoneal insulin delivery on growth hormone binding protein, insulin-like growth factor (IGF)-I, and IGF-binding protein-3 in IDDM.* Diabetologia, 1996. **39**(12): p. 1498-1504.
102. Georgopoulos, A. and C.D. Saudek, *Intraperitoneal insulin delivery decreases the levels of chylomicron remnants in patients with IDDM.* Diabetes Care, 1994. **17**(11): p. 1295-1299.
103. Beylot, M., et al., *Insulin-mediated glucose disposal in type 1 (insulin-dependent) diabetic subjects treated by continous subcutaneous or intraperitoneal insulin fusion.* Diabete et Metabolisme, 1987. **13**(4): p. 450-456.
104. Lassmann-Vague, V., et al., *SHBG (sex hormone binding globulin) levels in insulin dependent diabetic patients according to the route of insulin administration.* Hormone and Metabolic Research, 1994. **26**(9): p. 436-437.
105. Schnell , O., et al., *Continuous intraperitoneal insulin therapy via port-system in type 1 diabetes with delayed absorption of subcutaneously applied insulin [Kontinuierliche intraperitoneale insulintherapie mit portsystem bei typ 1 diabetikern mit subkutaner insulinaufnahmestorung].* Diabetes und Stoffwechsel, 1994. **3**: p. 51-55.
106. Duvillard, L., et al., *Comparison of apolipoprotein B100 metabolism between continuous subcutaneous and intraperitoneal insulin therapy in type 1 diabetes.* Journal of Clinical Endocrinology and Metabolism, 2005. **90**(10): p. 5761-5764.
107. Duvillard, L., et al., *No change in apolipoprotein AI metabolism when subcutaneous insulin infusion is replaced by intraperitoneal insulin infusion in type 1 diabetic patients.* Atherosclerosis, 2007. **194**(2): p. 342-347.
108. Guerci, B., et al., *Intraperitoneal insulin infusion improves the depletion in choline- containing phospholipids of lipoprotein B particles in type I diabetic patients.* Metabolism: Clinical and Experimental, 1996. **45**(4): p. 430-434.
109. Raccah, D., et al., *Intraperitoneal insulin administration does not modify plasminogen activator inhibitor 1 levels in IDDM patients.* Diabetes Care, 1994. **17**(8): p. 941-2.
110. Arnqvist, H., et al., *Higher circulating IGF-I bioactivity and total IGF-I with intraperitoneal insulin delivery than with CSII in type 1 diabetes.* Growth Hormone and IGF Research, 2010. **20**: p. S24-S25.
111. Hedman, C.A., et al., *Intraperitoneal insulin delivery gives higher circulating IGF-I activity than CSII in type 1 diabetes.* Diabetologia, 2009. **52 (S1)**: p. S376-S377.

112. Hedman, C.A., et al., *Intraperitoneal insulin delivery to patients with type 1 diabetes results in higher serum IGF-I bioactivity than continuous subcutaneous insulin infusion.* Clin Endocrinol (Oxf), 2014. **81**(1): p. 58-62.
113. Catargi, B., et al., *Glucose profiles in a type 1 diabetic patient successively treated with CSII using regular insulin, lispro and an implantable insulin pump.* Diabetes Metab, 2000. **26**(3): p. 210-4.
114. Colette, C., et al., *Effect of different insulin administration modalities on vitamin D metabolism of insulin-dependent diabetic patients.* Hormone and Metabolic Research, 1989. **21**(1): p. 37-41.
115. Jeandidier, N., et al., *Comparison of antigenicity of Hoechst 21PH insulin using either implantable intraperitoneal pump or subcutaneous external pump infusion in type 1 diabetic patients.* Diabetes Care, 2002. **25**(1): p. 84-8.
116. Arnqvist, H., et al., *OR9,53 Higher circulating IGF-I bioactivity and total IGF-I with intraperitoneal insulin delivery than with CSII in type 1 diabetes.* Growth Hormone & IGF Research, 2010. **20**: p. S24-S25.
117. van Dijk, P.R., et al., *Favourable serum calcification propensity with intraperitoneal as compared with subcutaneous insulin administration in type 1 diabetes.* Ther Adv Endocrinol Metab, 2020. **11**: p. 2042018820908456.

Paper II

BMJ Open Diabetes Research & Care

Intraperitoneal insulin administration in pigs: effect on circulating insulin and glucose levels

Ilze Dirnena-Fusini ,[1] Marte Kierulf Åm ,[1,2] Anders Lyngvi Fougner ,[3] Sven Magnus Carlsen,[1,2] Sverre Christian Christiansen[1,2]

To cite: Dirnena-Fusini I, Åm MK, Fougner AL, *et al*. Intraperitoneal insulin administration in pigs: effect on circulating insulin and glucose levels. *BMJ Open Diab Res Care* 2021;**9**:e001929. doi:10.1136/bmjdrc-2020-001929

► Supplemental material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2020-001929).

Preliminary data from this study were presented as an abstract and poster at the 12th International Conference on 'Advanced Technology and Treatment for diabetes' on 20–23 February 2019, Berlin, Germany.

Received 29 September 2020
Revised 26 November 2020
Accepted 2 January 2021

For numbered affiliations see end of article.

Correspondence to
Ilze Dirnena-Fusini;
ilze.dirnena-fusini@ntnu.no

ABSTRACT

Introduction The effect of intraperitoneal insulin infusion has limited evidence in the literature. Therefore, the aim of the study was to investigate the pharmacokinetics and pharmacodynamics of different intraperitoneal insulin boluses. There is a lack of studies comparing the insulin appearance in the systemic circulation after intraperitoneal compared with subcutaneous insulin delivery. Thus, we also aimed for a comparison with the subcutaneous route.

Research design and methods Eight anesthetized, non-diabetic pigs were given three different intraperitoneal insulin boluses (2, 5 and 10 U). The order of boluses for the last six pigs was randomized. Endogenous insulin and glucagon release were suppressed by repeated somatostatin analog injections. The first pig was used to identify the infusion rate of glucose to maintain stable glucose values throughout the experiment. The estimated difference between insulin boluses was compared using two-way analysis of variance (GraphPad Prism V.8). In addition, a trial of three pigs which received subcutaneous insulin boluses was included for comparison with intraperitoneal insulin boluses.

Results Decreased mean blood glucose levels were observed after 5 and 10 U intraperitoneal insulin boluses compared with the 2 U boluses. No changes in circulating insulin levels were observed after the 2 and 5 U intraperitoneal boluses, while increased circulating insulin levels were observed after the 10 U intraperitoneal boluses. Subcutaneously injected insulin resulted in higher values of circulating insulin compared with the corresponding intraperitoneal boluses.

Conclusions Smaller intraperitoneal boluses of insulin have an effect on circulating glucose levels without increasing insulin levels in the systemic circulation. By increasing the insulin bolus, a major increase in circulating insulin was observed, with a minor additive effect on circulating glucose levels. This is compatible with a close to 100% first-pass effect in the liver after smaller intraperitoneal boluses. Subcutaneous insulin boluses markedly increased circulating insulin levels.

Significance of this study

What is already known about this subject?

- After subcutaneous insulin boluses there is a dose-dependent increase in circulating insulin levels. This has until yet not been properly studied after intraperitoneal insulin delivery.

What are the new findings?

- The increase in circulating insulin levels as well as the glucose-lowering effect of intraperitoneally delivered insulin appears to be non-linear.
- We observed a close to 100% first-pass effect in the liver after the smaller (2 and 5 U) IP insulin boluses with hardly any insulin appearing in the systemic circulation concomitant with a major effect on circulating glucose levels.
- Larger (10 U) intraperitoneal insulin boluses increased systemic circulating insulin levels with only a minor further decrease in glucose levels.

How might these results change the focus of research or clinical practice?

- The present results underline the importance of the liver in glucose homeostasis, that a first-pass effect after a smaller intraperitoneal insulin dose approaches 100%, that good glucose control with normal circulating insulin levels could be possible, and that the glucose-lowering effect of insulin achieved in extrahepatic tissues throughout the body is minor compared with the hepatic effect.
- The present results underline that the algorithms in an artificial pancreas with intraperitoneal insulin delivery need to reflect the non-linear relationship between intraperitoneal insulin delivered and the subsequent effect on systemic circulating glucose levels.

INTRODUCTION

Insulin is the major hormone affecting the circulating blood glucose levels. An autoimmune destruction of the insulin-producing beta cells in the pancreas is the cause of diabetes mellitus type 1 (DM1). Thus, patients with DM1 are totally dependent on an external supply of insulin. Usually, insulin is supplied subcutaneously, either by multiple daily insulin injections or by continuous subcutaneous insulin infusion (CSII) by an insulin pump. From a theoretical point of view, intraperitoneal delivery of insulin mimics the normal physiology more closely than subcutaneous insulin delivery.[1] Animal studies suggest that insulin administration

in the portal vein is comparable to endogenous insulin delivery from the pancreas and mimics the normal physiological portal and systematic insulin levels and effects in the liver.[2] However, as intraportal insulin delivery in humans probably carries an unacceptable risk of complications, continuous intraperitoneal insulin infusion has been applied instead of CSII in patients with either severe subcutaneous insulin resistance or brittle diabetes, and with some improvement of HbA1c.[3]

Intraperitoneally administrated insulin reaches maximum circulating insulin levels faster and decreases faster than after subcutaneous insulin delivery.[3 4] Intraperitoneal insulin administration also seems to decrease the risk of hypoglycemia.[5]

The last decade has seen multiple attempts to make an artificial pancreas (AP), that is, a fully automated delivery of insulin in patients with DM1. So far, almost all attempts are based on the double subcutaneous approach, that is, both continuous glucose measurements (CGM) and insulin delivery in subcutaneous tissue. Unfortunately, this approach is hampered by significant delays both in subcutaneous CGM and in particular in the glucose-lowering effect of subcutaneously delivered insulin.[1] Thus, until yet, only hybrid APs are available on the marked. Currently, patients must inform their hybrid AP about the carbohydrate content of their meals and the device will estimate the bolus of insulin to be given.

Aiming to reduce the delays inherent in a double subcutaneous AP and to be able to make a true AP without the need for multiple daily interventions by the users, our research group works on a double intraperitoneal approach for an AP.[1]

To develop the control algorithms for such an AP with intraperitoneal delivery of insulin we need detailed information on the dynamics of intraperitoneal insulin boluses and the effect on glucose levels in the systemic circulation. With that aim, we performed an animal study with frequently repeated measurements of insulin and circulating glucose levels after intraperitoneal insulin boluses of different sizes.

RESEARCH DESIGN AND METHODS

Animals

Main trial

Between January 2017 and August 2018, eight juvenile, non-diabetic, cross-bred pigs (50% Landswine, 50% Yorkshire) approximately 3 months of age (one male (36.2 kg) and seven females (39.5±2.7 kg)) were brought from the same local farmer approximately 1 week before the trials and acclimatized to the staff and new environment. Whenever possible, the pigs were kept in groups in a common stall (11.2 m^2) with concrete floor covered with woodchips. In every stall, a heating lamp was provided. In the facility, a day-night photoperiod (night: 21:00–05:00, dawn: 05:00–07:00, day: 07:00–19:00, dusk: 19:00–21:00) was maintained at 22°C and a relative humidity of 45%±5%. The pigs were fed standard food (Format Vekst 100, Felleskjøpet, Norway) and fresh water was available *ad libitum*. Food was removed 17 hours before the start of the trial while water was available until anesthesia was initiated.

Additional trial

We also performed an additional trial to investigate subcutaneous insulin dynamics and effect on blood glucose levels, performed under similar conditions. For more details, see online supplemental material section 'Additional trial'. In short, between May 2019 and January 2020, three juvenile, non-diabetic, cross-bred male pigs (50% Landswine, 50% Yorkshire) approximately 3 months of age weighing 39.5±4.3 kg were brought from the same local farmer and kept in the conditions as in the main trial.

Intervention and randomization

In the main trial, the first pig was used to obtain information about optimal infusion rate of glucose to maintain stable glucose levels throughout the trial days. The second pig was used to confirm the chosen infusion rate. The remaining six pigs were used to study the pharmacokinetics and pharmacodynamics of intraperitoneally delivered insulin boluses. However, all pigs received boluses of 2, 5 and 10 U of insulin (1 U/s) in the upper right quadrant of the intraperitoneal cavity. The order of insulin boluses for the first two pigs was 2, 5 and 10 U. The order of boluses for the last six pigs was randomized.[6] There were at least 2 hours and 30 min between each bolus.

In the additional trial, two pigs received 10 U subcutaneous insulin boluses and one pig received a 5 U subcutaneous insulin bolus. All subcutaneous insulin boluses were performed as the first bolus of the day.

Anesthesia

In both the main trial and the additional trial, anesthesia was maintained by intravenous infusion of midazolam (0.5 mg/kg/hour) (Accord Healthcare, Middlesex, UK) and fentanyl (7.5 µg/kg/hour) (Actavis Group, Hafnarfjordur, Iceland) and by inhalation of isoflurane (0.5%–2%) (Baxter AS, Oslo, Norway). More detailed information regarding the anesthesia protocol is provided in the online supplemental material.

Surgical procedure

In both trials, an intra-arterial line was placed in the left carotid artery for blood sampling and monitoring of physiological parameters and an intravenous line was placed in the left internal jugular vein for glucose and fluid infusions. Both catheters were inserted through the same surgical opening.

The catheter for intraperitoneal delivery of insulin by an Animas Vibe insulin pump (Animas, West Chester, Pennsylvania, USA) was inserted through 2–3 cm long caudal to the umbilicus incision in the abdominal wall. The tip of the catheter was inserted intraperitoneally in the upper right region but was not fixed in the stationed

position. To avoid coagulation, 150 IU of heparin (LEO Pharma, Ballerup, Denmark) was injected into the intraperitoneal space.

At the end of all trial days, and still under full anesthesia, the pigs were euthanized. More detailed information is provided in the online supplemental material (Surgical procedure).

Endogenous insulin and glucagon secretion

To suppress the endogenous insulin and glucagon secretion, all pigs received the somatostatin analogs octreotide (Sandostatin 200 µg/mL, Novartis Europharm, UK) and pasireotide (Signifor 0.3 mg/mL, Novartis Europharm).

The somatostatin analogs were injected 1 hour before the first insulin bolus of the day. 0.4 mg octreotide was injected intravenously and 0.3 mg pasireotide was injected subcutaneously. The octreotide injections were repeated hourly and the pasireotide injections were repeated every 3 hours during the trial.

Glucose level

To prevent hypoglycemia, a continuous venous glucose infusion was provided through the left internal jugular vein and was continued for the duration of the experiment. The blood glucose levels were kept in the range of 4.5–5.5 mmol/L before each insulin bolus was given.

In the first pig, we tested different glucose infusion rates to identify a suitable rate. In the remaining seven pigs, a constant glucose infusion rate of 8 g/hour was used throughout the experiments.

In the additional trial, the glucose infusion rate was increased in one of the pigs during two separate periods due to hypoglycemia. Additionally, all pigs received an intraperitoneal glucagon bolus (150 µg) 40 min after the insulin bolus.

Accordingly, only the circulating insulin levels from the additional trial were compared with the mean circulating insulin levels from the main trial, that is, the blood glucose values from the additional trial were not compared directly to the results from the main trial due to increased glucose infusion rate.

Insulin boluses

At the start of every trial day, a fresh insulin analog (100 U/mL, NovoRapid, Novo Nordisk, Denmark) was inserted in an insulin pump (Animas Vibe).

In the main trial, all pigs (n=7) received three insulin boluses (2, 5 and 10 U) in the upper right intraperitoneal space. In the additional trial, insulin boluses were injected into the subcutaneous tissues in the left side of the neck. Two pigs received 10 U boluses and one pig received a 5 U bolus.

Glucose and insulin measurements

For both trials, arterial blood samples for glucose analysis were collected in heparinized syringes (LEO Pharma). Samples were analyzed on a Radiometer ABL 725 blood gas analyzer (Radiometer Medical, Brønshøj, Denmark). All blood samples were placed on ice immediately after extraction from the pigs. Most samples were analyzed consecutively, but some samples were stored on ice for a maximum of 20 min before analysis.

In the main trial, samples were collected 10, 5 and 1 min prior to the first somatostatin analog injection, every 10 min for the first hour after Sandostatin injection, every minute for the first 10 min after the insulin bolus, and every fifth minute thereafter for the next 110 min.

In the additional trial, samples for blood glucose measurements were collected 10, 5 and 1 min prior to first somatostatin analog injection and prior to starting the glucose infusion. Subsequent blood glucose samples were collected 2 min after insulin boluses and thereafter every 5 min for the next 118 min.

In both trials, blood samples for insulin analysis were stored on ice for at least 10 min before centrifugation (10 min at 2.000× rpm in a refrigerated centrifuge). Plasma was collected from the samples immediately after centrifugation and transferred into empty Eppendorf Tubes and temporarily stored at −20°C. At the end of each trial day, plasma samples were stored at −80°C.

Plasma insulin was analyzed as singles by Iso-Insulin ELISA kit (10-1128-01, Mercodia, Uppsala, Sweden). Suppression of endogenous insulin secretion was verified by analyses of Porcine Insulin ELISA (10-1200-01, Mercodia) according to the manufacturer's protocol. The results were converted from mU/L to pmol/L by a conversion factor 6, as recommended by the manufacturer. The lowest detectable insulin concentration for the Iso-Insulin ELISA kit was <3.0 mU/L (18 pmol/L) and for Porcine Insulin ELISA was <2.3 mU/L (13.8 pmol/L).

Statistical analysis

From the main trial, data from the last seven pigs were used for statistical analysis. We assumed the glucose levels at −5 min to be the baseline glucose levels at the start before each bolus (online supplemental figure S1).

Delta values collected from the main trial with different insulin boluses (2, 5 and 10 U) in the pigs were analyzed. In order to estimate possible significant differences in circulating insulin and blood glucose levels after IP insulin boluses, a two-way repeated measures analysis of variance was performed. Treatment and time were the sources of variation. Tukey's multiple comparisons test was used for distinguishing comparisons between different insulin boluses. All non-measurable insulin values were set at 18 pmol/L. All treatments are compared as models; therefore, comparison between unequal groups is allowed. Statistics were performed with GraphPad Prism V.8 Statistics. All values are given as mean±SD, unless stated otherwise. Differences between the groups were considered significant if $p \leq 0.05$.

RESULTS

Pilot experiment

The first pig was used for a pilot experiment as we had no information about the necessary glucose infusion rate

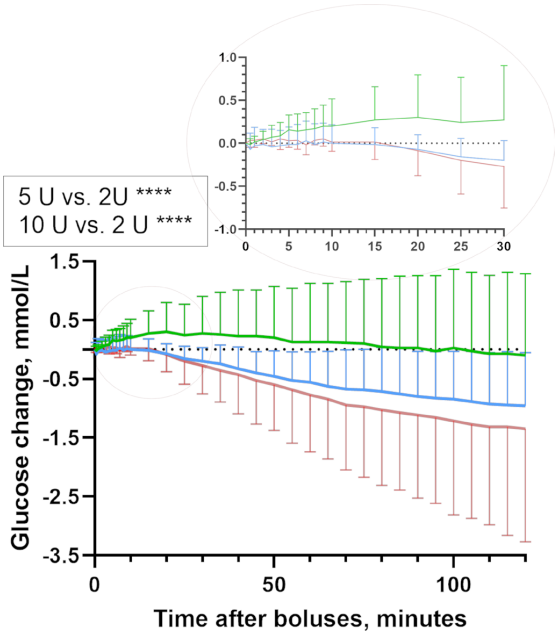


Figure 1 Glucose dynamics. Estimated blood glucose change for the 120 min after intraperitoneal insulin delivery in pigs (mean, SD). The 5 and 10 U intraperitoneal bolus gave significantly higher glucose elevations compared with 2 U intraperitoneal bolus. Insulin boluses: 2 U intraperitoneal insulin bolus (n=7, green line), 5 U intraperitoneal insulin bolus (n=7, blue line) and 10 U intraperitoneal insulin bolus (n=7, red line).

to achieve acceptable glucose values throughout the trial days. Based on the results from the first pig, a continuous glucose infusion of 8 g/hour (200 mg/mL at 40 mL/hour) was used throughout the rest of the trial.

Octreotide and pasireotide had the expected effect on insulin levels, as there were no detectable endogenous insulin levels (<13.8 pmol/L) during the experiments. Therefore, the same protocol was used for all experiments.

Glucose level

In the main trial, the mean blood glucose level at the start of the 2, 5 and 10 U insulin boluses was 5.07±0.11, 5.38±0.06 and 5.31±0.06 mmol/L, respectively (online supplemental figure S1A).

The estimated mean blood glucose level after the 5 and 10 U intraperitoneal insulin boluses was significantly decreased (mean±SE) by 0.48±0.07 and 0.61±0.07 mmol/L (95% CI 0.31 to 0.65 and 95% CI 0.44 to 0.79, respectively, p<0.0001) compared with the mean blood glucose level after the 2 U insulin boluses (figure 1). However, the mean blood glucose level after the 10 U intraperitoneal insulin boluses changed only by 0.13±0.07 mmol/L (95% CI −0.04 to 0.30, p=0.184) and was not different from the mean blood glucose level after the 5 U intraperitoneal insulin boluses.

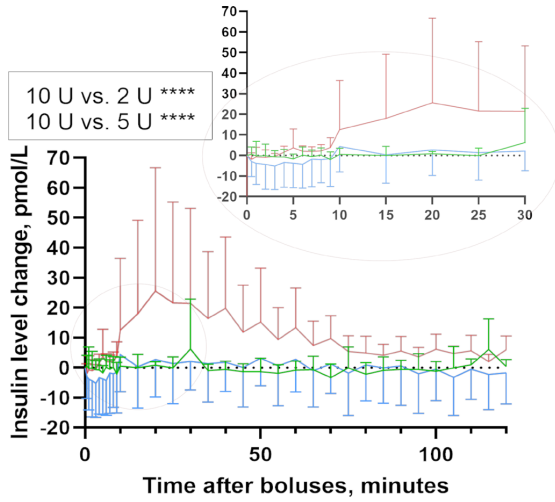


Figure 2 Insulin dynamics. Estimated insulin change for the 120 min after intraperitoneal insulin delivery in pigs (mean, SD). The 10 U intraperitoneal bolus gave significantly higher insulin elevation compared with 2 and 5 U intraperitoneal boluses. Insulin boluses: 2 U intraperitoneal insulin bolus (n=7, green line), 5 U intraperitoneal insulin bolus (n=7, blue line) and 10 U intraperitoneal insulin bolus (n=7, red line).

Insulin level

No difference in circulating mean insulin level was observed after the 2 and 5 U intraperitoneal insulin boluses while after the 10 U intraperitoneal insulin boluses, the mean insulin level started to increase after 10 min. The mean insulin level after the 10 U intraperitoneal insulin boluses was significantly increased by (mean±SE) 7.89±1.07 and 8.37±1.07 pmol/L compared with the mean insulin level after the 2 and 5 U intraperitoneal insulin boluses (95% CI 1.99 to 5.37 and 95% CI 5.86 to 10.89, respectively, p<0.0001) (figure 2). Mean insulin levels after 5 U compared with 2 U intraperitoneal insulin boluses were not different (p=0.89).

In the additional trial, the insulin level was increased 5 min after 5 U subcutaneous insulin bolus (n=1) and the mean insulin level was increased 5 min after 10 U subcutaneous insulin boluses (n=2) (online supplemental figure S4).

In the main trial, all insulin samples were run in singles with a coefficient of variation (CV) <5%. Interassay CV for Porcine Insulin was 4.1%, 4.3% and 3.3% for 5.04, 17.6 and 55.4 mU/L standards, respectively. Interassay CV for Iso-Insulin was 4.9% and 4.7% for 9.84 and 60.7 mU/L standards, respectively.

Comparison of intraperitoneal and subcutaneous insulin boluses

The mean circulating insulin level increased more after subcutaneous insulin boluses compared with intraperitoneal insulin boluses (p value not calculated due to low numbers) (online supplemental figures S2A,B, S5 and S6). Further, after the 10 U intraperitoneal insulin

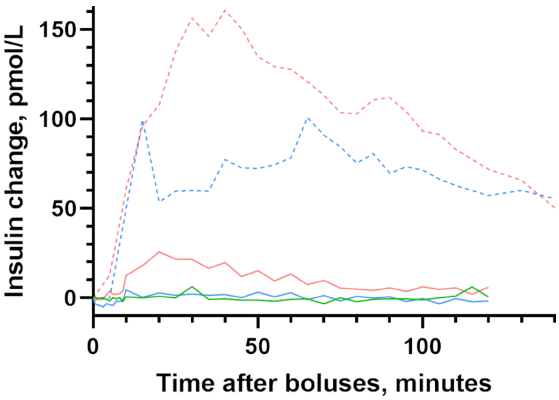


Figure 3 Insulin change after intraperitoneal and subcutaneous insulin boluses. Estimated insulin changes for the 120 min after intraperitoneal insulin delivery and for the 140 min after subcutaneous insulin delivery in pigs. Statistical testing was not provided based on limited number of included animals. Insulin boluses: 2 U intraperitoneal insulin bolus (n=7, green line), 5 U intraperitoneal insulin bolus (n=7, blue line), 10 U intraperitoneal insulin bolus (n=7, red line), 5 U subcutaneous insulin bolus (n=1, blue dashed line) and 10 U subcutaneous insulin bolus (n=2, red dashed line).

boluses, the mean circulating insulin level was close to baseline after 80 min, whereas after the 5 and 10 U subcutaneous insulin boluses, the insulin levels were still elevated after 140 min (figure 3).

DISCUSSION

The results of this animal trial indicate that: (1) after intraperitoneal insulin delivery there is a threshold dose before insulin appears in the systemic circulation, and (2) there is clear non-linear relationship between the intraperitoneal insulin dose and its glucose-decreasing effect. This is in contrast to the effect of subcutaneous insulin boluses seen in humans, where a linear association between increasing insulin doses and decreasing blood glucose is observed.[7]

In the additional trial, higher circulating insulin levels were observed after the subcutaneous compared with the intraperitoneal insulin boluses. Markedly increased circulating insulin levels after subcutaneous delivery had no effect or a much smaller effect on circulating insulin levels after intraperitoneal delivery of the same insulin bolus.

Previous human studies on intraperitoneal insulin infusion in humans did not report any major difference between circulating insulin levels when intraperitoneal was compared with either subcutaneous insulin boluses (0.2 U/kg)[8] or insulin infusion (5 U for 30 min followed by 2 U/hour for 3 hours).[9] One possible explanation may be that our data are generated from anesthetized pigs while the previous data are from human studies. Our data are also limited by the small number of pigs and insulin boluses used and need to be confirmed in additional studies with preferably a larger number of animals. We observed in our pig trials that both 2 and 5 U intraperitoneal insulin boluses did not increase the mean circulating insulin level, while the 10 U intraperitoneal insulin bolus significantly increased the mean circulating insulin level. This indicates that more or less all intraperitoneally delivered insulin, at least after the two smaller boluses, is absorbed into the portal circulation and that there is a substantial hepatic first-pass effect absorbing more or less all insulin delivered to the liver from the portal vein.[10]

A hepatic first-pass effect of up to 80% has been shown in an in vitro model.[11] Our data are consistent with that observation. Actually, our data indicate a close to 100% first-pass effect of insulin in the liver before the mechanism for hepatic insulin absorption is saturated. The liver is the major organ involved in glucose homeostasis. Our observation that there is hardly any difference in the glucose-lowering effect after the 5 and 10 U intraperitoneal insulin boluses is probably explained by this first-pass effect in the liver when the insulin delivery from the portal vein reaches a certain level. When this level of insulin is reached, the liver cannot absorb more glucose per unit time, that is, the hepatic capacity for glucose disposal is saturated and further increase in total body glucose disposal can only be achieved in extrahepatic tissues such as muscle and fat. This is illustrated by the fact that despite doubling the intraperitoneal insulin boluses from 5 to 10 U hardly any further decrease in circulating glucose is observed. This is compatible with the liver being saturated with insulin after a 5 U intraperitoneal bolus and no further effect on hepatic glucose disposal is achievable despite increasing intraperitoneal insulin doses. Similar results were observed in human studies where higher insulin doses were provided during the intraperitoneal insulin treatment without any increase in hypoglycemic events, as compared with the subcutaneous insulin delivery.[12]

Increasing the intraperitoneal insulin boluses to 10 U means that circulating insulin levels increase while the glucose-lowering effect is minimally increased. This illustrates that what can be achieved by insulin-mediated glucose disposal by other tissues such as fat and muscle is quite limited compared with the hepatic effect.[13–15]

We cannot exclude that the order of the boluses may influence the results. First, we were not able to look into this due to the low number of animals in our study, but we observed that during the trial day (8 hours), the amount of intraperitoneal fluid increased. Therefore, we hypothesize that increased amount of intraperitoneal fluid may reduce the speed of insulin absorption as previously suggested.[16] Second, as we gave the largest bolus (10 U) first, we trespassed the saturation in the liver, and the excess was distributed peripherally, and there may then be a larger chance that the next boluses of 2 and 5 U will be distributed peripherally as well, because of a possible lasting effect of the 10 U bolus on the hepatic insulin saturation. If so, the opposite may be the issue, when we

give 2 U as the first bolus of the day. In that case, theoretically a smaller proportion of the subsequent bolus of 5 or 10 U will be distributed peripherally, because most of that insulin bolus will be bound in the liver, because the previous 2 U insulin bolus far from saturated the liver. However, we do not know how long insulin exerts its effect in the liver and the hepatic saturation is repealed.

The fact that the more or less linear relationship between insulin dose and effect on blood glucose levels observed after subcutaneous insulin delivery is quite different when insulin is delivered intraperitoneally, illustrates that the steering algorithms for an AP with subcutaneous insulin delivery cannot be transferred directly to an AP with intraperitoneal insulin delivery. This means that the mathematical model used in the controller (or the simulator we would test the controller on) should probably include information about this strong nonlinearity. It is noteworthy that, when given intraperitoneally, a substantial effect on blood glucose levels can be achieved without any increase in circulating insulin levels and that a doubling of a medium insulin dose hardly induces further glucose-lowering effect.

Strengths and limitations of the study

The strengths of our trial are (1) frequent blood sampling before and for a relatively long period after each of the insulin boluses, (2) randomized order of boluses (in 6 out of 7 pigs), (3) equal age and gender of the pigs, (4) verified complete suppression of endogenous insulin secretion, (5) equal glucose levels at the initiation of the experiments and equal glucose infusion rate which makes the observed glucose effects more trustworthy (main trial), and (6) data from intraperitoneal insulin boluses were compared with subcutaneous insulin boluses with similar sampling times and similar protocol through the trials.

Among the limitations are (1) weights of the animals varied somewhat while insulin boluses were fixed, (2) limited number of included animals, especially in the additional trial with subcutaneous insulin boluses, (3) animals were anesthetized during the study, therefore obtained data do not reflect awakening animal dynamics of insulin absorption and effects, and (4) during the experiments animals accumulated different amounts of intraperitoneal fluid possibly affecting insulin absorption and effect.

CONCLUSIONS

In pigs, small to medium intraperitoneal insulin boluses (2 and 5 U) decrease circulating glucose levels without increasing insulin levels in the systemic circulation. By increasing the insulin bolus further (to 10 U), we observed a major increase in circulating insulin levels while only a minor additional lowering of blood glucose levels was observed. This is compatible with a close to complete first-pass effect in the liver after small to medium-sized intraperitoneal insulin boluses.

Author affiliations
[1]Department Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway
[2]Department of Endocrinology, St Olavs Hospital Universitetssykehuset i Trondheim, Trondheim, Norway
[3]Department of Engineering Cybernetics, Faculty of Information Technology and Electrical Engineering, Norwegian University of Science and Technology, Trondheim, Norway

Acknowledgements The animal experiments were conducted at the Comparative Medicine Core Facility (CoMed), Norwegian University of Science and Technology (NTNU). We thank Senior Engineer Oddveig Lyng, CoMed, NTNU, for assistance during the animal experiments. We thank Associate Professor Øyvind Salvesen, Faculty of Medicine and Health Sciences, NTNU, for statistical assistance.

Contributors IDF completed the trial, collected and analyzed the data, wrote and edited the manuscript and is the guarantor of the work. MKÅ completed the trial, analyzed the data, and reviewed and edited the manuscript. ALF, SMC and SCC contributed to the discussion, and reviewed and edited the manuscript. All authors contributed to the development of protocol.

Funding The Norwegian Research Council (NRC) is funding the Double Intraperitoneal Artificial Pancreas project (project number 248872/070). The study is also supported by a scholarship from the Central Norway Regional Health Authority (grant number 2014/23166) and Norwegian Medical Association Johan Selmer Kvanes Endowment. CoMed is funded by the Faculty of Medicine and Health Sciences at NTNU and the Central Norway Regional Health Authority.

Disclaimer The funding sources had no role in collection, analysis or interpretation of data.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Norwegian Food Safety Authority (FOTS number 12948) and was in accordance with, The Norwegian Regulation on Animal Experimentation and Directive 2010/63/EU on the protection of animals used for scientific purposes. All experiments were performed following the guidelines of good laboratory practice.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the animal study are included in the article or uploaded as supplemental information.

ORCID iDs
Ilze Dirnena-Fusini http://orcid.org/0000-0002-6632-808X
Marte Kierulf Åm http://orcid.org/0000-0001-8245-6893
Anders Lyngvi Fougner http://orcid.org/0000-0001-7583-5027

REFERENCES

1 Christiansen SC, Fougner AL, Stavdahl Øyvind, *et al*. A review of the current challenges associated with the development of an artificial pancreas by a double subcutaneous approach. *Diabetes Ther* 2017;8:489–506.
2 Farmer TD, Jenkins EC, O'Brien TP, *et al*. Comparison of the physiological relevance of systemic vs. portal insulin delivery to evaluate whole body glucose flux during an insulin clamp. *Am J Physiol Endocrinol Metab* 2015;308:E206–22.

3 Dirnena-Fusini I, Åm MK, Carlsen SM. The metabolic effects of continuous Intra-Peritoneal insulin infusion, a systematic review (ePoster). *ATTD* 2020.
4 Schade DS, Eaton RP, Friedman NM, *et al*. Normalization of plasma insulin profiles with intraperitoneal insulin infusion in diabetic man. *Diabetologia* 1980;19:35–9.
5 Fischer U, Fritze K, Freyse EJ. Insulin therapy on the peritoneal route: effects on glucose control in experimental insulin dependent diabetes. *Exp Clin Endocrinol* 1990;95:11–21.
6 Suresh K. An overview of randomization techniques: an unbiased assessment of outcome in clinical research. *J Hum Reprod Sci* 2011;4:8–11.
7 Heise T, Nosek L, Klein O, *et al*. Insulin degludec/insulin aspart produces a dose-proportional glucose-lowering effect in subjects with type 1 diabetes mellitus. *Diabetes Obes Metab* 2015;17:659–64.
8 Micossi P, Cristallo M, Librenti MC, *et al*. Free-insulin profiles after intraperitoneal, intramuscular, and subcutaneous insulin administration. *Diabetes Care* 1986;9:575–8.
9 Schade DS, Eaton RP, Friedman N, *et al*. The intravenous, intraperitoneal, and subcutaneous routes of insulin delivery in diabetic man. *Diabetes* 1979;28:1069–72.
10 Izzo JL, Bartlett JW, Roncone A, *et al*. Physiological processes and dynamics in the disposition of small and large doses of biologically active and inactive 131-I-insulins in the rat. *J Biol Chem* 1967;242:2343–55.
11 Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. *Endocr Rev* 1998;19:608–24.
12 Dassau E, Renard E, Place J, *et al*. Intraperitoneal insulin delivery provides superior glycaemic regulation to subcutaneous insulin delivery in model predictive control-based fully-automated artificial pancreas in patients with type 1 diabetes: a pilot study. *Diabetes Obes Metab* 2017;19:1698–705.
13 Ishida T, Chap Z, Chou J, *et al*. Differential effects of oral, peripheral intravenous, and intraportal glucose on hepatic glucose uptake and insulin and glucagon extraction in conscious dogs. *J Clin Invest* 1983;72:590–601.
14 Ferrannini E, Bjorkman O, Reichard GA, *et al*. The disposal of an oral glucose load in healthy subjects. A quantitative study. *Diabetes* 1985;34:580–8.
15 Moore MC, Cherrington AD, Cline G, *et al*. Sources of carbon for hepatic glycogen synthesis in the conscious dog. *J Clin Invest* 1991;88:578–87.
16 Schade DS, Eaton RP, Davis T, *et al*. The kinetics of peritoneal insulin absorption. *Metabolism* 1981;30:149–55.

Online supplementary materials

Title ”Intraperitoneal insulin administration in pigs: Effect on circulating insulin and glucose levels”

1 RESEARCH DESIGN AND METHODS

1.1 Anaesthesia

The pigs were premedicated with an intramuscular injection of 4 mg diazepam (Stesolid®, Actavis Group, Hafnarfjordur, Iceland), 160 mg azaperone (Stresnil®, Eli Lilly Regional Operations GmbH, Austria) and 750 mg ketamine (Ketalar®, Pfizer AS, Norway), while in a stall. The pigs were carried to the operation room and weighed. An aurical vein was cannulated and anaesthesia was induced with intravenous injections of 1 mg atropine (Takeda AS, Asker, Norway), 150 – 250 µg fentanyl (Actavis Group, Hafnarfjordur, Iceland), 75 – 125 mg thiopental (VUAB Pharma AS, Roztoky, Czech Republic) and 150 – 250 mg ketalar (Ketalar®, Pfizer AS, Norway). The same method was used in our previous animal trials [1].

The pigs were intubated in the lateral position and mechanically ventilated and monitored on an anaesthesia machine (Aisys, GE Healthcare Technologies, Oslo). Anaesthesia was maintained by intravenous infusion of midazolam (0.5 mg/kg/h) (Accord Healthcare Limited, Middlesex, UK) and fentanyl (7.5 µg/kg/h) (Actavis Group, Hafnarfjordur, Iceland) and by inhalation of isoflurane (0.5 – 2 %) (Baxter AS, Oslo, Norway). Room temperature was around 20 degrees Celsius. The body temperature of the pigs was monitored, and a heating blanket was used when necessary.

The pigs received two IV infusions of antibiotic (Cefalotin, Villerton Invest SA, Luxembourg) during the experiments; 2 g immediately after the pigs were anaesthetised and 1 g after 4 hours. Heparin (150 IE) (LEO Pharma A/S, Ballerup, Denmark) were injected in the peritoneal space through the opening for catheter insertion. Fluid balance was achieved by continuous IV infusion of Ringer’s acetate with individual adjustments to achieve stable blood pressure. The pigs also received IV fluid through antibiotics, glucose infusion and when the catheters were flushed after every blood sample.

1.2 Additional trial.

The additional trials were performed for a non-related study.

In contrast to the main study, two of the pigs in the additional study received an increased glucose infusion rate 20 and 70 minutes after the SC insulin bolus, respectively. All three pigs were also given an intraperitoneal (IP) glucagon bolus 40 minutes after the SC insulin injection.

The total fluid loss during the experiments is not known, but estimates suggest that the pigs were in positive fluid balance, even at the lower infusion regimen.

1.3 Surgical procedure

The pig was scrubbed with chlorhexidine (20 mg/ml) (Sage Products, The Netherlands) and covered with an operation blanket. An intra-arterial line was placed in the left carotid artery for blood sampling and monitoring of physiological parameters and an IV line was placed in the left internal jugular vein for glucose and fluid infusions. Both catheters were inserted through the same cut-down.

Catheter for IP insulin infusion by an insulin pump was inserted through a 2 – 3 cm long caudal-umbilicus incision in the abdominal wall. The tip of the catheter was inserted in the upper right IP region but was not fixed in the stationed position. To avoid coagulation heparin (150 IE) (LEO Pharma A/S, Ballerup, Denmark) was injected in IP space. Opening was closed with medical clamps and catheter was fixed with tape. The bladder was exposed through a small, low laparotomy for the insertion of a bladder catheter. Both cuts were made with a thermocauter to minimise bleeding into the abdominal cavity.

At the end of the experiments, and under full anaesthesia, the pigs were euthanised with an IV overdose of pentobarbital (minimum 100 mg/kg) (pentobarbital NAF, Apotek, Lørenskog, Norway).

2 RESULTS

2.1 Main trial

2.1.1 Raw blood glucose levels after IP insulin boluses

Raw blood glucose levels were measured for 120 minutes after administration of insulin boluses in pigs (n = 7). Fig S1a presents data from all time points, whereas Fig S1b shows specifically data from first 30 minutes.

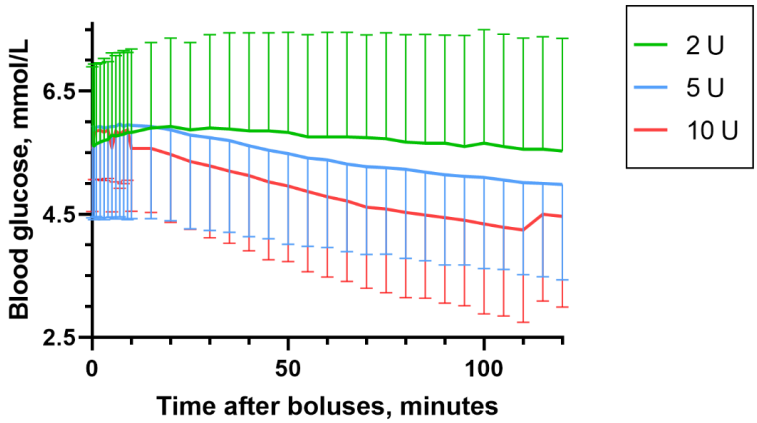


Figure S1a. Blood glucose levels after IP insulin boluses.

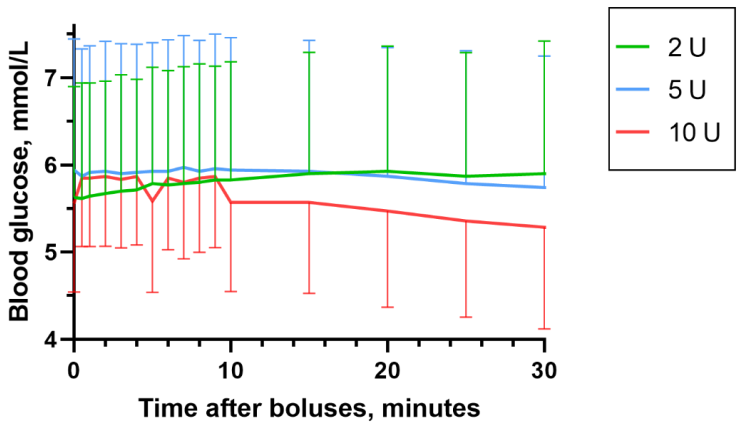


Figure S1b. Blood glucose levels for the first 30 minutes after IP insulin boluses.

2.1.2 Raw insulin levels after IP insulin boluses

Raw insulin levels were measured for 120 minutes after administration of insulin boluses in pigs (n = 7). Fig S2a presents data from all time points, whereas Fig S2b shows specifically data from first 30 minutes.

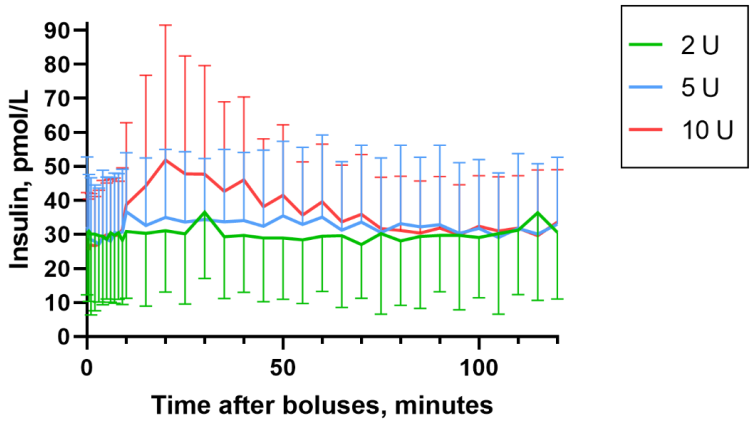


Figure S2a. Insulin levels after IP insulin boluses.

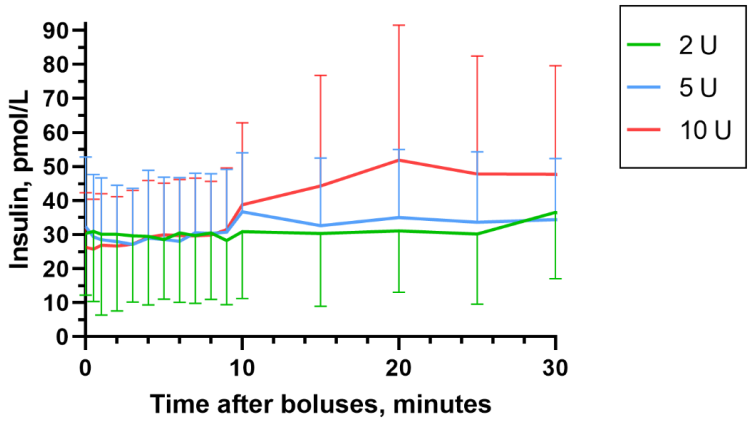


Figure S2b. Insulin levels for the first 30 minutes after IP insulin boluses.

2.2 Additional trial

2.2.1 Raw blood glucose levels after SC insulin boluses

Data from raw blood glucose levels are presented in Fig S3. The glucose infusion rate was increased in one of the pigs during two separate periods due to hypoglycaemia. Additionally, all pigs (n = 3) received an IP glucagon bolus (150 µg) 40 minutes after the insulin bolus.

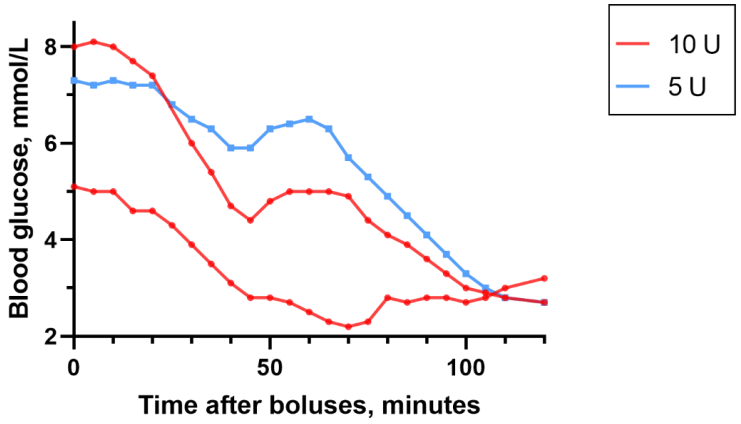


Figure S3. Blood glucose levels after SC insulin boluses.

2.2.2 Raw insulin levels after SC insulin boluses

Data from raw insulin levels are presented in Fig S4.

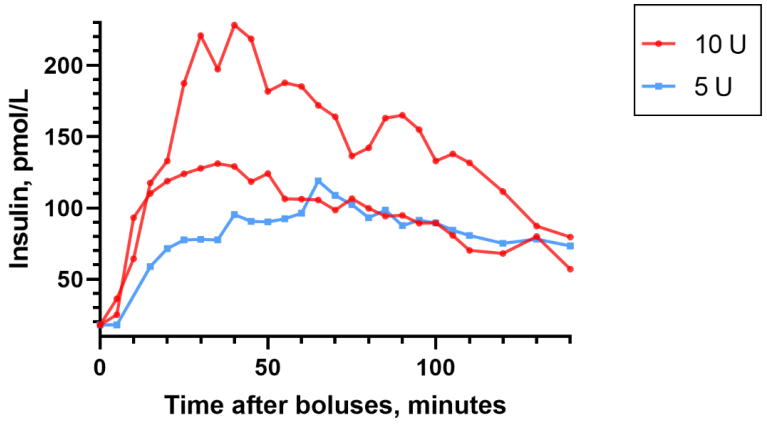


Figure S4. Insulin levels after SC insulin boluses.

Duration of presented measurements differ between blood glucose and insulin levels due the start of glucose infusion in the pigs 120 minutes after insulin boluses.

2.2.3 Insulin delta values after SC insulin boluses

Insulin delta values are presented in Fig S5.

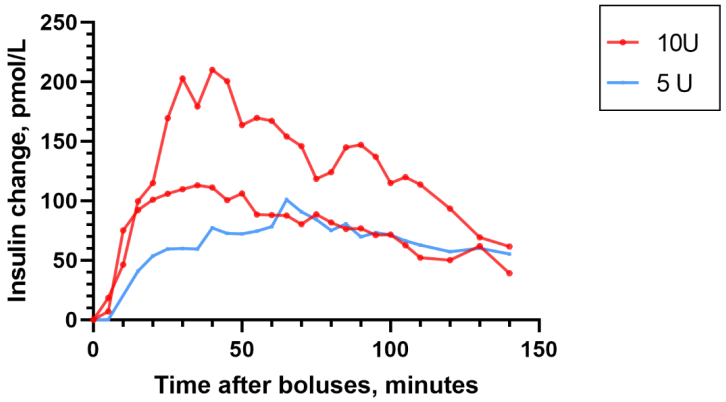


Figure S5. Insulin levels change after SC insulin boluses.

2.3 Comparison between Intraperitoneal and subcutaneous insulin boluses

2.3.1 Raw data comparison between IP and SC insulin boluses

Raw data comparison between IP insulin boluses (n = 7) and SC insulin boluses is presented in Fig S6.

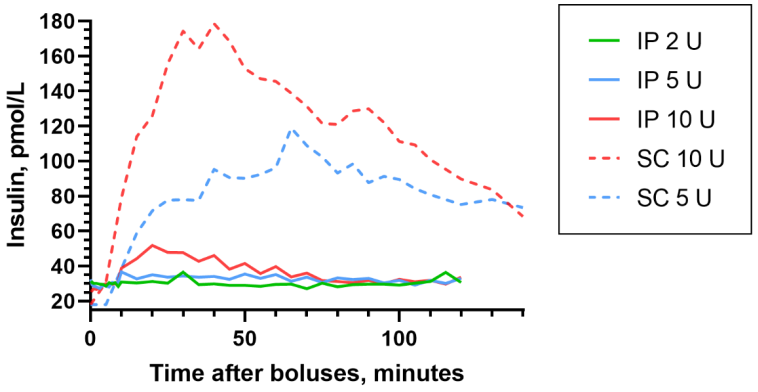


Figure S6. Insulin levels after IP and SC insulin boluses.

REFERENCES

1. Åm, M.K., et al., *Intraperitoneal and subcutaneous glucagon delivery in anaesthetized pigs: effects on circulating glucagon and glucose levels.* Sci Rep, 2020. **10**(1): p. 13735.

Paper III

BMJ Open Diabetes Research & Care

Intraperitoneal, subcutaneous and intravenous glucagon delivery and subsequent glucose response in rats: a randomized controlled crossover trial

Ilze Dirnena-Fusini,[1] Marte Kierulf Åm,[1,2] Anders Lyngvi Fougner,[3] Sven Magnus Carlsen,[1,2] Sverre Christian Christiansen[1,2]

To cite: Dirnena-Fusini I, Åm MK, Fougner AL, *et al*. Intraperitoneal, subcutaneous and intravenous glucagon delivery and subsequent glucose response in rats: a randomized controlled crossover trial. *BMJ Open Diab Res Care* 2018;**6**:e000560. doi:10.1136/bmjdrc-2018-000560

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2018-000560).

ID-F and MKÅm are joint first authors.

Data from this study were presented as an abstract and poster at the 11th International Conference on 'Advanced Technology and Treatment for Diabetes', February 14–17, 2018, Vienna, Austria.

Received 28 May 2018
Revised 31 August 2018
Accepted 6 October 2018

For numbered affiliations see end of article.

Correspondence to
Ilze Dirnena-Fusini;
ilze.dirnena-fusini@ntnu.no

ABSTRACT

Objective Hypoglycemia is a frequent and potentially dangerous event among patients with diabetes mellitus type 1. Subcutaneous glucagon is an emergency treatment to counteract severe hypoglycemia. The effect of intraperitoneal glucagon delivery is sparsely studied. We performed a direct comparison of the blood glucose response following intraperitoneally, subcutaneously and intravenously administered glucagon.

Research design and methods This is a prospective, randomized, controlled, open-label, crossover trial in 20 octreotide-treated rats. Three interventions, 1 week apart, in a randomized order, were done in each rat. All 20 rats were given intraperitoneal and subcutaneous glucagon injections, from which 5 rats were given intravenous glucagon injections and 15 rats received placebo (intraperitoneal isotonic saline) injection. The dose of glucagon was 5 µg/kg body weight for all routes of administration. Blood glucose levels were measured before and until 60 min after the glucagon/placebo injections.

Results Compared with placebo-treated rats, a significant increase in blood glucose was observed 4 min after intraperitoneal glucagon administration (p=0.009), whereas after subcutaneous and intravenous glucagon administration significant increases were seen after 8 min (p=0.002 and p<0.001, respectively). In intraperitoneally treated compared with subcutaneously treated rats, the increase in blood glucose was higher after 4 min (p=0.019) and lower after 40 min (p=0.005) and 50 min (p=0.011). The maximum glucose response occurred earlier after intraperitoneal compared with subcutaneous glucagon injection (25 min vs 35 min; p=0.003).

Conclusions Glucagon administered intraperitoneally gives a faster glucose response compared with subcutaneously administered glucagon in rats. If repeatable in humans, the more rapid glucose response may be of importance in a dual-hormone artificial pancreas using the intraperitoneal route for administration of insulin and glucagon.

Significance of this study

What is already known about this subject?

- Glucagon is usually given subcutaneously in patients with diabetes mellitus type 1 to treat severe hypoglycemia.
- Glucagon has also been used in dual-hormone artificial pancreas with some improvement in glucose control.

What are the new findings?

- Glucagon injected intraperitoneally gives a higher glucose response 4 min after administration and affects blood glucose for a shorter period compared with subcutaneous injection.

How might these results change the focus of research or clinical practice?

- The present results should encourage research on the feasibility of combined intraperitoneal administration of insulin and glucagon as part of an artificial pancreas in humans.

INTRODUCTION

Patients with diabetes mellitus type 1 (DM1) are treated with either repeated or continuous subcutaneous delivery of insulin to counteract hyperglycemia. Improved glucose control is important, as chronic hyperglycemia may induce neuropathy, nephropathy, retinopathy, and cardiovascular diseases.[1 2] Achieving euglycemia is challenging due to slow absorption and delayed glucose-lowering effect of subcutaneously administrated insulin. This makes it difficult to achieve optimal postprandial glucose control without the risk of subsequent hypoglycemia.[3] Repeated and frequent episodes of hypoglycemia are associated with impaired neuroendocrine counter-regulation and symptom perception and deterioration of cerebral functions and may lead to hypoglycemia unawareness.[4] Therefore, the central nervous system's adaptation to frequent short-term hypoglycemias may contribute to the increased incidence of severe hypoglycemia.[5] Despite many small improvements in the treatment of DM1 during the last decades,

hypoglycemia remains a challenge for many patients with DM1.[6–8]

Glucagon is used for treating severe hypoglycemia when patients with DM1 are unconscious and unable to consume carbohydrates. The standard treatment for adults is an intramuscular, intravenous or subcutaneous injection of 1 mg of glucagon. Whether this dose is optimal for all routes of administration has hardly been studied.[9 10] The glucose increasing effect depends on the dose of the injected glucagon,[11] amount of liver glycogen[12] and baseline blood glucose level.[13] When studied in healthy men, there seems to be no major difference in the glucose effect between intramuscular and subcutaneous administration.[14 15] Recently, smaller glucagon doses have been used with success to avoid mild or impending hypoglycemia in children and adults.[6 11 16] Glucagon used as an emergency nasal spray and nasal powder has been launched as an alternative route of administration, providing a success rate in treating hypoglycemia similar to intramuscular injections.[17–19]

Recent work on algorithm-steered insulin delivery (ie, artificial pancreas (AP)) provides improvements to glucose regulation. Unfortunately, this automatically controlled (closed loop) delivery of insulin carries certain limitations, as nearly all recent developments depend on a double subcutaneous approach, that is, both glucose measurements and insulin delivery are in the subcutaneous tissue. The limitations are due to slow subcutaneous glucose dynamics secondary to both delayed and slow subcutaneous insulin absorption, which unavoidably lead to alternating periods of either a lack or excess of circulating insulin.[20] To solve the challenge of relative insulin excess, some research groups have incorporated glucagon as a counter-regulator in the AP system, to counteract imminent hypoglycemia, that is, a dual hormonal AP.[7 21 22] Despite achieving as low as 3% of time in hypoglycemic range during day and night closed loop control, hypoglycemia still remains a substantial daytime problem also in this subcutaneous dual-hormone approach.[23] Therefore, new routes should be explored to find better solutions for prevention of hypoglycemia.

Intraperitoneal glucagon administration has only been reported from a few animal studies.[24 25]

The main aim of this study was to compare the glucose increasing effect after subcutaneous and intraperitoneal delivery of glucagon, and to investigate the potential for intraperitoneal delivery of small doses of glucagon in an AP. Intravenous delivery of hormones is less realistic in free-living conditions. Therefore, intravenous route was only included in the study as an additional route to obtain more information on glucose dynamics after glucagon delivery, and not included as a main outcome in the paper. We hypothesized that the glucose response is faster after intraperitoneal compared with subcutaneous administration of glucagon. To investigate this hypothesis, we compared the immediate glucose response after intraperitoneal, subcutaneous and intravenous administration of glucagon in an animal model.

RESEARCH DESIGN AND METHODS

Pilot study

A pilot study was performed on 10 rats to refine the experimental protocol and to determine the glucagon dose to be used in the main study. Detailed explanation is available in the online supplementary material.

Animals

In the main study male Sprague Dawley rats (n=20) (initial weight 470–615 g; Janvier Labs, France), in groups of three, were kept in plastic solid bottom cages (515×381×256 mm, Tecniplast, Italy) on sawdust. The rats were acclimatized to the animal facility and maintained on 12-hour light–12-hour dark photoperiod at 20–24°C and a relative humidity of 55%±5%. They were fed expanded pellets (Special Diets Services RM1 for rats, UK) and fresh water was available ad libitum. To reduce stress and the possible effect of stress on glucose levels, the rats were trained to accept general handling and use of a restrainer (Harvard Apparatus, Holliston, USA) for 3 weeks prior to the start of experiments.

Intervention groups and randomization

The assignment to intervention groups (n=20) and the order of procedures in each rat were randomized by creating random permutations of treatment and intervention groups. The glucagon dose was 5 µg/kg body weight (BW) for all interventions except placebo. All rats (n=20) received intraperitoneal and subcutaneous injection of glucagon, 15 of the rats received placebo intraperitoneal injections of 1 mL/kg BW of isotonic saline. The volume of placebo injection (1 mL/kg BW) was similar to the intraperitoneal glucagon injection (approximately 500 µL). To obtain information also after intravenous delivery of glucagon, five of the rats were administered intravenous glucagon (see online supplementary tables 2a and b). There was at least 1 week between each test procedure on each rat. To avoid bias based on metabolic individualities, trials were performed in the 12-hour light period, and all procedures in each individual rat were done at approximately the same time as of the light cycle. Group size was determined by the resource equation method.[26]

Technical challenges

The rats were monitored for the entire sampling period (70 min) and surveyed for signs of stress. Except when the rats were anesthetized, they were kept in restrainers to facilitate blood sampling. Restrainers of two different sizes were tested before the start of the experiment. For most of the rats, the restrainers were either too large or too small. Thus, the larger restrainer was used for all rats, and a paper tissue was rolled up and taped vertically to the inside of the restrainer at a level behind the rat's shoulder, to prevent the smaller rats from turning around inside the restrainer.

Procedures

Food was removed 1 hour before the start of the procedure and water was available ad libitum. The individual

glucagon and octreotide doses were based on the animal's weight on the day of the procedure.

Endogenous glucagon secretion

To suppress the endogenous glucagon and insulin secretion during the procedures, all rats received two subcutaneous injections of 10 µg/kg BW octreotide (Sandostatin 200 µg/mL, Novartis Europharm, UK). The first injection was given approximately 30 min before the start of each procedure and the second at the time of glucagon/placebo injection. Octreotide was given subcutaneously in the neck, but not at the same location as the subcutaneous glucagon injection.

Anesthesia

To prevent accidental movements in the time of the procedure, the rats were anesthetized with isoflurane (Isoflurane, Baxter, Oslo, Norway; 5% IF, 95% air in chamber; 2% IF, 95% air on face mask) for two short intervals at the start of each procedure. During the first anesthesia period, a cut in the tail for collection of blood samples was made. During the second anesthesia period, an injection of glucagon or placebo was given. When required, additional anesthesia was provided to rats showing signs of stress while kept in the restrainer.

Glucagon challenge

Glucagon (Glucagon, Novo Nordisk, Denmark) was diluted by 0.9% NaCl to a concentration of 5 µg/mL and the rats were given 5 µg/kg BW. Glucagon solutions were kept in a refrigerator and used the same day they were made. Solutions were warmed to approximately body temperature just before administration. Subcutaneous glucagon was injected at the back of the neck, and intraperitoneal glucagon and placebo (an equal volume of 0.9% NaCl) in the lower part of the abdomen, with the rat held at an angle after its hind legs. Intravenous glucagon was given in the lateral tail vein that was not currently used for blood sampling.

Glucose measurement

After disinfecting the skin, a 6–9 mm cut was made with a straight-edged scalpel over the lateral tail vein two-thirds down the length of the tail for blood sampling. Samples were collected 10, 5 and 1 min prior to glucagon injection, and 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, and 60 min after the glucagon or placebo injections. Whenever needed, the vein was carefully reopened with the tip of the scalpel to ensure sufficient blood flow for sampling.

Blood for glucose analyses was collected directly in heparinized capillary tubes (35 µL, Clinitubes, Radiometer Medical ApS, Brønshøj, Denmark), and stored on ice for a maximum of 30 min before analysis on a blood gas analyzer (Radiometer ABL 725, Radiometer Medical ApS). To ensure sufficient blood flow for sampling, the vein was gently stroked from the base of the tail and toward the wound, and the first small drop of blood was removed. For the third intervention, both veins had been used for sampling at former trials, and the new cut was made proximal to the older cut. Occlusion of the rat's tail vein occurred in only one rat, and in this case the vein on the other side of the tail was used.

Animal welfare

The rats were given non-steroidalanti-inflammatory drugs (Metacam vet, Boehringer Ingelheim Vetmedica) 1 mg/kg BW as a single subcutaneous injection at the end of the two first procedures. A suture, to close the wound and stop the bleeding at the end of the procedure, was necessary in 19 cases. The wounds healed well after sampling regardless of the wound being sutured or not, and no wound infections were observed. After the third procedure, the rats were euthanized with an intravenous injection of pentobarbital (100 mg/kg) (Norges Apotekerforening, Norway) under isoflurane anesthesia.

Statistical analysis

The relationship between glucose levels and time was analyzed for all interventions using a mixed linear model with the combination of time and treatment as the fixed effect. The dependent variable was defined as log glucose concentration to achieve normal distribution. To account for multiple measurement series on each rat, rat identification was included as a random effect. To account for dependence within each series, the error term for each series was specified as a first-order autoregressive process AR (1) series accounting for minutes between measurements. Mean changes in glucose concentrations from −1 min to 2–60 min for the four treatments were compared using the Wald test. Maximum concentration and time until maximum concentration of the estimated model for the treatments were compared using the Mann-Whitney U test. To eliminate the effect of placebo intervention on the glucose response, the mean value of the 15 placebo interventions was subtracted from the mean value of the 20 subcutaneous and intraperitoneal interventions and the mean value of the 5 intravenous interventions at the given time points. All interventions are compared as models; therefore, comparison between unequal groups is allowed.[27] The software package R was used to analyze the data.[28] All values in the text are given as mean±SE of the mean, unless stated otherwise. Differences between the group means were considered statistically significant at a threshold of $p \leq 0.05$.

RESULTS

In general, the rats stayed calm during the experimental procedures. Thirteen incidents occurred during 60 procedures, in which the rats turned around inside the restrainers or showed signs of stress and consequently were taken out of the restrainer and repositioned. A similar number of incidences were found in all interventions (four during the intraperitoneal, four during the subcutaneous and three during the intravenous intervention). These incidents included two rats in whom stress was observed during three procedures (intraperitoneal, subcutaneous and intravenous). Two incidents of stress

were observed during the placebo procedure; however, no increase in blood glucose levels from baseline was observed (data not shown).

Additional anesthesia during blood sampling was needed during 10 intraperitoneal, 10 subcutaneous, 5 intravenous, and 5 placebo interventions, and the mean±SD time in anesthesia was 13.8±5.5 min (14.05±5.47, 13.55±4.37, 20±9.43 and 12±4.85, respectively). The rats were conscious for the rest of the 70 min procedure. After individually analyzing data from the 16 rats which received the longest duration of anesthesia (time in anesthesia 15–31 min, subcutaneous n=5, intraperitoneal n=5, placebo n=3 and intravenous n=3), only two rats (subcutaneous intervention, n=2) showed prolonged elevated glucose levels and no decrease of glucose values at the end of the intervention (at 60 min) (data not shown).

Glucose level

For calculation of glycemic state for rats at the beginning of the interventions, a mean baseline glucose was calculated according to the mean of three measurements preceding the intervention (–10, –5 and –1 min), and in addition a mean±SD in each intervention group was calculated. Blood glucose levels at the beginning of the intraperitoneal, subcutaneous, intravenous and placebo interventions were 6.72±0.90, 6.47±0.81, 6.17±1.12 and 6.51±0.81 mmol/L, respectively (see online supplementary figure 1).

Compared with placebo, glucose was significantly increased 4 min after intraperitoneal glucagon injection (p=0.009, n=20), and 8 min after subcutaneous (p=0.002, n=20) and intravenous (p<0.001, n=5) injections (figure 1).

Comparing intraperitoneal glucagon injections with subcutaneous, the glucose increase after intraperitoneal glucagon was significantly higher at 4 min (p=0.019) and significantly lower at 40 (p=0.005) and 50 min (p=0.011) (figure 1). Comparing intravenous glucagon injections with intraperitoneal, the glucose increase after intravenous injection was significantly higher at 20 min (p=0.001). At the other time points, no significant differences were observed.

An increase in glucose levels was seen after all three routes of glucagon delivery, but there was no significant difference (p=0.52) in absolute maximum blood glucose value after intraperitoneal glucagon injection (9.74 mmol/L) compared with subcutaneous injection (10.3 mmol/L). The estimated time until the maximum glucose value was significantly shorter (p=0.003) after intraperitoneal glucagon injection (25 min) versus subcutaneous glucagon injection (35 min) (see online supplementary figure 1).

DISCUSSION

The results of this study indicate that the glucose response in rats comes earlier when glucagon is injected

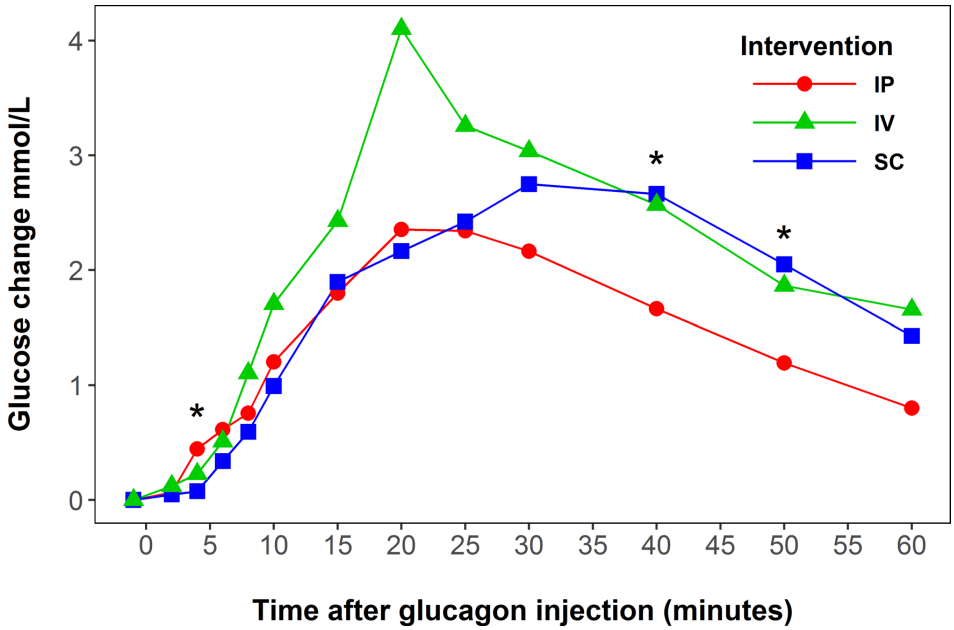


Figure 1 Estimated glucose delta values (mmol/L) after glucagon injection (5 µg/kg) in octreotide-treated rats. Green line represents intravenous (IV) intervention, blue line represents subcutaneous (SC) intervention, and red line represents intraperitoneal (IP) intervention. The glucose response for the placebo group has been subtracted from all groups presented in the figure. *Represents significant difference between intraperitoneal and subcutaneous of glucagon delivery. Note: For one rat in intraperitoneal intervention, delta values were calculated using the –5 minute measurement (–1 minute for other rats).

intraperitoneally than when injected subcutaneously. Second, the maximum effect of glucose increase appears earlier, and the glucose response diminishes faster after intraperitoneal compared with subcutaneous glucagon injection.

The peritoneal lining is highly vascularized, and the blood capillary density in the peritoneal lining varies between individuals (higher amount in infants (0–1 year) and adults, and lower amount in children).[29] There is no systematic variation in histological parameters in different parts of the peritoneum.[29 30] Compared with subcutaneous absorption, peritoneal absorption may be faster due to a shorter distance to reach the capillaries and easier diffusion into the bloodstream. From animal studies, we know that most of the intraperitoneally injected insulin enters the portal vein and passes the liver before entering the systemic circulation.[31–33] Consequently, after intraperitoneal delivery, glucagon probably reaches the liver both earlier and at a higher concentration and thereby promotes hepatic gluconeogenesis earlier as compared with subcutaneously injected glucagon. This is compatible with our observation of faster glucose increase after intraperitoneal injection of glucagon compared with other injection routes. It also fits well with the observed earlier maximum glucose response after intraperitoneally injected compared with subcutaneously injected glucagon. Interestingly, time until maximum blood glucose increase after subcutaneous injection in our animal model is similar to what is observed in humans with diabetes.[11]

Our finding of an earlier rise in blood glucose after intraperitoneally injected glucagon, compared with subcutaneous injection, is difficult to compare with previous studies as blood glucose was measured at different time points and intervals. In previous studies, blood glucose was only measured 20[24] and 30[25] min after glucagon injection. Moreover, the study by Zlotnik *et al*[25] provided only data of intraperitoneal glucagon injection.

In the present study, blood glucose was lower after intraperitoneal compared with subcutaneous injection of glucagon at time points 40 and 50 min. Fifty minutes after glucagon injection, a declining blood glucose was observed in all routes (intraperitoneal, subcutaneous and intravenous) of administration. This differs from a previous study in rats,[25] where after intraperitoneal injections of glucagon a significant rise in glucose levels was observed after 30, 60, 90 and 120 min. However, after 60–90 min, a flattening of the blood glucose curve was observed and at 120 min blood glucose subsequently decreased. This discrepancy between the previous and the present results may depend on the fact that in the previous study, rats were anesthetized during the whole procedure with isoflurane,[25] while in the present study we limited isoflurane use as much as possible (see online supplementary material). We also treated the rats with octreotide to inhibit endogenous release of insulin and glucagon during the experiments. Interestingly, in the previous study no difference in plasma glucose was observed in the control group, while in our pilot study, with extended use of isoflurane, we observed an increase in glucose levels (see online supplementary material). A glucose increasing effect of isoflurane has been described previously.[34]

Loxham *et al*[24] demonstrated results similar to our study, where, after intraperitoneal injection of glucagon in non-diabetic rats, the glucose response after intraperitoneal administration was higher after 20 min and lower after 45 min compared with subcutaneous administration. Baseline glucose levels were also similar to ours. However, the authors did not mention whether anesthetics were used.[24] Noteworthy, Loxham *et al*[24] suggested that different strains of rats may react differently to a sudden rise in counter-regulators (in this case glucagon), making comparison between different strains of rats difficult.

Our glucagon dose of 5 µg/kg BW was only 2.5% of the dose used by Loxham *et al*[24] (200 µg/kg) and only 1% of the dose used by Zlotnik *et al*[25] (50 µg/100 g). We do not have information about why these particular doses were chosen. Another aspect is that previous authors used naïve rats, whereas the rats in the present study were treated with octreotide.[35] Our study provides information about possible doses of glucagon with which glucagon saturation is reached (see online supplementary material). It seems that in our study a glucagon dose of 5 µg/kg BW was appropriate (see online supplementary material) based on the observed blood glucose increase of around 3 mmol/L in all injection routes.

Our study is not the first to explore the effect of smaller doses of glucagon. The effect of smaller doses than the standard 1 mg of glucagon (commonly used in cases of serious hypoglycemia) has recently been examined in humans.[6 11] Glucagon may induce nausea and vomiting, and these side effects may be related to the size of the injected dose and the subsequent higher levels of glucagon in the systemic circulation. In patients with DM1 there seems to be a dose–response relationship between subcutaneous glucagon doses ranging from 0.11 mg to 1.0 mg and the glucose response.[11] Mini-doses of glucagon are effective in treating mild to moderate hypoglycemic episodes in both children[6 36] as well as adults.[16]

The motivation for performing this study was to investigate and compare different administration routes of glucagon and explore if glucagon administration intraperitoneally would provide some benefits compared with subcutaneous injection, aspects of importance for the development of a dual hormonal AP. Ideally, a dual-hormone AP should prevent hypoglycemias with small and, if necessary, repeated doses of glucagon.[7 37] Minimizing the amount of exogenous glucagon needed to counteract hypoglycemia is important to avoid the depletion of liver glycogen, to reduce the side effects, such as nausea and vomiting, and to avoid reactive hyperglycemia.[11]

A small dip in glucose values was observed prior to the injection of glucagon or saline, that is, during all procedures (see online supplementary figure 1). The reason

for this is unclear, but the equal response during all procedures is an indication of consistency of the experimental protocol for all the procedures during the first part of the experiment and thereby a sign of quality of the present study.

Strengths and limitations of the study

The following are the strengths of the present study: (1) placebo-treated animals—the importance is illustrated by the fact that, although not significantly, the glucose levels tended to fluctuate also during the placebo procedures; (2) limited use of anesthesia, which may have major impact on glucose homeostasis; (3) systematic training of the rats to the procedures for weeks ahead of the test procedures (both pilot rats and trial rats) to minimize the stress response during the test procedures; and (4) randomization of the order of treatment in each rat.

The following are among the limitations of the present study: (1) With unguided injections into the abdominal cavity, we cannot be sure that all the glucagon or placebo was administrated in the peritoneal space. However, glucagon and placebo saline were injected by the same procedure in anesthetized rats (injection could be done without experiencing unexpected movements of rat); therefore, possible deviation from intraperitoneal delivery should be equal between groups. (2) A few rats were stressed during the procedures, which might affect the blood glucose levels. However, in rats receiving placebo intervention, under signs of stress, the blood glucose level did not increase significantly (see online supplementary material). (3) Additional anesthesia was needed for some rats in all interventions; however, as it was described in the results, prolonged increased glucose levels were observed only in 2 out of the 16 rats which were exposed to the longest duration of anesthesia in the main study. Therefore glucose level increase at the end of the intervention can be individual response, not anesthesia-induced. (4) Rats were fasted differently (between 1 and 3 hours) depending on the order of performing the procedure (intraperitoneal, subcutaneous, intravenous or placebo). However, to avoid bias, all experiments on the same rat were conducted at approximately the same time as of the light cycle.

CONCLUSION

Blood glucose increased faster when glucagon was injected in the peritoneal cavity compared with subcutaneous glucagon delivery in octreotide-treated rats. The maximum glucose response was reached earlier and the decline in glucose response was also faster. If repeatable in humans, a more rapid glucose response may be of importance in a dual-hormone AP using the intraperitoneal route for administration of insulin and glucagon.

Author affiliations
[1]Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU - Norwegian University of Science and Technology, Trondheim, Norway
[2]Department of Endocrinology, St Olavs Hospital, Trondheim, Norway
[3]Department of Engineering Cybernetics, Faculty of Information Technology and Electrical Engineering, NTNU - Norwegian University of Science and Technology, Trondheim, Norway

Acknowledgements The animal experiments were conducted at the Comparative Medicine Core Facility (CoMed), Norwegian University of Science and Technology (NTNU). CoMed is funded by the Faculty of Medicine and Health Sciences at NTNU and the Central Norway Regional Health Authority. We thank Associate Professor Øyvind Salvesen, Faculty of Medicine and Health Sciences, NTNU, for help with the statistical analysis, and Gita Nagarajah for her work on the glucose analysis.

Contributors ID-F and MKÅ completed the trial, collected and analyzed the data, wrote and edited the manuscript, and are the guarantors of the work. ALF, SMC and SCC contributed to the discussion, and reviewed and edited the manuscript. All authors contributed to the development of the protocol.

Funding The Norwegian Research Council (NRC) is funding the Double Intraperitoneal Artificial Pancreas project (project number 248872) as part of the Centre for Digital Life Norway (digitallifenorway.org). The study is also supported by a scholarship from the Central Norway Regional Health Authority (grant nr 2014/23166) and the Norwegian Medical Association Johan Selmer Kvanes Endowment. The funding sources had no role in the collection, analysis or interpretation of the data.

Competing interests None declared.

Patient consent Not required.

Ethics approval The study was approved by the Norwegian Food Safety Authority (FOTS-ID 10922) and was in accordance with 'The Norwegian Regulation on Animal Experimentation' and 'Directive 2010/63/EU on the protection of animals used for scientific purposes'.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Additional online data supplement is available.

REFERENCES

1. Shaikh M, Shaikh B, Shah NA. Comparative study on lipid changes in glycemic uncontrolled diabetes type 1 and type 2. *J Liaquat Uni Med Health Sci* 2009;8:201–4.
2. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. The DCCT Research Group. *Am J Med* 1991;90:450–9.
3. Guerci B, Sauvanet JP. Subcutaneous insulin: pharmacokinetic variability and glycemic variability. *Diabetes Metab* 2005;31:4S7–24.
4. Lingenfelser T, Overkamp D, Renn W, *et al*. Insulin-associated modulation of neuroendocrine counterregulation, hypoglycemia perception, and cerebral function in insulin-dependent diabetes mellitus: evidence for an intrinsic effect of insulin on the central nervous system. *J Clin Endocrinol Metab* 1996;81:1197–205.
5. Lingenfelser T, Renn W, Sommerwerck U, *et al*. Compromised hormonal counterregulation, symptom awareness, and neurophysiological function after recurrent short-term episodes of insulin-induced hypoglycemia in IDDM patients. *Diabetes* 1993;42:610–8.
6. Hartley M, Thomsett MJ, Cotterill AM. Mini-dose glucagon rescue for mild hypoglycaemia in children with type 1 diabetes: the Brisbane experience. *J Paediatr Child Health* 2006;42:108–11.
7. Haidar A, Rabasa-Lhoret R, Legault L, *et al*. Single- and dual-hormone artificial pancreas for overnight glucose control in type 1 diabetes. *J Clin Endocrinol Metab* 2016;101:214–23.
8. Jacobs PG, El Youssef J, Reddy R, *et al*. Randomized trial of a dual-hormone artificial pancreas with dosing adjustment during exercise compared with no adjustment and sensor-augmented pump therapy. *Diabetes Obes Metab* 2016;18:1110–9.
9. White M, Zacharin MR, Werther GA, *et al*. Intravenous glucagon in a deliberate insulin overdose in an adolescent with type 1 diabetes mellitus. *Pediatr Diabetes* 2016;17:66–9.
10. Blauw H, van Bon AC, Koops R, *et al*. Performance and safety of an integrated bihormonal artificial pancreas for fully automated glucose control at home. *Diabetes Obes Metab* 2016;18:671–7.

11. Blauw H, Wendl I, DeVries JH, *et al*. Pharmacokinetics and pharmacodynamics of various glucagon dosages at different blood glucose levels. *Diabetes Obes Metab* 2016;18:34–9.
12. Castle JR, Engle JM, El Youssef J, *et al*. Factors influencing the effectiveness of glucagon for preventing hypoglycemia. *J Diabetes Sci Technol* 2010;4:1305–10.
13. Rivera N, Ramnanan CJ, An Z, *et al*. Insulin-induced hypoglycemia increases hepatic sensitivity to glucagon in dogs. *J Clin Invest* 2010;120:4425–35.
14. Graf CJ, Woodworth JR, Seger ME, *et al*. Pharmacokinetic and glucodynamic comparisons of recombinant and animal-source glucagon after IV, IM, and SC injection in healthy volunteers. *J Pharm Sci* 1999;88:991–5.
15. Mühlhauser I, Koch J, Berger M. Pharmacokinetics and bioavailability of injected glucagon: differences between intramuscular, subcutaneous, and intravenous administration. *Diabetes Care* 1985;8:39–42.
16. Haymond MW, DuBose SN, Rickels MR, *et al*. Efficacy and safety of mini-dose glucagon for treatment of nonsevere hypoglycemia in adults with type 1 diabetes. *J Clin Endocrinol Metab* 2017;102:2994–3001.
17. Boido A, Ceriani V, Pontiroli AE. Glucagon for hypoglycemic episodes in insulin-treated diabetic patients: a systematic review and meta-analysis with a comparison of glucagon with dextrose and of different glucagon formulations. *Acta Diabetol* 2015;52:405–12.
18. Rickels MR, Ruedy KJ, Foster NC, *et al*. Intranasal glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 diabetes: a randomized crossover noninferiority study. *Diabetes Care* 2016;39:264–70.
19. Sherr JL, Ruedy KJ, Foster NC, *et al*. Glucagon nasal powder: a promising alternative to intramuscular glucagon in youth with type 1 diabetes. *Diabetes Care* 2016;39:555–62.
20. Christiansen SC, Fougner AL, Stavdahl Ø, *et al*. A review of the current challenges associated with the development of an artificial pancreas by a double subcutaneous approach. *Diabetes Ther* 2017;8:489–506.
21. Taleb N, Emami A, Suppere C, *et al*. Efficacy of single-hormone and dual-hormone artificial pancreas during continuous and interval exercise in adult patients with type 1 diabetes: randomised controlled crossover trial. *Diabetologia* 2016;59:2561–71.
22. Haidar A, Messier V, Legault L, *et al*. Outpatient 60-hour day-and-night glucose control with dual-hormone artificial pancreas, single-hormone artificial pancreas, or sensor-augmented pump therapy in adults with type 1 diabetes: an open-label, randomised, crossover, controlled trial. *Diabetes Obes Metab* 2017;19:713–20.
23. Russell SJ, El-Khatib FH, Sinha M, *et al*. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N Engl J Med* 2014;371:313–25.
24. Loxham SJ, Teague J, Poucher SM, *et al*. Glucagon challenge in the rat: a robust method for the in vivo assessment of Glycogen phosphorlyase inhibitor efficacy. *J Pharmacol Toxicol Methods* 2007;55:71–7.
25. Zlotnik A, Gruenbaum BF, Klin Y, *et al*. The effects of insulin, glucagon, glutamate, and glucose infusion on blood glutamate and plasma glucose levels in naive rats. *J Neurosurg Anesthesiol* 2011;23:323–8.
26. Charan J, Kantharia ND. How to calculate sample size in animal studies? *J Pharmacol Pharmacother* 2013;4:303–6.
27. Engle RF. wald, likelihood ratio, and lagrange multiplier tests in econometrics. In: . *Handbook of econometrics*. Elsevier, 1984: 775–826.
28. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2017.
29. Schaefer B, Bartosova M, Macher-Goeppinger S, *et al*. quantitative histomorphometry of the healthy peritoneum. *Sci Rep* 2016;6:21344.
30. Slater NJ, Raftery AT, Cope GH. The ultrastructure of human abdominal mesothelium. *J Anat* 1989;167:47–56.
31. Fischer U, Freyse EJ, Salzsieder E, *et al*. Artificial connection between glucose sensing and insulin delivery: implications of peritoneal administration. *Artif Organs* 1992;16:151–62.
32. Fritze K, Fischer U, Freyse EJ, *et al*. Intraindividual comparison of pharmacokinetics of insulin after intravenous, portal, subcutaneous and peritoneal administration. *Exp Clin Endocrinol* 1988;92:297–306.
33. Schade DS, Eaton RP, Davis T, *et al*. The kinetics of peritoneal insulin absorption. *Metabolism* 1981;30:149–55.
34. Wren-Dail MA, Dauchy RT, Blask DE, *et al*. Effect of isoflurane anesthesia on circadian metabolism and physiology in rats. *Comp Med* 2017;67:138–46.
35. Schmid HA, Brueggen J. Effects of somatostatin analogs on glucose homeostasis in rats. *J Endocrinol* 2012;212:49–60.
36. Hasan KS, Kabbani M. Mini-dose glucagon is effective at diabetes camp. *J Pediatr* 2004;144:834.
37. Castle JR, Engle JM, El Youssef J, *et al*. Novel use of glucagon in a closed-loop system for prevention of hypoglycemia in type 1 diabetes. *Diabetes Care* 2010;33:1282–7.

Supplementary material

Study prior study

Preceding the main trial, we tried to obtain missing information through additional experiments. We tried to define the lowest glucagon dose that could increase blood glucose level in euglycaemic octreotide rats. We aimed at a blood glucose increase of 1 – 3 mmol/l as compared to baseline. By the way of frequent blood sampling, we tried to obtain the exact time of blood glucose level increase and decrease as well as time until maximum blood glucose value. Therefore, blood samples were taken as often as it technically was possible, taking into account animal welfare. Therefore, blood samples were taken at -10, -5, -1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 50, 60 minutes, if not otherwise mentioned.

Animals

Male Sprague Dawley rats (n=10) (483–569 g; Janvier Labs, France) were kept in plastic solid bottom cages (515x381x256 mm, Techniplast, Italy) on sawdust in groups of three. They were acclimatized to the animal facility and maintained on 12 hours light – 12 hours dark photoperiod at 20-24°C and relative humidity 55 ± 5%. Food in the form of expanded pellets (Special Diets Services RM1 for rats, UK) and fresh water was provided and was available ad libitum. Rats were trained to accept general handling and use of a restrainer (Harvard apparatus, Holliston, USA) for three weeks prior to the start of experiments. In table 1 we show the intervention sessions after the start of the pilot study. There were at least 7 days in between each intervention, and all rats had at least 2 interventions.

Nr. of rat	**Intervention sessions**	**Nr. of rat**	**Intervention sessions**
1	1,5,7	6	2,6
2	1,5,9	7	3,7
3	1,5	8	3,7
4	2,6	9	4,8
5	2,6	10	4,8

Table 1. Rat inclusion in test procedures and days on inclusion.

Results

On the first session (6 of March), three rats received subcutaneous (SC) octreotide (to suppress the endogenous glucagon and insulin secretion) with dose 10 µg/kg and glucagon as an intraperitoneal injection (IP) where rat 1 received 25 µg/kg, rat 2 received 50 µg/kg and rat 3 received 100 µg/kg. Their blood glucose maximum value was 18.5 (rat 1), 15.2 (rat 2) and 17.4 mmol/L (rat 3), after 55, 60 and 55 minutes respectively. For comparison, baseline blood glucose value for all 3 rats were 5.0 ± 0.62 mmol/L (mean±SD).

On the second session (8 of march), we performed SC octreotide and IP glucagon injections in another three rats (rat 4, 5 and 6), we injected IP glucagon - 1µg/kg in rat 4 and 10 µg/kg in rat 5, and as control (placebo) we measured blood glucose without any injection in rat 6. We observed maximum glucose

value increase after 50 minutes, 17.9 mmol/L in rat 4 and 17.7 mmol/L in rat 5. However, unexpectedly, maximum blood glucose value in placebo rat (rat 6) was 11.1 mmol/L, and this maximum value was the last measurement, 60 minutes after intervention. Baseline blood glucose value for all 3 rats were 5.2 ± 0.64 mmol/L (mean±SD).

On the third session (9 of March), based on previous results, we repeated octreotide injection and glucagon dose 1 μg/kg in rat 7 but in this case subcutaneously (SC) and tried dose 10 times lower – 0.1 μg/kg injected IP in rat 8. Results again were not satisfying, maximum blood glucose reached 19.1 and 14.1 mmol/L, after 50 and 20 minutes, respectively. Baseline blood glucose value for SC rat (rat 7) 5.8 mmol/L and for IP rat (rat 8) it was 8.4 mmol/L.

On the fourth session (10 of March), rats 9 and 10 were used, octreotide was injected SC and glucagon dose 0.1 μg/kg in rat 9 and 0.05 μg/kg in rat 10 were injected IP, glucose maximum values were as last (60 minutes) measurements of the procedure (12.7 and 11.0 mmol/L, respectively). Baseline blood glucose values were 5.5 and 5.8 mmol/L, respectively.

On the fifth session (13 of March), we injected octreotide SC and glucagon dose 0.05 μg/kg IP in rat 1 and SC in rat 2, to observe if we can obtain different results between two injection sites. And we used placebo rat (rat 3) but this time we injected 0.5 ml physiological saline. This time we increased length of procedure (100 and 70 minutes instead of 60 minutes) for measurements after IP, SC glucagon injection and placebo, respectively. We observed similar maximum glucose value measurements as in previous days, 10.8 (rat 1) and 13.6 mmol/L (rat 2), at 80 and 70 minutes, respectively. However, maximum glucose value could not be measured for SC glucagon injection for the reason that last measurement was highest glucose value (70 min). In placebo rat (rat 3) maximum blood glucose value (13.1 mmol/L) was as well measured as last measurement (70 minutes), therefore maximum blood glucose value was not determined, again. Baseline glucose values for 3 rats were 5.96 ± 0.72 mmol/L (mean±SD).

On the sixth session (15 of March), we made simple interventions with rat 4,5 and 6, rats did not receive octreotide as injection, rat 4 did not get any manipulation, only blood samples were collected, rat 5 received isotonic saline IP and rat 6 received IP glucagon with dose 1 μg/kg. As affecting factor, anaesthetics – isoflurane was used, and blood samples were collected for one hour (every 10 min) for rat 4 and 5. For the rat 6 blood samples were taken as in protocol mentioned previous. In rat 4, blood glucose value increased from 7.3 mmol/L to 14.4 mmol/L. In rat 5, which received isotonic saline as IP injection glucose value increased from 8.1 to 14.1 mmol/L. Therefore, we suspected anaesthesia itself to affect blood glucose. A glucose increasing effect based on anaesthetics use is explained in literature.[1] In rat 6 maximum glucose value (11.4 mmol/L) was measured at 30 minutes, however octreotide was not injected, and insulin level was not measured as well, therefore, it is not adequate to interpret results from this test procedures.

On the seventh session (17 of March), we used 3 rats: Placebo rat (rat 7), who received as minimal isoflurane as it was possible (to make cut in tail for blood samples); placebo rat who received isoflurane and octreotide (rat 8); and rat who received intraperitoneally 25 μg/kg of glucagon (rat 1). In rats 7 and 8 blood glucose level increase were not observed. In rat 1, blood glucose increase was 13.0 mmol/L and was observed after 30 min from beginning of glucagon injection.

On the eight session (22 of March), we tried to identify the best glucagon dose to be used for the main trial. Therefore, we injected glucagon dose 25 μg/kg in rat 9 and tried lower dose – 10 μg/kg in rat 10. We observed maximum values 12.9 mmol/l after 50 minutes and maximum value 13.0 mmol/l at 25 minutes, respectively. We obtained similar blood glucose value increase with different glucagon dose.

On the ninth session (27 of March), we injected glucagon with dose 5 μg/kg in rat 2, and maximum blood glucose value 13.1 mmol/L was reached after 50 min. Therefore, after comparing our results with available literature, we decided for main study to use glucagon dose 5 μg/kg.

Discussion

The appropriate dose of injected glucagon was determined (pilot study) according to a 3 mmol/L increase in BG level. It is interesting to note that the pilot study unexpectedly showed blood glucose to rise prior to exogenous glucagon injection. Our explanation is that long exposure of isoflurane itself increases blood glucose level. This finding is in contrary to that of Zlotnik et al., who did not see differences in plasma glucose levels over time in placebo group (researchers used isoflurane for all the time of experiment).[2] We did not have the opportunity to find out if this was a consequence octreotide treatment itself, as our main trial did not have octreotide-naïve rats in comparison. However, after reviewing available literature, we found evidence of isoflurane`s effect on blood glucose rise.[1] Consequently we minimized isoflurane exposure time during the subsequent trial. After minimizing isoflurane to approximately 14 minutes per procedure a, blood glucose level changes were minimal in the placebo group.

Main study

Detailed information about animals and procedure are given in main article.

After appropriate glucagon dose was chosen, 20 new rats were used and assigned to intervention group and the order of procedure in each rat was randomized. Used block randomization is shown in Table 2.a and 2.b.

RAT ID	INTERVENTION: IV (1), SC (2), IP (3)		
12	1	3	2
13	2	3	1
21	3	1	2
22	1	2	3
23	2	1	3

Table 2.a Randomization of rats and treatments for intravenous (IV), subcutaneous (SC) and intraperitoneal (IP) intervention.

RAT ID	INTERVENTION: PLACEBO (1), SC (2), IP (3)		
31	2	1	3
32	3	1	2
33	1	2	3
41	2	3	1
42	3	1	2
43	1	2	3
51	3	1	2
52	1	2	3
53	3	2	1
61	3	1	2
62	2	3	1
63	2	3	1
71	1	3	2
72	3	2	1
73	2	1	3

Table 2.b. Randomization of rats and treatments for placebo (PL), subcutaneous (SC) and intraperitoneal (IP) intervention.

After randomization trial was started, 5 rats per day were used, with at least one week between repeated use of rat. Trial was started on 30th of March 2017 and finished on 26th of April 2017.

The highest glucose value increase (10.6 ± 2.3 mmol/L (±SD)) was observed 20 minutes after IV glucagon injection. After glucagon injection IP maximum glucose value 9.39 ± 1.42 mmol/L was reached after 30 minutes. After SC injection maximum glucose level 9.78±1.14 was reach after 40 minutes (see Figure 1 and Table 3).

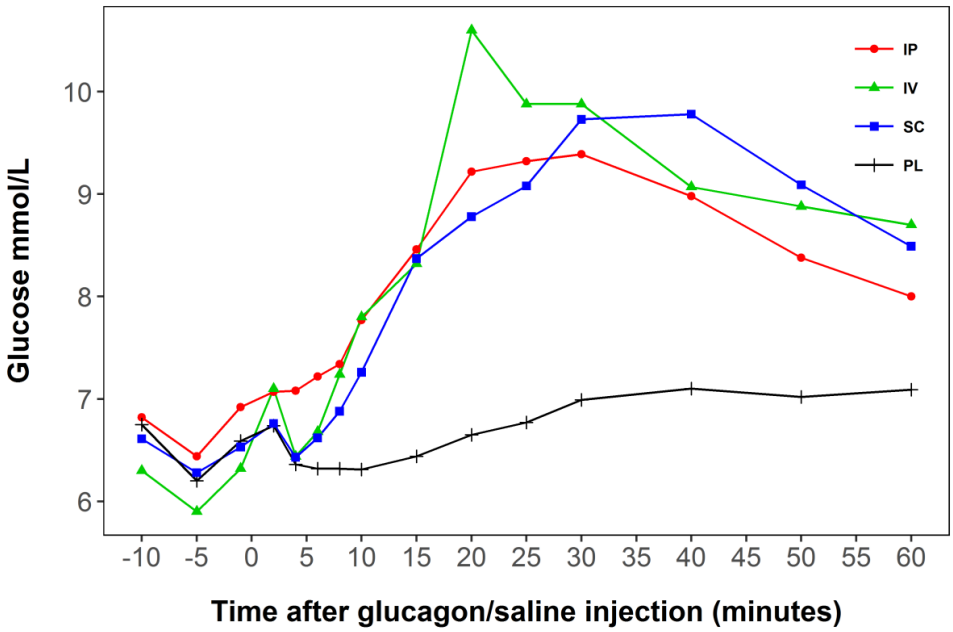


Figure 1. Raw data of blood glucose changes after glucagon/saline injection. Red line with circles represent mean blood glucose level (mmol/L) after intraperitoneal (IP) injection of 5 µg/kg BW of glucagon; green line with triangle represent mean blood glucose level (mmol/L) after intravenous (IV) injection of 5 µg/kg BW of glucagon; blue line with square represent mean blood glucose level (mmol/L) after subcutaneous (SC)) injection of 5 µg/kg BW of glucagon; black line with cross represent mean blood glucose level (mmol/L) after intraperitoneal isotonic saline injection (PL).

PROCEDURE	TIME	N	GLUCOSE	SD	SE	CI
IP	-10	20	6.82	0.9693	0.2167	0.4537
IP	-5	20	6.44	0.8041	0.1798	0.3763
IP	-1	19	6.92	1.208	0.2771	0.5822
IP	2	20	7.07	1.0225	0.2286	0.4786
IP	4	20	7.08	0.9966	0.2229	0.4664
IP	6	19	7.22	1.1246	0.258	0.542
IP	8	20	7.34	1.2365	0.2765	0.5787
IP	10	20	7.77	1.2499	0.2795	0.585
IP	15	19	8.46	1.2873	0.2953	0.6204
IP	20	20	9.22	1.1437	0.2557	0.5353
IP	25	20	9.32	1.2553	0.2807	0.5875
IP	30	20	9.39	1.4201	0.3175	0.6646
IP	40	20	8.98	1.5433	0.3451	0.7223
IP	50	20	8.38	1.276	0.2853	0.5972
IP	60	20	8	1.4157	0.3166	0.6626

PROCEDURE	TIME	N	GLUCOSE	SD	SE	CI
IV	-10	5	6.3	1.0954	0.4899	1.3602
IV	-5	5	5.9	1.3323	0.5958	1.6543
IV	-1	5	6.32	0.996	0.4454	1.2367
IV	2	3	7.1	1.6643	0.9609	4.1344
IV	4	5	6.44	1.4064	0.629	1.7463
IV	6	5	6.68	1.4498	0.6484	1.8002
IV	8	5	7.24	1.3686	0.612	1.6993
IV	10	5	7.8	1.3892	0.6213	1.725
IV	15	4	8.32	1.8191	0.9096	2.8946
IV	20	5	10.6	2.3054	1.031	2.8626
IV	25	5	9.88	1.7427	0.7794	2.1638
IV	30	5	9.88	1.3864	0.62	1.7214
IV	40	4	9.07	0.5909	0.2955	0.9403
IV	50	5	8.88	1.455	0.6507	1.8066
IV	60	5	8.7	1.2309	0.5505	1.5283
PL	-10	15	6.75	0.9133	0.2358	0.5058
PL	-5	15	6.2	0.6845	0.1767	0.3791
PL	-1	15	6.59	0.9456	0.2441	0.5236
PL	2	15	6.74	0.9046	0.2336	0.5009
PL	4	14	6.36	0.8409	0.2247	0.4855
PL	6	15	6.32	0.7504	0.1938	0.4156
PL	8	15	6.32	0.6971	0.18	0.3861
PL	10	15	6.31	0.8052	0.2079	0.4459
PL	15	15	6.44	0.7854	0.2028	0.4349
PL	20	15	6.65	0.7577	0.1956	0.4196
PL	25	15	6.77	0.7907	0.2042	0.4379
PL	30	15	6.99	0.6567	0.1696	0.3637
PL	40	15	7.1	0.7559	0.1952	0.4186
PL	50	15	7.02	0.6293	0.1625	0.3485
PL	60	14	7.09	1.0309	0.2755	0.5953
SC	-10	20	6.61	0.7993	0.1787	0.3741
SC	-5	20	6.28	0.8675	0.194	0.406
SC	-1	20	6.53	0.8945	0.2	0.4186
SC	2	19	6.76	0.9517	0.2183	0.4587
SC	4	20	6.43	0.7808	0.1746	0.3654
SC	6	20	6.62	0.6779	0.1516	0.3173
SC	8	20	6.88	0.7714	0.1725	0.361
SC	10	20	7.26	0.8381	0.1874	0.3922
SC	15	20	8.37	1.6374	0.3661	0.7663
SC	20	20	8.78	0.9743	0.2179	0.456
SC	25	19	9.08	1.1228	0.2576	0.5412
SC	30	20	9.73	1.2449	0.2784	0.5826
SC	40	19	9.78	1.1476	0.2633	0.5531
SC	50	20	9.09	1.1315	0.253	0.5296
SC	60	20	8.49	1.1348	0.2538	0.5311

Table 3. Mean raw data of intraperitoneal (IP), intravenous (IV), placebo (PL) and subcutaneous (SC) interventions. Time of samples is represented in minutes. Number of samples at particular time point is represented as N. Glucose is represented as mean value (mmol/L).

Reference

1. Wren-Dail MA, Dauchy RT, Blask DE, et al. Effect of Isoflurane Anesthesia on Circadian Metabolism and Physiology in Rats. *Comp Med* 2017;67(2):138-46. [published Online First: 2017/04/07]
2. Zlotnik A, Gruenbaum BF, Klin Y, et al. The effects of insulin, glucagon, glutamate, and glucose infusion on blood glutamate and plasma glucose levels in naive rats. *J Neurosurg Anesthesiol* 2011;23(4):323-8. doi: 10.1097/ANA.0b013e3182299b15 [published Online First: 2011/08/13]