Master's thesis

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Investigation of Differences in Appetite-Related Hormones and Subjective Feelings of Appetite Between Patients with Suboptimal- and Optimal Weight Loss Ten Years After Roux-en-Y Gastric Bypass Surgery

Master's thesis in Clinical Health Science
Supervisor: Siren Nymo
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Abstract (English)

Investigation of Differences in Appetite-Related Hormones and Subjective Feelings of Appetite Between Patients with Suboptimal and Optimal Weight Loss Ten Years After Roux-en-Y Gastric Bypass Surgery

Introduction: Roux-en-y gastric bypass (RYGB) is the most efficient method for sustained long-term weight loss maintenance. However, there is a sub-group of patients who experiences suboptimal weight loss (SWL). The aim was to investigate differences in appetite-related hormones and subjective appetite sensations between patients with SWL versus optimal weight loss (OWL) and association with weight loss (WL) outcome 10 years after RYGB.

Methods: Adult patients who underwent RYGB more than 10 years ago were recruited from the Bariatric Surgery Observation Study (BAROBS) in Central Norway. Participants with ≥ 50% excess weight loss (EWL) were categorised as OWL, whereas those with < 50% EWL were categorised as SWL. Blood samples for active ghrelin (AG), active glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and insulin were collected along with visual analogue scale (VAS) of 100 mm, which was used to assess subjective feelings for hunger, fullness, prospective food consumption (PFC) and desire to eat (DTE). Both blood and VAS were collected before and 2.5 hours after a meal at 15, 30, 45, 60, 90, 120, and 150 minutes postprandially. VAS was not collected at 45 min.

Results: A total of 33 participants (91% females) with a mean age of 50.8 ± 7.6 years and BMI of 35.4 ± 10.1 kg/m^{2}were included. The OWL group had higher basal and area under the curve (AUC) for AG plasma concentration (P = 0.047 and P = 0.004, respectively). Basal AG plasma concentration was positive correlated with %EWL (P = 0.053). For AG AUC there was a significant positive correlation with both %EWL and %TWL (P = 0.007 and P = 0.005, respectively), and a negative correlation with % weight regain (WR) (P = 0.033). For basal GLP-1 there was a significant negative correlation with %TWL (P = 0.045). For basal insulin there was a positive correlation with %WR (P = 0.003). The SWL group had a higher score for PFC in fasting state (P = 0.001). PFC in fasting state was higher in the SWL group (P = 0.001),and was positively correlated with %WR (P = 0.051), and negatively correlated with %EWL (P < 0.001) and %TWL (P < 0.001). Furthermore, AUC for DTE and PFC were both higher in the SWL group (P = 0.036 and P = 0.032, respectively).

Conclusion: SWL group had blunted AG plasma concentration before and after a meal. Blunted AG and higher basal GLP-1 plasma concentrations were associated with poorer WL outcomes.

The SWL group had a greater drive to eat before a meal, and a prolonged motivation to eat after a meal which may contribute to SWL after RYGB. However, longitudinal studies are needed.

Sammendrag (norsk)

Undersøkelse av forskjeller i appetittrelaterte hormoner og subjektiv appetitt opplevelse mellom pasienter med suboptimal og optimal vektnedgang 10 år etter Roux-en-Y Gastrisk Bypass kirurgi

Introduksjon: Roux-en-y gastrisk bypass (RYGB) er den mest effektive metoden for å oppnå vektnedgang på lang sikt. Likevel er det en undergruppe av pasientene som opplever suboptimal vektnedgang (SWL). Formålet med denne studien var å undersøke forskjeller i appetittrelaterte hormoner og subjektiv opplevelse av appetitt mellom pasienter med suboptimal versus optimal vektnedgang (OWL) 10 år etter RYGB.

Metoder: Voksne pasienter som har tatt RYGB for mer enn 10 år siden ble rekruttert fra Bariatric Surgery Observation Study (BAROBS) i Helse Midt-Norge. Deltakere som hadde gått ned ≥ 50% av overvekten (EWL) ble kategorisert som OWL, mens de som hadde gått ned < 50% av overvekten ble kategorisert som SWL. Blodprøver for aktiv ghrelin (AG), aktiv glucagon-like peptide-1 (GLP-1), peptide YY (PYY), og insulin ble tatt sammen med visual analogue scale (VAS) på 100 mm som ble brukt for å måle subjective opplevelser av sult, metthet, prospektivt matinntak (PFC) og lyst på mat (DTE). Begge innsamlingene ble gjort før og etter en standardisert frokost ved 15, 30, 45, 60, 90, 120, og 150 minutter. VAS ble ikke samlet ved 45 min.

Resultater: Totalt 33 deltakere (91% kvinner) med en gjennomsnittsalder på 50.8 ± 7.6 år, og BMI på 35.4 ± 10.1 kg/m^2 deltok. OWL-gruppen hadde høyere basalt og area under the curve (AUC) for plasmakonsentrasjon av AG (henholdsvis P = 0.047 og P = 0.004). Basal AG plasmakonsetrasjon hadde en positiv korrelasjon med %EWL (P = 0.053). For AG AUC var det signifikant positiv korrelasjon med både %EWL og %TWL (henholdsvis P = 0.007 og P = 0.005), og negativ korrelasjon med % vektgjennoppgang (WR) (P = 0.033). For basal GLP-1 var det en signifikant negativ korrelasjon med %TWL (P = 0.045). For basal insulin var det en positiv korrelasjon med %WR (P = 0.003). SWL-gruppen hadde høyere sum for PFC i fastende (P = 0.001). PFC I fastende var høyere i SWL-gruppen (P = 0.001), var positivt korrelert med %WR (P = 0.051), og negativt korrelert med %EWL og %TWL (P < 0.001 for begge). Videre var AUC for DTE og PFC begge høyere i SVT-gruppen (henholdsvis P = 0.036 og P = 0.032).

Konklusjon: SWL-gruppen hadde nedsatt plasmakonsentrasjon av AG før og etter et måltid. Nedsatt AG og høyere basal plasmakonsentrasjon for GLP-1 var assosiert med dårligere

vektutfall. SWL-gruppen hadde en sterkere motivasjon til å spise som også var synlig etter et måltid, noe som kan bidra til SWL etter RYGB. Det trengs flere longitudinelle studier.

Relevance

Roux-en-Y Gastric Bypass (RYGB) is a highly invasive procedure that can often be viewed by the patient as a last resort for WL. Therefore, it is important to find clues as to what causes suboptimal weight loss (SWL), as it has a heavy impact on medical, social, and economic aspects of the lives of the bariatric patients. This study contributes to find clues that can help improve clinical practice of RYGB, selection of patients for the procedure, and organizing the follow-up after the surgery.

Abbreviations

ADP:	air-displacement plethysmography
AG:	active ghrelin
AGLP-1	active GLP-1
AgRP:	agouti-related peptide
AgRP:	agouti-related peptide
AMPK:	adenosine monophosphate-activated protein kinase
AP:	area postrema
ARC:	arcuate nucleus
ARH:	arcuate nucleus of the hypothalamus
AUC:	area under the curve
BAROBS:	Bariatric Surgery Observation Study
BDNF:	brain-derived neurotrophic factor
BMI:	body mass index
CART:	cocaine- and amphetamine-regulated transcript
CCK:	cholecystokinin
CNRHA:	Central Norway Regional Health Authority
CNS:	central nervous system
CRH:	corticotropin-releasing hormone
CV:	coefficient of variation
CVD:	cardiovascular disease
DMN:	dorsomedial nucleus:
DPP-IV:	dipeptidyl peptidase IV
DTE:	desire to eat
DVC:	dorsoventral vagal complex
DVN:	dorsovagal neurons
EDTA:	ethylenediaminetetraacetic acid
EWL:	excess weight loss
FM:	fat mass

GHS-R1a:	growth hormone secretagogue receptor type 1a
GLP-1:	glucagon like peptide-1
GLP-2:	glucagon-like peptide-2
GOAT:	ghrelin O-acyltransferase
iAUC:	incremental area under the curve
L-cells:	enteroendocrine L cells of the gastrointestinal tract
LHA:	lateral hypothalamic area
MAPK:	mitogen-activated protein kinase
MCH:	melanin-concentrating hormone
ME:	median eminence
NAc:	nucleus accumbens
NPY:	neuropeptide Y
NTS:	nucleus of the tractus solitarius
NWR:	no weight regain
ObeCe:	center of obesity
OFC:	orbitofrontal cortex
OWL:	optimal weight loss
OXM:	oxyntomodulin
PfC:	pre-frontal cortex
PFC:	prospective food consumption
PKA:	protein kinase A
pm/mL:	Picomoles Per Litre
POMC:	pro-opiomelanocortin
PP:	pancreatic polypeptide
PVN:	paraventricular nucleus
PYY:	peptide YY
RYGB:	Roux-en-Y gastric bypass
SF-1:	steroidogenic factor-1
SWL:	suboptimal weight loss
TG:	total ghrelin

TRH:	thyrotropin-releasing hormone
TWL:	total weight loss
VAS:	visual analogue scale
VMN:	ventromedial nucleus
VSt:	ventral striatum
VTA:	ventral tegmental area
WL:	weight loss
WR:	weight regain
Y2R:	Y2 receptor

Innhold

1.0 Background

1.1 Introduction

Obesity is a serious health issue that holds a wide range of comorbidities such as cancers, cardiovascular diseases (CVD), hypertension, stroke, sleep apnea, gallbladder disease, dyslipidemia and type-2 diabetes (1). Worldwide the prevalence of obesity has increased by 28% for adults and 47% for children between the year 1980 and 2013 (1). In Norway more than 20% of the population presents with obesity – with the largest increase over time present in the youngest age groups (2). The obesity epidemic has increased over time, which can be explained in two ways. Firstly, through evolution the human body's physiology seems to protect more against weight loss (WL) than weight gain, to protect against starvation (3). Secondly, the changes of environment, meaning food surplus and availability, and social and psychological factors in modern society have a profound impact on eating habits (3). Obesity and overweight also have a substantial negative effect on emotional well-being, self-esteem, and psychological health (4). According to a review by González-Muniesa et al. (2017), alongside health complications, obesity also has a negative impact on psychological factors, mood, and cognitive function (4). Up to 70% of inter-individual body weight variation can be attributed to genes (4) and so it is assumed that the genetic make-up of each individual possibly predisposes to obesity. There is a large interindividual variation in comorbidity related to obesity, and it is possible to be metabolically healthy, i. e. not presenting with metabolic syndrome, although present with severe obesity (5). When it comes to fat distribution, it is mainly fat accumulated in the visceral area that increases the risk of metabolic complications (4).

A modest WL of 5-10% is associated with significant health benefits and a reduction or resolution of obesity comorbidities (6, 7). There are several approaches to WL treatment, like pharmacotherapy, life-style intervention, and bariatric surgery. In a study by Kushner et. al. (2014) (8), it is stated that because of little training, strict drug laws, and biased attitudes towards obesity, less than 3% of individuals who have obesity are treated by prescription medication because of its modest effect on WL (9). Life-style interventions are effective in the short-term but long-term results vary (10). The best results in WL programs are seen when combining physical activity and dietary intervention (10-13).

Bariatric surgery is considered the most efficient treatment method for sustained WL maintenance in patients with severe obesity (1, 9, 10, 14-17), with an effect superior to conservative treatment like lifestyle interventions (16, 17). Bariatric surgery can yield a total

weight reduction of up to 38% 1 year post-operatively, and most patients experience resolution of type 2 diabetes, hypertension, hyperlipidemia, and obstructive sleep apnea (14). The most common form of bariatric surgery has been the Roux-en-Y Gastric Bypass (RYGB) (17, 18). A systematic review by O'brien et al. (2006) (19) showed that RYGB yields an average of 67% excess weight loss (EWL) at 1 and 2 years after surgery, and 58% EWL at 5 years. A more recent systematic review by the same author (2019) (20), showed that RYGB yields an average of 55.4% EWL more than 10 years after surgery.

Weight regain (WR) over time is also a concern with patients undergoing RYGB. The Swedish Obese Subjects Study (SOS-study), the largest non-randomised intervention trial comparing WL outcomes in a group of over 4000 surgical and non-surgical subjects, reported a mean WR of 34% (at a ten year follow-up) from NADIR weight loss (14). Furthermore, there exists a sub-group of up to 30% of patients who experience suboptimal weigh loss (SWL) and/or early WR after RYGB (21-23). In a study by Hawkins et al. (2017), it was found that for 1087 patients who underwent RYGB in 1985-2004, the mean EWL was 57% in the 617 patients present for a 10-year follow-up, but there was a subgroup of 10% of the patients who presented with WL failure (≤ 0% reduction in excess body weight) (21). SWL has been defined as <50% EWL or a WR of ≥15% (23). The reasons for SWL after RYGB are not fully understood (17), but studies have shown that psychological processes are likely a factor (22). The fact that pre-RYGB psychopathology is associated with SWL (22) is important to take into consideration.

RYGB is referred to as a metabolic surgery, both because of the changes in appetite-related hormones, and physiological changes like increased insulin sensitivity and reduced nutrient absorption seen after surgery (24). Although the mechanisms behind sustained WL post-RYGB are not fully understood, the procedure's effect on WL most likely is multifactorial, and it is suggested that WL mainly happens as a result of blunted appetite and that this in turn leads to reduced energy intake (25).

1.2 Theoretical Background

1.2.1 Appetite Regulation

Humans typically eat when they have a feeling of hunger or a desire to eat food. This causes an episodic eating pattern, where we typically refrain from eating when we do not feel hungry. Appetite regulation is complex, involving both homeostatic and non-homeostatic factors affecting the drive to actively search for, choosing the type of, and ingesting food (26). Non-homeostatic factors as environmental factors involving colour, smell and ambient setting, and

also social factors play a profound role in affecting the amount of food consumed. However, in this study the focus is on the homeostatic system.

A figure visualising the gut-brain axis as the control system for food intake can be seen below in Figure 1.

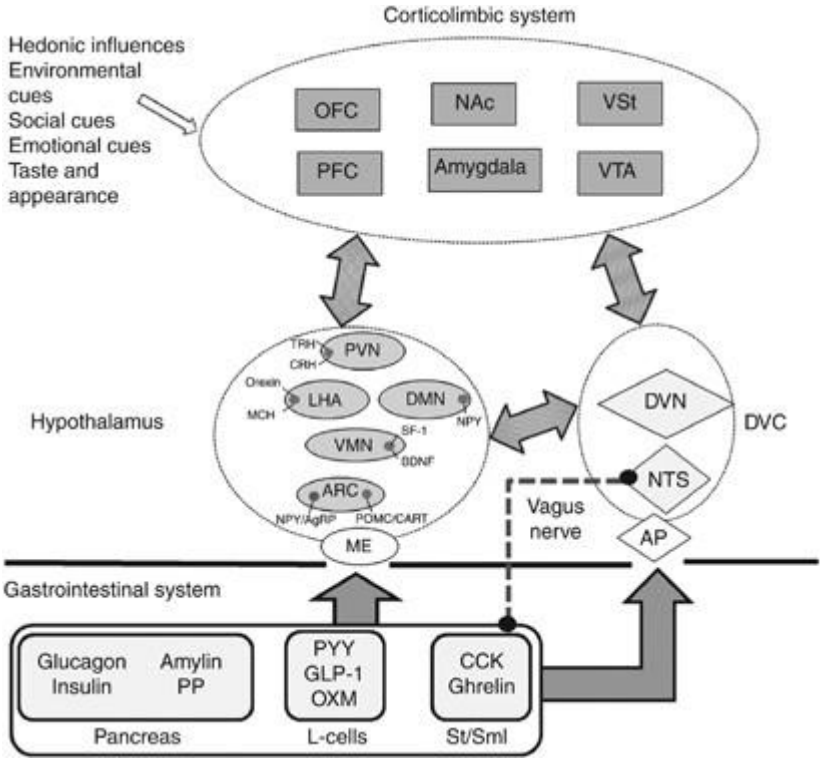

Figure 1. AP: area postrema. ARC: arcuate nucleus. BDNF: brain-derived neurotrophic factor. CCK: choleycystokinin. CRH: corticotropin-releasing hormone. DMN: dorsomedial nucleus. DVC: dorsoventral vagal complex. DVN: dorsovagal neurons. GLP-1: glucagon like peptide-1. L-cells: enteroendocrine L cells of the gastrointestinal tract. LHA: lateral hypothalamic area. MCH: melanin-concentrating hormone. ME: median eminence. NAc: nucleus accumbens. NTS: nucleus of the tractus solitarius. NPY/AgRP: neuropeptide Y and agouti-related peptide. OFC: orbitofrontal cortex. OXM: oxyntomodulin. PFC: pre-frontal cortex. POMC/CART: pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript. PVN: paraventricular nucleus. PP: pancreatic polypeptide. PYY: peptide YY. SF-1: steroidogenic factor-1. St/SmI: stomach and small intestine. TRH: thyrotropin-releasing hormone. VMN: ventromedial nucleus. VSt: ventral striatum. VTA: ventral tegmental area.

A study by Hussain et al. (2013) (27) visualised the gut-brain axis with a detailed figure (Figure 1). The gut brain axis is described as the control system for food intake, influenced by both the homeostatic and non-homeostatic system (27).

The homeostatic appetite control system is communicating with the central nervous system (CNS) (28, 29). More specifically two neuronal populations in the arcuate nucleus of the hypothalamus (ARH) seem to play an important role in energy balance. These two populations

are the pro-opiomelanocortin (POMC), and neuropeptide Y or agouti-related peptide (NPY and AgRP, respectively) which have separate qualities as anorexigenic (appetite-suppressing) and orexigenic (appetite-increasing), respectively. These neurons receive input through neurotransmitters and hormones from both the CNS and from peripheral organs via the blood-brain barrier to produce a central command for feeding behaviour (28). The orexigenic hormone ghrelin, released from gastric mucosa has been shown to supress POMC neurons whilst exciting NPY/AgRP neurons by indirect mechanisms (28). The possible anorexic effects of exciting the POMC neurons and the suppression of NPY/AgRP neurons are not fully understood, but it is considered that the anorexigenic effects of satiety-inducing hormones are mediated by their effects on these neurons (28).

When it comes to satiety and meal termination, it seems that the hypothalamus is unlikely to be the only deciding organ (29). Rather, processing of satiety signals leading to meal termination happens in the hindbrain, where the signals are conveyed through the afferent fibers of the vagus nerve from the upper gastrointestinal tract (29). Signals of satiety start out as chemical or mechanical stimulus from food ingestion in the stomach pouch and small intestine, followed by humoral signals resulting from neuroendocrine cells reacting to nutrients. The signals reach the nucleus tractus solitarius (NTS) through the vagus nerve, where the information is integrated along with neural input from oral taste receptors. The NTS is located in the caudal area of the brain stem (29), and the process behind meal termination has been demonstrated to take place even without the influence of the hypothalamus in rats (30).

1.2.2 Appetite-Related Hormones

Ghrelin, first discovered in 1976 by Bowers et al. (1980) (31), is a hormone with orexigenic qualities. The hormone originates in the mucosa of the upper gastric tract (26, 28), and is modified by ghrelin O-acyltransferase (GOAT) to become acylated ghrelin/active ghrelin (AG) that can stimulate appetite through the blood-brain barrier. AG uses two different routes to convey signals to the CNS (32). In the vagal pathway, the hormone attaches itself to the growth hormone secretagogue receptor type 1a (GHS-R1a) that has been synthesised in the nodose ganglion of the vagal afferent nerve and transported to the stomach (32). As a result of the attachment with AG, electrical activity in the vagal afferent nerve is suppressed. This signal reaches the NTS where synapses are connected to NPY/AgRP neurons in the ARC, and noradrenaline is released to activate these neurons (32). The endocrine pathway for AG to convey signals to the hypothalamus is by traveling through the blood in the circulation system

and crossing the blood-brain barrier. Close to the ARC, the median eminence in the hypothalamus has neurons fenestrated with capillaries that AG reaches and binds to (33). Basal plasma concentrations of ghrelin are lower in individuals who have obesity vs normal weight individuals, and is shown to normalise as a result of WL (34-36).

Glucagon-like peptide-1 (GLP-1) was first in 1983, along with Glucagon-like peptide-2 (GLP-2), identified in rodents in a study by G.I. Bell et. al. (1983) (37), who subsequently the same year identified the two peptides in humans (38). GLP-1 has anorexigenic qualities in that it induces satiety, and slows down gastric emptying (39), and is secreted after ingested carbohydrates reach the small intestine (39) and from the mechanical distention of the stomach pouch postprandially (40). GLP-1 is an incretin hormone, a gut peptide secreted after nutrient intake that stimulates insulin secretion (41). GLP-1 is produced in L-cells mostly in the small and large intestine with a higher density in the ileum and gradually lower density towards the duodenum. Some expression of GLP-1 is also found in the colon and rectum (42). Centrally, GLP-1 is also produced in neurons of the NTS (43). GLP-1 suppresses food intake by activating the GLP-1 receptor (GLP-1R) in the NTS that in turn inhibits signalling of the fuel-sensing enzyme AMP-activated protein kinase (AMPK), increases protein kinase A (PKA) activity, and increases phosphorylation of p44/42 mitogen-activated protein kinase (MAPK) (43). Basal plasma concentrations of GLP-1 have been suggested to be indifferent between people with obesity vs normal weight individuals. However, a lower postprandial GLP-1 response in individuals with obesity can be seen (34, 35).

Peptide YY (PYY) was discovered in 1982 in a study by Tatemoto et al (1982) (44), and was later stated as belonging to the same family of peptides as pancreatic polypeptide (PP) and NPY, namely the PP family (45). Although PYY has a role in regulating insulin-related mechanisms, more notably PYY has anorexigenic qualities (46, 47). The peptide is released postprandially in an amount proportionally to the amount of calories ingested (48). PYY is secreted in distal gut L-cells, but has a longer half-life of approximately 8 minutes (47, 49). It is not entirely clear how PYY affects appetite but its effect through the blood-brain barrier and centrally have both been suggested (47, 50). PYY is an agonist to the Y2 receptor (Y2R) (51) which in turn is highly expressed in NPY neurons (52). It is suggested that postprandially released PYY reaches the ARH through the circulatory system and crosses the blood-brain barrier to inhibit food intake by activating the Y2R (53). Furthermore, it is suggested that PYY regulates appetite centrally in the dorsal vagal complex (DVC), mediated by the vagus nerve (54). The relationship between basal and postprandial plasma concentrations of PYY and obesity is

unclear. However, WL has been shown to decrease both basal and postprandial PYY concentrations (34, 36).

Insulin was first discussed in 1909 by De Meyer and was thought to be secreted in the pancreas to suppress blood sugar after ingestion of food (55). (56). Insulin is naturally secreted in humans by β-cells of the pancreas bound to maintaining glycemic homeostasis with the primary regulator of secretion being circulating blood glucose (57). Although β-cells are highly adaptable to change in metabolic demand, prolonged over-nutrition can tire the cells as well as cause insulin resistance. Over time, β-cells can turn dysfunctional and eventually fail completely (57). Insulin resistance/type-2 diabetes is a common comorbidity to obesity, as people with obesity often have higher than normal blood glucose levels, leading to the tiring of the β-cells (58).

1.2.3 Appetite-Related Hormones and RYGB

The mechanisms behind sustained WL after RYGB are not fully understood, but changes in the secretion of appetite related hormones are thought to be decisive factors (59, 60).

The secretion of the orexigenic hormone AG has previously been shown to decrease post-surgery (61, 62). In the randomised controlled trial by Schmidt et al. (2016) a decrease in AG was found that may be involved in early appetite suppression post-RYGB (62). Schmidt et al.'s finding is contrary to a study by Christou et al. (2005), where no relationship between plasma AG concentration and SWL post-RYGB was found (63). Studies regarding AG and WL results after RYGB have long shown controversial results (64, 65). There are several studies showing that both basal and postprandial levels of ghrelin are decreased after RYGB compared to controls (66-75), while other studies show no change (76-78) or even increased ghrelin levels (79-81) after RYGB. Most likely, differences in surgical methods as well different laboratorial methods and duration of follow-up periods in studies are the reasons for the controversial results regarding ghrelin changes after RYGB (64). According to a recent systematic review by Xu et al. (2019) (82), decreased ghrelin levels are seen short-term, and increased ghrelin levels are seen long-term after RYGB.

Exaggerated postprandial secretion of the satiety inducing gut hormones GLP-1 and PYY after RYGB seems to play an important role in WL results (59, 60, 62, 79, 83). It is suggested that the increased GLP-1 and PYY response is a result of faster exposure from ingested food to the small intestine, and absorption of nutrients (25). A study by le Roux et. al. (2007) found that

the attenuated appetite after RYGB was associated with elevated PYY and GLP-1 concentrations and that the appetite would return if these gut hormones were inhibited (84).

Another study by Santo et. al. (2016) examining WR after RYGB found reduced postprandial release of GLP-1 in those participants experiencing WR (85).

A review by Dimitriadis et al. (2017) (59) mention that increased insulin secretion after bariatric surgery might produce weight gain. Several studies have demonstrated that insulin response to a meal has been decreased after WL following RYGB. Specifically this happens in the way that a more rapid insulin response with a subsequent steep fall in insulin plasma concentrations is seen in glucose tolerant individuals after a meal (86-88). Furthermore, the same group of studies shows that β-cell sensitivity to glucose is significantly increased after RYGB, and often a remission of type 2 diabetes is seen. Even though insulin does influence appetite, it is mostly its regulatory effects on glucose that is discussed regarding changes in insulin secretion and sensitivity after RYGB.

It remains to be ascertained if differences in WL outcomes 10 years after RYGB can be explained by differences in the postprandial release of satiety hormones.

1.2.4 Subjective Feelings of Appetite

Concepts like hunger, appetite and satiety are used by people as a way of describing the range of sensations that predict their normal eating behaviour (89). This means that the meaning behind the use of for example the term hunger likely differs between individuals, making it challenging to measure. It also suggests that the best measure is repeated within-subjects measures rather than between-subjects measures (89). Some of the most common measures for subjective feelings of appetite are done with visual analogue scale (VAS) and questions regarding the feeling of hunger, fullness, desire to eat, and prospective food consumption (how much you think you can eat) (89-92). The questions are asked to capture somatic sensations, motivations and judgments the subject has on their appetite over a given time period (90).

1.2.5 Subjective Feelings of Appetite and RYGB

A study by Stano et al. (2017) used VAS to assess differences in hunger and fullness pre- and post-RYGB. They found that postprandial peak fullness was higher, and the subjects reached peak fullness faster after surgery compared to before (93). Hunger scores in fasting state were not different from pre-RYGB, but hunger was more supressed in response to food, with solid

food being more suppressive than liquid food (93). Cazzo et. Al. (2017) found similar results regarding satiety, in that the pre-RYGB group reported lower fullness and was less satisfied postprandially compared to after RYGB, although in contrary to the study by Stano et al. (2017) (93) which found an increased basal hunger after RYGB (94). Furthermore, the group had a higher mean score on postprandial PFC than after surgery (94). There was also found significant correlation between post-RYGB levels of GLP-1 and the satiety aspects assessed by VAS (94). Other studies have also found increased basal hunger after RYGB (95, 96). In the study by Thirlby et al. (2006) (95) it seems that only the participants characterised by predisposing genes for obesity experienced a decrease in basal hunger after RYGB (95). Furthermore, this study also found that satiety seems to be stronger and maintained for a prolonged time after RYGB (95). A study by Halliday et al. (2019) (75) found that subjective feelings of hunger, fullness, PFC and DTE were all changed in a manner of decreasing energy intake after RYGB.

No study has to our knowledge investigated whether there are differences in subjective feelings of appetite between those with SWL and optimal WL after RYGB.

1.3 Aim and Hypothesis

Based on the knowledge that gut hormones play an important role in appetite, it is hypothesised that exaggerated secretion of satiety hormones post-surgery is crucial for sustained WL post-RYGB. Therefore, it is expected that within the sub-group presenting with suboptimal- or WL failure post-RYGB there will be a lower postprandial secretion of GLP-1 and PYY compared to the group experiencing optimal WL. It is also expected that those with SWL after RYGB present with lower postprandial feelings of fullness compared to the optimal WL group.

The main aim of this study was, therefore, to compare appetite-related hormones and subjective appetite markers between patients with suboptimal versus optimal WL 10 years after RYGB.

The secondary aims of this study were to:

- Compare appetite-related hormones and subjective appetite variables between those experiencing weight regain (WR) and those with no weight regain (NWR)
- Assess if there was an association between the independent variables and %EWL, %WR and %TWL.

2.0 Methods

2.1 Study Design

This was a cross sectional case control study. The study took place at St. Olavs Hospital, Forsyningssenteret, ObeCe (Center of Obesity). The participants were divided into two groups; those experiencing SWL and those with optimal WL results. A WL of < 50 % EW was used as criteria for SWL (97, 98). Anthropometrics were performed with the participants in fasting, and subjective markers and appetite-related hormones were measured before and continually for 2.5 hours after breakfast. The breakfast was in the form of a 200 ml meal replacement shake (Diben drink, Fresenius Kabi, Norway). Drink contents (200 ml): 300 kcal, 15 g protein, 14 g fat, 26.2 g carbohydrates, 4 g fiber (appendix III). Participants were instructed to consume the whole shake within 15 minutes after taking the first sip.

2.2 Participants

Adult participants who have had RYGB more than 10 years ago were recruited from BAROBS (the Bariatric Surgery Observation Study), a clinical observational study in Central Norway Regional Health Authority (CNRHA), aiming to investigate the effect on health, WL and nutritional aspects 10 years after bariatric surgery.

Patients who experienced severe surgery-related complication such as leakage within 30 days of operation and revisional surgery have received medical dietary treatment, and those who were pregnant or were breast feeding, and patients who had developed medical conditions known to effect body weight or appetite were not included in the study.

The participants were invited to the research unit for one day. A consent form was sent to the participants who accepted the invitation, along with information about the study (appendix IV). The study was approved by the Regional Committee for Medical Research Ethics in Norway (REK) together with the BAROBS study with number 2017/1828-21.

2.3 Anthropometric Measurements

All measurements were done with the participants in fasting with an empty bladder. Body composition for each participant was measured with an air-displacement plethysmography (ADP) device (BodPod, COSMED, Italy). Height (in cm) and weight (in kg) for each participant was measured with a stadiometer (Seca 217, SECA, Hamburg, Germany) and a digital flat scale (seca 876, SECA, Hamburg, Germany), respectively. Participants were

asked to remove clothing and jewellery and leave underwear on before the ADP and weight measurement. Height measurement was measured with participants standing with bare feet.

2.4 Appetite Markers

Both appetite-related hormones and subjective feelings of appetite were collected. Appetite related hormones were collected through blood samples taken in the fasting state and after the breakfast at 15, 30, 45, 60, 90, 120, and 150 minutes (AG, insulin, active GLP-1 and PYY). Blood was collected in 4 ml EDTA tubes and 1 ml of blood was then taken from the EDTA tube for each participant and moved to a cryotube. Here, a 20 µl mixture of inhibitors (10 µl of DPP-IV (Merck Millipore, Germany) and 10 µl of Pefabloc (Roche Diagnostic, Germany)) was added immediately to the cryotube blood. The cryotubes were then centrifuged at 1000 G for 10 min at 18°C. Plasma was then pipetted from the centrifuged cryotubes into new cryotubes and frozen at -80°C until analysis. Plasma concentrations of AG, GLP-1, total PYY and insulin were analysed using human metabolic hormone magnetic bead panel (MILLIPLEX® MAP Kit, Merck KGaA, Germany). The intra-assay coefficient of variation (CV) was <10% and the inter-assay CV was <20% for AG, GLP-1 and PYY, and <10% and <15% for insulin, respectively.

Subjective feelings of appetite (hunger, fullness, desire to eat and prospective food consumption), were collected in fasting and postprandially at 15, 30, 60, 90, 120 and 150 minutes using a 100-mm visual analogue scale (VAS) (89). The VAS questions were as follows: "How hungry do you feel?", "How full do you feel?", "How much food do you think you can eat?", "How much food do you want to eat?". The questions on the forms handed to the participants were translated to Norwegian to ensure everyone could understand the question in their own language.

2.5 Statistical Analysis

All statistical analyses were carried out using SPSS 25 (SPSS Inc., Chicago, IL) and statistical significance assumed at $P < 0.05$. Differences between groups were assessed by comparing central tendencies between SWL and OWL, and WR and NWR groups in both fasting and postprandially for appetite-related hormones and subjective feelings of appetite. For appetite-related hormones basal concentrations and AUC (area under the curve) and iAUC (incremental area under the curve) for concentrations where compared between groups.
For subjective feelings of appetite mm VAS in fasting and AUC/iAUC for mm VAS was compared between groups. Independent samples t-tests were used for comparing normally

distributed data, and Mann-Whitney U tests for non-normally distributed data. Correlation analyses were performed with Pearson correlation for normally distributed data, and Spearman correlation for non-normally distributed data.

AUC and iAUC for plasma concentrations of appetite-related hormones and mm VAS for subjective feelings of appetite were calculated from 0 to 150 minutes using the trapezoid rule with imputed data for missing values. The method for imputation was to first calculate means for each time point at group level, then calculate what percentage the mean of one time point is relative to the next time point. For example, the SWL group the formula looks like this: $(\text{mean}SWL_{15min}*100)/\text{mean}_{30min} = x_{SWL15min}$. So, to impute for a participant in the SWL group with a missing value at the 15 min. time point ($P_{SWL15min}$), we calculate that as $x_{SWL15min} * P_{SWL30min}$ = imputed value for $P_{SWL15min}$.

2.6 Power Calculation and Sample Size Estimation

The sample size for this study was based on a study by le Roux et. al. (2007) (84), and estimated by a power calculation using Stata (StataCorp LLC, USA), where levels in 3-hour postprandial GLP-1 response within post-operative subjects to RYGB was measured. After a standard meal of 400 kcal, the estimated GLP-1 concentration for the poor WL group was ~3500 pmol/L*min and for the successful WL group was ~9000 pmol/L*min. For a SD of 567, a power of 90% and a significance level of 5%, the estimated sample size needed in order to observe a difference was 4 subjects. Since there is limited comparable literature and the reference study contains few subjects, it is reasonable to assume that more participants are needed.

3.0 Results

3.1 Participants

A flowchart of the study can be seen in Figure 2. A total of 67 participants were contacted to join the study and a total of 33 participants (91% females) were included in the study.

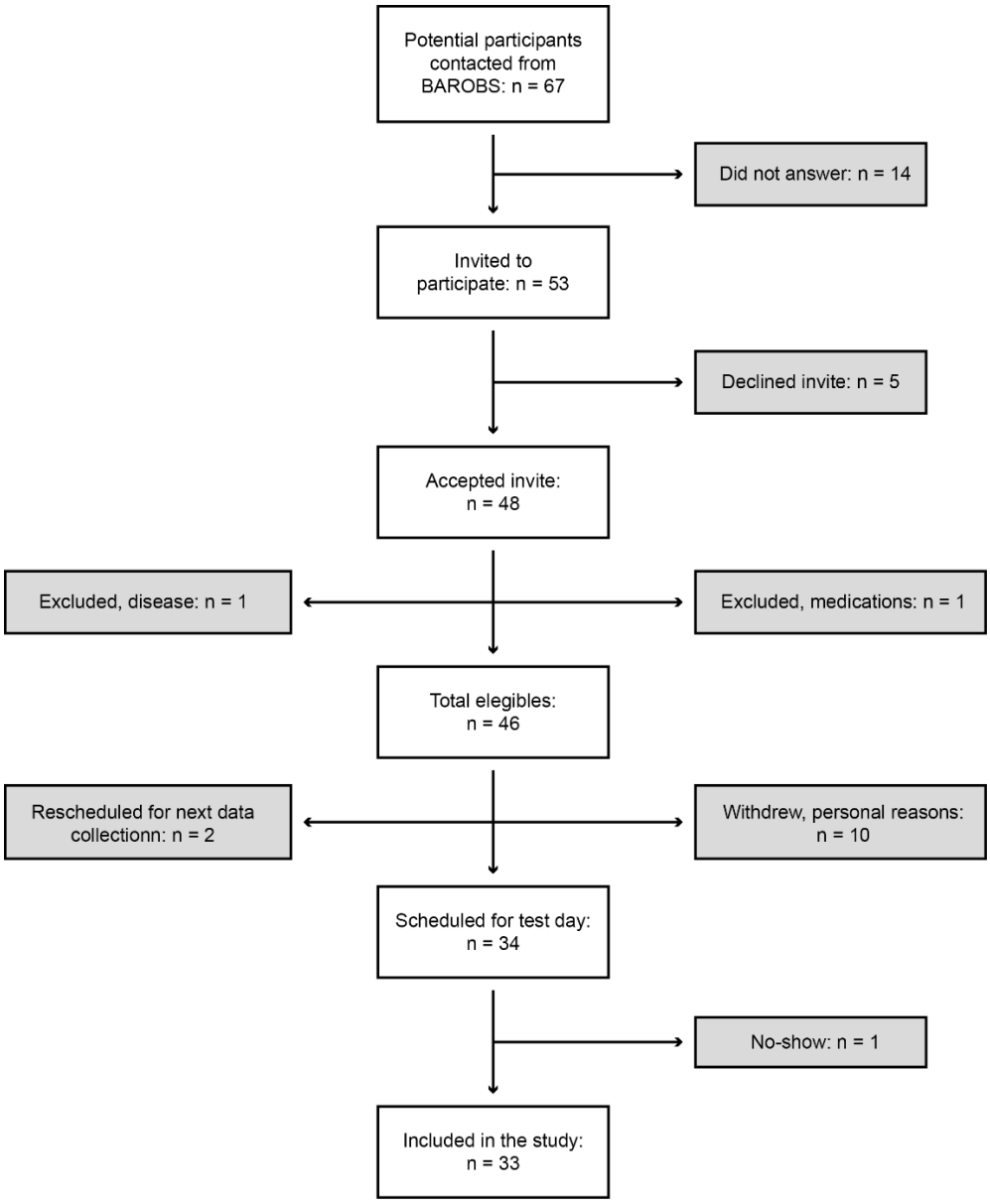

Figure 2. Flowchart of the study.

Descriptive characteristics of the participants are presented in Table 1.

Table 1. Descriptive characteristics for all participants, optimal- and suboptimal weight loss groups.

	All (n = 33)	**SWL (n = 18)**	**OWL (n = 15)**	**P-value**
Age (years)	50.8 ± 7.6	49.1 ± 5.4	52.8 ± 9.4	0.193
Height (cm)	166.2 ± 9.1[s]	168.6 ± 8.4	166.0 ± 6.9[s]	0.375
Weight (kg)	99.7 ± 30.7	122.9 ± 20.2	71.9 ± 11.7	< 0.001
BMI (kg/m^2)	35.4 ± 10.1	41.5 ± 5.8[s]	26.0 ± 3.5	< 0.001
% FM	43.7 ± 10.7	51.5 ± 4.9	35.0 ± 8.5	< 0.001
% EWL	51.7 ± 44.4	16.4 ± 18.9	94.1 ± 23.4	< 0.001
% TWL	21.0 ± 17.7	7.4 ± 9.0	37.5 ± 9.5	< 0.001
% WR	18.5 ± 21.3	22.1 ± 24.0[s]	5.4 ± 15.9	0.001

Data presented as mean ± SD. Values denoted by [s] are non-normal and presented with median ± IQR. P-values are for the difference between OWL and SWL. OWL: optimal weight loss. SWL: suboptimal weight loss. BMI: body mass index. % TWL: % total weight loss. % EWL: % excess weight loss. % WR: % weight regain. % FM: % fat mass of total body weight. % FFM: % fat free mass of total body weight.

No significant differences in terms of age, height and BMI were found between the SWL- and OWL groups. The SWL group had a higher % FM and WR compared to the OWL group ($P < 0.001$ for both). The OWL group had a higher % EWL ($P < 0.001$) compared to the SWL group.

3.2 Appetite-Related Hormones

The plasma concentration of appetite-related hormones over time in the OWL and SWL groups can be seen in Figure 3. One participant was excluded from GLP-1 analyses because of extreme values across all time points.

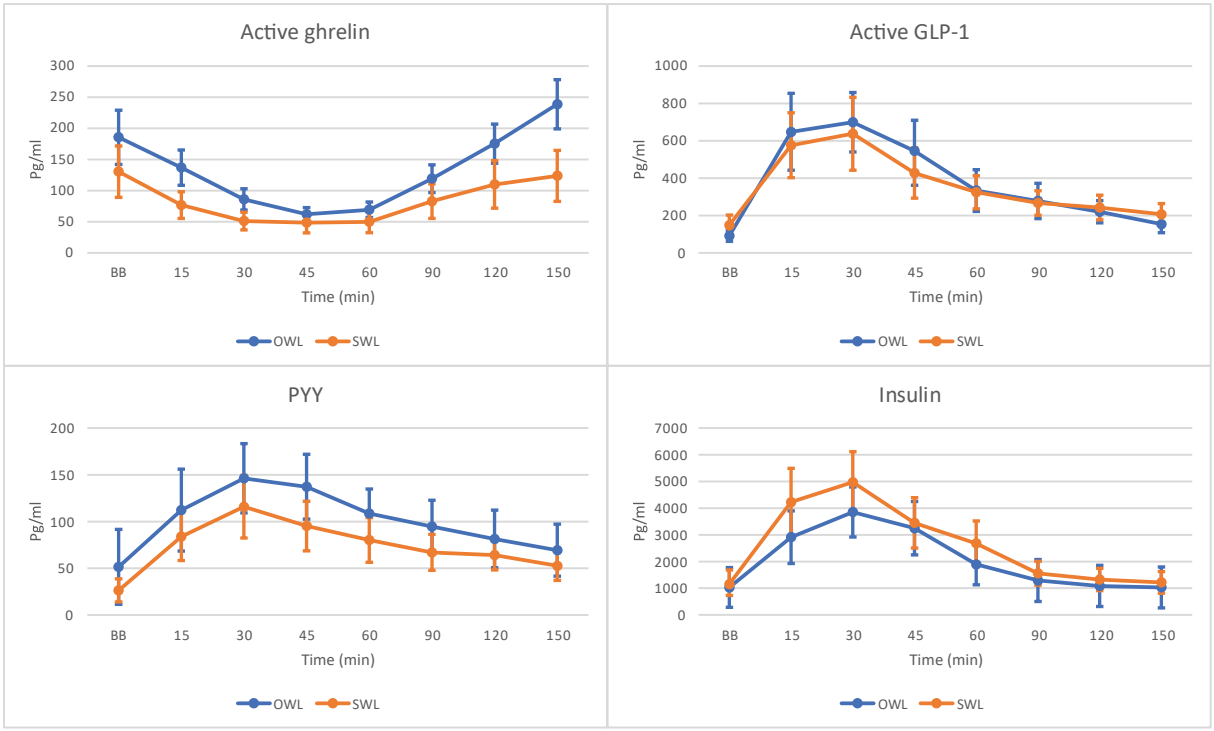

Figure 3. Plasma concentration of appetite-related hormones over time in response to a meal for suboptimal- and optimal weight loss groups. Data presented as means and confidence intervals at each time point. pg/ml: picograms per millilitre. OWL: optimal weight loss. SWL: suboptimal weight loss. AG: active ghrelin. AGLP-1: active glucagon-like peptide-1. PYY: peptide YY.

Basal plasma concentrations for objective appetite makers is presented in Table 2.

Table 2. Basal plasma concentrations for appetite-related hormones in suboptimal- and optimal weight loss groups and differences between groups.

	SWL (n = 18)	**OWL (n = 15)**	**P-value**
AG (pg/mL)	**110.9 ± 88.1**	**177.6 ± 145.4**	**0.047**
GLP-1 (pg/mL)	120.6 ± 178.4[s]	73.2 ± 95.5[s]	0.180
PYY (pg/mL)	18.9 ± 9.9	15.6 ± 59.0	0.350
Insulin (pg/mL)	1070.5 ± 867.9	466.3 ± 817.3	0.112

Data presented as mean ± SD or median ± IQR for basal blood concentration in pg/mL for both groups separately. Median values denoted by [s], are presented for non-normally distributed data. SWL: suboptimal weight loss group. OWL: optimal weight loss group. AG: active ghrelin. GLP-1: active glucagon-like peptide-1. PYY: peptide YY. Pg/mL: picogram per millilitre.

There was a significantly higher basal blood concentration of AG in the OWL group vs the SWL group (P = 0.047). No differences between the groups were found for basal GLP-1, PYY, or insulin.

Postprandial values of objective appetite markers are presented as AUC and iAUC in Table 3.

Table 3. Postprandial plasma concentrations for appetite-related hormones in suboptimal- and optimal weight loss groups, and differences between groups presented as AUC and iAUC values.

	SWL (n = 18)	**OWL (n = 15**	**P-value**
AG AUC (pg/mL)	**12400 ± 7871**	**19833 ± 5482**	**0.004**
AG iAUC (pg/mL)	-5753 ± 5468[s]	-12566 ± 13431[s]	0.060
GLP-1 AUC (pg/mL)	51505 ± 24262	53758 ± 23682	0.793
GLP-1 iAUC (pg/mL)	24286 ± 29144[s]	40820 ± 29730[s]	0.071
PYY AUC (pg/mL)	11155 ± 5748	14974 ± 7285	0.102
PYY iAUC (pg/mL)	6188 ± 8809[s]	9709 ± 8381[s]	0.169
Insulin AUC (pg/mL)	333021 ± 296444[s]	207831 ± 187419[s]	0.089
Insulin iAUC (pg/mL)	190097 ± 123208	133122 ± 79762	0.134

Data presented as mean ± SD or median ± IQR of AUC for blood concentration in pg/mL for both groups. Median values denoted by [s], are presented for non-normally distributed data. SWL: suboptimal weight loss group. OWL: optimal weight loss group. AG: active ghrelin. GLP-1: active glucagon-like peptide-1. PYY: peptide YY. AUC: area under the curve. iAUC: incremental area under the curve.

AG AUC was significantly higher for the OWL group vs the SWL group (P = 0.004). There was also a trend towards a higher AG iAUC for the SWL group vs the OWL group (P = 0.060). For GLP, PYY and insulin AUC no difference was found between SWL and OWL groups, but for GLP-1 iAUC there was a trend towards a difference between the groups (P = 0.071) with GLP-1 iAUC being lower for SWL. No difference was found for PYY or Insulin iAUC between groups.

Comparing Basal and Postprandial Plasma Concentrations Between Weight Regain and No Weight Regain Groups

The WR group had significantly higher basal insulin concentration compared to the NWR group (P = 0.001). No differences were found between the groups regarding basal plasma concentrations of AG, GLP-1, or PYY. Plasma concentrations of Insulin AUC were significantly higher in the WR group compared to the NWR group (P = 0.001). No difference was found between the groups regarding AUC or iAUC for any of the appetite-related hormones.

Correlations Analyses Between Appetite-Related Hormones and Weight Loss Results

Scatterplots for correlation analyses between basal plasma concentrations of AG and WL outcomes can be seen in Figure 4.

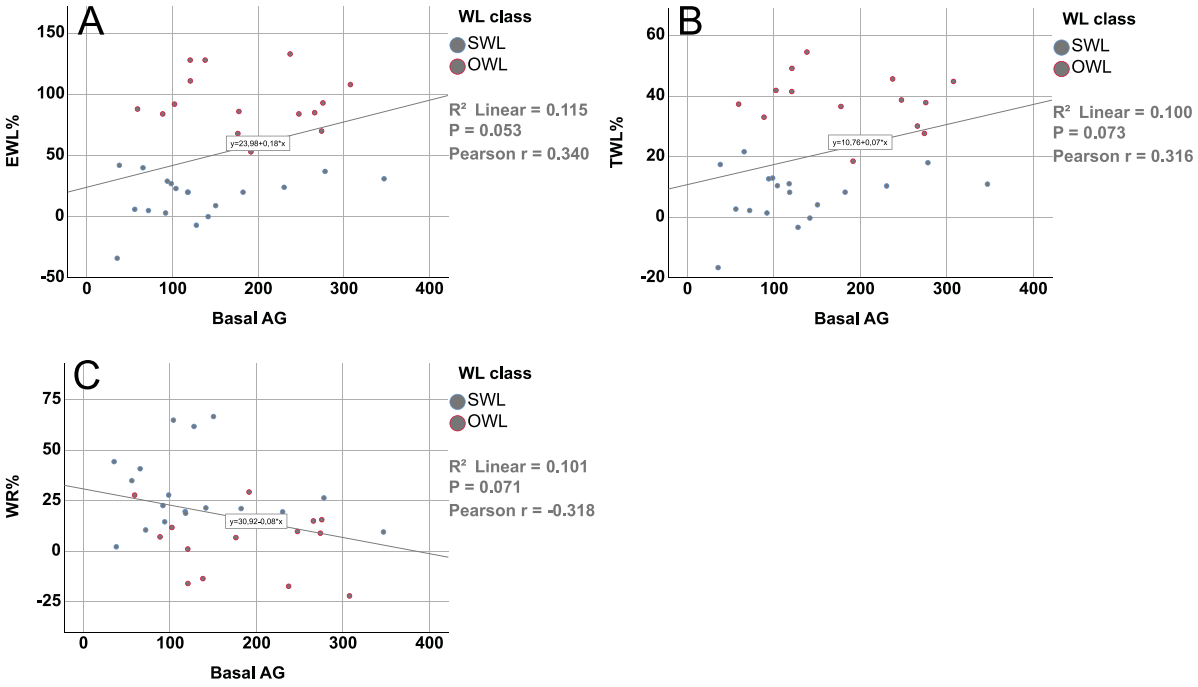

Figure 4. Scatterplots for correlation between basal active ghrelin concentrations and excess weight loss (A), total weight loss (B), and weight regain (C). EWL: excess weight loss. TWL: total weight loss. WR: weight regain. AG: active ghrelin. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss.

There was a trend towards a positive correlation between basal AG concentration and % EWL (A) (Pearson r = 0.340, n = 33, P = 0.053) and % TWL (B) (Pearson r = 0.316, n = 33, P = 0.073), and a trend towards a negative correlation with % WR (C) (Pearson r = -0.318, n = 33,

P = 0.071). This shows that those experiencing the largest EWL and TWL, and the least WR after RYGB have the highest AG concentrations in the fasting state.

Scatterplots for correlation analyses between AUC for plasma concentrations of AG and WL outcomes can be seen in Figure 5.

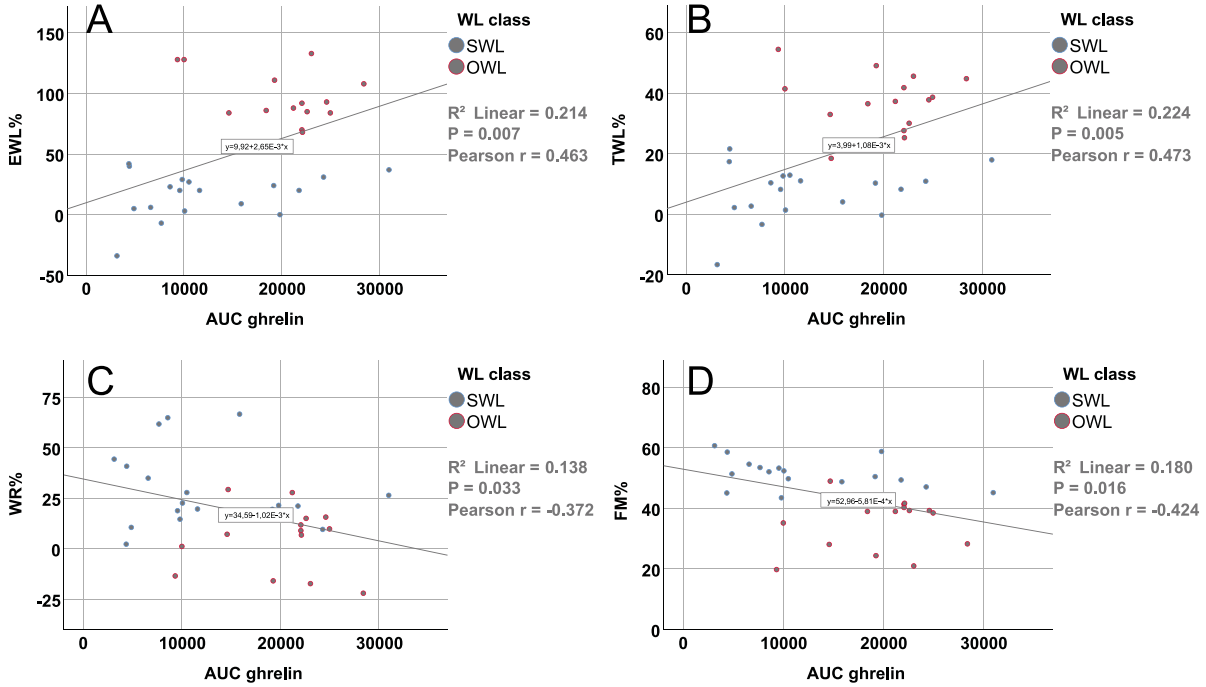

Figure 5. Scatterplots for correlation between AUC for active ghrelin concentrations and excess weight loss (A), total weight loss (B), weight regain (C), and fat mass (D). EWL: excess weight loss. TWL: total weight loss. WR: weight regain. FM: fat mass. AUC: area under the curve. AG: active ghrelin. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss

For AG AUC there was a significant positive correlation with both % EWL (A) and % TWL (B) (Pearson r = 0.463, n = 33, P = 0.007 and Pearson r = 0.473, P = 0.005, respectively), and a negative correlation with % WR (C) (Pearson r = -0.372, n = 33, P = 0.033) and % FM (D) (Pearson r = -0.424, n = 32, P = 0.016). No correlation was found between AG iAUC and % % EWL, % TWL, % WR or % FM. Similar to the basal levels of ghrelin, those experiencing the most EWL and TWL, and the least amount of WR after RYGB also have higher postprandial concentrations of AG.

Scatterplots for correlation analyses between basal plasma concentrations of GLP-1 and WL outcomes can be seen in Figure 6

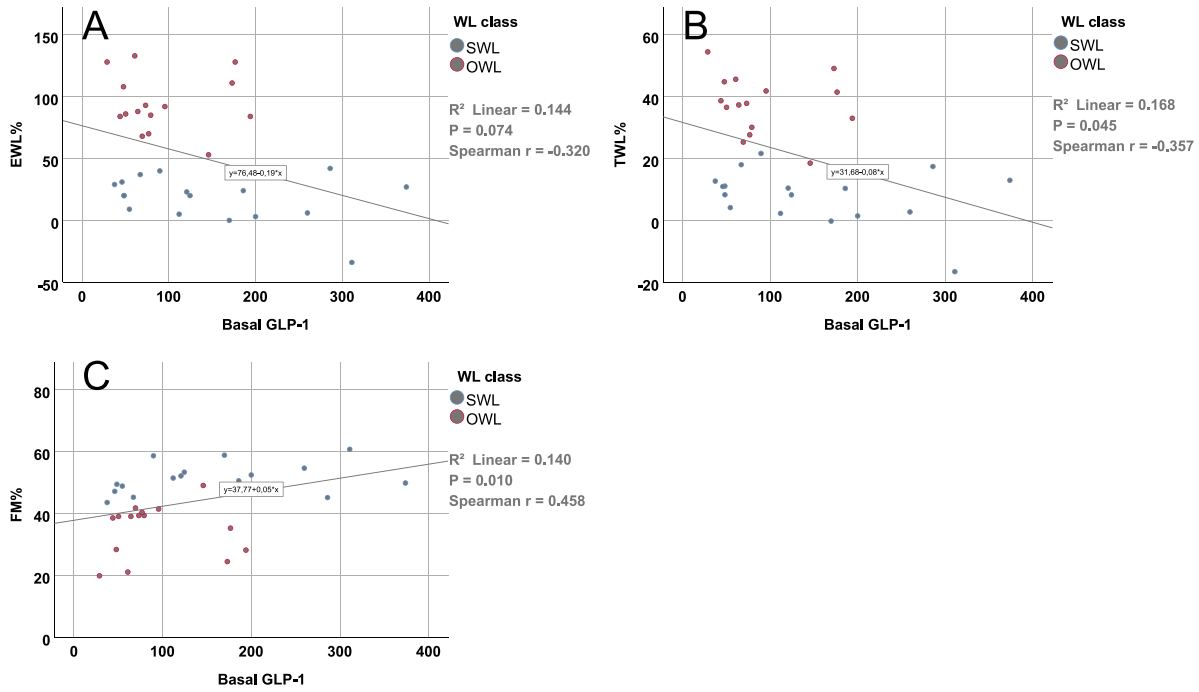

Figure 6. Scatterplots for correlation between basal GLP-1 concentrations and excess weight loss (A), total weight loss (B), and fat mass (C). EWL: excess weight loss. TWL: total weight loss. WR: weight regain. FM: fat mass. GLP-1: glucagon-like peptide-1. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss.

For basal GLP-1 there was a trend towards a negative correlation with % EWL (A) (Spearman r = -0.320, n = 32, P = 0.074) and a significant negative correlation with % TWL (B) (Spearman r = -0.357, n = 32, P = 0.045). Basal GLP-1 also had a positive correlation with % FM (C) (Spearman r = 0.458, n = 31, P = 0.010). No correlation was found between basal GLP-1 and % WR. Furthermore, there were no correlations between GLP-1 AUC or iAUC and either % EWL, % TWL, % WR or % FM. These results show that those who experienced the least amount of EWL and TWL had the highest concentrations of GLP-1 in fasting conditions. This is also reflected by the higher relative FM within the subjects with higher basal GLP-1 in fasting.

Scatterplot for correlation analysis between basal plasma concentrations of insulin and WR can be seen in Figure 7.

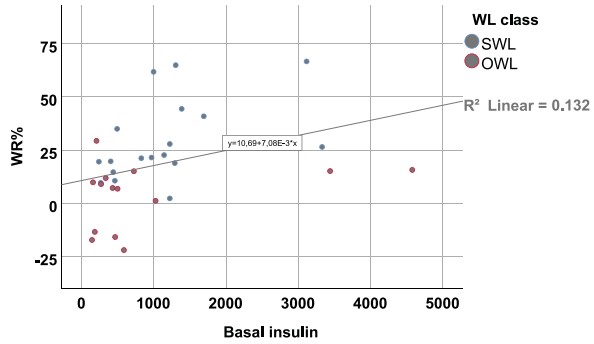

Figure 7. Scatterplot for correlation between basal insulin concentrations and weight regain. WR: weight regain. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss.

For basal insulin there was a positive correlation with % WR (Spearman r = 0.504, n = 33, P = 0.003). No correlation was found between basal insulin and % EWL, or % TWL.

Scatterplots for correlation analyses between insulin AUC and WL outcomes can be seen in Figure 8.

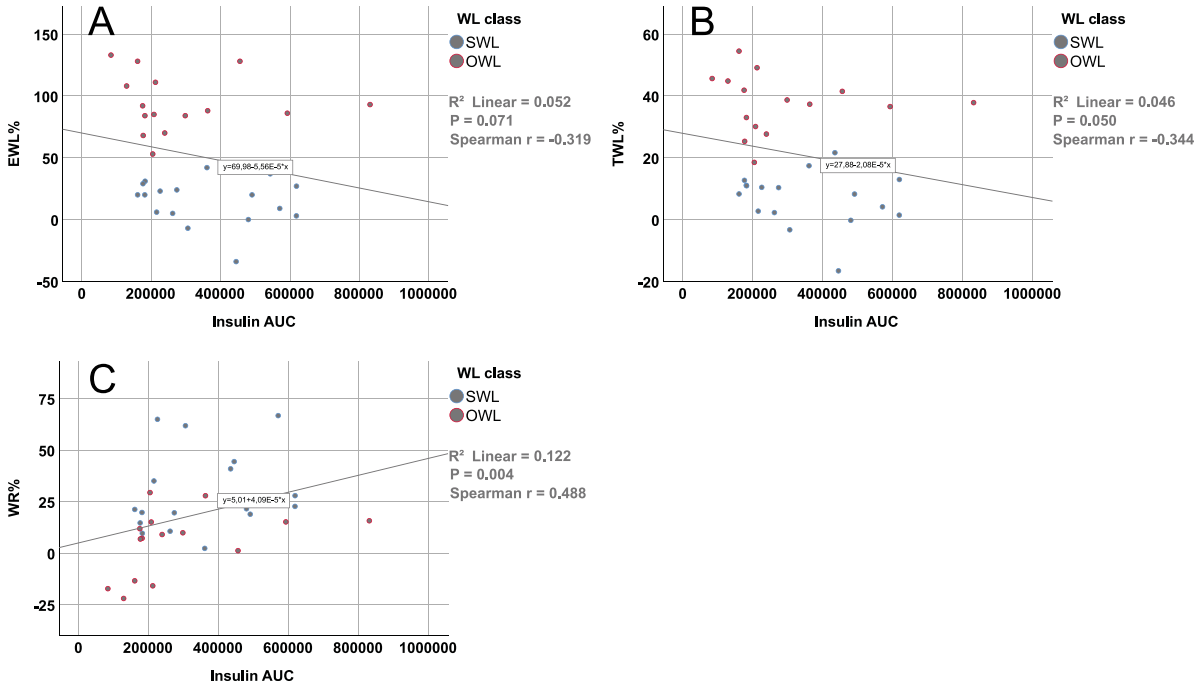

Figure 8. Scatterplots for correlation between AUC for insulin concentrations and excess weight loss (A), total weight loss (B), and weight regain (C). EWL: excess weight loss. TWL: total weight loss. WR: weight regain. FM: fat mass. AUC: area under the curve. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss.

AUC insulin trended towards a negative correlation with % EWL (A) (Spearman r = -0.319, n = 33, P = 0.071), and was significantly negatively correlated with % TWL (B) (Spearman r = -0.344, n = 33, P = 0.050. Furthermore, insulin AUC was positively correlated with % WR (C) (Spearman r = 0.488, n = 33, P = 0.004).

Scatterplot for correlation analysis between iAUC for plasma concentrations of insulin and TWL can be seen in Figure 9.

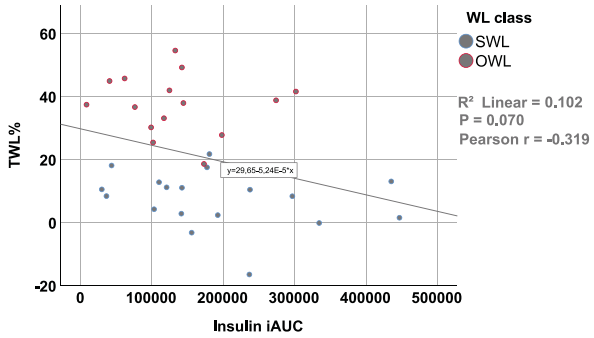

Figure 9. Scatterplot for correlation between iAUC for insulin concentrations and total weight loss. TWL: total weight loss. iAUC: incremental area under the curve. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss.

Insulin iAUC trended towards a negative correlation with % TWL (A) (Pearson r = -0.319, P = 0.070). No correlation was found between insulin iAUC and % EWL or % WR.

No correlation was found between basal PYY (basal, AUC and iAUC) and any of the WL categories.

3.3 Subjective Feelings of Appetite

Line diagrams for mm VAS for subjective feelings of appetite at each time point can be seen in Figure 10.

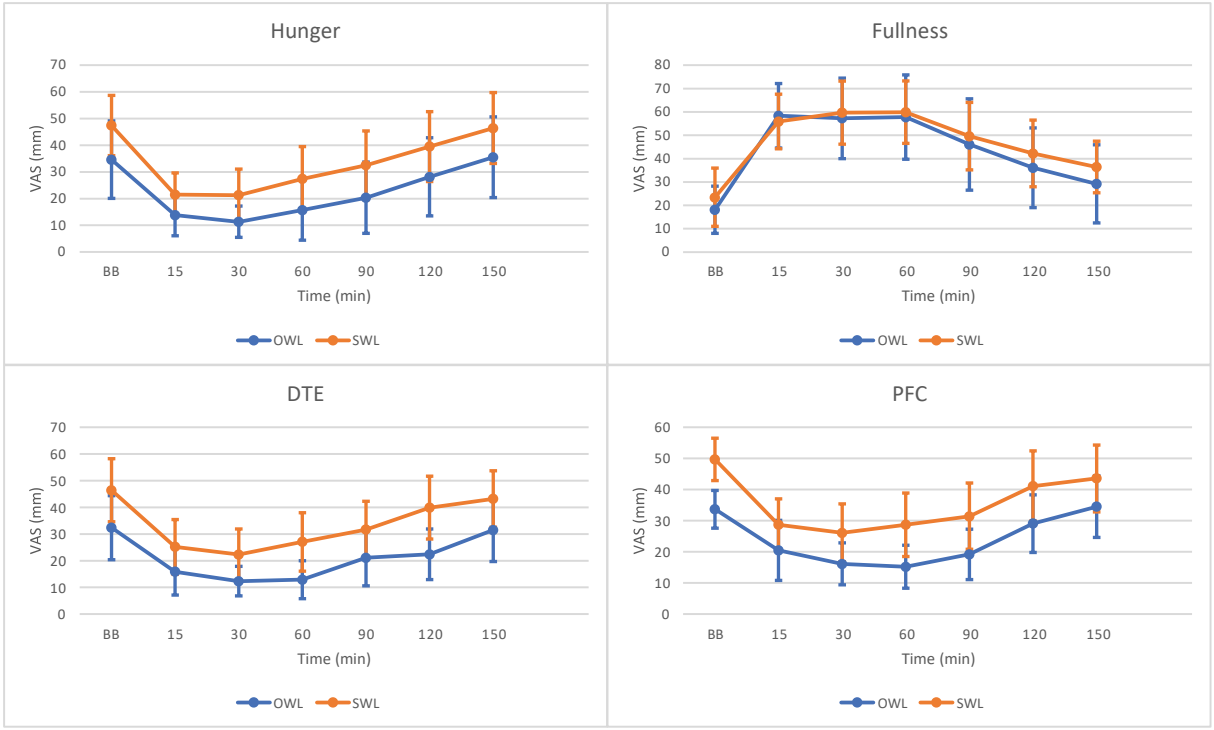

Figure 10. Subjective feelings of appetite over time in response to a meal for suboptimal- and optimal weight loss groups. Data presented means and confidence intervals at each time point. VAS: visual analogue scale. OWL: optimal weight loss. SWL: suboptimal weight loss. DTE: desire to eat. PFC: prospective food consumption.

Basal VAS scores on subjective feelings of appetite are presented in Table 4.

Table 4. Subjective feelings of appetite in fasting state for suboptimal- and optimal weight loss groups, and differences between groups.

	SWL (n = 18)	**OWL (n = 15)**	**P-value**
Hunger (mm)	47.4 ± 22.7	34.6 ± 26.2	0.142
Fullness (mm)	15.0 ± 51.0[s]	13.0 ± 27.0[s]	0.690
DTE (mm)	46.4 ± 23.7	32.4 ± 21.7	0.089
PFC (mm)	**49.7 ± 13.6**	**33.7 ± 11.0**	**0.001**

Data presented as mean ± SD or median ± IQR of fasting visual analogue scale scores. Median values denoted by [s], are presented for non-normally distributed data. OWL: optimal weight loss group. SWL: suboptimal weight loss group. PFC: prospective food consumption. DTE: desire to eat.

There was a significantly higher score in fasting PFC in the SWL vs the OWL group (P = 0.001). No differences were found between the groups regarding basal hunger, fullness, or DTE.

Postprandial values of subjective feelings of appetite are presented as AUC and iAUC in Table 5.

Table 5. Subjective feelings of appetite in suboptimal- and optimal weight loss groups, and differences between groups in AUC and iAUC values.

	SWL (n = 18)	**OWL (n = 15)**	**P-value**
Hunger AUC (mm)	4836 ± 3144	3180 ± 2841	0.126
Hunger iAUC (mm)	-1882 ± 3024	-2481 ± 3490	0.601
Fullness AUC (mm)	7456 ± 3401	6936 ± 4081	0.692
Fullness iAUC (mm)	3601 ± 3817	4840 ± 3377	0.336
DTE AUC (mm)	**4834 ± 2903**	**2923 ± 1880**	**0.036**
DTE iAUC (mm)	-1855 ± 2913	-2266 ± 2913	0.676
PFC AUC (mm)	**5081 ± 2616**	**3344 ± 1815**	**0.032**
PFC iAUC (mm)	-1699 ± 3872[s]	-1523 ± 5175[s]	0.772

Data presented as mean ± SD or median ± IQR of AUC and iAUC for visual analogue scale scores in mm for both groups. Median values denoted by [s], are presented for non-normally distributed data. SWL = suboptimal weight loss group. OWL = optimal weight loss group. PFC: prospective food consumption. DTE: desire to eat. AUC: area under the curve. iAUC: incremental area under the curve.

There was a significantly higher AUC for DTE and PFC for the SWL group vs the OWL group (P = 0.036 and P = 0.032, respectively). No differences were found between the groups regarding AUC for hunger or fullness. Furthermore, no differences were found between the groups regarding iAUC for any of the subjective appetite measures.

Comparing Fasting and Postprandial Visual Analogue Scale Scores for Subjective Feelings of Appetite Between Weight Regain and No Weight Regain Groups

No differences were found between the WR and NWR groups regarding fasting or postprandial subjective feelings of appetite.

Correlations Analyses Between Subjective Feelings of Appetite and Weight Loss Results

Scatterplots for correlation analyses between fasting PFC and WL outcomes can be seen in Figure 11.

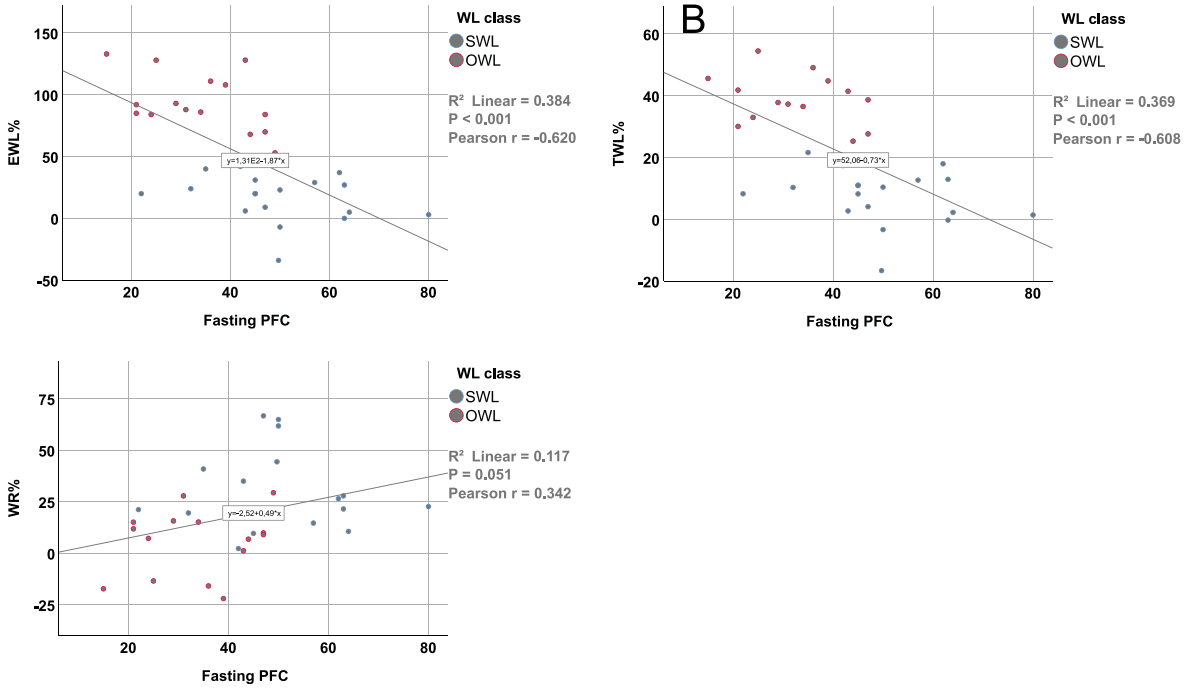

Figure 11. Scatterplots for correlation between basal PFC and excess weight loss (A), total weight loss (B), and WR (C). EWL: excess weight loss. TWL: total weight loss. WR: weight regain. PFC: prospective food consumption. WL: weight loss. SWL: suboptimal weight loss. OWL: optimal weight loss.

Fasting feelings of PFC had a negative correlation with %EWL (A) (Pearson r = -0.620, n = 33, P < 0.001) and %TWL (B) (Pearson r = -0.608, n = 33, P < 0.001), and trended towards a positive correlation with %WR (C) (Pearson r = 0.342, n = 33, P = 0.051). No correlation was found between any other categories of subjective feelings of appetite in fasting or postprandially and %EWL, %TWL, and %WR. These results show that those with a higher drive to eat while in fasting conditions experienced lower EWL and TWL after RYGB.

4.0 Discussion

The main aim of this study was to investigate if there were differences in the plasma concentration of appetite-related hormones and/or subjective appetite feelings between patients with suboptimal versus optimal WL 10 years after RYGB. The secondary aims of this study were to assess associations between the independent variables and the different WL outcomes.

Main Findings

The main findings were that the SWL group had lower plasma concentration of AG both basal and postprandially. Furthermore, correlation analyses showed that those experiencing the highest WL had both higher basal and postprandial AG concentrations.

The SWL group also trended towards a lower postprandial GLP-1 concentrations.

The drive to eat (the feelings of PFC) was higher both in fasting and postprandially for the SWL group. Furthermore, the SWL group also had a higher DTE postprandially. Correlation analyses showed that the higher drive to eat (the feelings of PFC) in fasting was associated with poorer WL outcomes.

When dividing the participants into WR and NWR groups the only difference observed was a higher concentration of insulin both in fasting and postprandially in the WR group, likely because of differences in body weight and FM.

Appetite-Related Hormones

There was a higher AG plasma concentration in fasting in the OWL group compared to the SWL group. Furthermore, higher basal AG concentrations were positively associated with % EWL and TWL as well as trending towards an association with lower WR. Furthermore, with AG AUC there was a positive association between both EWL and TWL, and negative association with WR. These results suggest that higher basal concentrations of AG in the OWL group probably result from a normalisation of AG after WL. Lower ghrelin concentrations have been found in obese individuals, and WL can cause ghrelin levels to increase and normalise (34-36). However, this is not always the case after WL following RYGB, since some studies have found a reduction in AG plasma concentrations after RYGB (61, 62). In the study by Falken et al. (2011) (61), ghrelin was measured in its total quantity and not in the active/acylated form as in the present study. Furthermore, the changes were only observed postprandially 3 days post-surgery, and not at 2 months or 1 year (61). Also in the study by Schmidt et al. (2016)

(62), only postprandial changes were found towards lower total ghrelin (TG) concentrations with measurements at 11 weeks after RYGB. The role of AG in the context of WL results after RYGB has been widely studied with controversial results (64, 66-81). Changes in ghrelin concentrations after RYGB have been proposed to be linked to the following factors: follow-up time in the study, WL outcomes, and surgical technique (64). However, it seems that shorter follow-up time is the most prominent factor linking together the studies that have found a decrease in ghrelin concentrations after RYGB. Firstly, the 3 studies by Frühbeck et al. (all from 2004) (67-69) concluded that TG changes depended on the procedure's change on the fundus as it contains cells secreting ghrelin (68, 69), and that changes were not determined by WL as they had compared similar WL results between different procedures and only found decreased TG after RYGB (67). However, the studies had short follow-up periods of 24 hours (68), and 6 months (where follow-up time differed vs the other procedures) (67, 69). Furthermore, several other studies that observed decreased ghrelin have done measurements at 30 minutes, 30 days, or 2-5 months after RYGB (70, 72-75).

Contrary to the findings in the above-mentioned studies, other studies with longer follow-up periods show results similar to the present study. The study by Korner et al. (2005) (78), with a follow-up of ~3 years (35 ± 5 months), found that fasting and postprandial TG and AG concentrations were similar between RYGB patients and weight-matched or lean controls. In another study by Stoeckli et al. (2004) (66), with a follow-up period of 2 years, no significant change in AG was found. This was similar to the study by Karamanakos et al. (2008) (77), who measured AG, and the study by Kruljac et al. (2016) (76), who measured TG, where no significant changes in ghrelin were found at 1 year after RYGB in either study. In the study by Tsouristakis et al. (2019) (79), they also found no TG change at 1 year, but TG concentrations increased throughout 2-4 years after RYGB. However, a study by Holdstock et al. (2003) (81) found a AG increase of 62% 1 year after surgery, and 44 % already after 6 months. Similarly, ghrelin was found to be increased both at 6 months and 1 year after surgery in the study by Alamuddin et al. (2017) (80). Importantly, a recent systematic review by Xu et al. (2019) (82) performed meta-analyses on the changes in ghrelin levels after RYGB and concluded that TG levels where decreased up until 3 months after surgery and increased after 3 months. Furthermore, gastric pouch volume, alimentary limb length and biliopancreatic limb length were not associated with changes in ghrelin levels (82).

It is worth noting that the practise of measuring either AG or TG varies greatly between studies, and can account for differences in findings.

Associations between both higher basal and AUC for levels of AG and better WL outcomes more than 10 years post-surgery is an important finding in the present study that requires more research.

Plasma concentrations of GLP-1 were similar in both groups, except regarding GLP-1 iAUC, showing there was a trend towards a higher postprandial release within the OWL group compared to the SWL group. This might indicate that the OWL group experiences a higher satiating effect after a meal compared to the SWL group. Furthermore, this shows that it might be the ratio of postprandial release compared to basal levels of GLP-1 that dictates the satiating effect of GLP-1 since there was no difference in GLP-1 AUC between the groups. Higher plasma concentration of GLP-1 postprandially has been described as a metabolic improvement after RYGB, and the importance of this as an indicator of increased satiety has been stressed in several studies (59-61, 79, 83).

Regarding PYY, no differences between the SWL or OWL groups, or relationships with WL results after RYGB were found in either fasting or postprandially. In previous studies, an increase in both basal and postprandial PYY has been found after RYGB (79, 83).

The review study by Dimitriadis et al. (2017) (59) discussed that increased posprandial GLP-1 after RYGB is possibly a result of the anatomical changes after RYGB that causes more intact nutrients to reach the ileum. The earlier review by Münzberg et al. (2015) (60) rather dismissed GLP-1 changes after RYGB as important, because the procedure done on mice with deficient GLP-1 receptors still managed to decrease energy intake. In the study by Falken et al. (2011) (61), during a 1-year follow up, they observed no changes in basal GLP-1 but postprandial concentrations were significantly increased, with a gradual increase until the end of the follow-up period (1 year). In the study by Shankar et al. (2017) (83), with a 4 week long follow-up after RYGB, showed that postprandial GLP-1 and PYY (iAUC) levels were increased compared to before the surgery. Furthermore, postprandial PYY was increased only at week 2, while GLP-1 was increased at both week 2 and 4. Basal GLP-1 was unchanged, but basal PYY was increased at 1-week after RYGB (83). The study by Tsouristakis et al. (2019) (79) found increased basal and postprandial PYY levels that were maintained 4 years after RYGB, vs 6 months before surgery. However, levels of PYY did not correlate with WL after surgery (79), which is in line with the present study. It is suggested by Tsouristakis et al. (79) (2019) that an important aspect of increased PYY plasma concentrations after RYGB is that it may be involved in increasing insulin sensitivity, independent of WL outcome (79), which indicates that PYY

has an important role in the remission of diabetes that is often seen after RYGB. Differences in test meal size, composition, and texture vary between studies and will likely account for some of the differences in findings.

None of the mentioned studies found a relationship between GLP-1 and WL outcomes. However, previous studies show that a change in postprandial GLP-1 after RYGB is to be expected, and the results in the present study show that lower postprandial GLP-1 levels may contribute to SWL. Furthermore, because of the relationship between GLP-1 and FM, it remains to be ascertained if lower postprandial release of GLP-1 is caused by the poor results from RYGB, or if decreased GLP-1 release has a causal effect on SWL after RYGB.

There were no differences when comparing basal and postprandial plasma concentrations of insulin between the SWL and OWL groups, which was unexpected considering the differences in weight (see Table 1.) between the two groups. However, basal insulin concentration was positively associated with WR. Furthermore, higher insulin AUC was associated with lower TWL, higher WR and trended towards an association with lower EWL, while postprandial plasma concentration trended towards an association with lower TWL. This coincides with a hypothesis presented in a review by Dimitriadis et al. (2017) (59), where they stated that the GLP-1-induced incretin effect might be in part responsible for the improved glucose tolerance after bariatric surgery, and that increased insulin secretion might be expected to produce weight gain rather than weight loss. This is most likely related to the role of insulin in facilitating the storing of energy from blood glucose and the fact that insulin secretion is decreased after RYGB while GLP-1 is increased. According to several studies, this happens as a result of increased incretin effect from increased postprandial GLP-1 (86-88). In the study by Jacobsen et al. (86) (2012), they measured insulin response to glucose and a mixed meal the last month before and on three separate days within 2 weeks after the RYGB procedure. They found increased insulin secretion postprandially and an increased insulin sensitivity. This demonstrates that insulin changes after surgery happen rapidly and before noteworthy WL has been achieved. However, in the study by Bradley et al. (87) (2012) they compared insulin concentrations before and after 20% WL from RYGB vs laparoscopic adjustable gastric banding (LAGB). They concluded that weight loss itself is primarily responsible for increased insulin sensitivity and glucose tolerance, as there was no difference between the two procedures (87).

There is a possibility that early changes in insulin concentrations after RYGB is an important precursor for achieving OWL. Importantly, the previous studies on insulin changes after RYGB

had a follow-up period lasting between 2 weeks and 1 year. This is significantly shorter than the present study, where there was found a link between both higher basal and postprandial insulin levels and higher WR. This indicates that the positive changes in insulin towards diabetes remission/prevention seen shortly after RYGB may be lost because of WR.

Subjective Feelings of Appetite

In the present study there were no differences between the SWL and OWL groups in either fasting or postprandial feeling of hunger. Based on earlier research, changes in hunger both in fasting and postprandially were expected differently from the presented study. Other studies have found a decrease in fasting hunger and/or postprandial hunger after RYGB (93-96). In the study by Stano et al. (2017) (93), using a 150 mm VAS, they found that hunger was more suppressed postprandially 1 year after RYGB while fasting hunger was unchanged. They tested with both solid and liquid meals and found that though liquid meals trended towards causing more discomfort and satiety, solid meals showed a trend towards a greater suppression of hunger (93). The prospective study by Thirlby et al. (2006) (95) measured hunger before and at least 3 months after RYGB. They observed a decrease in the subjective feelings of hunger in the subjects after the surgery. The method they used was a 9-point Likert, which is similar to the VAS used in the present study, except the Likert scale ranges from 1-9 (1 = extremely full, and 9 = extremely hungry) while in the present study, the VAS ranged from 0-100 mm (95). Similar to the present study they tested both before and after a meal (after an overnight fast), which in this case was a Snickers bar (95) vs a liquid meal in the present study. Similar to the results in the present study, they did not find any association between hunger and WL outcomes. They suggested that the reduction in feeling of hunger in fasting after RYGB might be linked to genetic predisposing for obesity (95). Importantly, the vast difference in follow-up periods in the present study compared to the literature may account for different findings.

There were no differences between the SWL and OWL groups regarding fullness in fasting or postprandially. In previous studies, feelings of postprandial fullness have been observed to increase after RYGB, and this is believed to facilitate WL (93-95). According to the study by Stano et al. (2017) (93), the meal texture affects the satiating effect of a meal. They found that a liquid meal 1 year after RYGB induced greater fullness with a faster peak fullness, along with greater discomfort vs a solid meal with the same nutrient and calorie content (600 kcal). The liquid meal used in the present study had half the calorie content compared with the that study (93) and may account for the different findings. Furthermore the time passed between the

operation and performing the meal test likely affects the results, where 10 years passed in the present study vs 1 year in the Stano et al. (2017) (93) study. From the present study it cannot be concluded that changes in fullness are connected to WL results after RYGB. More studies investigating the possible link between SWL and postprandial fullness are needed.

No differences were seen between the SWL and OWL groups regarding DTE in fasting or postprandially. The knowledge on DTE after RYGB exist is limited, however a study by Ochner et al. (2012) (99) has looked at desire to eat in response to visual and audial stimuli of high and low caloric food before and after RYGB. They found that DTE was lower in response to stimuli regarding high calorie food (E.g. picture of- or hearing the name chocolate brownie) one month after RYGB vs one month before. They were asked the question "On a scale from 0 to 100, how much did what you just saw/heard make you want to eat, zero being "not at all" and 100 being "very much"", which the participants answered verbally (99). Another study by Cazzo et al. (2017) (94) found that feeling of satisfaction was higher postprandially after RYGB compared to before. A study by Halliday et al. (2019) (75) found a reduction in DTE after participants had reached a 10 kg WL (after ~30 days) after RYGB. They measured DTE with a food cravings questionnaire using questions such as "I have an intense desire to eat one of my favourite foods" and asked the participants to rate from 1-5 how much they agreed with the statement. They also used visual stimuli evaluations where they were shown pictures of different types of food and answered with a 100 mm VAS on desire to eat for each picture. The reduction in DTE was only seen in fasting state. Both these studies had a very short follow-up time and they used different assessment tools compared to the presented study which can explain differences in outcomes.

The SWL group had a significantly higher score in fasting PFC compared to the OWL. The study by Halliday et al. (2019) (75) compared PFC in two groups after a WL of 10 kg either with dietary induced WL (~10 kg WL in ~43 days) or after RYGB (~10 kg WL in ~30 days) (75). The RYGB group had a reduction in postprandial PFC while the dietary WL group had increased PFC. This suggests that changes in PFC after RYGB may be a predictor for greater long-term WL outcomes after RYGB vs dietary induced WL. The results in the present study, in light of the previous study by Halliday et al. (2019) (75), suggest that the participants experiencing SWL after RYGB might have a greater drive to eat before a meal, leading to greater food intake. This indicates that PFC may contribute to SWL after RYGB.

Strengths/Limitation (Including Methodological Limitations)

The present study has several strengths. Measurements of body composition and appetite were done using gold standard validated methods. Both subjective and objective appetite was measured, in fasting and in response to meal. Nevertheless, the present study has also some limitations that should be emphasised.

Phase of menstrual cycle was not taken into consideration. This is of potential importance given that postprandial GLP-1 secretion, hunger feelings and insulin have been shown to change throughout the menstrual cycle (100).

Another limitation in this study is the use of a multi kit to analyse the hormones instead of optimised assay for each hormone, which is less accurate. Moreover, this study might have been underpowered to detect differences in subjective feelings of appetite. Furthermore, investigations of the role of PYY regarding remission of diabetes after RYGB should be done.

Finally, this study had a cross-sectional design, which does not make it possible to tell if the differences in appetite are either a cause or a consequence of RYGB or WL.

Practical Implications

These results are of great importance for patients and healthcare professionals to be aware of individual differences in the homeostatic and subjective appetite system, in that those experiencing SWL after RYGB could have a less sensitive appetite system with lower AG before a meal and lower GLP-1 after a meal. Furthermore, patients could be screened for subjective feelings of appetite, and made aware that a greater drive to eat is associated with SWL after RYGB.

Further Research

Longitudinal studies with control groups of people who did not undergo RYGB with the same BMI as our participants preoperatively are needed. Causal relationships between appetite-related hormones and WL outcomes should be investigated thoroughly in longitudinal clinical trials, and hormones should be measured with optimised methods for each hormone. Furthermore, a focus on the hedonic system and psychological factors related to actual energy intake and choices of types of food is needed. Further research has to ensure enough power to detect differences in subjective feelings of appetite.

5.0 Conclusion

SWL was associated with lower basal and postprandial levels of AG concentrations in plasma, and lower postprandial GLP-1 plasma concentrations. Lower basal and postprandial AG and higher basal GLP-1 plasma concentrations were associated with poorer WL outcomes. The SWL group had a greater drive to eat before a meal, and a prolonged motivation to eat after a meal which may contribute to SWL after RYGB, and this was also associated with poorer WL outcome more than 10 year after RYGB. Larger longitudinal clinical studies are needed to better understand the causality between appetite related hormones, appetite sensations, actual food intake and long-term weight loss maintenance after RYGB.

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Appendix I. Appetite-related hormones concentration tables

Table 12. Appetite-related hormones in fasting and changes over time postprandially.

Time (min)	BB	15	30	45	60	90	120	150
AG								
SWL	130.6 [89.2, 171.9]	76.8 [55.4, 98.2]	51.2 [36.9, 65.4]	48.7 [32.0, 65.4]	50.0 [32.3, 67.7]	83.0 [55.4, 110.7]	109.9 [71.7, 148.2]	123.8 [82.8, 164.7]
OWL	185.7 [142.0, 229.3]	137.0 [108.7, 165.2]	86.0 [68.9, 103.1]	62.0 [51.4, 72.7]	69.3 [56.9, 81.8]	119.2 [97.0, 141.4]	175.5 [143.9, 207.0]	238.7 [199.2, 278.1]
GLP-1								
SWL	149.0 [94.9, 203.1]	576.3 [402.6, 750.0]	638.0 [442.9, 833.2]	428.0 [293.1, 562.8]	325.1 [236.2, 413.9]	267.7 [202.4, 333.1]	243.3 [177.6, 308.9]	207.3 [150.4, 264.1]
OWL	91.9 [62.4, 121.5]	647.9 [442.5, 854.3]	699.8 [540.8, 858.8]	546.0 [361.7, 710.3]	334.4 [222.5, 446.4]	278.4 [183.8, 373.0]	220.7 [161.1, 280.4]	154.3 [108.1, 200.5]
PYY								
SWL	26.3 [13.9, 38.8]	83.9 [58.2, 109.6]	115.9 [68.7, 149.5]	95.3 [68.7, 121.8]	80.3 [56.3, 104.3]	66.9 [47.7, 86.2]	64.2 [48.2, 80.2]	52.6 [37.1, 68.2]
OWL	51.5 [11.4, 91.6]	112.3 [68.4, 156.1]	146.3 [109.2, 183.4]	137.2 [102.5, 172.0]	108.7 [108.7, 134.9]	94.7 [66.8, 122.7]	81.3 [50.5, 112.2]	69.3 [41.6, 97.1]
Insulin								
SWL	1156 [725, 1686]	4225 [2960, 5490]	4970 [3821, 6119]	3448 [2501, 4394]	2681 [1839, 3524]	1559 [1113, 2005]	1326 [907, 1745]	1219 [810, 1627]
OWL	1028 [282, 1773]	2912 [1926, 3897]	3855 [2917, 4793]	3248 [2249, 4247]	1892 [1126, 2658]	1291 [506, 2076]	1085 [315, 1857]	1031 [261, 1801]

Data presented as means in pg/mL at each time point with confidence intervals. SWL: suboptimal weight loss. OWL: optimal weight loss. BB: before breakfast. AG: active ghrelin. GLP-1: active glucagon-like peptide-1. PYY: peptide YY. Pg/mL: picogram per millilitre.

Table 13. Basal blood concentrations for appetite-related hormones in weight regain- and no weight regain groups and differences between groups.

	WR (n = 19)	**NWR (n = 14)**	**P-value**
AG (pg/mL)	145.8 ± 75.8	1689.0 ± 96.7	0.445
GLP-1 (pg/mL)	105.1 ± 127.5[s]	73.0 ± 128.3[s]	0.184
PYY (pg/mL)	18.9 ± 41.5[s]	15.6 ± 18.3[s]	0.592
Insulin (pg/mL)	**1221.6 ± 1634.6[s]**	**435.7 ± 275.3[s]**	**0.001**

Data presented as mean ± SD or median ± IQR for basal blood concentration in pg/mL for both groups separately. Median values denoted by [s], are presented for non-normally distributed data. WR = weight regain. NWR = no weight regain. AG: active ghrelin. GLP-1: active glucagon-like peptide-1. PYY: peptide YY. Pg/mL: picogram per millilitre.

Table 14. Blood concentrations for appetite-related hormones in weight regain- and no weight regain groups, and differences between groups in AUC and iAUC values.

	WR (n = 19)	**NWR (n = 14)**	**P-value for difference**
AG AUC (pg/mL)	14809 ± 7624	17094 ± 8028	0.412
AG iAUC (pg/mL)	-8899 ± 5969	-9708 ± 8465	0.749
GLP-1 AUC (pg/mL)	54201 ± 22420	50453 ± 25802	0.664
GLP-1 iAUC (pg/mL)	33662 ± 18311	34954 ± 22112	0.858
PYY AUC (pg/mL)	13666 ± 5551	11839 ± 8050	0.473
PYY iAUC (pg/mL)	8779 ± 4535	7613 ± 5209	0.489
Insulin AUC (pg/mL)	**408385 ± 190693**	**220901 ± 97064**	**0.001**
Insulin iAUC (pg/mL)	173738 ± 129245	151254 ± 72823	0.508

Data presented as mean ± SD or median ± IQR of AUC for blood concentration in pg/mL for both groups. Median values denoted by [s], are presented for non-normally distributed data. WR: weight regain. NWR: no weight regain. AG: active ghrelin. GLP-1: active glucagon-like peptide-1. PYY: peptide YY. AUC: area under the curve. iAUC: incremental area under the curve.

Appendix II. Subjective feelings of appetite tables

Table 15. Subjective feelings of appetite in fasting and changes over time postprandially.

Time (min)	**BB**	**15**	**30**	**60**	**90**	**120**	**150**
Hunger							
SWL	47.4 [36.1, 58.7]	21.5 [13.4, 29.6]	21.3 [11.6, 31.1]	27.4 [15.2, 39.5]	19.6 [19.6, 45.4]	39.5 [26.4, 52.6]	46.4 [33.2, 59.7]
OWL	34.6 [20.1, 49.1]	13.8 [6.1, 21.5]	11.3 [5.5, 17.2]	15.7 [4.4, 27.1]	20.3 [7.0, 33.7]	28.1 [13.5, 42.8]	35.5 [20.4, 50.6]
Fullness							
SWL	23.4 [11.0, 36.0]	55.9 [44.2, 67.6]	59.7 [46.2, 73.2]	59.9 [46.5, 73.3]	49.6 [35.2, 64.1]	42.2 [28.0, 56.5]	36.4 [25.4, 47.5]
OWL	18.1 [8.0, 28.2]	58.4 [44.6, 72.2]	57.3 [40.0, 74.5]	57.8 [39.7, 75.9]	46.1 [26.5, 65.6]	36.1 [19.0, 53.2]	29.2 [12.5, 45.9]
DTE							
SWL	46.4 [34.6, 58.2]	25.2 [14.9, 35.4]	22.3 [12.8, 31.9]	27.1 [16.1, 38.0]	31.7 [21.1, 42.3]	39.9 [28.1, 51.7]	43.2 [32.6, 53.7]
OWL	32.4 [20.4, 44.4]	15.9 [7.1, 24.7]	12.3 [6.8, 17.9]	12.9 [5.8, 20.0]	21.1 [10.6, 31.7]	22.4 [12.9, 31.9]	31.5 [19.7, 43.3]
PFC							
SWL	49.7 [42.9, 56.5]	28.7 [20.5, 37.0]	26.1 [16.8, 35.4]	28.7 [18.5, 38.9)	31.4 [20.8, 42.1]	41.1 [29.9, 52.4]	43.6 [32.8, 54.3]
OWL	33.7 [27.6, 39.7]	20.5 [10.8, 30.1]	16.1 [9.4, 22.9]	15.2 [8.3, 22.1]	19.2 [11.1, 27.3]	29.1 [19.8, 29.4]	34.5 [24.6, 44.4]

Data presented as means in mm at each time point with confidence intervals. SWL: suboptimal weight loss. OWL: optimal weight loss. BB: before breakfast. PFC: prospective food consumption. DTE: desire to eat.

Table 16. Subjective feelings of appetite in fasting state for weight regain- and no weight regain groups, and differences between groups.

	WR (n = 19)	NWR (n = 14)	P-value for difference
Hunger (mm)	47.4 ± 33.0[s]	50.0 ± 49.0[s]	0.884
Fullness (mm)	16.0 ± 37[s]	13.5 ± 21[s]	0.546
DTE (mm)	34.0 ± 31[s]	46.5 ± 34[s]	0.702
PFC (mm)	45.0 ± 18[s]	42.5 ± 22[s]	0.304

WR = weight regain. NWR = no weight regain. Data presented as mean ± SD or median ± IQR of basal visual analogue scale scores in mm for both groups. Median values denoted by [s], are presented for non-normally distributed data.

Table 17. Subjective feelings of appetite in weight regain- and no weight regain groups, and differences between groups in AUC and iAUC values.

	WR (n = 19)	NWR (n = 14)	P-value for difference
Hunger AUC (mm)	4006 ± 2970	4190 ± 3334	0.868
Hunger iAUC (mm)	-2645 ± 3288	-1489 ± 2082	0.313
Fullness AUC (mm)	7290 ± 3853	7123 ± 3558	0.900
Fullness iAUC (mm)	4242 ± 3843	4058 ± 3440	0.888
DTE AUC (mm)	3413 ± 2370[s]	4099 ± 4348[s]	0.689
DTE iAUC (mm)	-2036 ± 3128	-2049 ± 2262	0.989
PFC AUC (mm)	4291 ± 2259	4292 ± 2708	1.000
PFC iAUC (mm)	-2293 ± 2651	-1770 ± 2380	0.563

WR = weight regain. NWR = no weight regain. Data presented as mean ± SD or median ± IQR of AUC and iAUC for visual analogue scale scores in mm for both groups. Median values denoted by [s], are presented for non-normally distributed dat

Appendix III. Diben Drink specifications

NUTRITIONAL COMPOSITION	Per 100ml	Per 200ml
ENERGY kcal(KJ)	150 (630)	300 (1260)
PROTEIN g	7.5	15
CARBOHYDRATE g	13.1	26.2
of which sugars g	2.5	5
of which lactose g	≤0.5	≤1.0
FAT g	7	14
of which saturated fatty acids g	1.7	3.4
of which MCT g	1.2	2.4
of which monounsaturated fatty acids g	3.8	7.6
of which polyunsaturated fatty acids g	1.5	3
of which EPA g	0.038	0.076
of which DHA g	0.016	0.032
FIBRE g	2	4
SALT g (Na x 2.5)	0.16[1], 0.18	0.32 [1], 0.36
WATER ml	79	158
OSMOLARITY mosmol/l	350-390**	350-390**
OSMOLALITY mosmol/kg H2O	440-490**	440-490**
MINERALS AND TRACE ELEMENTS	**Per 100ml**	**Per 200ml**
SODIUM mg (mmol)	65 (2.8) [1] 70(3.1)	130(5.6) [1,] 140(6.2)
POTASSIUM mg (mmol)	130 (3.3)	260 (6.6)
CHLORIDE mg (mmol)	55 (1.6)	110 (3.2)
CALCIUM mg (mmol)	150 (3.7)	300 (7.4)
PHOSPHORUS mg (mmol)	95 (3.1)	190 (6.2)
MAGNESIUM mg (mmol)	15 (0.6)	30 (1.2)
IRON mg	2	4
ZINC mg	1.5	3
COPPER µg	300	600
MANGANESE mg	0.4	0.8
IODINE µg	30	60
CHROMIUM µg	10	20
MOLYBDENUM µg	15	30
FLUORIDE mg	0.2	0.4
SELENIUM µg	10	20
VITAMINS AND OTHER NUTRIENTS*	**Per 100ml**	**Per 200ml**
VITAMIN A µgRE	170	340
of which β- carotene µg RE	50	100
VITAMIN D_3 µg	2	4
VITAMIN E mgαTE	3	6
VITAMIN K_1 µg	16.7	33.4
VITAMIN B_1 mg	0.23	0.46
VITAMIN B_2 mg	0.32	0.64
NIACIN mg	1.50(2.76 mgNE)	3(5.52 MG NE)
VITAMIN B_6 mg	0.33	0.66
VITAMIN B_{12} µg	0.6	1.2
PANTOTHENIC ACID mg	1.2	2.4
BIOTIN µg	7.5	15
FOLIC ACID µg	50	100
VITAMIN C mg	15	30
CHOLINE* mg	26.7	53.4

TYPICAL FATTY ACID PROFILE	g Per 100ml
C6:0 Caproic acid	0.002
C8:0 Captylic acid	0.67
C10:0 Capric acid	0.46
C12:0 Lauric acid	0.004
C14 Myristic acid	0.014
C16 Palmitic acid	0.28
C16:1 n-7 Palmitoleic acid	0.017
C18 Stearic acid	0.14
C18:1 n-9 Oleic acid	3.51
C18:2 n-6 Linoleic acid	1.02
C18:3 n-3 α-Linolenic acid	0.39
C20:5 n-3 Eicosapentaenoic acid	0.038
C22:6 n-3 Docosahexaenoic acid	0.016
Other - 3 fatty acids from fish	0.005

TYPICAL AMINO ACID PROFILE	g Per 100ml
ESSENTIAL	
Histidine	0.22
Isoleucine	0.44
Leucine	0.76
Lysine	0.64
Methionine	0.22
Phenylalanine	0.38
Threonine	0.37
Tryptophan	0.08
Valine	0.54
CONDITIONALLY ESSENTIAL	
Cysteine	0.05
Tyrosine	0.41
Taurine	n.s.
Glycine	0.14
Arginine	0.28
Glutamine	0.57
Proline	0.76
NON-ESSENTIAL	
Aspartic acid and asparagine	0.72
Glutamic acid	0.79
Alanine	0.25
Serine	0.46

TYPICAL CARBOHYDRATE PROFILE	g Per 100ml
Glucose	0.16-0.17**
Fructose	1.9
Maltose	0.21-0.22**
Saccharose	n.s.
Lactose	≤0.5
Oligosaccharides and Polysaccharides	5.35
Starch	5.25

n.s= not specified
1. Vanilla flavour
**depending on flavour

Appendix IV. Information about the study with consent form

HELSE NORD-TRØNDELAG

BAROBS-HOLD VEKTA

Forespørsel om deltakelse i forskningsprosjektet

Suboptimalt vekttap 10-15 år etter gastrisk bypass – Undersøkelse av potensielle årsaker til utilstrekkelig vedlikehold av vekttap etter fedmekirurgi (BAROBS-Suboptmal weight loss) SUB10WL

I forbindelse med at du deltok i BAROBS-studien i Helse Midt-Norge, ga du oss lov til å ta kontakt med deg angående oppfølgingsstudier. Dette er et spørsmål om å delta i videre forskning etter gastrisk bypass. Vi vil undersøke ulike faktorer, som kan være forklaringsmekanismer (kosthold, fysisk aktivitet, appetitt, energiforbruk, spiseadferd, søvn, mikrobiotika) for at fedmeoperasjon gir ulikt vekttap på kort og lang sikt. Selv om fedmekirurgi er den beste metoden for å holde vekttapet over tid, er det noen som ikke oppnår den forventede vektreduksjonen, kunnskap om hvorfor vekttapet og vektoppgang er forskjellig er begrenset. En mindre del av pasientene vil erfare redusert vekttap eller tidlig vektoppgang dvs mindre enn 50 % av overvekten. En bedre forståelse om hvorfor det er slik også etter fedmekirurgi vil være av stor betydning for enkelt mennesket og samfunnsøkonomien.

Hva innebærer PROSJEKTET?

I denne studien kaller vi inn 3 ulike grupper deltagere; en gruppe som gikk ned i vekt 50 % eller mer ved gastrisk bypass og har gått opp mindre enn 15 %, den andre gruppe deltagere som gikk ned mindre enn 50 % av overvekten og/ eller har gått opp mer enn 15 % av vekttapet og den tredje gruppen er en kontrollgruppe, fra venteliste til fedmekirurgi, som har den samme BMI som de andre to gruppe pre-operativt.

Hovedmålet med studien er å undersøke potensielle årsaker til SUB optimalt vekttap 10-15 år etter fedmekirurgi. Derfor vil vi undersøke:

- Veiing og kroppssammensetning
- Appetitt hormoner, følelse og hedonisk appetitt
- Indirekte kalorimetri og aktivitetsindusert energiforbruk
- Blodprøver for appetitthormoner, blodsukker og alkohol
- Tarmflora (avføringsprøver)
- Spørreskjema

Prosjektet går ut på at du blir innkalt til en dag (4-5 timer) med undersøkelser som nevnt over. Du må møte fastende, fordi noen av undersøkelsene skal gjøres i fastende tilstand.

I prosjektet vil vi innhente og registrere opplysninger om deg fra BAROBS-studien. Vi vil se på helseopplysninger, spørreskjemaopplysninger og blodprøver for å sammenstille dette med de nye opplysningene, for å finne svar på forskningsspørsmålet vårt.

Mulige fordeler og ulemper

Utilstrekkelig vekttap etter fedmekirurgi har stor medisinsk, sosial og økonomisk betydning for deltageren. Resultater fra denne studien vil være nyttig for fremtidens pasienter og helsesystem. Deltakelse kan også gjøre at du blir bedre kjent med appetitt mekanismene i kroppen din. Du vil også få informasjon om din kroppssammensetning og energi behov. Studien anses ikke som risikabel, men undersøkelsene innebærer noen blodprøver og at du må levere en avføringsprøve, som kan oppleves ubehagelig for noen deltaker.

Frivillig deltakelse og mulighet for å trekke sitt samtykke

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte studiekoordinator Siren Nymo, telefon 74098014/ 99514188, siren.nymo@ntnu.no.

Hva skjer med OPPLYSNINGENE om deg?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet i hensikten med prosjektet. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene.

Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste. Det er kun studiekoordinator Siren Nymo og masterstudenter som er involvert i prosjektet, som har tilgang til denne listen.

Prosjektleder har ansvar for den daglige driften av forskningsprosjektet og at opplysninger om deg blir behandlet på en sikker måte. Etter planen avsluttes prosjektet 31.12.2022. Av dokumentasjonshensyn skal opplysningene likevel bevares inntil 31.12.2027 før de slettes eller anonymiseres.

Hva skjer med prøver som blir tatt av deg?

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer, eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste.

Det er kun autorisert personell knyttet til prosjektet, som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Biobank

Blodprøvene for analyser av appetitthormoner og avføringsprøver som blir tatt vil bli lagret i en forskningsbiobank ved Institutt for Klinisk og Molekylær Medisin (NTNU). Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Overlege Torstein Rø er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Forsikring

Du er dekket gjennom Norsk pasientskadeerstatning jfr. Pasientskadelovens § 1.

Utlevering av opplysninger til andre

Ved å delta i prosjektet, samtykker du også til at avidentifiserte opplysninger om deg kan utleveres til andre forskere innen fedmeforskning. Alle forskningsprosjekt må godkjennes av Regional komite for medisinsk og helsefaglig forskningsetikk og andre offentlige instanser som loven krever.

Avføringsprøver vil bli sendt til Universitetet i Oslo for analyse av tarmflora, og blodprøver vil bli sendt til København Universitet for analyse av appetitt regulerende hormoner.

Koden som knytter deg til dine personidentifiserende opplysninger vil ikke bli utlevert.

Økonomi

Det gis ingen økonomisk kompensasjon for å delta i studien, men du slipper å betale egenandel for undersøkelsene som blir utført. Du kan sende reiseregning til HELFO for dekning av reiseutgifter.

Godkjenning

Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk ref. 2017/1828/REK sørøst B.

Dersom du mener at opplysninger om deg eller biologisk materiale fra deg ikke behandles i samsvar med forskriften kan du henvende deg til Datatilsynet eller Statens helsetilsyn.

Dataansvarlig for prosjektet:
St. Olavs Hospital v/ adm. dir., post@stolav.no

Forskningsansvarlig:
Kirurgisk klinikk St. Olavs hospital v/Klinikksjef Birger Henning Endreseth, e-post: Birger.Henning.Endreseth@stolav.no

Prosjektledelse: Regionalt senter for fedmeforskning og innovasjon professor Bård Kulseng, e-post: bard.kulseng@stolav.no, telefonnummer 72 82 54 08.

Helse Nord-Trøndelag v/adm. dir. er dataansvarlige for utleveringen av opplysninger til prosjektet.

Du har rett til å klage på behandlingen av dine opplysninger til Datatilsynet.

KONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet kan du ta kontakt med studiekoordinator Siren Nymo, 74098014/99514188, siren.nymo@ntnu.no

Personvernombud ved institusjonen er Fredrik Høie Jordet, fredrikhoie.jordet@helse-nordtrondelag.no

Jeg samtykker til å delta i BAROBS-HOLDVEKTA

Jeg har lest gjennom informasjonen om forskningsprosjektet «BAROBS-HOLDVEKTA» og samtykker til at data om meg som samles inn i prosjektet brukes til fedmeforskning. Jeg er kjent med at opplysninger samles i en felles regional forskningsdatabase og at tilsvarende gjelder for data som hentes fra min pasientjournal/lokalt kvalitetsregister for fedmekirurgi.

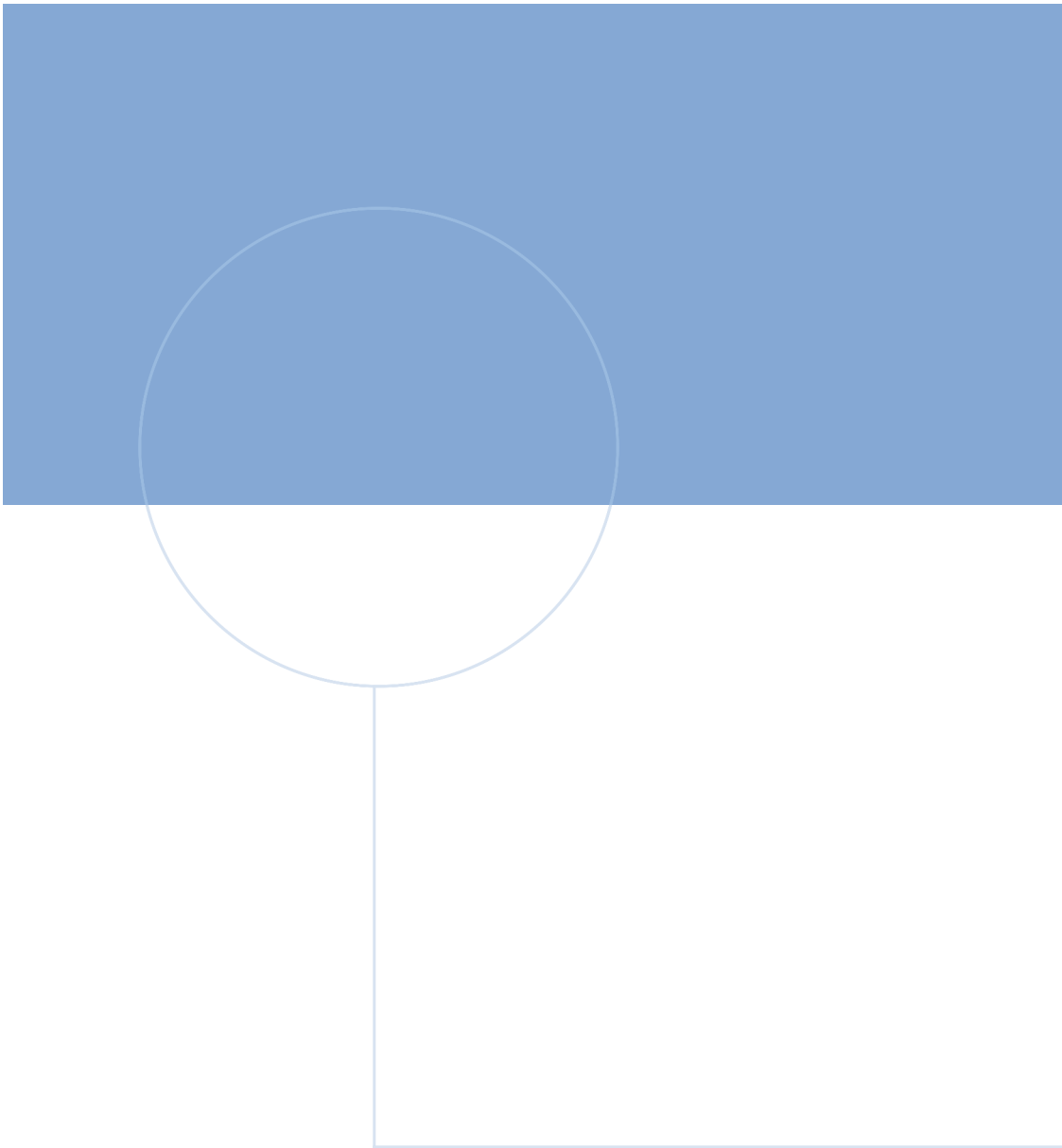
Jeg er kjent med at registrerte opplysninger oppbevares til prosjektet avsluttes, og slettes eller anonymiseres seinest 31.12.2027.

Jeg er kjent med mine rettigheter til innsyn, endring og sletting av opplysninger som er samlet inn.

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver

Jeg bekrefter å ha gitt informasjon om prosjektet

Sted og dato	Signatur
	Rolle i prosjektet



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