Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

Karina Stakvik

Appetite suppression during ketogenic diets: Do fecal short-chain fatty acids play a role?

Master's thesis in Clinical Health Science - Obesity and Health

May 2021

NTNU
Kunnskap for en bedre verden

Karina Stakvik

Appetite suppression during ketogenic diets: Do fecal short-chain fatty acids play a role?

Master's thesis in Clinical Health Science - Obesity and Health
Supervisor: Catia Martins
Co-supervisor: Jessica Ann Røknes
May 2021

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

NTNU
Norwegian University of
Science and Technology

Abstract

Introduction: Ketogenic diets (KDs) have the ability to suppress the increase in appetite otherwise seen with diet-induced weight loss (WL). The exact mechanisms involved remains unknown, but changes in gut microbiota (GM)/short-chain fatty acids (SCFA) have been suggested as a potential link. Therefore, the main aim of this thesis was to evaluate if changes in fecal SCFA concentrations are associated with appetite suppression during WL induced by a ketogenic low energy diet (LED). A secondary aim was to investigate the impact of WL induced by a ketogenic LED on GM and SCFA.

Method: This study is a longitudinal study with repeated measurements. 83 healthy adults with obesity (BMI: 34.9±3.5 kg/m^2) underwent an 8-week powder-based LED (1000 kcal/day), with a carbohydrate content ranging from 70-130 g/day, followed by a 4-week refeeding and weight stabilization phase. Body weight (BW) and composition, subjective appetite feelings and plasma concentration of appetite hormones (both fasting and postprandial), fasting β-hydroxybutyric acid (BHB) plasma concentration, GM and fecal SCFA were measured at three timepoints (baseline (BL), week 9 (W9) and week 13 (W13)). Data are shown as estimated marginal means ± SEM.

Results: Participants lost 14±1.3 kg at W9 ($P<0.001$) and maintained it at W13. Plasma BHB increased from BL to W9 (0.8±0.0 mmol/L) and was no longer different from BL at W13. Fasting and postprandial feelings of hunger did not change at W9, but both were higher than BL at W13 ($P<0.01$, $P<0.001$, respectively). Basal, but not postprandial AG increased from BL to W9 ($P<0.001$), and both basal and postprandial AG were higher than BL at W13 ($P<0.001$, $P<0,01$, respectively). Alistipes and Ruminococcaceae both increased from BL to W9 ($P<0.001$, $P<0.01$, respectively), and Ruminococcaceae decreased from W9 to W13 ($P<0.05$). Eubacterium rectale decreased from BL to W9 ($P<0.001$) and increased from W9 to W13 ($P<0.001$), however the relative composition of this group was lower at W13 compered to BL ($P<0.01$). Fecal concentration of acetic, propionic and butyric acids decreased from BL to W9 ($P<0.001$, for all) and increased from W9 to W13 ($P<0.05$, $P<0.01$, $P<0.001$, respectively), but values at W13 were still below BL ($P<0.01$, for all). The greater the decrease in butyric acid the higher the increase, or smaller the reduction, in basal AG ($r=-0.260$, $P=0.043$, $n=61$) under ketogenic conditions (W9).

Conclusions: This study suggest that WL induced by KD alters GM and SCFA production. However, fecal SCFA do not seem to play a role in the appetite suppression seen under ketogenic conditions. The exact molecular mechanisms mediating appetite suppression under KD remain unknown and more research is clearly needed in this field.

Sammendrag

Introduksjon: Ketogene dietter (KD) har evnen til å undertrykke økningen i appetitt som ellers ses med diettindusert vekttap (WL). De nøyaktige mekanismene som er involvert i dette forblir ukjent, men endringer i tarmmikrobiota (GM) / kortkjedede fettsyrer (SCFA) har blitt foreslått som en potensiell kobling. Derfor var hovedmålet med denne oppgaven å evaluere om endringer i fekale SCFA-konsentrasjoner er assosiert med appetittundertrykkelse under WL indusert av en ketogen lavenergidiet (LED). Et sekundært mål var å undersøke virkningen av WL indusert av en ketogen LED på GM og SCFA.

Metode: Denne studien er en longitudinell studie med repeterte målinger. 83 friske voksne med fedme (BMI: 34,9±3,5 kg/m2) gjennomgikk en 8-ukers pulverbasert LED (1000 kcal/dag), karbohydratinntaket varierte fra 70-130 g/dag, og var etterfulgt av en 4-ukers «refeeding» og vektstabiliseringsfase. Kroppsvekt og kroppssammensetning, subjektive appetittfølelser og plasmakonsentrasjon av appetitthormoner (både fastende og postprandial), fastende β-hydroxybutyric syre (BHB) plasmakonsentrasjon, GM og fekal SCFA ble målt på tre tidspunkter (baseline (BL) uke 9 (W9) og uke 13 (W13)). Data vises som estimert marginalt gjennomsnitt ±SEM**.**

Resultater: Deltakerne gikk ned 14±1,3 kg fra BL til W9 (P<0,001) og vekten ble opprettholdt frem til W13. Plasma BHB økte fra BL til W9 (0,8-0,0 mmol / L), ved W13 var BHB verdiene like som ved BL. Fastende og postprandial følelse av sult endret seg ikke under dietten (W9), men begge var høyere enn BL ved W13 (henholdsvis, P<0,01, P<0,001). Basal, men ikke postprandial AG økte fra BL til W9 (P<0,001), og både basal og postprandial AG var høyere ved W13 enn BL (henholdsvis, P<0,001, P<0,01,). Både Alistipes og Ruminococcaceae økte fra BL til W9 (henholdsvis, P<0,001, P<0,01), og Ruminococcaceae ble redusert fra W9 til W13 (P<0,05). Eubacterium rektale ble redusert fra BL til W9 (P<0,001) og økte fra W9 til W13 (P<0,001), men den relative sammensetningen av denne gruppen var lavere ved W13 sammenlignet med BL (P<0,01). Fekal konsentrasjon av eddiksyre, propionsyre og smørsyre ble redusert fra BL til W9 (P<0,001 for alle) og økte fra W9 til W13 (P<0,05, P<0,01, P<0,001), men verdiene ved W13 var fortsatt lavere enn BL verdier (P<0,01, for alle). Jo større reduksjon i smørsyre var jo høyere økning, eller mindre reduksjon, i basal AG (r=-0,260, P=0,043, n=61) under ketogene forhold (W9).

Konklusjon: Denne studien foreslår at WL indusert av KD endrer GM. Imidlertid ser ikke fekal SCFA ut til å spille en rolle i undertrykkelsen av appetitt sett under ketogene forhold. De nøyaktige molekylære mekanismene som formidler appetittundertrykkelse under ketogene forhold er fortsatt ukjente, og det er tydelig behov for mer forskning på dette feltet.

Acknowledgements

The past year has been a learning process like no other. I have learned about the academic world, and a lot about myself. It has been both challenging and frustrating from time to time, and I would not have been able to do this without all the help and support I have received the last year.

First of all, I would like to thank my main supervisor, Catia Martins, for giving me the opportunity to work on this project, for believing in me and for always being available to answer all my questions and guide me in the right direction. I am truly grateful for her valuable advice, guidance, and dedication of time throughout the entire process. I would also like to thank my secondary supervisor Jessica Ann Røknes for guidance and good advice and Knut Rudi for giving good advice on the gut microbiota.

Last, but not least, I would like to thank my children and the rest of my family and friends for their patience, support and encouragement throughout the past year, and my friends Kristin Karlsøen and Stine Nicoline Nygård for proofreading my thesis.

Jessheim, May 2021

Karina Stakvik

Abbreviations

AcAc - acetoacetate

AG - acylated ghrelin

ARC - arcuate nucleus

BL - baseline

BHB - β-hydroxybutyric acid

BMI - body mass index

BW - body weight

CHO - carbohydrate

CVD - cardiovascular disease

CNS - central nervous system

CCK - Cholecystokinin

CoA - coenzyme A

DAG - desacyl ghrelin

DTE - desire to eat

FFAR2 – free fatty acid receptor 2

FFAR3 – free fatty acid receptor 3

GLP-1 - glucagon-like peptide 1

GM - gut microbiota

GHS-R - growth hormone secretagogue receptor

IQR – Inter Quartile range

KDs – Ketogenic diets

KLCDs - ketogenic low carbohydrate diets

KB - ketone bodies

LED - low energy diets

NCD - noncommunicable diseases

NTNU – Norwegian university of science and technology

PAL – Physical activity level

PFC - prospective food consumption

POMC - Proopiomelanocortin

PYY - peptide YY

ObeCe - regional Center of Obesity Research and Innovation

RMR – resting metabolic rate

REK - regional ethics committee

SCFA – Short-chain fatty acids

SEM – standard error of the mean

T2DM - type 2 diabetes

VLEDs - very-low energy diets

VAS - visual analogue scale

WL - weight loss

WHO - World Health Organization

Contents

1.0 Background

1.1 Introduction

Obesity is a growing problem worldwide and its prevalence almost tripled since 1975 (1). More than 1.9 billion adults (18 years and older) were overweight, and over 650 millions of these were obese in 2016 (1). In Norway about 23% adults (almost one million people) over the age of 18 has obesity. Norway is one of the European countries that has had the largest increase in the incidence of obesity in recent years, which puts Norway above the average incidence of obesity for European countries (2).

Overweight and obesity are usually defined by body mass index (BMI) (3), and the World Health Organization (WHO) defines a BMI greater than or equal to 25 kg/m^2 as overweight and a BMI greater than or equal to 30 kg/m^2 as obesity (1). Obesity is a complex disease (4), and is considered a risk factor for noncommunicable diseases (NCD) such as type 2 diabetes (T2DM), cardiovascular disease (CVD), and some types of cancer (5,6). Weight gain and obesity are a result of chronic positive energy balance, with energy intake exceeding energy expenditure (7). A study from 2008 shows that energy expenditure has not declined since the 1980`s, suggesting that increased food consumption is the reason for obesity (8). According to the WHO obesity is preventable (1).

In order to achieve weight loss (WL), various methods can be used such as: bariatric surgery (9) and lifestyle interventions including different types of diets, increased physical activity and behavioral techniques (10). There is evidence that energy restricted diets are the most effective non-invasive method to achieve WL (11), and that intensive lifestyle intervention should be the first treatment choice for obesity (9).

Diet-induced WL is usually followed by an increase in appetite (feelings of hunger and ghrelin secretion) (12,13), which may compromise adherence to the diet and WL outcomes. However when WL is induced by ketogenic diets (KDs) the increase in hunger and ghrelin are absent (14). The absence of increase in appetite typically seen in KDs is believed to result from ketosis (14,15), but the mechanisms are not completely understood, and gut microbiota (GM)/short-chain fatty acid (SCFA) might be involved (16). The GM produces SCFA by fermentation of non-digestible carbohydrates (CHO), and the amount of SCFA produced, is largely determined by the amount of fermentable CHO in the diet (17). SCFA are thought to play a key role in increasing the host capacity to harvest excess energy from the diet (18), and therefore maybe appetite suppression after KDs can be explained by changes in SCFA (and/or GM).

1.2 Appetite and appetite regulation

Humans eat in episodes (meals and snacks) (19). In humans, appetite is controlled through integrated sequences of behaviors that are constantly changing (e.g., hunger, satiety, urge to eat) and more stable characteristics (e.g., hunger and swallowing) (20). People usually eat until they reach satiation (comfortably full), and the drive to eat is normally low right after a meal (satiety). The drive to eat builds up until the next eating episode, but the moment for the next episode is not only determined by internal factors, but also influenced by the hedonic appetite system and by environmental factors such as social pressure, sensory stimulation, tension reduction and boredom, food preferences and food availability (19,21). Hedonic appetite is the brain's desire to eat for pleasure despite a lack of physiological hunger (22).

The hypothalamus, in particular its arcuate nucleus (ARC), and the corticolimbic pathway in the central nervous system (CNS), both play an important role in feeding behavior in humans (23,24). Neuronal circuits in the limbic system mediate the motivational and reward aspects of feeding (26). Both food intake and energy expenditure tend to variate from a day-to-day basis, however under normal circumstances body weight (BW) is carefully regulated by the homeostatic system (25). The gut-brain axis controls appetite and satiety through neuronal and hormonal signals (long-term effect) (26). When nutrients reach the small intestine, the release of several satiety hormones is stimulated, such as cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide 1 (GLP-1). These peptides act as a negative feedback signal to terminate feeding and reduce meal size (26). Previously, it was common to distinguish between short-term and long-term regulation of appetite, but episodic (meal-to-meal) and tonic (days and weeks) signals seems to be more appropriate terms to use (21,27,28). Episodic signals are essentially inhibitory, but can be stimulating, and are generally generated by eating (27). These signals change according to eating patterns and most are associated with signaling satiety (27). Tonic signals originate from tissue stores, including adipose tissue, and exert a tonic pressure on the expression of appetite (27). The balance between tonic and episodic signaling and the balance between homeostatic and hedonic processes ultimately determines the will or reluctance of humans to eat or not eat (20).

In the regulation of energy balance, gut hormones act like short-term signals and primarily control satiety, hunger and satiation. On the other hand, adiposity signals (insulin and leptin) act as long term signals (25,29). Insulin is produced by the beta cells in the pancreas, and leptin is mainly synthesized by adipose tissue and the circulating concentrations of both leptin and insulin reflect the body fat content (25,29). Circulating insulin and leptin act on hypothalamus, by stimulating catabolic pathways at the same time as they inhibit anabolic pathways. These pathways have opposing effects on energy balance (29).

As mentioned earlier, the small intestine secretes gut hormones when nutrients reach the small intestine (26). The macronutrient composition of a meal has an impact on which satiety hormones are released from the gastrointestinal tract (26,30,31). CCK is secreted in response to long-chained fatty acids, small peptides and protein (amino acids) and act as a satiation signal on the CNS through receptors of the nervus vagus (26,30). The concentration of CCK is highest postprandially and reduces gradually during fasting (32). GLP-1 is one of the products of proglucagon and is a gut peptide and is secreted post- prandially in proportion to the amount of calories ingested (33). Activation of GLP-1 receptors in the gut and CNS is modulated by cholinergic signals from the vagus nerve and promotes satiety (34). The bioactive form of PYY is PYY_{3-36}, and is released after a meal in a dose-dependent manner to the protein content of the meal (35,36). PYY performs its satiating actions by inhibitory actions on the Y2 receptor in the ARC (37).

Ghrelin is known as the hunger-hormone due to its role in controlling appetite, but studies have shown that ghrelin exercises a wide range of functions in addition to regulation of food intake (38–40). Ghrelin is known as the only peripherally- derived orexigenic hormone that increases appetite and food intake, even though it original was discovered as the endogenous ligand of growth hormone secretagogue receptor (GHS-R) (39–43). Ghrelin is a gastric peptide hormone, mainly produced in the stomach and the concentration of ghrelin increases before a meal/fasting and decreases after a meal (40–42). There are two main isoforms of ghrelin in the circulation; desacyl ghrelin (DAG) and acylated ghrelin (AG) (44). DAG seems to account for more than 90% of total circulating ghrelin (45), however AG is the active form (from now on called ghrelin, if not other specified) that has an impact on the regulation on food intake and BW regulation (44,45). Ghrelin activates the ARC and stimulates appetite by central and peripheral pathways and through the vagus nerve. Ghrelin is secreted by the stomach and reaches the hypothalamus in the brain, by crossing the blood-brain barrier (39).

Appetite can also be measured by looking at subjective appetite feelings and actual food intake (19). To measure changes in appetite usually the subjective ratings of hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) are measured by visual analogue scale (VAS) (19,46). A VAS is typically a straight line (varying length) with extreme words anchored in both ends, for example for the question "how hungry are you", are normally anchored with "not hungry at all" in one end, and in the other end "I have never been more hungry" (46,47). The subjects make a mark on the line that corresponds with their feeling, and the quantification is done, by measuring the distance from the left end of the line to the mark (47).

1.3 Weight loss and changes in appetite

The majority of studies shows that WL in overweight and obese subjects leads to increased hunger, DTE (13,48–54) and PFC (13,49,55–57), and no change in postprandial fullness (13,49,52,55–57).

The increased appetite seen with diet-induced WL is likely to reflect a normalization to a lower BW (58,59). WL is also accompanied by changes in the basal and postprandial concentration of appetite-regulating hormones such as ghrelin, CCK, PYY and GLP-1 (49,60). The majority of studies show an upregulated ghrelin secretion (both basal and postprandial) as a respond to diet-induced WL (12,48,49,56,61–65), but a few show no changes in ghrelin (56,66,67). One study showed reduction in the postprandial levels of CCK, total PYY and no change in GLP-1 in individuals for up to one year after WL (49), and another study showed an increase in secretion of total GLP-1 and PYY ($_{3\text{-}36}$) as a response to WL (68). So, the impact of WL on the release of satiety peptides remains controversial, and seems to depend on which hormonal fractions are measured (49,60,68). Regardless, no study has ever shown a decrese in fullness feelings in the postpradial state with WL.

1.4 Ketogenic diets, ketosis and changes in appetite markers

Dietary interventions such as ketogenic low carbohydrate diets (KLCDs) and very-low energy diets (VLEDs) are both used in the treatment of obesity (14,69,70). They both induce ketosis, either by severely restricting CHO (14) or energy intake (69,70), respectively.

While diet-induced WL is usually followed by increased appetite (hunger feelings and ghrelin secretion), when WL is induced by KLCDs or VLEDs the increase in appetite is absent (14). Due to the growing evidence that KDs may suppress appetite, KDs may therefore be an important tool in weight management (12,14,16,71,72). The absence of increase in hunger and ghrelin (otherwise seen with WL) that is typically seen in KLCDs and VLEDs is believed (but not clinically approved) to result from ketosis (14,15).

After a few days of drastically reduced CHO intake (KLCDs, VLEDs or fasting), the body can no longer provide the body with enough glucose, and some cells in the body cannot use fatty acids as an energy source, such as red cells in the blood and in the medulla of the kidney and the CNS and is therefore forced to find alternative energy sources (16,73). An overproduction of coenzyme A (CoA) (seen in high-fat/low-CHO diets, fasting and type 1 diabetes), which is driven by low insulin levels, leads to a higher than normal production of ketone bodies (KB) (acetoacetate (AcAc), β-hydroxybutyric acid (BHB) and acetone), known as ketogenesis (16,73). KB can cross the blood brain barrier, and can therefore be used as an energy source for the CNS

(16). Ketogenesis occurs primarily in the liver (mitochondrial matrix), and the main KB produced in the liver is AcAc, however, BHB is the primary circulating KB (16,73).

The amount of KB generated and used as fuel is determined by (low) CHO availability and the action of insulin. Under normal conditions fasting serum level of BHB is below 0.1mmol/L (16,73). Nutritionally induced ketosis (defined as a BHB >0.3 mM) is a physiological status and ketonemia reaches maximum levels of 7-8 mmol/L without any changes in pH, and it is very important to differentiate it from the pathological keto acidosis seen in type 1 diabetes, where ketonemia can be over 20 mmol/L and a reduction in the pH values (16,73).

The exact mechanisms through which KDs suppress appetite remain unknown, however, ketosis is thought to be involved (12,14–16,71–75). Diets that include high-protein and low CHO seem to suppress appetite more than diets that include high-protein and medium amounts of CHO (76). Nymo and colleagues found that the suppression of appetite only lasted for as long as the participants were ketotic, and that with refeeding and reintroduction of CHO, there was a significant increase in hunger feelings and ghrelin secretion above baseline (BL) levels (74). Martins and colleagues also found recently that the higher the plasma concentration of BHB (fasting) under a VLED, the smaller the increase in ghrelin secretion and the larger the increase in the plasma concentrations of GLP-1 and CCK in the postprandial state (77). These findings add further evidence for the role of ketosis in modulating appetite, however more research is needed to confirm these findings and to elucidate the mechanisms through which ketosis modulates the secretion of gut peptides (77). KDs, have a contradictory role on GM, and changes in GM trough modulation of fecal SCFA production, have also been suggested as another mechanism that could lead to appetite suppression under KDs (78).

1.5 Gut microbiota and short-chain fatty acid

When we are born the body gets colonized by microbes, all parts that are in contact with the external environment get colonized, and the colon has the highest abundance of microbes (79). During the first year of life, the GM in humans develops to a more mature microbiota composition, and there are several factors that influence the GM in adults, such as use of antibiotics, the host`s genetic, hygiene and diet (3). Most of the microbes colonizing the human intestine belong to the phyla Firmicutes and Bacteroidetes and, to a lesser extent, Actinobacteria, Proteobacteria, Verrucomicrobia, Fusobacteria, and Euryarchaeota (79,80). The GM has emerged as an environmental factor that modulates the host's energy balance (79).

The human gut contains trillions of microbes. As enzymes in the small intestine cannot break down substrates such as dietary fiber and resistant starch, the microbes in the gut help the host to break down these substrates. SCFA are the main products of bacterial fermentation of non-

digestible CHO (80). Acetate, propionate and butyrate are the main SCFAs produced by bacterial fermentation, in the approximate molar ratio 60 : 20 : 20 (79,81). SCFAs, are thought to play a key role in increasing the host capacity to harvest excess energy from the diet (18).These metabolites and microbial products act as signaling molecules that modulate appetite, gut motility, energy uptake and storage, and energy expenditure (79).

There is a strong link between the diet, the GM and the effects on the host's metabolism (79). Some types of dietary components will favor some microbes but not others, and the diet will therefore strongly influence the GM composition. Additionally, the diet composition will also determine which metabolites are produced by the GM (79).

Besides being an energy source, it has been suggested that an increase in production of SCFA produced by the GM are involved in a number of metabolic processes, effecting both appetite regulation and energy homeostatic (80,81). These findings suggest that since microbially produced SCFAs act as ligands on both free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3) (formerly: GPR43, and GPR41, respectively), and stimulates individual G-proteins which triggers cellular responses via secondary messenger cascades (80,81). Physiological concentrations of SCFA activate both FFAR2 (preference acetate and propionate) and FFAR3 (preference propionate and butyrate), and these receptors have been shown to be expressed in multiple tissue sites in addition to the gut epithelium; adipose tissue, immune cells, skeletal muscle and within the peripheral nervous system (80,81). FFAR2 and FFAR3 are precent on the colonic endocrine L-cells, which secretes PYY and GLP-1, and therefore have a possible role in modulating energy intake (80,81). Through the release of PYY and GLP-1, SCFA may also modulate motility of the upper digestive tract, by delaying the gastric emptying of ingested food and prolongs the stimulation of mechanoreceptors and chemoreceptors in the gastrointestinal tract (81). Further, increased concentrations of propionate in the portal vein, would lead to an increased hepatic energy status, as the liver would take up propionate and stimulate hepatic gluconeogenesis (81). An increased hepatic energy status can modulate feeding behavior through the stimulation of hepatic vagal nerve afferents (81). SCFA from the gut who do not undergo hepatic metabolism, enter the peripheral circulation, and circulating SCFA (acetate and propionate) have a major regulatory role in both adipocyte function and metabolism and stimulate the secretion of leptin by activation of FFA2 (80,81). Acetate may have a direct effect on central appetite regulation, by crossing the blood-brain-barrier (81). In the brain, acetate is mainly taken up by hypothalamus, and act as an anorectic signal in the ARC, which leads to increased Proopiomelanocortin (POMC) (81). Further studies are needed to determine how the function of the GM can be altered to obtain long-term beneficial metabolic effects.

Both dietary composition and duration of caloric restriction have been shown to have the ability to significantly alter GM composition and health outcomes (17). However, there is some concern

that CHO-reduced diets can have a negative impact on GM. A decrease in gut microbiome diversity, concentration of butyrate-producing bacteria and butyrate in fecal samples have been reported in response to low-CHO, high protein diets (82,83). However, more research is needed on how KLCDs affects the GM.

There is a growing interest on the impact of macronutrients on GM composition, especially dietary fibers (78). There is evidence that dietary patterns that include a high intake of "microbiota accessible carbohydrate" (non-digestible CHO) and non-refined foods supports the growth of SCFAs (78). Although the effect of KDs on the gut microbiome is a hot topic in research, only a few experimental studies sought to explore the relationship between KDs and GM (78). The effects of KDs on gut microbiome have been studied in both humans and mice, with mixed results (78).

KDs have established themselves as a feasible and popular WL approach in the management of obesity. However, still little is known regarding the impact of KDs on the GM (78). Moreover, it is possible that the absence of increased appetite during KD can be mediated by changes in GM and/or SCFA. Therefore, the main aim of this study was to investigate if appetite suppression following KDs was modulated by changes in SCFA.

1.6 Aims and Hypothesis

The primary aim of this study was to evaluate if changes in fecal SCFA concentrations are associated with appetite suppression during WL induced by a ketogenic low energy diet (LED). A secondary aim was to investigate the impact of WL induced by a ketogenic LED on GM and SCFA.

The hypothesis for the primary aim was that changes in fecal SCFA concentrations played a role in appetite suppression during WL induced by a ketogenic LED. For the secondary aim, the hypothesis was that WL induced by a ketogenic LED was associated with non-beneficial changes in GM and SCFA.

2.0 Methods

2.1 Study design

This master thesis is part of a larger PhD project where individuals with obesity were randomized to three isocaloric LEDs 4128 kJ/day (1000 kcal/day), with a CHO content of 70, 100 or 130 g CHO/day for 8 weeks. However, for the purpose of this thesis all CHO groups were analyzed together. The present thesis represents, therefore a longitudinal study with repeated measures. Participants followed a 1000 kcal/day LED with a CHO content ranging from 70-130g/day for 8 weeks, followed by a refeeding and weight stabilization phase for 4 weeks.

2.2 Participants

This study included ninety-nine adults (18-65 years old) who were healthy volunteers, both men and women, with class I or II obesity (30 kg/m^2 < BMI > 40 kg/m^2). To meet the inclusion criteria the participants had to be weight stable (<2 kg variation in weight within the last three months), not dieting to lose weight and have a sedentary lifestyle (<150 min of physical activity/week) (29) at BL.
Participants who were pregnant, breast-feeding, dealing with drug or alcohol abuse within the last two years, took medication known to affect appetite or induce WL or were enrolled in another obesity treatment program were excluded from this study. In addition to the criteria listed above, those who had a history of psychological disorders, had bariatric surgery, metabolic diseases (such as hypo/hyperthyroidism and diabetes type 1 or 2), eating disorders, lactose intolerance, gastrointestinal (particularly cholelithiasis), kidney, liver, lung, CVD, rheumatoid arthritis, Crohn`s disease and malignancies were also excluded from the study. Moreover, those who consumed probiotics over the last six months and/or used antibiotics over the last three months prior to BL were not included in this study.

The recruitment of the participants was done in line with the guidelines presented by the Helsinki Declaration. Participants were recruited through newspaper advertisements, Facebook, announcements on the intranet of St. Olav's Hospital and Norwegian University of Science and Technology (NTNU), and posters and flyers that were placed in Trondheim. Upon recruitment and fulfillment of eligibility criteria, written informed consent was obtained from all participants enrolled in this study (see appendix I). Participation in this study was voluntary and participants were able to withdraw from the study at any time. The study was approved by the regional ethics committee (REK) (Ref.,2016/1297), and registered in Clinical trials.gov (NCT03287726).

2.3 Detailed protocol

2.3.1 Weight loss phase

The participants followed one of the three powder-based LEDs for eight weeks. They were given specific instructions on how to follow the LEDs (appendix II) and was encouraged to consume at least 2.5L water/or other non-energy drinks during this phase. The participants were allowed to add 100g of vegetables containing low amounts of CHO per day (see appendix III) and were asked to maintain their physical activity level (PAL) during the full duration of the study.

Diet intervention

Participants were randomized into one of three isocaloric LEDs, that included 1000 kcal/day with different amount of CHO: 70, 100 and 130g CHO/day. Protein intake was fixed 75 g/day for all groups and the fat intake for all groups was at a minimum of 20 g/day (included 11 g linoleic acid and 1.4 g α-linoleic acid). The macronutrient composition of the three LEDs can be seen in table 12, appendix IV. The LEDs were powder-based products and were specially made to meet the macronutrient requirements for this study with raw ingredients, made by Food Innovation AS. The calculations were based on the recommendations of the European Food and Safety Authority for adults (26). A standardized, adequate amount of dietary fiber was also included in the total amount of CHO intake in each group to avoid the potential side effect of constipation.

2.3.2 Weight-stabilization phase

After the WL-phase, participants underwent a four-week refeeding and weight stabilization phase. Participants received an individualized dietary prescription and counseling from a dietician, and the diets consisted of 50-60% of CHO, 15-30% protein and 20-30% fat, that matched their energy expenditure to maintained their weight. During week 9 and 10, participants gradually started introducing normal food, with only 2 and 1 meal replacement/day, respectively. At the same time, participants were asked to increase their consumption of fruits and vegetables, poultry, fish and lean meats, as well as limit their intake of dietary fats, fatty meats, sweets, pastries and desserts. Healthy eating guidelines provided can be seen in appendix V. Daily energy needs were calculated by multiplying their current resting metabolic rate (RMR) using indirect calorimetry, and PAL assessed using activity monitors.

2.3.3 Compliance

The participants were followed up on a weekly basis by a team of researchers and research nurses at the Regional Center of Obesity Research and Innovation (ObeCe) in Trondheim, Norway throughout the entire duration of the diet. Dietary compliance was assessed on weekly visits by weighing and the measurement of fasting KB both in the urine (AcAc) (using Ketostix, Bayer Corp, Elkhart, IN) and in blood (BHB) (using a capillary blood ketone meter, Freestyle Optium Neo, Abbott Diabetes Care Inc, Alameda, CA).

Participants were also asked to keep a paper-based food diary, detailing the daily foods and fluids consumed throughout the diet. Any side effects of the diets and BW were also recorded every week. All participants completed the weekly food diaries throughout the intervention and the diaries were discussed every week at follow up.

Participants were instructed to maintain their PAL levels during the 8 weeks. Physical activity was assessed by asking the participants to use Sensewear armbands for a 7 -day period, at BL, week four and week eight. Instruction for activity monitors can be seen in appendix VI. For the data to be considered valid, the participants had to wear the device for more than 4 days (including at least one weekend day), and more than 22.8 hours per day (95% of the time) (30).

To estimate the daily average energy and macronutrient intake reported during the WL phase, a web-based diet planner based on the Norwegian food composition table, *Kostholdsplanleggeren* (Norwegian Directorate of Health and food safety Authority Oslo, Norway) was used. Only diaries completed during weeks 2, 5 and 8 of the study were included in the analysis.

2.4 Data Collection

The data was collected at three timepoints: BL (week 0), week 9 (after eight weeks of the LED) and week 13 (after refeeding and weight stabilization). Participants were asked to not consume any alcohol, caffeine or nicotine and not to participate in any strenuous activity after 8:00 PM the night before. Participants were asked to meet at 7:30 am or 8:00 am after an overnighting fast (at least 10 h), with water allowed ad libitum. BW, body composition, subjective feelings of appetite, plasma concentration of AG, CCK, PYY, GLP-1, insulin and BHB (only measured in fasting) were measured at all time points. Participants was also asked to give a stool sample at all three timepoints for analysis of GM and SCFA. More information about these measurements is provided below.

2.4.1 Anthropometric measurements

Anthropometric measurements (BW, height, waist and hips) were measured using standard procedures. To measure BW, Seca 877 digital scale (SECA, Hamburg, Germany) was used. BW were measured after emptying the bladder, wearing underwear and without shoes and was rounded to the nearest 0.1 kg. Hip and waist circumference were measured at the widest point around the hip, and the midpoint between the iliac crest bone and the lowest rib, respectively using a metric measuring tape, and measures were rounded to the nearest 0.1 cm. Height was only measured at BL, using 217 stadiometer (SECA, Hamburg, Germany) without shoes, and was rounded to the nearest 0.5 cm.

2.4.2 Body composition

Body composition was measured with air displacement plethysmography (ADP) using (BodPod, COSMED, Italy). Prior to testing, the Bod Pod equipment was calibrated in two steps between participants and every morning. Participants were tested in the fasted state and were asked to take off jewelry and metals and only wear tight underwear and a Lycra swim cap. Participants were instructed to sit still, be quiet and breathe relaxed during the test. Two repeated measurements for each subject were performed. The Bod Pod model is based on the same principle as hydrostatic (under water) weighing, considered the gold standard (31), and is a two-compartment model that uses the whole-body densitometry to determine body composition (31). The technique relies on the physics of Boyles`s law, which states that pressure and volume vary inversely with one another (32). Body mass can be estimated from BW that is measured from a highly accurate scale and volume. Density is defined as mass (M_B) divided by volume (V_B) ($D_B = M_B/V_B$) (32). When body density is known, the Bod Pod uses known (or user-customized) densiometric equations. For this study the Brozek equation for lean and obese individuals ($FM\% = (4.57/D_B - 4.142) * 100$) was used to determine fat percentage (33). The following equations were used to calculate percentage of fat free mass (FFM), based on the fat mass (FM)% from the Bod Pod:

$$(FFM\ \% = 100 - FM\%)$$

$$FM = (FM\%)(M_B)\ /100\%$$

$$(FFM = M_B - FM)$$

2.4.3 Subjective feelings of appetite

To measure subjective feelings of hunger, fullness, DTE and PFC a 10-cm visual analogue scale was used. VAS is a validated method for measuring appetite (34). For the questions used in the VAS, see table 1. Measurements were done in fasting, immediately after consuming a standardized breakfast meal (see table 13, appendix VII) and then every 30 minutes, up to 2.5 hours (30, 60, 90, 120, 150 minutes). The breakfast meal consisted of oat-bread, butter, cheese, strawberry jam, orange juice and milk or yoghurt, providing approximately 500 kcal, 48 % carbohydrate 17% protein, 35% fat. Subjects had to eat the whole breakfast meal within 15 minutes.

Table 1. Questions assessing subjective appetite.

The following Questions were assessed using the VAS:	
"How hungry do you feel?"	(not hungry at all – never been hungrier)
"How full do you feel?"	(not full at all – very full)
"How much food do you think you can eat?"	(nothing – a lot)
"How much food do you want to eat?"	(nothing – a lot)

2.4.4 Appetite related hormones

Blood samples were taken fasting and over a period of 2.5 hours (30, 60, 90, 120, 150 minutes) to assess the release of appetite related hormones after consuming a standard breakfast (described above). Blood was collected in 3x4 EDTA tubes, spun in a centrifuge (1000 G for 10 min at 4°C), and frozen at –80°C for future analysis. Plasma samples collected were analyzed for insulin, AG, CCK, GLP-1, PYY using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore), BHB using a KB assay kit (Sigma- Aldrich Inc, St. Louis, MO) and CCK using an extensively characterized "in-house" RIA method (27). The intra-assay coefficient of variation (CV) was <10%, and the inter-assay CV was 20%. To reduce intra-assay variability, all samples from the same participants were analyzed in the same assay.

2.4.5 Stool samples

Stool samples were sent to the Norwegian University of Life Sciences (NMBU), Ås, Norway to be analyzed by the research group led by Professor Knut Rudi. Prior to analysis, all fecal samples were diluted 1:10 in stool DNA stabilizer (PSP Spin Stool DNA Plus Kit, Invitek Molecular) and stored at -80°C. For easier extraction the fecal samples were homogenized, and pulse centrifuged (1200 rpm for 8 seconds). From the 1:10 diluted samples, 300µl and 100µl aliquots were used for 16S rRNA sequencing and SCFA composition, respectively.

Short-Chain fatty acid analysis

The main fatty acids analyzed were: acetate, propionate, iso-butyrate, butyrate, iso-valerate and valerate. The aliquots were diluted 1:1 with MilliQ-water, and then 1:1 with an internal standard, containing 2% formic acid with 500µM 2-methylvaleric acid. Samples were centrifuged (13 000 rpm for 10 min). The supernatant was filtered with 0.2µM filter columns (VWR, USA) (10 000 rpm for 5 min). The eluate was transferred to gas chromatograph (GC) vials (VWR, USA) and applied to the gas chromatograph (Trace 1310 equipped with an autosampler, ThermoFisher Scientific) with ramping temperatures from 90°C to 150°C for 6 minutes and 150°C to 245°C for 1.9 minutes. 0.2µl was applied with a split injection to a Topaz 4.0mm drilled uniliner (Restek), using helium as

the carrier gas with 2.5ml/min column flow, 3 ml/min purge flow and 200 ml/min split flow. The column used was a Stabilwax DA 30m, 0.25mm ID, 0.25µM (Restek), with a flame ionization detector analyzing the analytes. The chromatograms were processed with the Chromeleon 7 software.

A standard with 300µM acetic acid, 12µM propionic acid, 8µM isobutyric acid, 12µM butyric acid, 8µM isovaleric acid, 8µM valeric acid, 25µM internal standard and 1% formic acid was applied twice in between every 10th sample to detect shifts or variabilities. All acids used were purchased from Sigma-Aldrich, Germany.

Gut microbiota/ 16S rRNA

For gut microbiota analyses, a selection of primers was used to cover the most important bacteria in the gut. These include: Bacterial species (or groups) that show marked changes, changes in the relations of the phyla Bacteroidetes and Firmicutes (proportion %, ratio), Bacteroides group, Clostridium clusters (Ruminococcaceae, Lachnospiraceae), Akermansia (and/or its relatives), Roseburia spp. and Eubacterium rectale subgroup, Bifidobacteria, Lactobacilli, Sulfate reducing bacteria, Ruminococcus, Methanobrevibacter (Archaebacteria), Faecalibacterium prausnitzii (and/or its relatives), Prevotella species, Veillonella, Rikenellaceae (Alistipes). Principal component scores were first evaluated to identify which taxonomic groups of the microbiota present would best explain the variation in microbiota present in the samples collected during the ketogenic diet-induced WL intervention.

The 16S rRNA data were analyzed with Quantitative Insights Into Microbial Ecology (QIIME) pipeline (50,51). QIIME was used to assemble forward and reverse reads and split them into their respective samples. The reads were checked for chimeras and removed, and OTUs with a 97% or higher 16S rRNA ident were created and assigned taxonomy by the SILVA database[34]. Two sequencing runs were performed resulting in 30 878 312 ssDNA fragments. The cut-off was set at 5 000 dsDNA fragments, resulting in 352 samples with sufficient depth and quality.

Bacterial cells in fecal sample aliquots were disrupted using 0.2g <106µm acid-washed glass beads (Sigma-Aldrich, Germany), 0.2g 425-600µm acid-washed glass beads (Sigma-Aldrich, Germany) and 2×2.5-3.5mm acid-washed glass beads before being processed twice on a FastPrep 96 (MP Biomedicals, USA) at 1800rpm for 40 seconds. The samples were centrifuged at 13 000 rpm for 5 minutes before DNA was extracted using LGC Mag Midi Nucleic acid extraction kit (LGC genomics, UK). The V3 to V4 region of 16S rRNA was amplified using PRK341F and PRK806R primers[32] at 95°C for 15 minutes followed by 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds, before a final step at 72°C for 7 minutes. Cycles were increased to 30 for meconium. Reactions contained 2µl DNA template with 1× HotFirePol Blend Master Mix Ready to Load (Solis

BioDyne, Germany) and 0.2µm PRK forward and reverse primers. Samples were purified using 1× Sera Mag beads to the DNA volume, following AMPure's protocol on a Biomek 3000 (Beckman Coulter, USA).

Index PCR was performed with a combination of 16 forward and 30 reverse modified PRK primers with Illumina indexes. Samples were amplified at 95°C for 5 minutes followed by 10 cycles of 95°C for 30 seconds, 55°C for 60 seconds, and 72°C for 45 seconds, before a final step of 72°C for 7 minutes. Each reaction consisted of 1× FirePol Master Mix Ready to Load (Solis BioDyne, Germany), 0.2µM forward & reverse primers, nuclease free-water (VWR, USA) and 1µl DNA. The DNA concentration was quantified following Qubit's protocol, normalized and pooled on a Biomek 3000. The pooled sample was split in two for quantification and sequencing. Samples for quantification were first subjected to droplet generation using BioRad QX200™ – Droplet Generator, before being amplified at 95°C for 5 minutes followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds before the last two steps at 4°C for 5 minutes and 90°C for 5 minutes before quantification on BioRad QX200 – Droplet Reader. The reactions contained 1× Super mix for EvaGreen (BioRad, USA), 0.2µM Illumina colony forward & reverse primer, 2.4µl DNA template and PCR water. The second part of the sample was diluted to 6 pM DNA with 15% PhiX following Illumina's instructions, with the exception of using nuclease-free water instead of Tris and sequenced on Illumina MiSeq.

2.5 Power calculation

This master thesis is part of a larger study aimed to identify the maximum CHO intake still associated with appetite suppression under LED. A simulation-based multilevel power calculation was performed since participants were to be randomized into three isocaloric groups with varying CHO intake. 75 participants were deemed necessary to obtain at least 80 % power to detect a change in mean differences of hunger (0-20mm) between groups at a significance level of $P<0.05$. Since a dropout of 25% is commonly seen in this type of studies, the aim was, therefore, to include 100 participants.

No formal power calculation was done for this study as this is a completely novel research question. This study should be seen as a pilot study that can guide power calculations in future studies.

2.6 Statistical analysis

All statistical analyses in this study was performed using IBM SPSS Statistics 26, and statistical significance was defined as $P<0.05$, unless otherwise specified. For the specific aims of this thesis, all groups were merged and the randomization was, therefore, not accounted for in the analyses. Participants were considered completers if GM data was present at BL and W9. Q-Q plots and Kolmogorov-Smirnov test were used to check for normality.

Data is presented as estimated marginal means ± standard error of the mean (SEM), except for BL characteristics, food consumption, GM and correlation analysis. For BL characteristics and food consumption, data is presented as mean ± SD, and as median ± inter quartile range (IQR) for GM. To examine differences between completers and non-completers for BL characteristics, independent sample t-tests were used, and for the data that was not normally distributed a Mann-Whitney U-test was performed. For normal distributed data, a linear mixed-effects model was used to analyze repeated measurements (including fixed effects for time). For the data that were not normally distributed Wilcoxon signed rank test was performed.

To perform correlation analysis Spearman's correlation was used, as one or both variables were not normally distributed, unless something else is specified. Correlation analysis was performed between BHB at W9 and 1) changes in appetite markers and SCFA from BL to W9 and 2) taxonomic groups at W9. Correlation analysis was also preformed between changes in appetite markers (W9-BL), changes in SCFA (W9-BL), and taxonomic groups at W9. A correlation coefficient of 0.10 is thought to represent a weak (or small) association; a correlation coefficient of 0.30 is considered a moderate correlation; and a correlation coefficient of 0.50 or larger is thought to represent a strong or large correlation.
Multiple linear regression was also performed. Changes in appetite markers (W9-BL) were the dependent variables, and changes in SCFA (W9-BL), BHB at W9 and taxonomic groups at W9 were the predictors. A model was also built using the taxonomic groups at W9 as the dependent variables and BHB at W9 as the predictor. The models were adjusted for the following confounders: CHO and fiber (g/day), except for the models between changes in appetite markers and BHB where the confounders were age, sex and FM loss kg. Extreme values, defined as any data values which lie more than 3.0 times interquartile range above the third quartile or below the first quartile, were identified and removed from the dataset.

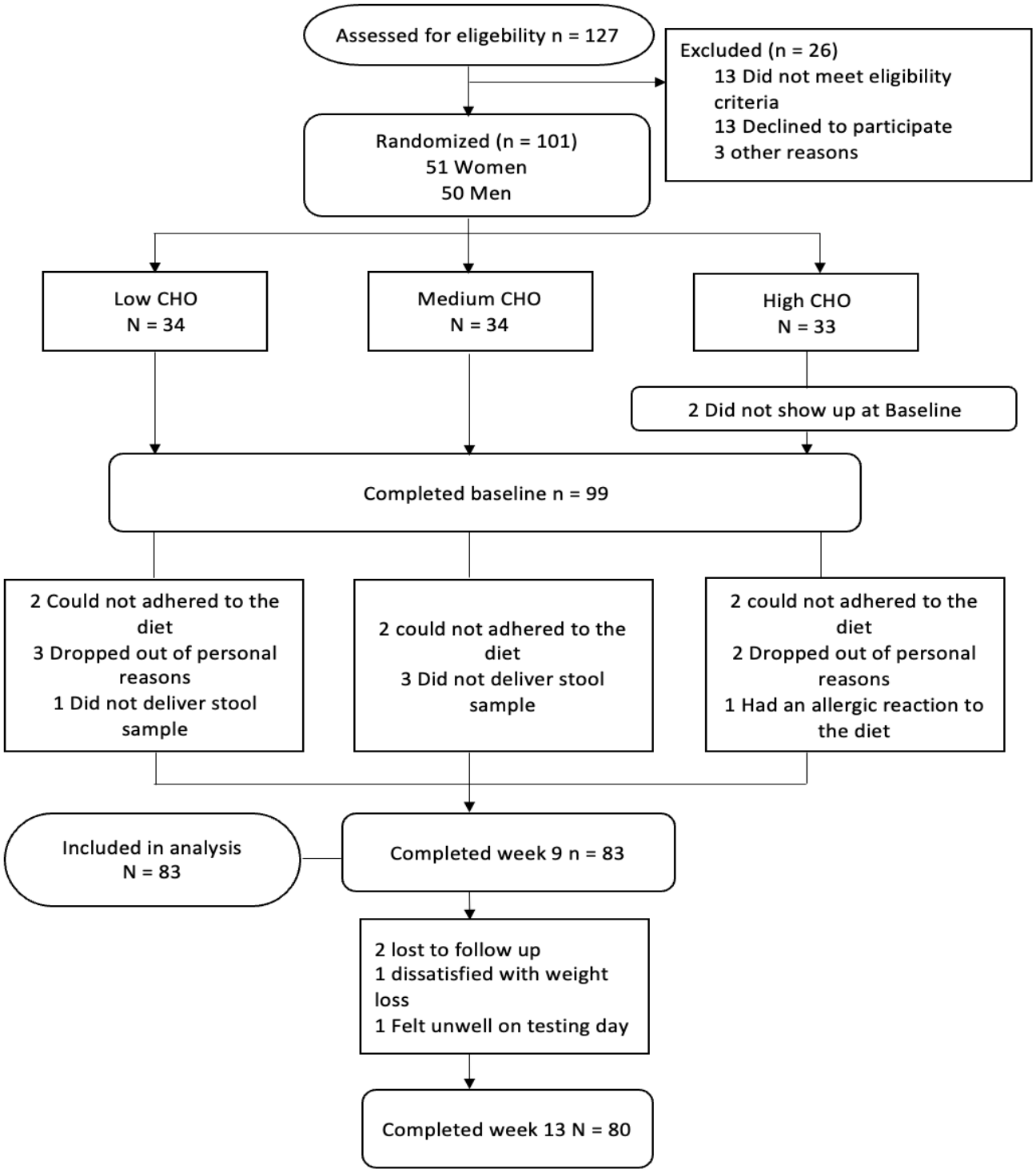
Total and incremental area under the curve (tAUC and iAUC, respectively) were calculated for subjective feelings of appetite and AG, PYY, GLP-1, CCK, and insulin from 0 to 150 minutes using the trapezoidal rule. Incremental AUC (iAUC) was calculated as tAUC- fasting x time. If there was not significant changes over time for basal appetite markers, tAUC was used to measure changes in postprandial state. If there were significant changes over time for basal appetite markers, this was adjusted for, by using iAUC.

3.0 Results

3.1 The study population

Ninety-nine out of one-hundred-and-one participants that were recruited, started the LEDs. Sixteen participants did not complete the intervention or were excluded. Reasons for dropouts and exclusions are shown in Figure 1.

Figure 1. Flowchart of the study



The general characteristics of the participants can be seen in Table 2.

Table 2. Characteristics of the participants at baseline

	All Participants (N=99)	Completers (N= 83)	Non-Completers (N=16)	P- value
Age (years)	44.8±9.7	44.8±9.3	45.1±11.8	0.580
Height (cm)	173.7±8.8	173.8±9.1	172.9±7.6	0.721
BW (kg)	105.2±16.0	104.9±16.0	106.9±15.9	0.649
BMI (kg/m^2)	35.1±3.5	34.9±3.5	36.0±3.1	0.239
Hip (cm)	116.3±8.7	116.0±8.7	117.8±8.5	0.456
Waist (cm)	112.3±11.0	112.3±11.0	112.0±11.5	0.901
FM (kg)	44.2±9.2	43.6±9.2	47.3±8.6	0.132
FM (%)	42.2±6.3	41.8±6.3	44.5±5.9	0.109
FFM (kg)	61.0±11.3	61.3±11.3	59.3±11.5	0.669
FFM (%)	57.9±6.2	58.4±6.1	55.6±6.1	0.094

Data presented as mean ± SD. BW: Body weight. BMI: Body mass index. FM: Fat mass. FFM: Fat free mass. P values for the comparison between completers and non-completers.

Eighty-three out of ninety-nine participants completed the study, with an average age of 45±9 years and a BMI of 35±4 kg/m^2. There were no significant differences between the completers and non-completers for any of the anthropometric variables measured.

3.2 Body composition and BHB

BW and composition and BHB plasma concentration over time are presented in table 3.

Table 3. BW, body composition and BHB over time

	Baseline	Week 9	Week 13
BW (kg)	104.9±1.3	90.8±1.3***	91.1±1.3***
BMI (kg/m^2)	34.6±0.4	30.2±0.4***	30.3±0.4***
Hip (cm)	115.8±0.9	107.8±0.9***	107.4±0.9***
Waist (cm)	112.5±1.0	102.2±1.0***	101.0±1.0***
FM (kg)	43.4±1.0	32.5±1.0***	-32.0±1.0***
FM (%)	41.8±0.6	35.8±0.6***	34.9±0.6***
FFM (kg)	61.5±0.6	58.2±0.6***	59.2±0.6***
FFM (%)	58.5±0.6	64.2±0.6***	65.1±0.6***
BHB Plasma (mmol/l)	0.1±0.0	0.8±0.0***	0.1±0.0

*Data presented as estimated marginal means ± SEM. BW: Body weight. BMI: Body mass index. FM: Fat mass. FFM: Fat free mass. BHB: β-hydroxybutyric acid. Symbols denote significant changes over time (***P<0.001).*

A significant main effect of time (P<0.001, for both) was found for both BW and BMI. Participants lost on average 14±1 kg from BL to W9 (P<0.001) and maintained their BW from W9 to W13. An average reduction of 4 kg/m^2 in BMI was seen between BL and W9 (P<0.001) and this was maintained at W13.

There were reductions in hip and waist circumference (8±1 and 10±1 cm, respectively) from BL to W9 (P<0.001, for both) and this was maintained at W13. A significant main effect of time (P<0.001, for all) was found for both FM (kg and %) and FFM (kg and %). FM (kg and %) and FFM (kg) decreased (11±0 kg and 3±0 kg, respectively) from BL to W9 (P<0.001). FM (kg) was maintained from W9 to W13, while FM (%) decreased (P<0.05) and FFM (kg) increased (P<0.001) from W9 to W13. FFM (%) increased from BL to W9 (6±0 %, P<0.001) and increased further from W9 to W13 (P<0.001).

BHB plasma concentration increased from BL to week 9 (0.8±0 mmol/L) (P<0.001) and decreased from W9 to W13 (P<0.001). There was no difference in BHB plasma concentration between BL and W13.

3.3 Food consumption

The average energy and macronutrient intake during the first 8 weeks is presented in table 4.

Table 4. Mean values and range of actual energy and macronutrient intake

	Mean±SD	Range
Energy (kcal/day)	1077.0±144.5	813.7 – 1259.3
Protein (g/day)	65.8±8.1	50.4 – 76.3
Protein (E%)	24.8±0.6	24 - 26
Fat (g/day)	40.3±4.6	33.2 – 46.4
Fat (E%)	35.0±8.1	25 - 46
Carbohydrate (g/day)	102.2±35.7	52.9 – 153.0
Carbohydrate (E%)	36.9±8.8	26 - 49
Fiber (g/day)	14.4±1.2	11.2 – 18.1
Fiber (E%)	3.3±0.7	1.9 – 5.4

Data presented as mean ± SD and range. E% = Energy percentage.

The average energy intake was 1077±144 kcal/day. The average daily intake of protein, fat, CHO and fiber was 66, 40, 102 and 14 g/day, respectively or 25, 35, 37 and 3 E%, respectively.

3.4 Subjective feelings of appetite

Subjective feelings of appetite over time are presented in table 5.

Table 5. Subjective feelings of appetite over time

		Baseline	Week 9	Week 13
Hunger				
	Basal (mm)	26.9±3.2	32.9±3.0	37.8±3.4*
	iAUC (mm*min)	2917.2±373.7	3803.7±358.9	4421.0±408.7**
Fullness				
	Basal (mm)	16.9±2.6	23.0±2.4	17.3±2.8
	tAUC (mm*min)	7653.8±311.7	8847.1±299.1***	8544.5±332.2*
DTE				
	Basal (mm)	37.7±3.6	45.0±3.4	43.7±3.9
	tAUC (mm*min)	2565.4±208.0	2354.1±201.4	2702.0±222.1
PFC				
	Basal (mm)	40.0±3.0	33.4±2.8	40.9±3.2
	tAUC (mm*min)	3301.6±221.9	2468.3±214.9***	2942.3±233.5

*Data presented as estimated marginal means ± SEM. DTE: Desire to eat. PFC: Prospective food consumption. iAUC: Incremental area under the curve. tAUC: Total area under the curve. Symbols denote significant changes over time (*P<0.05, **P<0.01, ***P<0.001).*

A significant main effect of time (P<0.05, P<0.01 and P<0.001, respectively) was found for basal and iAUC hunger and tAUC fullness. A significant increase in both basal and iAUC feelings of hunger was seen between BL and W13 (P<0.01, P<0.001 respectively). There were no changes in subjective feelings of DTE over time. iAUC fullness increased from BL to W9 (P<0.001) and was higher at W13 compared with BL (P<0.05). tAUC PFC decreased from BL to W9 (P<0.001), and increased from W9 to W13 (P<0.05), with values at W13 no longer different from BL.

3.5 Appetite-related hormones

Plasma concentration of appetite-related hormones over time can be seen in table 6.

Table 6. Plasma concentration of appetite-related hormones over time

	Baseline	Week 9	Week 13
AG			
Basal (pg/ml)	291.4±49.3	372.9±49.3***	402.6±53.0***
iAUC (pg/ml*min)	16425±3093.4	20214±2985.4	21994±3376.6**
Total PYY			
Basal (pg/ml)	115.4±12.7	103.4±12.6***	109.4±12.9
iAUC (pg/ml*min)	4424.8±524.3	5636.9±498.4**	4404.7±600.4
Total GLP-1			
Basal (pg/ml)	162.1±7.0	137.0±6.9***	132.8±7.2***
iAUC (pg/ml*min)	9016±973.9	12554±952.5***	11887±1026.8***
CCK			
Basal (pmol/l)	0.9±0.1	0.8±0.1	0.8±0.1
tAUC (pmol/l*min)	442.8±23.7	351.1±23.2***	404.8±24.4
Insulin			
Basal (mmol/l)	841.4±39.6	476.6±38.5***	536.8±42.5***
iAUC (mmol/l*min)	328482±18465	254169±17860***	212436±20036***

*Data presented as estimated marginal means ± SEM. iAUC: Incremental area under the curve. tAUC: Total area under the curve. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant changes over time (*P<0.05**P<0.01, ***P<0.001).*

Basal AG increased from BL to W9 (P<0.001). At W13 both basal AG and iAUC AG were higher compared to BL (P<0.001, P<0.01, respectively).

No main effect of time was found for basal CCK (P=NS). A significant main effect of time (P<0.001, for all) was found for tAUC CCK, basal GLP-1 and iAUC GLP-1. Basal PYY and GLP-1 both decreased (P<0.001 for both) from BL to W9, and basal GLP-1 was lower at W13 compared to BL (P<0.001). tAUC CCK decreased from BL to W9 (P<0.001) and increased from W9 to W13 (P<001), however there were no difference in tAUC CCK at W13 compared whit BL. iAUC PYY and GLP-1 both increased from BL to W9 (P<0.05, P<0.001, respectively) however iAUC GLP-1 was higher at W13 compared to BL (P<0.001).

A significant main effect of time (P<0.001, for both) was found for basal and iAUC insulin. Insulin, both basal and iAUC, decreased from BL to W9 (P<0.001, for both). And both basal and postprandial (iAUC) plasma insulin concentration were lower at W13 compared to BL (P<0.001 for both).

3.6 Gut microbiota

The composition of important taxonomic groups of microbiota over time can be seen in table 7.

Table 7. The composition of important taxonomic groups of microbiota over time.

	Baseline	Week 9	Week 13
Bacteroides	0.193±0.18	0.203±0.20	0.196±0.23
Alistipes	0.057±0.08	0.080±0.06***	0.070±0.07
Blautia	0.021±0.03	0.025±0.03	0.016±0.02
Eubacterium rectale	0.054±0.08	0.005±0.01***	0.038±0.04**
Faecalbacterium	0.065±0.05	0.053±0.07	0.063±0.06
Ruminococcaceae	0.010±0.05	0.021±0.07**	0.018±0.05

*Data presented as median ± IQR. Symbols denote significant changes over time (**P<0.01, ***P<0.001).*

Alistipes and Ruminococcaceae both increased from BL to W9 (P<0.001, P<0.01), and Ruminococcaceae decreased from W9 to W13 (P<0.05), but there were no changes at W13 compared to BL for both Alistipes and Ruminococcaceae. Eubacterium rectale decreased from BL to W9 (P<0.001) and increased from W9 to W13 (P<0.001), however the relative composition of this group was lower at W13 compered to BL (P<0.01).

There were no changes in Bacteroides, Blautia and Faecalbacterium over time.

3.7 Short-chain fatty acid

Fecal concentration of SCFA over time is shown in table 8.

Table 8. Fecal concentration of SCFA over time

	Baseline	Week 9	Week 13
Acetic Acid (µM)	46468±2950	22578±2862***	31887±3292**
Propionic Acid (µM)	16004±1605	7357±1547***	10573±1821**
Isobutyric Acid (µM)	2834±110	2513±105*	2418±123*
Butyric Acid (µM)	19960±1074	8230±1031***	14181±1201**
Isovaleric Acid (µM)	4035±153	3772±146	3700±173
Valeric Acid (µM)	3634±110	2941±104***	3236±124*

*Data presented as estimated marginal means ± SEM. Symbols denote significant changes over time (*P<0.05, **P<0.01, ***P<0.001).*

A significant main effect of time (P<0.001, for all except isobutyric P<0.01) was found for acetic, propionic, butyric, isobutyric and valeric acid. No main effect of time was found for isovaleric acid (P=NS). Acetic, propionic and butyric acid decreased from BL to W9 (P<0.001, for all) and increased from W9 to W13 (P<0.05, P<0.01, P<0.001, respectively). However acetic, propionic and butyric acid were still lower at W13 compared to BL (P<0.01, for all). Isobutyric and valeric acid also decreased from BL to W9 (P<0.05, P<0.001, respectively) and were lower at W13 compared to BL (P<0.05, for both).

3.8 Correlation analysis

3.8.1 Correlation between BHB and changes in appetite

Simple correlation between BHB plasma concentration W9 and changes in appetite markers (from BL to W9) can be seen in table 9.

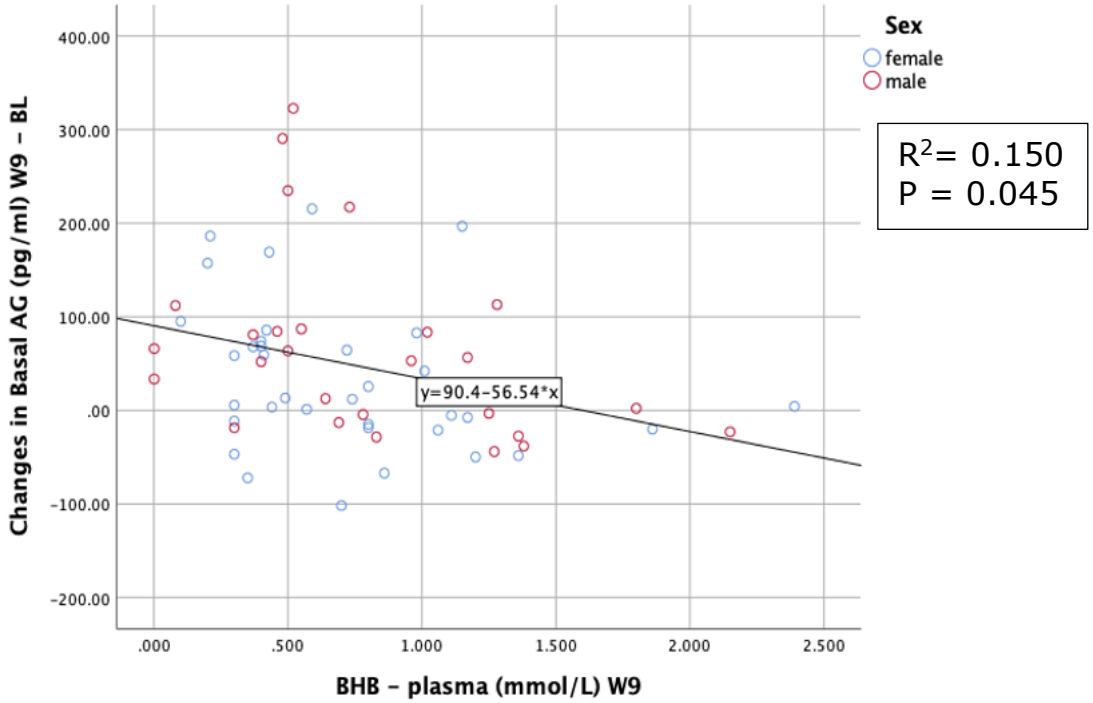
Table 9. Correlation coefficients between BHB W9 and changes in appetite markers between BL and W9

	r	P-value
Basal Hunger	-0.051	0.650
iAUC Hunger	0.061	0.590
Basal Fullness	-0.133	0.239
tAUC Fullness	0.120	0.283
Basal DTE	0.168	0.135
tAUC DTE	-0.034	0.768
Basal PFC	0.111	0.322
tAUC PFC	0.050	0.656
Basal AG	-0.393	**0.001****
iAUC AG	-0.094	0.450
Basal PYY	-0.300	**0.009***
iAUC PYY	0.171	0.150
Basal CCK	-0.016	0.891
iAUC CCK	-0.012	0.918
Basal GLP-1	-0.013	0.911
iAUC GLP-1	0.160	0.180

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlations at *P<0.05, **P<0.01.*

An inverse moderate correlation was seen between BHB plasma concentration at W9 and changes in basal AG from BL to W9 (r=-0.393, P=0.001, n=74). The higher the plasma concentration of BHB at W9, the smaller the increase, or the larger the reduction, in basal AG concentration from BL to W9. After adjusting for FM loss (kg), age and sex, BHB plasma concentration at W9 was still a significant predictor of changes in basal AG (P<0.05) and the regression model explained 15% of the variation (P<0.05). Scatterplots shown in figure 2.

Figure 2. Scatterplot for the association between BHB at W9 and changes in basal AG (W9 – BL)



AG: Acylated Ghrelin. W9: week 9. BL: Baseline. BHB: β-hydroxybutyric acid.

An inverse moderate correlation was also seen between BHB plasma concentration at W9 and changes in basal PYY from BL to W9 (r=-0.300, P=0.009, n=74). However, after adjusting for FM loss (kg) age and sex, BHB was no longer a significant predictor of changes in basal PYY.

3.8.2 Correlation between BHB and the composition of important taxonomic groups

Simple correlation between BHB W9 and the composition of important taxonomic groups W9 can be seen in table 10.

Table 10. Correlation coefficients between BHB W9 and the composition of important taxonomic groups W9

	r	P-value
Bacteroides	0.030	0.788
Alistipes	0.001	0.993
Blautia	-0.188	0.097
Eubacterium rectale	-0.161	0.153
Faecalbacterium	-0.229	**0.040***
Ruminococcaceae	-0.086	0.447

*Symbols denote significant correlations (*P<0.05).*

A weak inverse correlation was found between BHB plasma concentration at W9 and Faecalbacterium W9 (r=-0.229, P=0.04, n=81). However, after adjusting for CHO (g) and fiber (g), BHB plasma concentration was no longer a significant predictor of changes in Faecalbacterium W9.

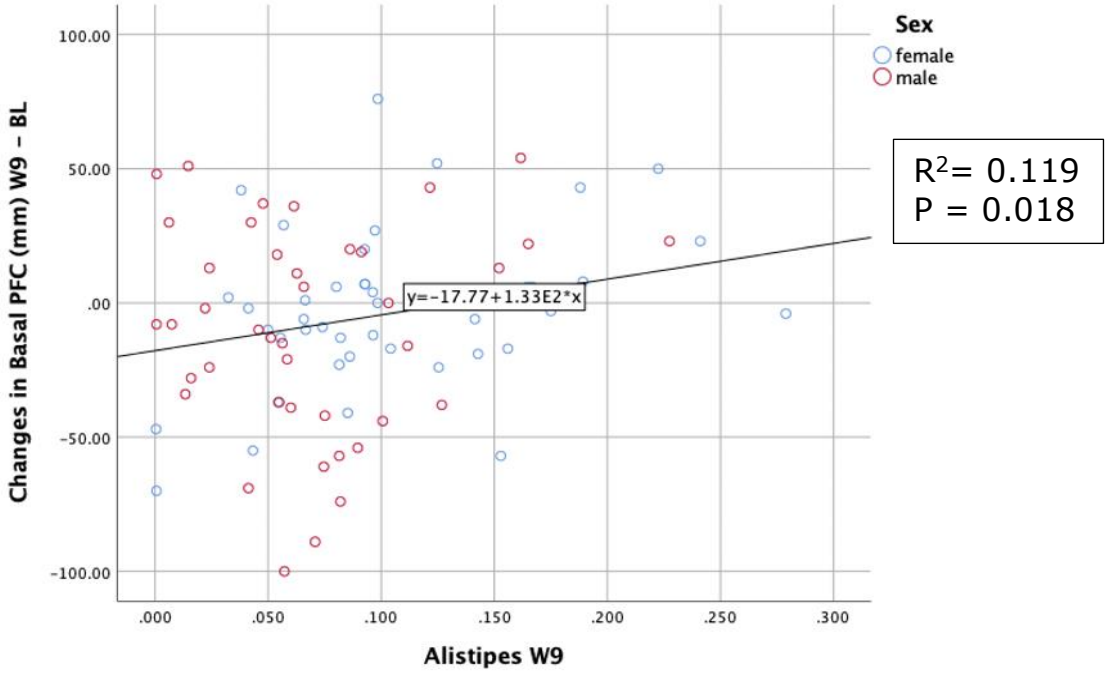
3.8.3 Correlation between the composition of important taxonomic groups and changes in appetite

Simple correlation between the composition of important taxonomic groups and changes in appetite W9 can be seen in appendix VIII (table 14-19).

A weak positive correlation was seen between Alistipes W9 and changes in tAUC fullness from BL to W9 (r=0.219, P=0.047, n=83), and between Blautia W9 and changes in tAUC CCK from BL to W9 (r=0.237, P=0.042, n=74). However, after adjusting for CHO (g) and fiber (g), Alistipes and Blautia at W9 were no longer significant predictors of changes in either tAUC fullness or CCK.

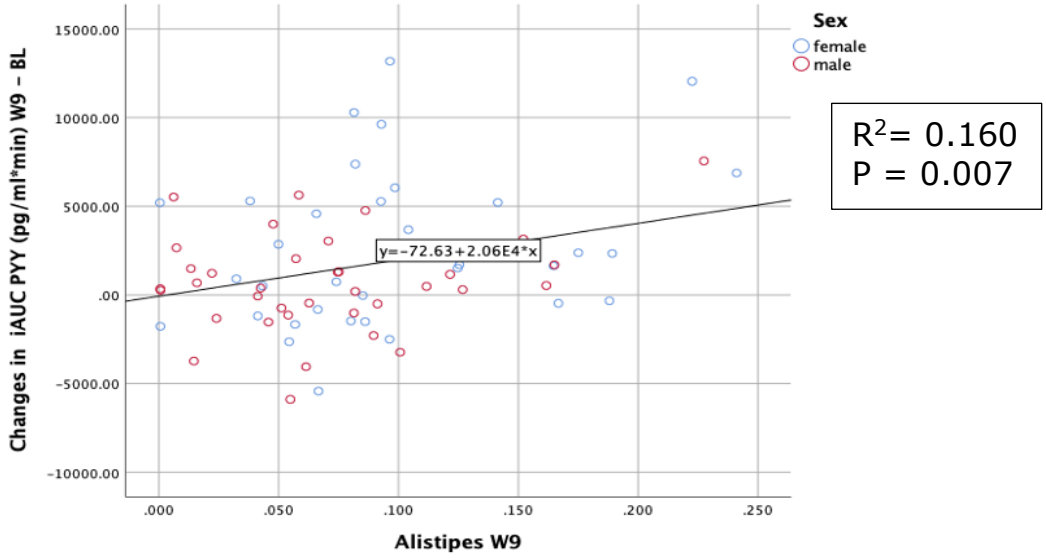
There was no correlation between Alistipes W9 and changes in basal PFC and iAUC PYY from BL to W9. After adjusting for CHO (g) and fiber (g) the regression model showed that Alistipes W9 explained respectively 12% and 16% of the variation of the changes in basal PFC and iAUC PYY from BL to W9 (P<0.05, P<0.01, respectively). The greater the decrease in Alistipes at W9, the smaller the increase, or larger the reduction in in basal PFC and iAUC PYY. Scatterplots can be seen in figure 3 and 4.

Figure 3. Scatterplot for the association between Alistipes W9 and changes in basal PFC (W9–BL).



W9: week 9. BL: Baseline. PFC: Prospective food consumption.

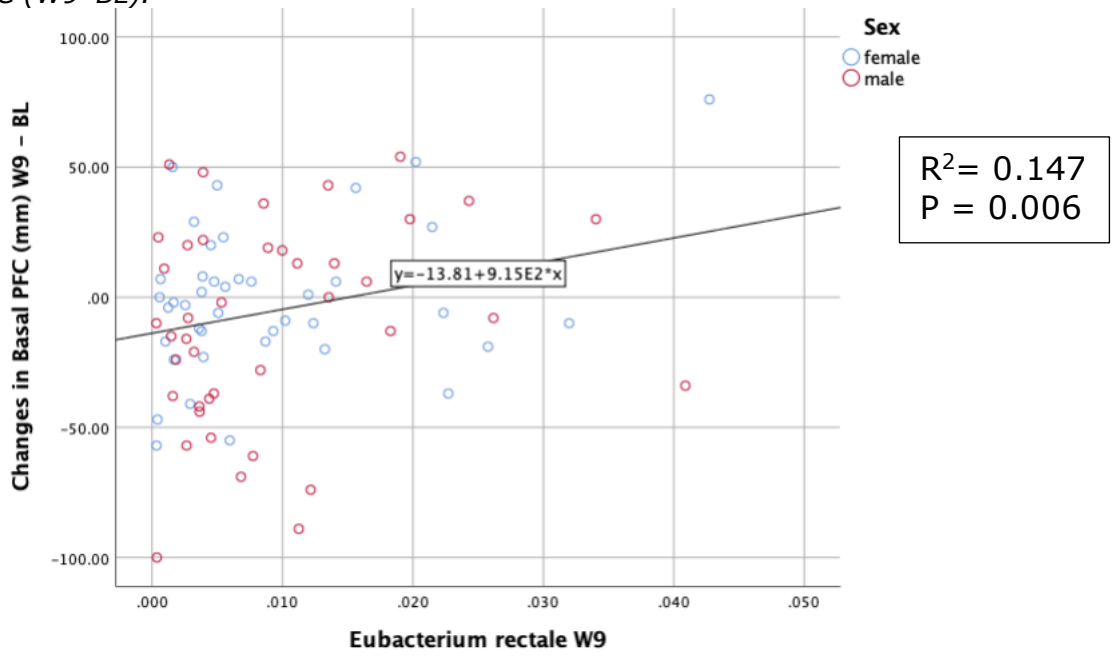
Figure 4. Scatterplot for the association between Alistipes W9 and changes in iAUC PYY (W9–BL)



W9: week 9. BL: Baseline. PYY: iAUC: incremental area under the curve.

A weak positive correlation was seen between Eubacterium rectale W9 and changes in basal PFC from BL to W9 (r=0.220, P=0.047, n=82). The greater the decrease in Eubacterium rectale, the larger the increase, or smaller the reduction in in basal PFC. The regression model showed that Eubacterium rectale at W9 explained 12% of the variation in the changes in basal PFC (P<0.01) after adjusting for CHO (g) and fiber (g). Scatterplot shown in figure 5.

Figure 5. Scatterplot for the association between Eubacterium rectale W9 and changes in basal PFC (W9–BL).

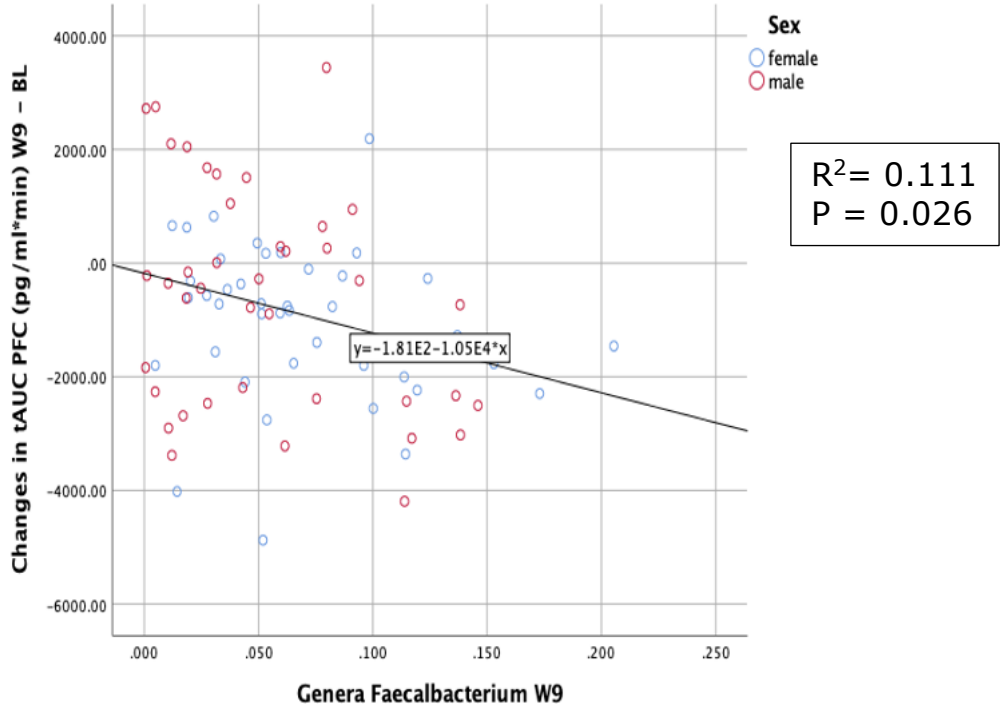


W9: week 9. BL: Baseline. PFC: Prospective food consumption.

There was a trend for an inverse correlation between both Blautia and Eubacterium rectale at W9 and changes in iAUC hunger from BL to W9 (P=0.051, for both) .

A weak inverse correlation was seen between Faecalbacterium W9 and changes in tAUC PFC from BL to W9 (r=-0.261, P=0.018, n=82). The larger the decrease in Faecalbacterium, the larger the increase, or smaller the reduction in tAUC PFC. After adjusting for CHO (g) and fiber (g), the regression model showed that Faecalbacterium W9 explained 11% of the variation of the changes in tAUC PFC from BL to W9 (P<0.05). Scatterplot shown in figure 6.

Figure 6. Scatterplot for the association between Faecalbacterium W9 and changes in tAUC PFC (W9 – BL)



PFC: Prospective food consumption. tAUC: total area under the curve. W9: week 9. BL: Baseline.

A weak inverse correlation was seen between Faecalbacterium W9 and changes in iAUC hunger from BL to W9 (r=-0.221, P=0.044, n=83). However, after adjusting for CHO (g) and fiber (g), Faecalbacterium was no longer a significant predictor of changes in iAUC hunger

3.8.4 Correlation between BHB and SCFA

Simple correlation between BHB W9 and changes in SCFA from BL to W9 can be seen in table 11.

Table 11. Correlation coefficients between BHB W9 and changes in SCFA from BL to W9

	r	P-value
Acetic acid (µM)	-0.052	0.645
Propionic acid (µM)	0.009	0.940
Isobutyric acid (µM)	-0.119	0.291
Butyric acid (µM)	0.006	0.957
Isovaleric acid (µM)	-0.044	0.694
Valeric acid (µM)	-0.030	0.796

There was no correlation between BHB W9 and changes in SCFA from BL to W9.

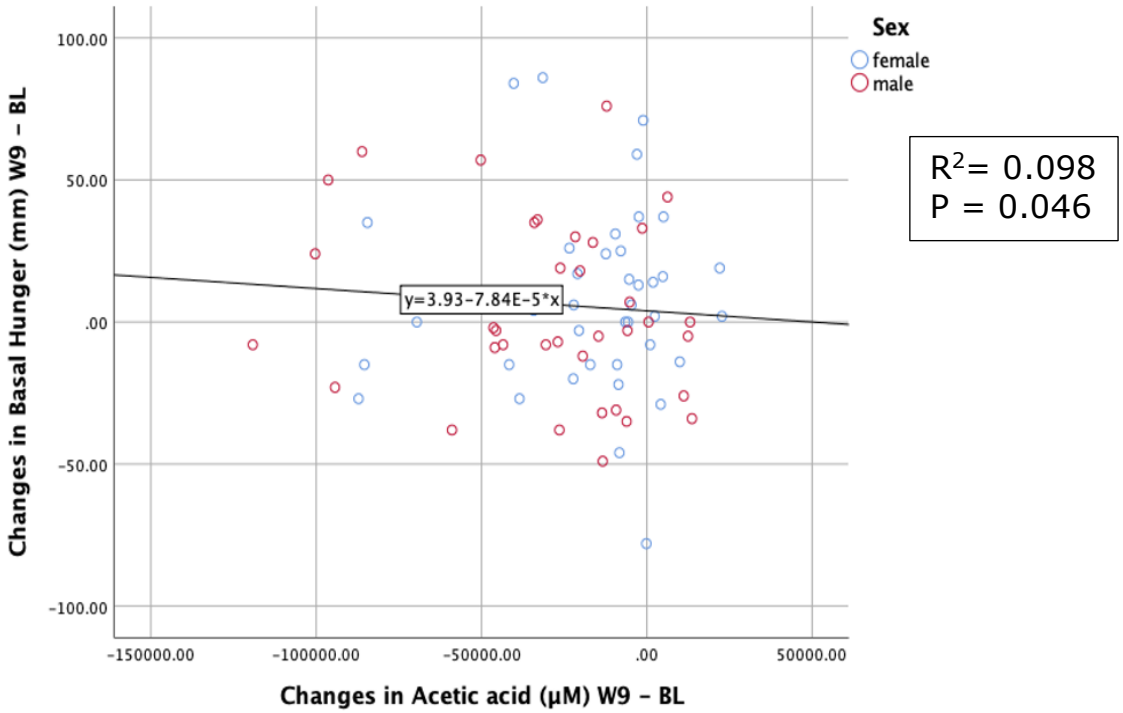
3.8.5 Correlation between SCFA and appetite markers

Simple correlation between SCFA and changes in appetite W9 can be seen in appendix IX, (table 20-25).

A weak inverse correlation was seen between changes in acetate and changes in iAUC insulin from BL to W9 ($r=-0.234$, $P=0.030$, $n=75$). However, after adjusting for CHO (g) and fiber (g), changes in acetate was no longer a significant predictor of changes in iAUC insulin.

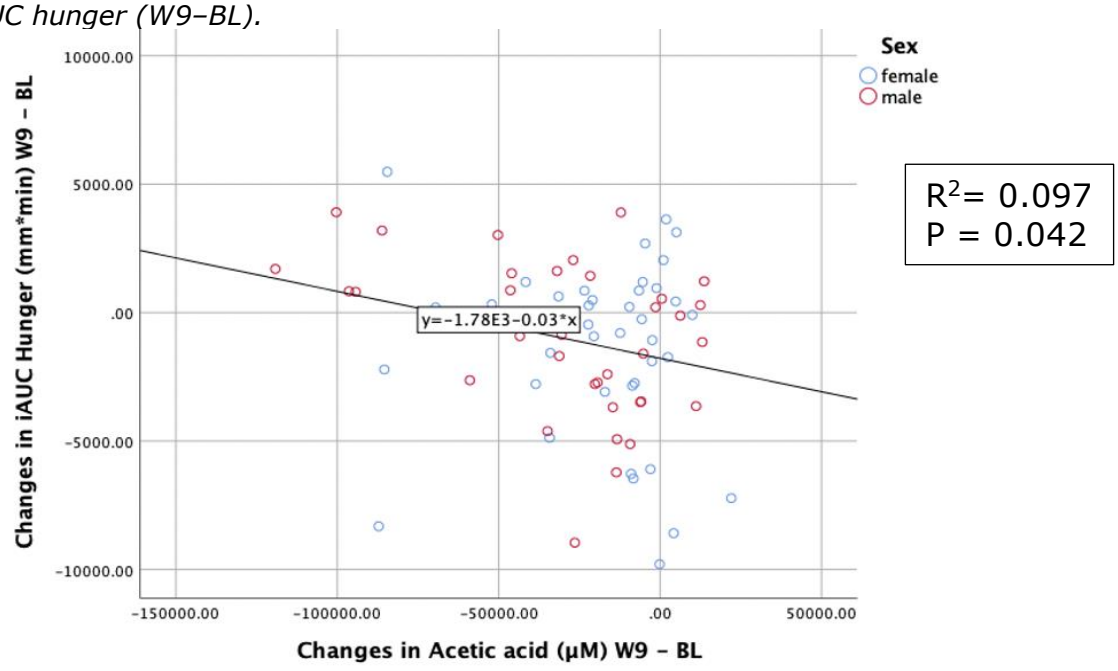
There was no correlation between changes in acetate and changes in both basal and iAUC hunger from BL to W9. However, after adjusting for CHO (g) and fiber (g) the regression model showed that changes in acetic acid from BL to W9 explained respectively 10% of the variation in the changes in basal and iAUC hunger from BL to W9 ($P<0.05$, for both). The larger the decrease in acetic acid, the higher the increase, or the smaller the reduction in both basal and iAUC hunger. Scatterplots can be seen in figure 7 and 8.

Figure 7. Scatterplot for the association between changes in acetic acid (W9-BL) and changes in basal hunger (W9–BL).



W9: week 9. BL: Baseline.

Figure 8. Scatterplot for the association between changes in acetic acid (W9-BL) and changes in iAUC hunger (W9–BL).

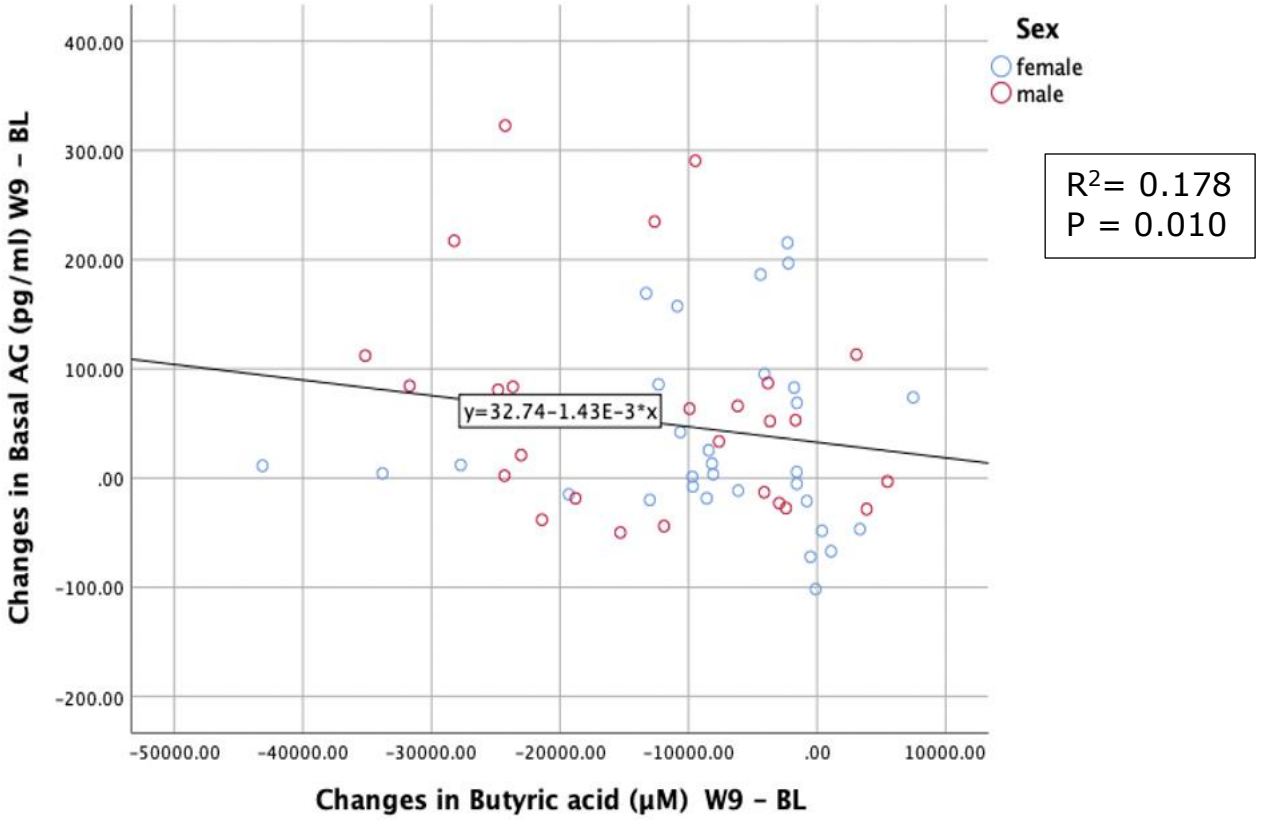


W9: week 9. BL: Baseline. iAUC: Incremental area under the curve.

A weak inverse correlation between changes in propionic acid and changes in tAUC DTE (r=0.234, P=0.037, n=80), a weak positive correlation between propionic acid and changes in iAUC insulin (r=0.240, P=0.038, n=75) and a moderate positive correlation between propionic acid and changes in iAUC PYY (r=0.306, P=0.009, n=72) was seen from BL to W9. However, after adjusting for CHO (g) and fiber (g), changes in propionic acid from BL to W9 were no longer a significant predictor of changes in tAUC DTE, iAUC insulin and iAUC PYY.

A weak inverse correlation between changes in butyric acid and changes in basal AG (r=-0.260, P=0.043, n=61) was seen from BL to W9. The greater the reduction in butyric acid, the larger the increase, or lower the reduction in basal AG. After adjusting for CHO (g) and fiber (g), the regression model showed that changes in butyric acid from BL to W9 explained 18% of the variation of the changes in basal AG from BL to W9 (P<0.05). Scatterplot shown in figure 9.

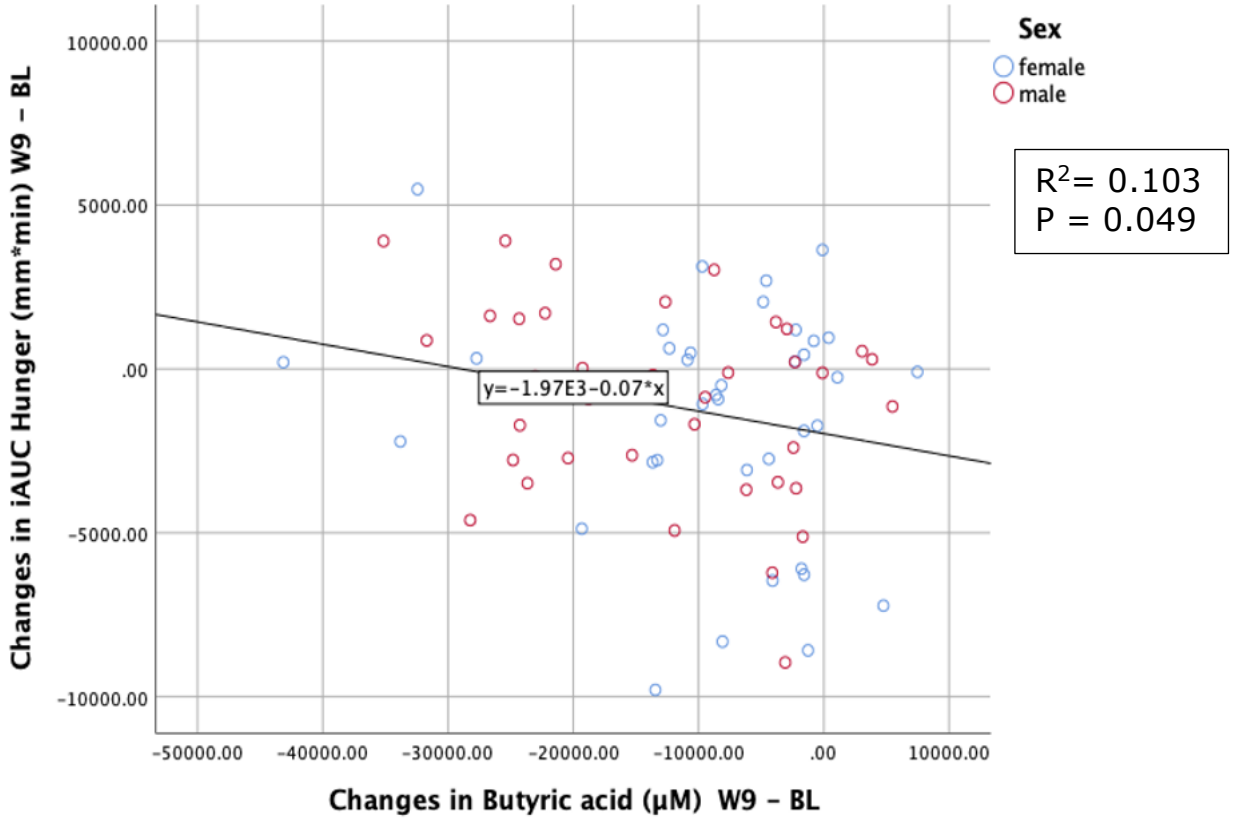
Figure 9. Scatterplot for the association between changes in butyric acid (W9 – BL) and changes in basal AG (W9 – BL)



W9: week 9. BL: Baseline.

There was no correlation between changes in butyric acid and changes in iAUC hunger from BL to W9. After adjusting for CHO (g) and fiber (g) the regression model showed that changes in butyric acid from BL to W9 explained 10% of the variation of the changes in iAUC hunger from BL to W9 (P<0.05). The greater the reduction in butyric acid, the higher the increase, or smaller the reduction in iAUC hunger. Scatterplot can be seen in figure 10.

Figure 10. Scatterplot for the association between changes in butyric acid (W9-BL) and changes in iAUC hunger (W9–BL).



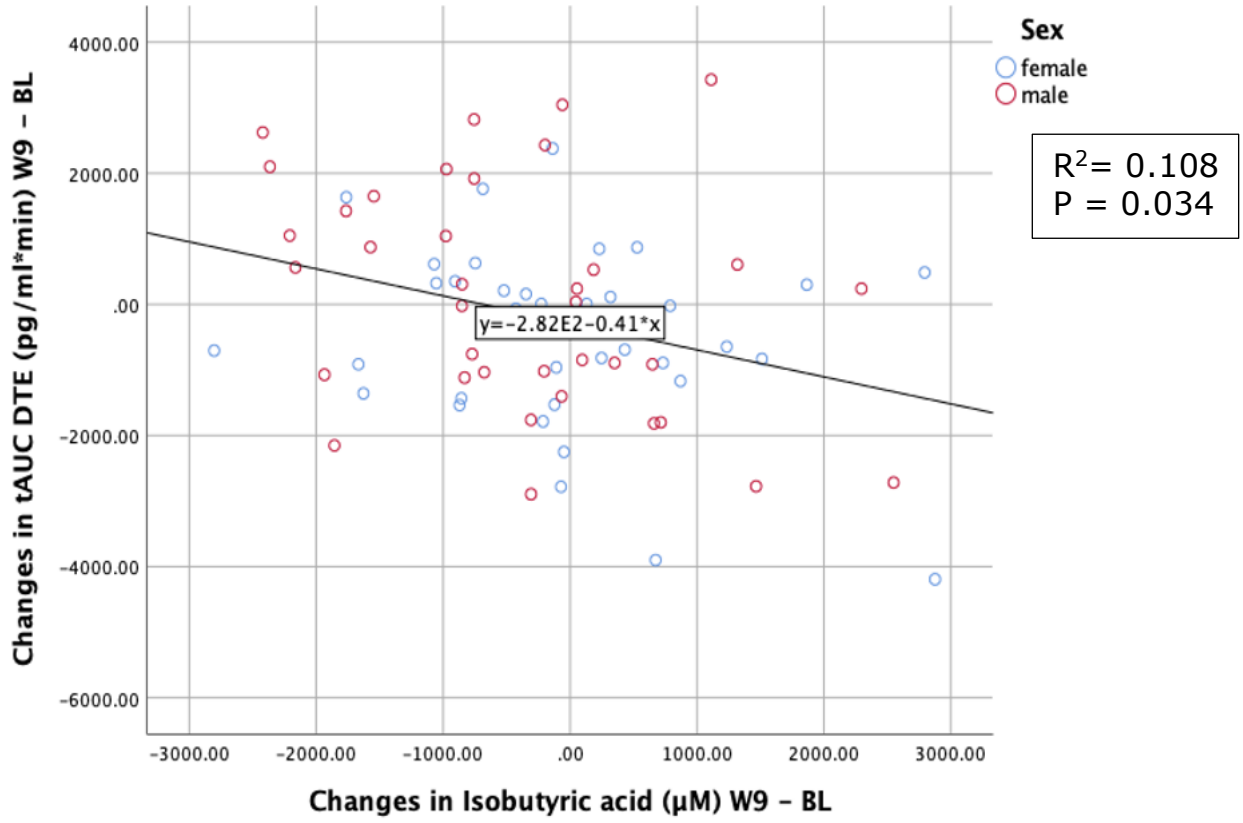
W9: week 9. BL: Baseline. iAUC: incremental area under the curve. DTE: Desire to eat.

A weak inverse correlation between changes in isobutyric acid and changes in tAUC DTE (r=0.280, P=0.013, n=79) and a weak positive correlation between changes in isobutyric acid and changes in iAUC PYY (r=0.257, P=0.013, n=71) was seen from BL to W9. However, after adjusting for CHO (g) and fiber (g), changes in isobutyric acid from BL to W9 were no longer a significant predictor of changes in tAUC DTE and iAUC PYY.

There was a weak positive correlation between CHO W9 and changes in isobutyric acid (data not shown) from BL to W9 (r=0.255, P=0.021, n=81), regression model showed that CHO W9 explained 6% of the variation in changes in isobutyric acid from BL to W9 (P<0.05).

There was no correlation between changes in isobutyric acid and changes in tAUC DTE from BL to W9. However, after adjusting for CHO (g) and fiber (g) the regression model showed that changes in isobutyric acid from BL to W9 explained 11% of the variation of the changes in tAUC DTE from BL to W9 (P<0.05). The greater the reduction in isobutyric acid the higher the increase, or smaller the reduction in tAUC DTE. Scatterplot can be seen in figure 11.

Figure 11. Scatterplot for the association between changes in isobutyric acid (W9-BL) and changes in tAUC DTE (W9–BL).



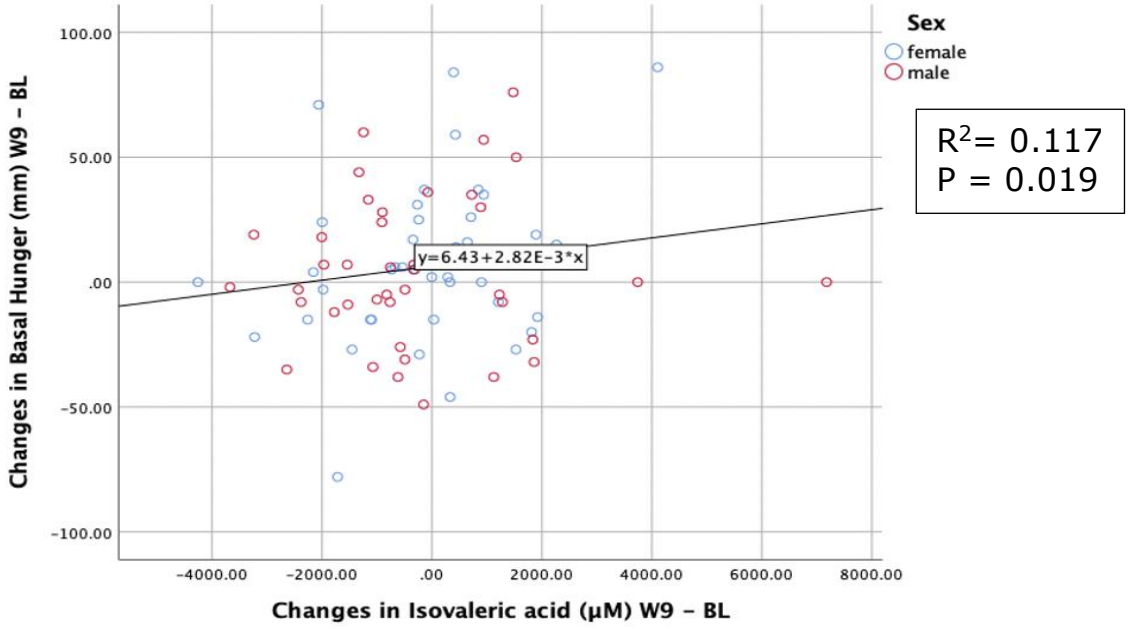
W9: week 9. BL: Baseline. tAUC: total area under the curve. DTE: Desire to eat.

A weak inverse correlation between changes in isovaleric acid and changes in tAUC DTE (r=0.298, P=0.007, n=81) and a weak positive correlation between isovaleric acid and tAUC fullness (r=0.249, P=0.023, n=83) was seen from BL to W9. However, after adjusting for CHO (g) and fiber (g), the regression analysis showed that changes in isovaleric acid from BL to W9 were no longer a significant predictor of changes in tAUC DTE and tAUC fullness from BL to W9.

There was no correlation between changes in isovaleric acid and changes in basal hunger and iAUC AG from BL to W9. However, after adjusting for CHO (g) and fiber (g) the regression model showed that changes in isovaleric acid from BL to W9 explained respectively, 12% and 13% of the variation of the changes in basal hunger and iAUC AG from BL to W9 (P<0.05, for both). The

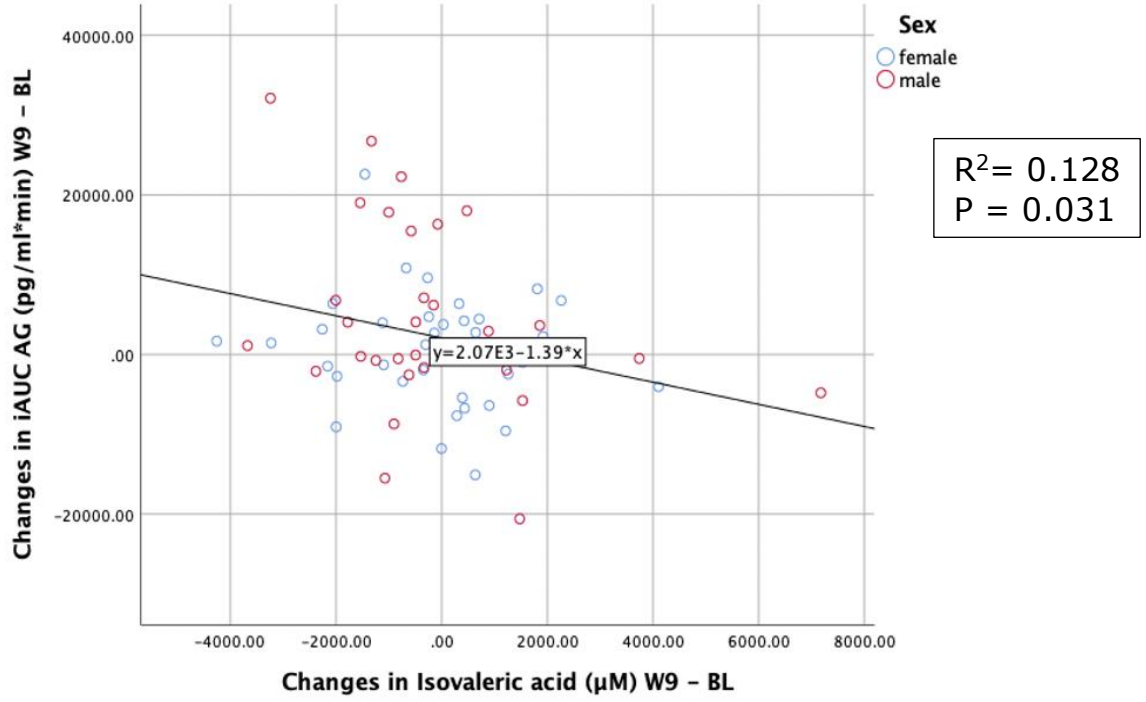
greater the reduction in isovaleric acid, the higher the increase, or smaller the reduction in basal hunger and iAUC AG. Scatterplots can be seen in figure 12 and 13.

Figure 12. Scatterplot for the association between changes in isovaleric acid (W9-BL) and changes in basal hunger (W9–BL).



W9: week 9. BL: Baseline.

Figure 13. Scatterplot for the association between changes in isovaleric acid (W9-BL) and changes in iAUC AG (W9–BL).

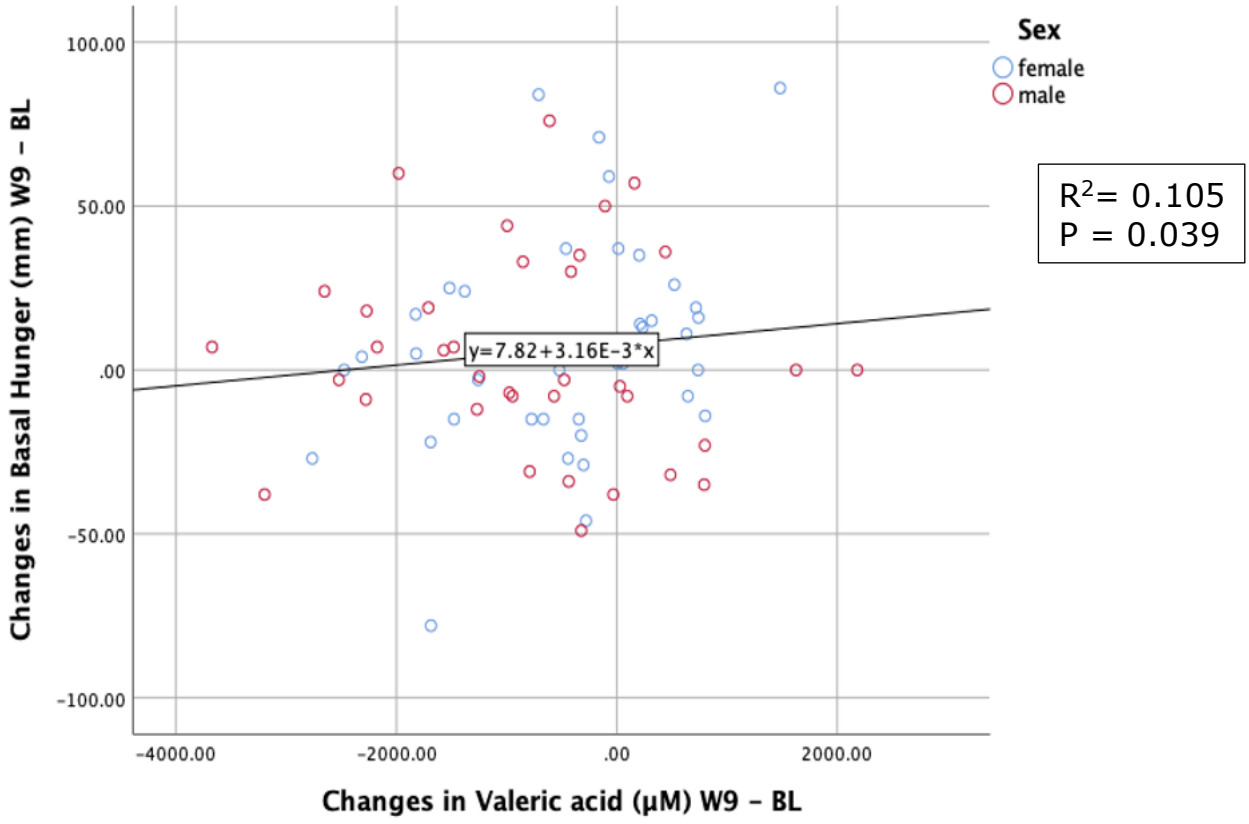


W9: week 9. BL: Baseline. iAUC: incremental area under the curve.
AG: Acylated ghrelin.

A weak inverse correlation between changes in valeric acid and changes in tAUC DTE (r=-0.239, P=0.037, n=77) and a weak positive correlation between changes in valeric acid and changes in tAUC fullness (r=0.236, P=0.036, n=79) and iAUC PYY (r=0.241, P=0.046, n=69) was seen from BL to W9. However, after adjusting for CHO (g) and fiber (g), the regression analysis showed that changes in valeric acid from BL to W9 were no longer a significant predictor of changes in tAUC DTE, tAUC fullness and iAUC PYY from BL to W9.

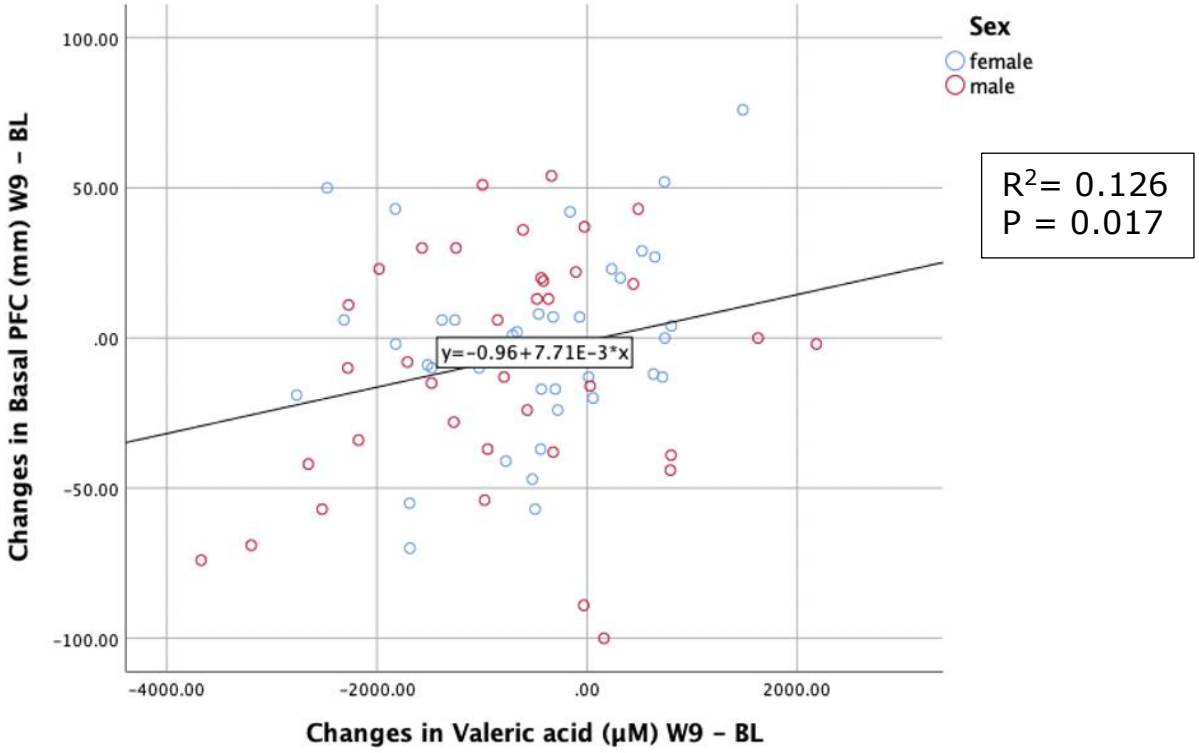
There was no correlation between changes in valeric acid and changes in basal hunger, PFC and AG from BL to W9. However, after adjusting for CHO (g) and fiber (g) the regression model showed that changes in valeric acid from BL to W9 explained respectively, 12%, 13% and 14% of the variation of the changes in basal hunger, PFC and AG from BL to W9 (P<0.05, for all). The greater the decrease in valeric acid, the higher the increase, or smaller reduction in basal hunger, PFC and AG. Scatterplots can be seen in figure 14, 15 and 16.

Figure 14. Scatterplot for the association between changes in valeric acid (W9-BL) and changes in basal hunger (W9–BL).



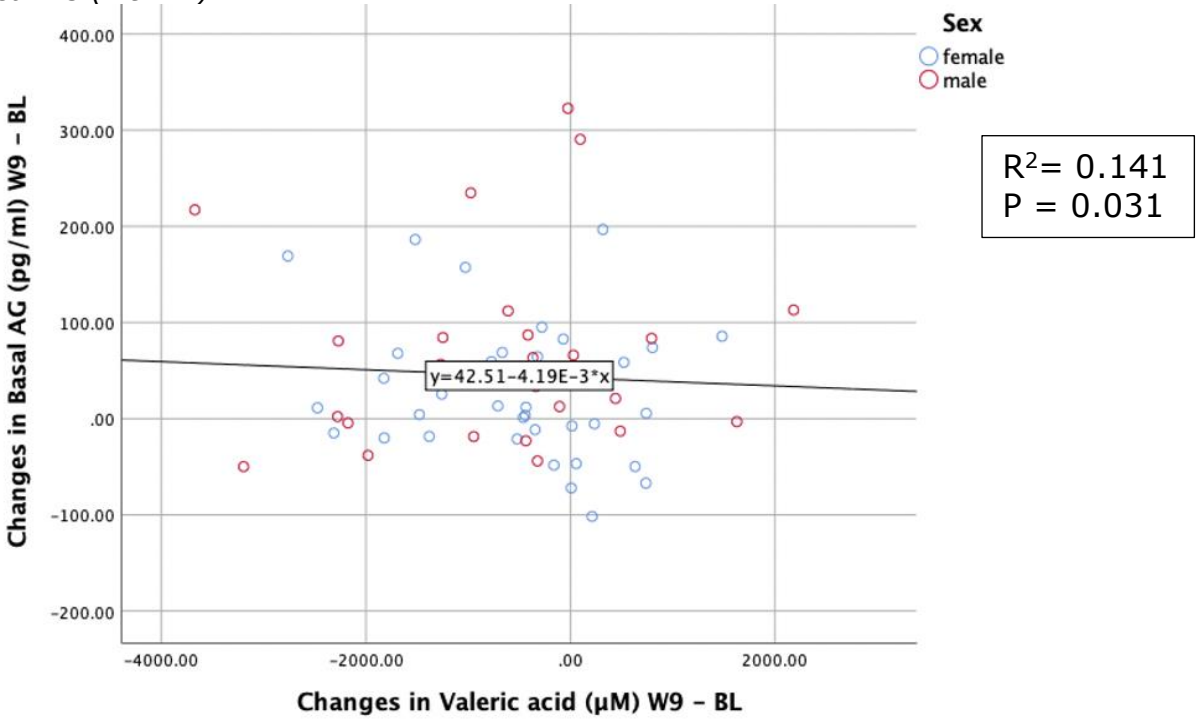
W9: week 9. BL: Baseline.

Figure 15. Scatterplot for the association between changes in valeric acid (W9-BL) and changes in basal PFC (W9–BL).



W9: week 9. BL: Baseline. PFC: Prospective food consumption.

Figure 16. Scatterplot for the association between changes in valeric acid (W9-BL) and changes in basal AG (W9–BL).



W9: week 9. BL: Baseline. AG: Acylated ghrelin.

4.0 Discussion

The primary aim of this study was to evaluate if changes in fecal SCFA concentration are associated with appetite suppression during WL induced by a ketogenic LED. The hypothesis was that changes in fecal SCFA concentrations played a role in appetite suppression during WL induced by a ketogenic LED. A secondary aim was to investigate the impact of WL induced by a ketogenic LED on GM and SCFA, and the hypothesis was that WL induced by a ketogenic LED was associated with non-beneficial changes in GM and SCFA.

During the eight weeks on the LED, participants lost an average of 14kg and were under nutritional-induced ketosis (BHB 0.8±0.0 mmol/L). During ketosis, a more favorable appetite profile was seen (W9), with no change in basal or postprandial subjective feelings of hunger or postprandial AG, and an increase in postprandial PYY, which disappeared when participants came out of ketosis (W13). Surprisingly there was an increase in basal AG during WL under ketosis (normally not seen during nutritional-induced ketosis). Additionally, this study showed a decrease in both SCFA-producing bacteria's in the GM and levels of SCFA with WL under ketosis (W9), and during refeeding levels increased, despite levels still lower at W13 than BL. These findings add to the evidence that WL induced by a KD has the ability to alter GM. Moreover, the results of this study do not support a role of SCFA in suppressing appetite during ketosis. Under ketosis (W9), the greater the decrease in fecal concentration of acetate and butyrate was, the higher the increase, or smaller the reduction in subjective basal (only acetate) and postprandial feelings of hunger. Furthermore, the greater the decrease in butyric acid was, the higher the increase, or smaller the reduction in basal AG during ketosis. However, the inverse association between butyric acid and basal AG was weak.

Under ketotic conditions the present study reported no change in basal or postprandial feelings of hunger during WL compared to BL. Which is not in line with the meta-analysis from Gibson et al. showing a decrease in feelings of hunger in the postprandial state (14). This may be explained by differences in CHO intake: from 70- to 130 g/day for the present study, versus 42- to 52g/day (14), and/or the magnitude of WL (14 kg in present study, versus ~6 kg in Gibson et al.). After refeeding and out of ketosis, the feelings of hunger both in fasting and postprandially increased significantly above BL values. These results are in line with Nymo et al. (12) and Sumithran et al. (13) findings. Moreover, the present study reported an increase in postprandial fullness in and out of ketosis, which are in line with a few studies (14,84,85). However, Sumithran et al.(13) and Nymo et al. (12) reported no change in postprandial fullness, after a KD (49). Furthermore the present study (n=83) reported no change in fasting DTE and PFC after 8 weeks of a ketogenic LED, and is in line with Sumithran et al. (13). Doucet and colleagues (51) (n=17) showed an increase in both fasting DTE and PFC after diet induced WL (non -ketogenic). This may be explained by differences in diet-intervention and/or sample size. Under ketosis the present study

showed no changes in postprandial DTE and a decrease in postprandial PFC, after WL (W9) compared to BL, which is in line with both Nymo et al. (12) and Sumithran et al. (13).

Surprisingly the present study showed a significant increase in basal AG when participants were under ketosis, which is not in line with the majority of studies, who reported no changes in basal AG when following a KD (12,13,86,87). This may be explained by the difference in total energy and CHO content of the diets (all studies used VLED, the present study LED). Sumithran and colleges stated that the participants who were not ketotic (BHB<0.3 mmol/L) had significantly increased basal AG concentrations during WL (13). Moreover, both basal and postprandial AG concentrations were significantly higher than BL values after refeeding and out of ketosis, this is in keeping whit the majority of the studies reporting values greater than BL after the refeeding period (out of ketosis) (12,13,86). Furthermore, the present study found a significant moderate inverse correlation between BHB at W9 and changes in basal AG from BL to W9. The stronger the degree of ketosis W9, the larger the reduction, or smaller the increase in basal AG was. This is in line with Sumithran et al, who reported a significant inverse correlation between BHB and postprandially AG.

In ketosis (W9) the present study reported, a decrease in basal PYY, and an increase in postprandial PYY compared to BL. Both Sumithran et al.(13) and Iepsen et al. (68) reported a decrease in both basal and postprandial PYY in ketosis. However, some studies also reported no change in both basal and postprandial PYY (12,86,88) in and out of ketosis. Moreover, under WL and in ketosis the present study and Sumithran et al. (13) reported a decrease, and Diepvens et al. (89) reported no change in basal GLP-1. However, in ketosis the present study reported an increase, Sumithran et al. (13) and Diepvens et al. (89) reported no change, and Adam et al. (90) reported a decrease in postprandial GLP-1 during WL. Furthermore, after refeeding period and out of ketosis, the present study reported no change in both basal and postprandial GLP-1. Which is in line with the majority of studies who shows no change in basal (12,53,68,86,89,91) and postprandial (12,49,86,89,91) GLP-1 after refeeding (out of ketosis). The divergent results for PYY and GLP-1 both in fasting and after a meal under ketosis may be explained by different hormonal fractions being measured.

Under ketosis the majority of studies including the present study reported no change (12,54,89), and two studies reported a decrease (13,86) in basal CCK, and after refeeding and out of ketosis the majority of studies showed no change (12,54,86,89), in basal CCK. However, one study reported an increase (89) and another study showed lower concentration compared to BL (13) in basal CCK after refeeding and out of ketosis. Moreover, some studies, including the present one, reported a decrease (12,86), and three studies reported no change (13,54,89) in postprandial CCK during WL under ketosis. After refeeding and out of ketosis studies showed no change (present study,12,86), an increase (54), or a decrease (13) in postprandial CCK. The inconsistent

may be explained by sample size, assay specificity, different study design and length of refeeding period. Furthermore, the present study shows a decrease in both basal and postprandial insulin concentration during WL, both in and out of ketosis. These findings are in line with the majority of studies that reported a decrease in basal (12,13,68,85–88) and postprandial (12,13,54,68,86,88) insulin concentration both in ketosis and after refeeding (out of ketosis) compared to BL.

Despite limited studies in the field, Paoli et al. have recently published a review investigating the interactions between KDs and GM (78), with mixed results. The present study reported no change in Blautia, Faecalbacterium and Bacteroides both in and out of ketosis, which is not in line with the results from Paoli et al. review, where three studies reported an increase in Bacteroides, and one study reported a decrease in Faecalbacterium under ketosis (78). Moreover, the present study reported a reduction in Eubacterium rectale under ketosis, and an increase in Eubacterium rectale during refeeding period and out of ketosis, with values at W13 still lower than BL. These findings are in line with Lindefeldt et al. (included in Paoli et al. review) who reported a decrease in Eubacterium rectale during ketosis (92). Furthermore, the present study also reported an increase in Alistipes and Ruminococcaceae during WL (in ketosis), but after refeeding and out of ketosis, the values were similar to BL. The differences in the results may be explained by several factors; the review mentioned above, included 9 studies from both humans (6) and animals (3), three of the studies were done in children, and the majority of the studies were done in subjects who had epilepsy/or other conditions, which can limit the generalization to the overall population. Moreover, differences in the taxonomic classification of bacteria's/species used could also explain the differences in the results. In the future, a "standardization" of the use of taxonomic ranks would make the results more easily comparable and generalizable.

Additionally, the present study showed associations between some taxonomic groups and a few appetite markers. The greater the decrease in Alistipes and Eubacterium rectale was, the smaller the increase, or larger the reduction in basal PFC and postprandial PYY (only Alistipes) under ketosis, suggesting a link between fecal concentrations of Alistipes and Eubacterium rectale and changes in basal PFC and postprandial PYY during ketosis. Moreover, the larger the decrease in Faecalbacterium was, the larger the increase, or smaller the reduction, in postprandial PFC under ketosis, suggesting a non-beneficial link between concentrations of Faecalbacterium and postprandial PFC during ketosis.

A secondary aim was to investigate the impact of WL induced by a ketogenic LED on GM and SCFA. GM plays a central role in the host metabolism and is highly sensitive to the host dietary intake (93). WL induced by KD reduces the GM diversity (78,93), and Duncan et al. showed that a reduction in dietary intake of CHO in subjects with obesity leads to a decrease in fecal concentrations of both butyrate- producing bacteria and butyrate in feces (82). The main SCFAs

producing bacteria is Ruminococcaceae (acetate), Eubacterium rectale and Faecalibacterium prausnitzii (butyrate) and Bacteroidetes (propionate) (94,95). A reduction in SCFA- producing bacteria was expected as a result of WL on a KD (78,82,93), as well as a reduction in fecal SCFA concentrations (82). Even though the present study showed an increase in Ruminococcaceae, a main acetate producer, it also found a decrease in Eubacterium rectale, a SCFA producing bacteria, and in the fecal concentration of the main SCFA (acetic, propionic and butyric acid) after WL under ketosis. This is in line with the literature, and may be a result of reduced intake of CHO and/or energy. These results strengthen the evidence that WL induced with KD can alter GM. The metabolic mechanisms are still unclear (93), and more research is needed in humans to investigate the metabolic pathways.

A recent review conducted by Sowah et al. (2019) concluded that WL induced by KD has a lowering or neutral effect on SCFA concentrations in feces (96). About 95% of the SCFA present in the human colon lumen are acetate, propionate and butyrate (81).The present study reported a decrease in acetate, butyrate and propionate during WL when in ketosis (W9), and an increase during refeeding (W13) and out of ketosis, however the values at W13 were still lower compared with BL values. For acetate these results are in the line with the majority of studies in Sowah et al. review, where five of the nine studies reported a reduction in acetate fecal concentration after WL in ketosis, two studies (who compared diet-induced WL with different amounts of CHO) showed a stronger decrease in acetate in the low CHO groups compared to the groups with higher amounts of CHO, and three studies reported no change in acetate after WL in ketosis (96). Moreover, three of the studies in Sowah and colleges review (who compared diet-induced WL whit different amounts of CHO), reported a decrease in butyrate in the groups with low CHO and medium CHO, and no change in butyrate for the other groups after WL and in ketosis (96). Three other studies from the same review reported no change in butyrate, and a decrease in butyrate in 2 studies (96) during WL (in ketosis), which are in line with what the present study reported. The present study also reported a decrease in propionate after WL and in ketosis which is in line with four of the studies from the mentioned review, however four other studies in the review reported no change in propionate after WL in ketosis (96). The difference in the findings for both butyrate and propionate may be explained by different types of diets (amounts of both energy and CHO), the sample size (in the review, sample size varied from 19 to 91 participants), and the majority of the studies in Sowah et al. review includes only men (5 out of 9) (96).

This study found both a decrease and increase in SCFA-producing bacteria's in the GM and reduced levels of fecal SCFA during WL under ketosis, which increased (Ruminococcaceae decreased) during refeeding (out of ketosis). These findings add to the evidence that WL induced by a KD, has the ability to alter GM. Moreover, the present study showed that the greater the decrease in acetate (under ketosis), the higher the increase, or smaller the reduction in basal and postprandial feelings of hunger. Furthermore, the greater the reduction in fecal concentration in

butyrate during WL (in ketosis), the higher the increase, or smaller the reduction, in postprandial feelings of hunger in ketosis (from BL to W9). A weak inverse correlation was also seen between changes in fecal concentrations of butyrate and basal AG under ketosis. The greater the reduction in butyrate, the higher the increase, or smaller the reduction, in basal AG. Even though these findings strengthen the evidence that acetate and butyrate are appetite suppressant, which are in line with the literature (80,81), it do not support a role for fecal acetate and butyrate on appetite suppression under ketosis. These results suggests that other mechanisms are likely to be involved in appetite suppression during a KD. More studies in humans and with a larger sample size, including both men and women, are needed to further investigate the association between SCFA and appetite suppression during ketosis and to examine other potential mechanisms mediating appetite suppression under ketogenic conditions.

Strength and limitations

The present study has several strengths. The main strength of this study was that repeated measurements were performed at all three timepoints (BL: before starting the LED, W9: during WL and in ketosis and W13: after refeeding and out of ketosis). As the participants had the same WL in (W9) and out (W13) of ketosis, makes it possible to separate the effect of ketosis from the effect of WL. The sample size of this study is larger than many previous studies and includes both men and women in a balanced way. Both subjective and objective measurements of appetite were assessed, both in fasting and postprandially (after a test meal). Subjective appetite was measured by VAS, which is a valid method. Compliance with the diet was monitored weekly and was good. This study used the 16S rRNA, which is the best method for GM, and gives the possibility to investigate the correlation between microbiome and metabolome. To adjust for the increase risk of type 1 error, Bonferroni adjustments were used for the multiple time comparisons. In the regression analyses adjustments were done for known confounders of GM, SCFA and BHB.

This study also has some limitations. To measure objective appetite markers; insulin, AG, CCK, GLP-1 and PYY a multiplex kit was used, which is likely to result in less accurate and precise measurements than optimized assays for each individual hormone. Moreover, the consumption of permitted vegetables, which were consumed in addition to the LED products, was not taken into account in the analysis.

Practical implications and further research

This study suggests that SCFA are not involved in appetite suppression during WL induced by a ketogenic LED. This study is unique in this field, done in healthy humans and with a large sample size. There is limited research in this field (specially in humans), and for the same reasons as mentioned above, many of the studies have limitations, therefore further research should be done in healthy humans, with bigger sample sizes to further look at the associations between SCFA and appetite suppression during ketosis and to examine other potential mechanisms that can explain appetite suppression under ketogenic conditions.

5.0 Conclusions

This study suggest that WL induced by KD alters GM and SCFA production. However, fecal SCFA do not seem to play a role in the appetite suppression seen under ketogenic conditions. The exact molecular mechanisms mediating appetite suppression under KD remain unknown and more research is clearly needed in this field.

References

1. Obesity and overweight [Internet]. [cited 2021 Mar 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
2. Aamo AAW, Lind LH, Myklebust A. OVERVEKT OG FEDME I NORGE: OMFANG, UTVIKLING OG SAMFUNNSKOSTNADER. :50.
3. Overvekt og fedme - NHI.no [Internet]. [cited 2021 Mar 12]. Available from: https://nhi.no/kosthold/overvektfedme/overvekt-og-fedme/?page=1
4. Kyle TK, Dhurandhar EJ, Allison DB. Regarding Obesity as a Disease: Evolving Policies and Their Implications. Endocrinol Metab Clin North Am. 2016 Sep;45(3):511–20.
5. Non communicable diseases [Internet]. [cited 2021 Mar 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases
6. Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB. Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. Curr Obes Rep. 2015 Sep;4(3):363–70.
7. Hall KD, Guo J. Obesity Energetics: Body Weight Regulation and the Effects of Diet Composition. Gastroenterology. 2017 May;152(7):1718-1727.e3.
8. Westerterp KR, Speakman JR. Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. International Journal of Obesity. 2008 Aug;32(8):1256–63.
9. Kushner RF. Weight loss strategies for treatment of obesity. Prog Cardiovasc Dis. 2014 Feb;56(4):465–72.
10. Hassan Y, Head V, Jacob D, Bachmann MO, Diu S, Ford J. Lifestyle interventions for weight loss in adults with severe obesity: a systematic review. Clin Obes. 2016 Dec;6(6):395–403.
11. SMETHERS AD, ROLLS BJ. DIETARY MANAGEMENT OF OBESITY: CORNERSTONES OF HEALTHY EATING PATTERNS. Med Clin North Am. 2018 Jan;102(1):107–24.
12. Nymo S, Coutinho SR, Jørgensen J, Rehfeld JF, Truby H, Kulseng B, et al. Timeline of changes in appetite during weight loss with a ketogenic diet. Int J Obes (Lond). 2017 Aug;41(8):1224–31.
13. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Ketosis and appetite-mediating nutrients and hormones after weight loss. European Journal of Clinical Nutrition. 2013 Jul;67(7):759–64.
14. Gibson AA, Seimon RV, Lee CMY, Ayre J, Franklin J, Markovic TP, et al. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. Obes Rev. 2015 Jan;16(1):64–76.
15. Deemer SE, Plaisance EP, Martins C. Impact of ketosis on appetite regulation—a review. Nutrition Research. 2020 May 1;77:1–11.
16. Paoli A. Ketogenic Diet for Obesity: Friend or Foe? Int J Environ Res Public Health. 2014 Feb;11(2):2092–107.
17. Dao MC, Everard A, Clément K, Cani PD. Losing weight for a better health: Role for the gut microbiota. Clinical Nutrition Experimental. 2016 Apr 1;6:39–58.
18. Barrea L, Muscogiuri G, Annunziata G, Laudisio D, Pugliese G, Salzano C, et al. From gut microbiota dysfunction to obesity: could short-chain fatty acids stop this dangerous course? Hormones. 2019 Sep 1;18(3):245–50.
19. de Graaf C, Blom WA, Smeets PA, Stafleu A, Hendriks HF. Biomarkers of satiation and satiety. The American Journal of Clinical Nutrition. 2004 Jun 1;79(6):946–61.
20. Perspective on the Central Control of Appetite - Blundell - 2006 - Obesity - Wiley Online Library [Internet]. [cited 2021 Mar 12]. Available from: https://onlinelibrary.wiley.com/doi/full/10.1038/oby.2006.298
21. Harrold JA, Dovey TM, Blundell JE, Halford JCG. CNS regulation of appetite. Neuropharmacology. 2012 Jul;63(1):3–17.
22. Espel-Huynh HM, Muratore AF, Lowe MR. A narrative review of the construct of hedonic

hunger and its measurement by the Power of Food Scale. Obes Sci Pract. 2018 Feb 28;4(3):238–49.
23. Münzberg H, Qualls-Creekmore E, Yu S, Morrison CD, Berthoud H-R. Hedonics Act in Unison with the Homeostatic System to Unconsciously Control Body Weight. Front Nutr [Internet]. 2016 Feb 15 [cited 2021 Mar 12];3. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4753312/
24. Shin AC, Zheng H, Berthoud H-R. An expanded view of energy homeostasis: Neural integration of metabolic, cognitive, and emotional drives to eat. Physiol Behav. 2009 Jul 14;97(5):572–80.
25. MacLean PS, Higgins JA, Giles ED, Sherk VD, Jackman MR. The role for adipose tissue in weight regain after weight loss. Obes Rev. 2015 Feb;16(Suppl 1):45–54.
26. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. Endocrinol Metab Clin North Am. 2008 Dec;37(4):811–23.
27. Hopkins M, Blundell J, Halford J, King N, Finlayson G. The Regulation of Food Intake in Humans. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [cited 2021 Mar 12]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK278931/
28. Dong CX, Brubaker PL. Ghrelin, the proglucagon-derived peptides and peptide YY in nutrient homeostasis. Nat Rev Gastroenterol Hepatol. 2012 Dec;9(12):705–15.
29. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000 Apr;404(6778):661–71.
30. Geliebter A, Ochner CN, Aviram-Friedman R. Appetite-Related Gut Peptides in Obesity and Binge Eating Disorder. Am J Lifestyle Med. 2008 Jul;2(4):305–14.
31. Murphy KG, Dhillo WS, Bloom SR. Gut Peptides in the Regulation of Food Intake and Energy Homeostasis. Endocrine Reviews. 2006 Dec 1;27(7):719–27.
32. Beglinger C, Degen L, Matzinger D, D'Amato M, Drewe J. Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. Am J Physiol Regul Integr Comp Physiol. 2001 Apr;280(4):R1149-1154.
33. Shah M, Vella A. Effects of GLP-1 on appetite and weight. Rev Endocr Metab Disord. 2014 Sep;15(3):181–7.
34. Parkinson JRC, Chaudhri OB, Kuo Y-T, Field BCT, Herlihy AH, Dhillo WS, et al. Differential patterns of neuronal activation in the brainstem and hypothalamus following peripheral injection of GLP-1, oxyntomodulin and lithium chloride in mice detected by manganese-enhanced magnetic resonance imaging (MEMRI). NeuroImage. 2009 Feb 1;44(3):1022–31.
35. Grandt D, Schimiczek M, Beglinger Ch, Layer P, Goebell H, Eysselein VE, et al. Two molecular forms of Peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1–36 and PYY 3–36. Regulatory Peptides. 1994 May 5;51(2):151–9.
36. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metabolism. 2006 Sep 1;4(3):223–33.
37. Acosta A, Hurtado MD, Gorbatyuk O, La Sala M, Duncan D, Aslanidi G, et al. Salivary PYY: A Putative Bypass to Satiety. PLoS One [Internet]. 2011 Oct 10 [cited 2021 Mar 12];6(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189958/
38. Pradhan G, Samson SL, Sun Y. Ghrelin: much more than a hunger hormone. Curr Opin Clin Nutr Metab Care. 2013 Nov;16(6):619–24.
39. Delporte C. Structure and Physiological Actions of Ghrelin. Scientifica (Cairo) [Internet]. 2013 [cited 2021 Mar 12];2013. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3863518/
40. Ibrahim Abdalla MM. Ghrelin – Physiological Functions and Regulation. Eur Endocrinol. 2015 Aug;11(2):90–5.
41. Patterson M, Bloom SR, Gardiner JV. Ghrelin and appetite control in humans—Potential application in the treatment of obesity. Peptides. 2011 Nov 1;32(11):2290–4.

42. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, et al. Ghrelin increases food intake in obese as well as lean subjects. International Journal of Obesity. 2005 Sep;29(9):1130–6.
43. Howick K, Griffin BT, Cryan JF, Schellekens H. From Belly to Brain: Targeting the Ghrelin Receptor in Appetite and Food Intake Regulation. Int J Mol Sci [Internet]. 2017 Jan 27 [cited 2021 Mar 12];18(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343809/
44. Delhanty PJD, Neggers SJ, Lely AJ van der. MECHANISMS IN ENDOCRINOLOGY: Ghrelin: the differences between acyl- and des-acyl ghrelin. European Journal of Endocrinology. 2012 Nov 1;167(5):601–8.
45. Asakawa A. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut. 2005 Jan 1;54(1):18–24.
46. Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. British Journal of Nutrition. 2000 Oct;84(4):405–15.
47. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. International Journal of Obesity. 2000 Jan;24(1):38–48.
48. Melby CL, Paris HL, Foright RM, Peth J. Attenuating the Biologic Drive for Weight Regain Following Weight Loss: Must What Goes Down Always Go Back Up? Nutrients [Internet]. 2017 May 6 [cited 2021 Mar 12];9(5). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5452198/
49. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-Term Persistence of Hormonal Adaptations to Weight Loss [Internet]. http://dx.doi.org/10.1056/NEJMoa1105816. Massachusetts Medical Society; 2011 [cited 2021 Mar 12]. Available from: https://www.nejm.org/doi/10.1056/NEJMoa1105816
50. Drapeau V, King N, Hetherington M, Doucet E, Blundell J, Tremblay A. Appetite sensations and satiety quotient: Predictors of energy intake and weight loss. Appetite. 2007 Mar 1;48(2):159–66.
51. Doucet E, St-Pierre S, Alméras N, Tremblay A. Relation between appetite ratings before and after a standard meal and estimates of daily energy intake in obese and reduced obese individuals. Appetite. 2003 Feb 1;40(2):137–43.
52. Seimon RV, Taylor P, Little TJ, Noakes M, Standfield S, Clifton PM, et al. Effects of acute and longer-term dietary restriction on upper gut motility, hormone, appetite, and energy-intake responses to duodenal lipid in lean and obese men. The American Journal of Clinical Nutrition. 2014 Jan 1;99(1):24–34.
53. Verdich C, Toubro S, Buemann B, Lysgård Madsen J, Juul Holst J, Astrup A. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety—effect of obesity and weight reduction. International Journal of Obesity. 2001 Aug;25(8):1206–14.
54. Chearskul S, Delbridge E, Shulkes A, Proietto J, Kriketos A. Effect of weight loss and ketosis on postprandial cholecystokinin and free fatty acid concentrations. The American Journal of Clinical Nutrition. 2008 May 1;87(5):1238–46.
55. Anton SD, Han H, York E, Martin CK, Ravussin E, Williamson DA. Effect of calorie restriction on subjective ratings of appetite. Journal of Human Nutrition and Dietetics. 2009;22(2):141–7.
56. Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome | The American Journal of Clinical Nutrition | Oxford Academic [Internet]. [cited 2021 Mar 12]. Available from: https://academic.oup.com/ajcn/article/86/6/1603/4649820
57. Doucet E, Imbeault P, St-Pierre S, Alméras N, Mauriège P, Richard D, et al. Appetite after weight loss by energy restriction and a low-fat diet–exercise follow-up. International Journal of Obesity. 2000 Jul;24(7):906–14.
58. Martins C, Dutton GR, Hunter GR, Gower BA. Revisiting the Compensatory Theory as an explanatory model for relapse in obesity management. The American Journal of Clinical Nutrition.

2020 Nov 11;112(5):1170–9.
59. DeBenedictis JN, Nymo S, Ollestad KH, Boyesen GA, Rehfeld JF, Holst JJ, et al. Changes in the Homeostatic Appetite System After Weight Loss Reflect a Normalization Toward a Lower Body Weight. J Clin Endocrinol Metab. 2020 Apr 17;105(7):e2538–46.
60. Nymo S, Coutinho S, Eknes P, Vestbostad I, Rehfeld J, Truby H, et al. Investigation of the long-term sustainability of changes in appetite after weight loss. Int J Obes (Lond). 2018;42(8):1489–99.
61. Sumithran P, Proietto J. The defence of body weight: a physiological basis for weight regain after weight loss. Clinical Science. 2012 Oct 31;124(4):231–41.
62. Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, et al. Weight loss increases circulating levels of ghrelin in human obesity. Clin Endocrinol (Oxf). 2002 Feb;56(2):203–6.
63. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med. 2002 May 23;346(21):1623–30.
64. Strohacker K, McCaffery JM, MacLean PS, Wing RR. Adaptations of leptin, ghrelin or insulin during weight loss as predictors of weight regain: a review of current literature. Int J Obes (Lond). 2014 Mar;38(3):388–96.
65. Hill BR, Rolls BJ, Roe LS, De Souza MJ, Williams NI. Ghrelin and peptide YY increase with weight loss during a 12-month intervention to reduce dietary energy density in obese women. Peptides. 2013 Nov;49:138–44.
66. Beck EJ, Tapsell LC, Batterham MJ, Tosh SM, Huang X-F. Oat beta-glucan supplementation does not enhance the effectiveness of an energy-restricted diet in overweight women. Br J Nutr. 2010 Apr;103(8):1212–22.
67. Crujeiras AB, Goyenechea E, Abete I, Lage M, Carreira MC, Martínez JA, et al. Weight regain after a diet-induced loss is predicted by higher baseline leptin and lower ghrelin plasma levels. J Clin Endocrinol Metab. 2010 Nov;95(11):5037–44.
68. Iepsen EW, Lundgren J, Holst JJ, Madsbad S, Torekov SS. Successful weight loss maintenance includes long-term increased meal responses of GLP-1 and PYY3-36. Eur J Endocrinol. 2016 Jun;174(6):775–84.
69. Sweeting AN, Caterson ID. Approaches to obesity management. Internal Medicine Journal. 2017;47(7):734–9.
70. Clinical effectiveness of very-low-energy diets in the management of weight loss: a systematic review and meta-analysis of randomized controlled trials - Parretti - 2016 - Obesity Reviews - Wiley Online Library [Internet]. [cited 2021 Mar 12]. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/obr.12366
71. Paoli A, Bosco G, Camporesi EM, Mangar D. Ketosis, ketogenic diet and food intake control: a complex relationship. Front Psychol [Internet]. 2015 Feb 2 [cited 2021 Mar 12];6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4313585/
72. Gibson AA, Sainsbury A. Strategies to Improve Adherence to Dietary Weight Loss Interventions in Research and Real-World Settings. Behav Sci (Basel) [Internet]. 2017 Jul 11 [cited 2021 Mar 12];7(3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5618052/
73. Paoli A, Rubini A, Volek JS, Grimaldi KA. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. Eur J Clin Nutr. 2013 Aug;67(8):789–96.
74. Masood W, Annamaraju P, Uppaluri KR. Ketogenic Diet. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [cited 2021 Mar 12]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK499830/
75. Stubbs BJ, Cox PJ, Evans RD, Cyranka M, Clarke K, de Wet H. A Ketone Ester Drink Lowers Human Ghrelin and Appetite. Obesity (Silver Spring). 2018 Feb;26(2):269–73.
76. Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. Am J Clin Nutr. 2008 Jan;87(1):44–55.

77. Martins C, Nymo S, Truby H, Rehfeld JF, Hunter GR, Gower BA. Association Between Ketosis and Changes in Appetite Markers with Weight Loss Following a Very Low-Energy Diet. Obesity. 2020;28(12):2331–8.
78. Paoli A, Mancin L, Bianco A, Thomas E, Mota JF, Piccini F. Ketogenic Diet and Microbiota: Friends or Enemies? Genes (Basel) [Internet]. 2019 Jul 15 [cited 2021 Apr 7];10(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6678592/
79. Heiss CN, Olofsson LE. Gut Microbiota-Dependent Modulation of Energy Metabolism. J Innate Immun. 2018;10(3):163–71.
80. Byrne CS, Chambers ES, Morrison DJ, Frost G. The role of short chain fatty acids in appetite regulation and energy homeostasis. Int J Obes (Lond). 2015 Sep;39(9):1331–8.
81. Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? Proceedings of the Nutrition Society. 2015 Aug;74(3):328–36.
82. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol. 2007 Feb;73(4):1073–8.
83. Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. The American Journal of Clinical Nutrition. 2011 May 1;93(5):1062–72.
84. Johnston CS, Tjonn SL, Swan PD, White A, Hutchins H, Sears B. Ketogenic low-carbohydrate diets have no metabolic advantage over nonketogenic low-carbohydrate diets. The American Journal of Clinical Nutrition. 2006 May 1;83(5):1055–61.
85. Ratliff J, Mutungi G, Puglisi MJ, Volek JS, Fernandez ML. Carbohydrate restriction (with or without additional dietary cholesterol provided by eggs) reduces insulin resistance and plasma leptin without modifying appetite hormones in adult men. Nutrition Research. 2009 Apr 1;29(4):262–8.
86. Lyngstad A, Nymo S, Coutinho SR, Rehfeld JF, Truby H, Kulseng B, et al. Investigating the effect of sex and ketosis on weight-loss-induced changes in appetite. Am J Clin Nutr. 2019 Jun;109(6):1511–8.
87. Mohorko N, Černelič-Bizjak M, Poklar-Vatovec T, Grom G, Kenig S, Petelin A, et al. Weight loss, improved physical performance, cognitive function, eating behavior, and metabolic profile in a 12-week ketogenic diet in obese adults. Nutrition Research. 2019 Feb 1;62:64–77.
88. Moran LJ, Noakes M, Clifton PM, Wittert GA, Le Roux CW, Ghatei MA, et al. Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome. The American Journal of Clinical Nutrition. 2007 Dec 1;86(6):1603–10.
89. Diepvens K, Soenen S, Steijns J, Arnold M, Westerterp-Plantenga M. Long-term effects of consumption of a novel fat emulsion in relation to body-weight management. International Journal of Obesity. 2007 Jun;31(6):942–9.
90. Adam TCM, Lejeune MPGM, Westerterp-Plantenga MS. Nutrient-stimulated glucagon-like peptide 1 release after body-weight loss and weight maintenance in human subjects. British Journal of Nutrition. 2006 Jan;95(1):160–7.
91. Adam-Perrot A, Clifton P, Brouns F. Low-carbohydrate diets: nutritional and physiological aspects. Obesity Reviews. 2006;7(1):49–58.
92. Lindefeldt M, Eng A, Darban H, Bjerkner A, Zetterström CK, Allander T, et al. The ketogenic diet influences taxonomic and functional composition of the gut microbiota in children with severe epilepsy. NPJ Biofilms Microbiomes [Internet]. 2019 Jan 23 [cited 2021 Apr 20];5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6344533/
93. Ang QY, Alexander M, Newman JC, Tian Y, Cai J, Upadhyay V, et al. Ketogenic Diets Alter the Gut Microbiome Resulting in Decreased Intestinal Th17 Cells. Cell. 2020 Jun 11;181(6):1263-1275.e16.
94. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiology Letters. 2009 May 1;294(1):1–8.

95. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. Environmental Microbiology. 2017;19(1):29–41.
96. Sowah SA, Riedl L, Damms-Machado A, Johnson TS, Schübel R, Graf M, et al. Effects of Weight-Loss Interventions on Short-Chain Fatty Acid Concentrations in Blood and Feces of Adults: A Systematic Review. Adv Nutr. 2019 Jul;10(4):673–84.

Appendix I. Consent form

Forespørsel om deltakelse i et forskningsprosjekt

Hvilken mengde karbohydrat kan man spise og samtidig redusere sult, men øke metthetsfølelse?

Bakgrunn og hensikt

Dette er en forespørsel til deg om å delta i en forskningsstudie med utgangspunkt i en 8-ukers lavkalori diett hvor karbohydrat inntaket vil variere mellom deltakerne etterfulgt av en 4 ukers fase hvor målet er vektstabilisering og 9 måneder oppfølging. Problemsstillingene i studien er:

- Hva er det maksimale inntaket karbohydrater man kan innta og samtidig undertrykke appetitten under en lavkalori diett?
- Hvordan påvirkes appetitt hormonene som regulerer appetitt i diettens aktive fase?
- Hvordan påvirkes blodkomponenter, inflammasjon og immunsystemet
- Hvordan probiotika (melkesyrebakterier som kan ha gunstig helse effekt) påvirke vedlikehold av vekttap

NTNU, Norges teknisk-naturvitenskapelige universitet er ansvarlig for studien.

Hva innebærer studien?

Studien går over en 8-ukers diettperiode hvor inntaket av karbohydrater vil variere mellom deltakerne. Deltakere skal spise et variert utvalg av mat/diett produkter (milkshakes & supper) som tilsvarer et daglig energiinntak på 1000 kcal, fordelt over tre grupper med forskjellig karbohydrat inntak. Vi tar sikte på å oppnå i gjennomsnitt 8-10 % vekttap. Etter diett-perioden gjennomfører alle deltagerne en 4-ukers vekt-stabiliseringsfase, hvor man gradvis går over fra diett-produkter til å spise vanlig mat.

Det vil være ukentlig oppfølging fra forskere ved NTNU som gjennomgår kostdagboken din. Veiing inngår som en del av denne prosessen. Alle deltakerne vil også måtte avgi blod og urinprøver hver uke under diettfasen, og avføringsprøver på begynnelsen av studie (baseline), uke 9 (etter diettfase), uke 13 (etter vektstabiliseringsfase), 6 måneder og 12 måneder.

I uke 13, blir deltakerne randomisert (plassert tilfeldig) til å ta probiotika eller placebo daglig i totalt 9 måneder. Deltakerne skal møte månedlig til oppfølging ved Regionalt senter for fedmeforskning.

Undersøkelsene i studien foregår ved oppstart, uke 8, uke og 12 og ved 6 og 12 måneder. Oppfølgingen omfatter blodprøver, blodtrykksmåling, avføringsprøver, målinger av energibehov, vekt og livvidde, kroppssammensetning med BodPod (air displacement plethysmography) og BIA (Bioelectrical impedance analysis), bruk av aktivitetsarmbånd, samt utfylling av diverse spørreskjemaer.

Mulige fordeler og ulemper
Fordelen med deltakelse i studien er å oppnå mulig vektreduksjon og vedlikehold av den tapte vekta. I tillegg forbedrer deltakere helsen uten kirurgiske inngrep. Deltakelse kan også gjøre at du blir bedre kjent med mekanismene i kroppen din som påvirker appetitten. Dessuten vil du spare kostnader på mat i studiens diettfase (diettproduktene får du gratis i studien) og får probiota (eller placebo) gratis. Behandlingen anses ikke som risikabel, men siden undersøkelsene innebærer blodprøvetaking, kan noen deltakere oppleve dette som litt ubehagelig.

Hva skjer med prøvene og informasjonen om 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.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, vennligst undertegn samtykkeerklæringen på siste side. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte studiekoordinator Jessica Røkenes, som nås på telefon 46 77 02 40.

Studien er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk REK Sør-Øst B.

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*
Ytterligere informasjon om personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A – Utdypende forklaring av hva studien innebærer

Kriterier for deltakelse
De som kan delta i denne studien må
1. ha BMI mellom 30 og 40 kg/m^2,
2. være mellom 18 og 65 år,
3. ha et ønske om å gå ned i vekt ved hjelp av diett,
4. være relativt vektstabil de siste tre månedene (< 2 kg variasjon),
5. ikke være på diett i de siste tre måneder,
6. være frisk,
7. være inaktiv (ikke trene/mosjonere regelmessig)
8. Ikke har tatt probiotika i løpet av de siste 6 måneder før start av studie
9. ikke har tatt antibiotika i løpet av de siste 3 måneder før start av studie

Kvinner må dessuten enten være over menstruerende alder eller benytte p-piller eller andre hormonell-prevensjonsmetoder.

Bakgrunn for studien
Lavkalori dietter er en relativt sikker metode for å gå ned i vekt og gir også et raskt vekttap. Slike dietter kan gi vekttap på 8-10% i løpet av 8 uker. Dette kan også gi bedring i overvekts relaterte sykdommer og risiko faktorer. Vi vet at lavkalori dietter som er lav på karbohydrater kan indusere ketose, en tilstand som antas å forårsake undertrykkelse av appetitt. Det antas at ketose oppstår når forbruket av karbohydrater er lavt. Det lave forbruket av karbohydrater fører ofte til en begrensning av matvarer som frukt, grønnsaker, melkeprodukter, helkorn/fullkorn og belgfrukter som er gunstig for en persons helse. Den maksimale mengden karbohydrater i en lavkalori diett som er forbundet med ketose er derimot ukjent. Mengden karbohydrater man kan spise før man trigger appetittfølelsen, når man er i ketose, er også midlertidig usikkert. Det er behov for mer kunnskap om hvordan ketose fungerer, og hvordan vi kan innlemme mer karbohydrater i en lavkalori diett må undersøkes videre.
Dessuten vet vi at probiotika kan hjelpe med vekttap, men få studier har sett på vekttap vedlikehold.

Hovedhensikt med denne studien er å sammenligne undertrykkelse av appetitt gjennom en 8-ukers lavkalori diett hos pasienter som deltar i tre diett program med ulik mengde karbohydrat inntak.

Vi vil også se nærmere på hvordan den hormonelle appetitt reguleringen endres i diettens aktive fase. Appetitt er et komplisert samspill av blant annet hormoner som både stimulerer og reduserer matlysten, og vi vil følge utviklingen i disse i løpet av de ukene som dietten varer. Det er hittil gjort lite forskning på dette.

I tillegg skal det også undersøkes hvis daglig inntak av probiotika, sammenlignet med placebo, har en påvirkning på vekttap vedlikehold.

Undersøkelser
Som del av studien vil du måtte møte fastende og gjennomgå ulike undersøkelser før du start studie, slutten av uke 8 og 12 og 1 år oppfølging (totalt vil dette ta cirka 2,5 – 4 timer).

- Veiing og kroppsmassemåling
- Måling av kroppssammensetning med BodPod (Air displacement plethysmography) og BIA (Bioelectrical Impedance Analysis)
- Blodprøver
    - Måling av appetitt hormoner og ketoner i blod (for å måle ketose)
    - Måling av blodkomponenter inklusive inflammatoriske markører og immun funksjon (leukocytt responser)
- Indirekte kalorimetri (måling av energibehov)
- Blodtrykk (systolisk og diastolisk)
- Spørreskjema
- Urinprøver (også ukentlig fram til uke 12) og avføringsprøver (baseline, Uke 9, Uke 13, 6 måneder, 12 måneder)

I enkelte perioder av studien må du gå med et spesielt armbånd som registrerer din fysiske aktivitet. Varighet er en uke. Dette skjer før diett start, uke 4, 8 og 12 og 6 og 12 måneder.

Tidsskjema for intervensjonsperioden (12 uker) - felles for alle
Du vil få utdelt et variert utvalg av mat/diett produkter (milkshakes, supper) tilsvarende et daglig energiinntak på 1000 kcal med forskjellige makro-næringsstoff fordeling. Du skal utelukkende spise disse produktene imens du er i diettens aktive fase (8 uker) (standardisert for alle), men du oppfordres til å drikke rikelig vann (minst 2,5 liter) og eventuelt kalorifri drikke i tillegg. Du vil så få time hos en forsker hver uke for ukentlig oppfølging. Gjennomgang av kostdagbok, veiing og

urin-og avføringsprøver er en del av diettfasen. Overgangen fra diett-produkter til normal-kost vil skje gradvis i løpet av studieuke 9 og 10.

Studiedeltakerens ansvar
Det er studiedeltakerens ansvar å møte til avtalt tid, og det er av stor betydning for at kvaliteten på studien skal bli så god som mulig.

Kompensasjon og egenandel
Det gis ingen premiering for å delta i studien, men du vil få diettproduktene i diettens aktive fase og probiotika (eller placebo) gratis. Vi kan dessverre ikke gi kompensasjon for reiseutgifter. Det er viktig å standardisere dietten slik at alle spiser samme mengde energi.

Kapittel B – Personvern, biobank, økonomi og forsikring

Personvern

Ulike opplysninger om deg vil registreres som en del av dette prosjektet. Alle opplysninger som registreres om deg er konfidensielle. Ingen utenforstående forskere vil ha tilgang til dataene.

Vi vil benytte et internettbasert system for å samle inn spørreskjemadata. Dette betinger at du har tilgang til en datamaskin eller iPad. Rapporteringssystemet krypterer svarene dine slik at det ivaretar kravene til personvern.

NTNU ved administrerende direktør er databehandlingsansvarlig.

Biobank

Det biologiske materialet som blir tatt vil bli lagret i den spesifikke forskningsbiobanken "Ketosis study" ved Institutt for Kreftforskning og Molekylær Medisin (NTNU). Materialet vil bli analysert for ulike metabolitter/hormoner som er involvert i appetitt regulering, blodkomponenter, inflammatoriske markører og immunologisk funksjon. Instituttleder Professor Magne Børset er ansvarlig for denne forskningsbiobanken. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyse resultater inngår i biobanken. 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.

Økonomi

Studien finansieres av midler fra NTNU.

Forsikring
Studiedeltakerne omfattes av Norsk pasientskadeforsikring, jf. pasientskadelovens §1.

Informasjon om utfallet av studien

Du er berettiget til å motta informasjon om utfallet av studien.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix II. Instructions on how to follow the LED

Veiledning – LED

I de neste 8 ukene skal du følge en LED (lav kalori diett) uten å endre ditt fysiske aktivitetsnivå.

Nedenfor er et eksempel på hvordan du kan legge opp kostholdet ditt de neste 8 ukene:

Måltid	**Mat**
Frokost	**1** shake (sjokolade, jordbær eller vanilje)
Lunsj	**1** suppe (tomat eller kylling) * det er mulighet for å tilsette litt grønnsaker med lavt innhold av karbohydrater til suppen (beskrevet senere)
Mellommåltid	**1** shake (sjokolade, jordbær eller vanilje)
Middag	**1** suppe (tomat eller kylling) * det er mulighet for å tilsette litt grønnsaker med lavt innhold av karbohydrater til suppen (beskrevet senere)
Mellommåltid	**1** shake (sjokolade, jordbær eller vanilje)

Dette er kun ment som et forslag. Du kan planlegge dagene dine som du selv ønsker, men du må få i deg **5 pakker per dag**. (2 supper og 3 shaker)

Viktige faktorer å ta i betraktning:

- Du bør spise all maten som er forskrevet **(5 pakker/dag)**, selv om du ikke føler deg sulten.
- Du behøver ikke å ta noe vitamin eller mineraltilskudd, da dietten inneholder de mikronæringsstoffene som kroppen din trenger.
- Det bør ikke brukes kokende vann i suppene, da suppene klumper seg av dette. Vent litt før du heller i det varme vannet over suppen din.
- Du kan tilføre opptil 50g grønnsaker per suppe per dag. Grønnsakene du har lov til å spise til suppen er de som vokser over bakken (f.eks. blomkål, brokkoli, salat, tomat, agurk, squash, aubergine), og de kan være rå eller kokt.
- Du kan ikke drikke alkohol, brus eller naturlige frukt juicer. Du kan drikke ta, kaffe og andre drikker uten kalorier. Det anbefales at du drikker minst 2,5 liter vann per dag.
- Vær obs på at vann med smak kan inneholde kalorier. Det anbefales derfor at du holder deg til Farris naturell, eller drikker vanlig vann tilsatt sitronskiver (ikke sitronekstrakt). Ved tvil, les innholdet bakpå flasken. Hvis innholdsfortegnelsen viser at drikken inneholder kalorier (energi/kcal/kj per 100 ml) kan denne ikke drikkes.
- Det er normalt og forventet at du de første dagene (i noen tilfeller den første uken) vil føle deg sulten (det er en normal respons fra kroppen), men sultfølelsen forsvinner stort sett etter et par dager. Det er svært viktig at du holder deg til planen.
- Sukkerfri tyggegummi og sukkerfrie pastiller **(maks 3-4 stk/dag)** er tillatt hvis ønskelig. Dette må noteres i kostdagboken.

Kostholdsdagbok:

Dato: _____ Ukedag: _____ Navn: _____

Husk å også ta med annet, f.eks tyggis, drikke med smak, kaffe eller generelle kommentarer

Måltid	Anbefalt matinntak	Faktisk inntak og kommentarer
Frokost	**1** shake eller suppe *Du kan tilsette litt grønnsaker med lavt innhold av karbohydrater.	● Variant Kommentarer: _____
Lunsj	**1** shake eller suppe *Du kan tilsette litt grønnsaker med lavt innhold av karbohydrater.	● Variant Kommentarer: _____
Mellommåltid, dag	**1** shake eller suppe *Du kan tilsette litt grønnsaker med lavt innhold av karbohydrater.	● Variant Kommentarer: _____
Middag	**1** shake eller suppe *Du kan tilsette litt grønnsaker med lavt innhold av karbohydrater.	● Variant Kommentarer: _____
Mellommåltid, kveld	**1** shake eller suppe *Du kan tilsette litt grønnsaker med lavt innhold av karbohydrater.	● Variant Kommentarer: _____

Appendix III. Vegetables that was allowed

Grønnsaksliste:

Du kan spise 100 gram av disse grønnsakene i tillegg til måltidene.
Grønnsakene merket med * kan du kun spise 50 gram av da disse inneholder mere stivelse og energi.

- Agurk
- Alfalfaspire
- Artiskokk
- Asparges
- Aubergine
- Bambusskudd
- Basilikum
- Bladsalat
- Blomkål
- Brokkoli
- Brønnkarse
- Crispisalat
- Endivesalat
- Feltsalat
- Fennikel
- Gressløk
- Grønn paprika
- Grønnkål
- Hjertesalat
- Isbergsalat
- Kinakål
- Raddichio salat
- Rapidsalat
- Reddik
- Romano salat
- Ruccola salat
- Sopp (alle typer)
- Spinat
- Squash/ Zucchini
- Stangselleri
- Tomat (Cherry og vanlig)
- Vårløk

* Hodekål
* Ingefærrot
* Løk (alle typer)
* Koriander
* Rosmarin

Disse grønnsakene er IKKE lov å spise:

- Avokado
- Søtpotet
- Potet
- Bønner
- Erter
- Gresskar
- Kål
- Mais

Hvis det er noen grønnsaker som er ikke på listen som du har lyst til å spise, men er usikker om det er lov, så ta kontakt med oss eller sjekk på matvaretabellen.no om det inneholder mindre enn 3 gram karbohydrater eller ikke.

Appendix IV. Macronutrient composition of the three LEDs

Table 12. Macronutrient composition of the three LEDs

	Low		**Medium**		**High**	
	Pr 275 g/5 Servings (50 g/each)/day	E%	Pr 275 g/5 Servings (50 g/each)/day	E%	Pr 275 g/5 Servings (55 g/each)/day	E%
Energy kj	4282.5		4032.5		4215.7	
Energy kcal	1027.5		972.5		1023	
Fat g	48	42.6 %	32	30.2 %	21.2	19.1 %
Saturated fat g	5.7		3.5		1.8	
Carbohydrate g	69.5	27.6 %	99.8	42.1 %	132.3	53.3 %
Mono- and Disaccharides g	55.5		75.8		108.1	
Sugar	0		0		0	
Starch	0.3		0.3		0.3	
Fiber g	14.25		10.5		10.2	
Protein g	73.7	29.3 %	72	30.4 %	73.5	29.8 %
Salt mg	4918.8		3742.5		3610.8	

Appendix V. Healthy guidelines

Retningslinjer for vedlikehold av vekten:

Dette heftet er kun en liten hjelp til dere for å se eksempler på hvordan dere kan legge opp måltidene etter at dere er ferdig med pulverkuren. Det anbefales en gradvis nedgang i bruk av produktene. I uke 9 er dette satt til 2 produkter om dagen. I uke 10 er det anbefalt å ta 1 produkt om dagen både for kvinner og menn.

Her har vi laget et eksempel på hvordan dere kan legge opp måltidene i løpet av en dag. Dere trenger ikke følge dette slavisk, da det kun er ment som en liten hjelp frem til dere får eget kostholdsopplegg fra ernæringsfysiolog.

Her er noen generelle kostholdsråd som er greie å tenke på for å legge opp et sunt kosthold:

- Se etter matvare merket med nøkkelhull.

- Sammenliknet med andre matvarer av samme type, oppfyller produkter med nøkkelhull ett eller flere av disse kravene:
  - Mindre og sunnere fett
  - Mindre sukker
  - Mindre salt
  - Mer kostfiber og fullkorn

- Spis minst fem porsjoner grønnsaker, frukt og bær hver dag. Dvs. 2 porsjoner frukt og 3 porsjoner grønnsaker. En porsjon tilsvarer 100 gram. 1 dl juice tilsvarer en av fem om dagen.

Bilde er ett eksempel på hvordan man kan oppfylle kravet om fem om dagen.

- Spis grove kornprodukter hver dag. Brødet bør ha minst tre eller fire kakestykker

- La magre meieriprodukter være en del av det daglige kostholdet. F.eks. ekstra lettmelk, mager cottage cheese, mager kesam, norvegia lett ost.
- Spis fisk til middag to til tre ganger i uken. Bruk også gjerne fisk som pålegg.
- Velg magert kjøtt og magre kjøttprodukter. Begrens mengden bearbeidet kjøtt og rødt kjøtt (f.eks. kjøttdeig, farse)
- Velg matoljer (rapsolje, solsikkeolje, olivenolje), flytende margarin og myk margarin fremfor hard margarin og smør.
- Velg matvarer med lite salt og begrens bruken av salt i matlagingen og på maten. Vær obs på posesupper, sauser, frokostblandinger osv da disse kan inneholde mye salt/sukker.
- Unngå mat og drikke med mye sukker til hverdags
- Velg vann som tørstedrikk
- Ha en god balanse mellom hvor mye energi du får i deg gjennom mat og drikke, og hvor mye du forbruker gjennom aktivitet.

Menyforslag

UKE 9 – valgfritt med 2 produkter om dagen for kvinner (anbefales), og 3 produkter for menn (anbefales).

Frokost:

- Alternativ 1: 1 dl musli eller havregryn (kok gjerne) med ekstra lettmelk/vann
- Alternativ 2: 2 skiver grovt brød eller 3 grove knekkebrød med magert kjøttpålegg, ost eller egg. Litt smør eller lettmargarin. Bruk så mye paprika, agurk og tomat du ønsker.
- Alternativ 3: 1 shake (valgfri type)

Mellommåltid: 1 frukt eller 100 gr grønnsaker (f.eks. sukkerert, gulrot, tomat)

Lunsj:

- Alternativ 1: blandet salat med 125-150gr kylling (1 filet), kjøtt eller fisk med 1 ss oljedressing.
- Alternativ 2: 2 skiver grovt brød eller 3 grove knekkebrød med magert kjøttpålegg, ost eller egg. Tynt lag med lettmargarin. Bruk så mye paprika, agurk og tomat som du ønsker.
- Alternativ 3: 1 shake, **eller** 1 suppe (valgfri type)

Middag:

- Legg opp 125-150gr fisk/kjøtt, 2 små poteter, 50gr fullkornris eller fullkornspasta og grønnsaker etter tallerkenmodellen.

Mellommåltid: 1 frukt eller 100gr grønnsaker (f.eks. sukkererter, gulrot, tomat)

Kveldsmat:

- Alternativ 1: 1 kopp te, 2 grove knekkebrød med mager ost eller skinke, tynt lag med lettmargarin og gjerne tomat, paprika, agurk eller andre grønnsaker.
- Alternativ 2: 1 grovt knekkebrød med magert pålegg (mager ost, skinke, makrell i tomat og lignende), 1 liten yoghurt og 1 glass melk.
- Alternativ 3: 1 shake **eller** 1 suppe (valgfri type)

UKE 10 – valgfritt med 1 produkt om dagen for kvinner og menn (anbefales)

Frokost:

- Alternativ 1: 1 dl müsli eller havregryn (kok gjerne) med ekstra lettmelk.

- Alternativ 2: 2 skiver grovt brød eller 3 grove knekkebrød med magert kjøttpålegg, ost eller egg. Litt smør eller lettmargarin. Bruk så mye paprika, agurk og tomat du ønsker.
- Alternativ 3: 1 shake (valgfri type)

Mellommåltid: 1 frukt eller 150gr grønnsaker (f.eks. sukkererter, gulrot, tomat)

Lunsj:

- Alternativ 1: blandet salat med 125-150gr kylling (1 filet), kjøtt eller fisk med 1 ss oljedressing.
- Alternativ 2: 2 skiver grovt brød eller 3 grove knekkebrød med magert kjøttpålegg, ost eller egg. Tynt lag med lettmargarin. Bruk så mye paprika, agurk og tomat du ønsker.
- Alternativ 3: 1 shake **eller** 1 suppe (hvis denne ikke er tatt tidligere på dagen)

Middag:

- Legg opp 125-150gr fisk/kjøtt, 2 små poteter, 50gr fullkornris eller fullkornspasta og grønnsaker etter tallerkenmodellen.

Mellommåltid: 1 frukt eller 150gr grønnsaker (f.eks. sukkererter, gulrot, tomat)

Kveldsmat:

- Alternativ 1: 1 kopp te, 2 grove knekkebrød med mager ost eller skinke, tynt lag med lettmargarin og gjerne tomat, paprika, agurk eller andre grønnsaker.
- Alternativ 2: 1 grovt knekkebrød med magert pålegg (mager ost, skinke, makrell i tomat og lignende), 1 liten yoghurt og 1 glass melk.
- Alternativ 3: 1 shake **eller** 1 suppe (hvis denne ikke er spist tidligere på dagen)

Appendix VI. User manual for activity monitor

Brukermanual for SenseWare armbånd

1. Armbånd & sensor tåler ikke vann, ta den av når du dusjer, bader (etc)

2. Elektromagnetiske forstyrrelser: skal du i CT-scan eller lignende må armbåndet tas av. Dette informerer som regel helsepersonell om.

3. Armbåndet skal være på minst 7 dager, ta det av etter den 8. dagen.

4. Armbåndet må være på hele døgnet - også når du sover. Tas kun av maks 1 time per dag (f.eks. når du dusjer).

5. Armbåndet skrur seg på når du tar det på, og skrur seg av når du tar det av. Du trenger hverken å trykke på sensoren eller lade den i den perioden du skal bruke båndet.
6. Tørk av synlig skitt eller svette i det tidsrommet du tar av armbåndet (max 1 time av per 24 timer).
7. Ta med deg båndet tilbake til oss neste gang du skal innom, men sørg for at du har brukt det sammenhengende i minst 7 dager før.
8. Bruk armbåndet på din *ikke-dominante* arm, dvs. er du høyrehendt, bruk den på venstre overarm

Appendix VII. Standardized test meal

Table 13. Breakfast macronutrient composition

	W/milk	W/yoghurt	E%
Energy kj	2486.9	2563.5	
Energy kcal	**594.4**	**62.7**	
Fat g	**23.2**	**24.6**	**35%**
Saturated fat g	13.7	15.7	
Carbohydrate g	**69.2**	**69.2**	**49%**
Mono- & Disaccharides g	38.7	39.8	
Sugar g	10.8	18.2	
Starch g	27.2	27.6	
Fiber g	3.9	3.6	
Protein g	**23.9**	**22**	**16%**
Salt	1.6	1.6	

Appendix VIII. Simple correlation between taxonomic groups and changes in appetite at W9

Table 14. Correlation coefficients between Bacteroides and changes in appetite W9

	r	P-value
Basal Hunger	-0.033	0.770
iAUC Hunger	-0.037	0.740
Basal DTE	0.024	0.833
tAUC DTE	-0.010	0.931
Basal PFC	-0.021	0.852
tAUC PFC	-0.088	0.434
tAUC Fullness	0.017	0.979
Basal AG	0.005	0.971
iAUC AG	-0.021	0.864
Basal Insulin	-0.005	0.969
iAUC Insulin	-0.139	0.232
iAUC PYY	0.185	0.112
iAUC GLP-1	-0.033	0.778
tAUC CCK	-0.138	0.235

iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin.

Table 15. Correlation coefficients between Alistipes W9 and changes in appetite from BL to W9

	r	P-value
Basal Hunger	0.070	0.531
iAUC Hunger	0.050	0.653
Basal DTE	0.098	0.379
tAUC DTE	0.118	0.299
Basal PFC	0.191	0.084
tAUC PFC	-0.029	0.797
tAUC Fullness	0.219	**0.047***
Basal AG	-0.065	0.600
iAUC AG	-0.178	0.147
Basal Insulin	0.004	0.973
iAUC Insulin	0.119	0.307
iAUC PYY	0.215	0.064
iAUC GLP-1	0.039	0.736
tAUC CCK	-0.091	0.433

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05*

Table 16. Correlation coefficients between Blautia and changes in appetite W9

	r	P-value
Basal Hunger	-0.044	0.694
iAUC Hunger	-0.218	0.051
Basal DTE	0.112	0.319
tAUC DTE	0.066	0.561
Basal PFC	0.117	0.298
tAUC PFC	0.060	0.600
tAUC Fullness	-0.159	0.157
Basal AG	0.169	0.179
iAUC AG	0.231	0.062
Basal Insulin	0.045	0.705
iAUC Insulin	-0.030	0.799
iAUC PYY	-0.180	0.128
iAUC GLP-1	-0.121	0.306
tAUC CCK	0.237	**0.042***

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05.*

Table 17. Correlation coefficients between Eubacterium rectale and changes in appetite W9

	r	P-value
Basal Hunger	-0.068	-0.546
iAUC Hunger	-0.216	0.051
Basal DTE	0.077	0.490
tAUC DTE	-0.066	0.559
Basal PFC	0.220	**0.047***
tAUC PFC	-0.118	0.295
tAUC Fullness	-0.071	0.525
Basal AG	0.192	0.120
iAUC AG	-0.018	0.883
Basal Insulin	0.016	0.889
iAUC Insulin	0.125	0.287
iAUC PYY	0.038	0.747
iAUC GLP-1	-0.055	0.637
tAUC CCK	0.197	0.091

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05.*

Table 18. Correlation coefficients between Faecalbacterium and changes in appetite W9

	r	P-value
Basal Hunger	-0.003	0.977
iAUC Hunger	-0.221	**0.044***
Basal DTE	-0.007	0.952
tAUC DTE	-0.133	0.236
Basal PFC	-0.091	0.411
tAUC PFC	-0.261	**0.018***
tAUC Fullness	0.072	0.519
Basal AG	-0.054	0.664
iAUC AG	-0.094	0.446
Basal Insulin	0.108	0.353
iAUC Insulin	0.039	0.739
iAUC PYY	-0.133	0.255
iAUC GLP-1	-0.129	0.268
tAUC CCK	0.087	0.457

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05.*

Table 19. Correlation coefficients between Ruminococcaceae and changes in appetite W9

	r	P-value
Basal Hunger	-0.012	0.914
iAUC Hunger	0.092	0.413
Basal DTE	-0.102	0.362
tAUC DTE	0.130	0.252
Basal PFC	-0.093	0.406
tAUC PFC	0.091	0.481
tAUC Fullness	-0.138	0.217
Basal AG	-0.160	0.197
iAUC AG	-0.121	0.328
Basal Insulin	0.055	0.640
iAUC Insulin	0.130	0.265
iAUC PYY	-0.186	0.112
iAUC GLP-1	0.027	0.818
tAUC CCK	-0.091	0.431

iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin.

Appendix IX. Simple correlation between SCFA and changes in appetite between BL and W9

Table 20. Correlation coefficients between changes in acetic acid and changes in appetite from BL to W9

	r	P-value
Basal Hunger	-0.012	0.917
iAUC Hunger	-0.189	0.094
Basal DTE	-0.026	0.819
tAUC DTE	-0.036	0.749
Basal PFC	0.119	0.289
tAUC PFC	0.012	0.917
tAUC Fullness	0.125	0.262
Basal AG	-0.103	0.416
iAUC AG	-0.070	0.572
Basal Insulin	0.162	0.166
iAUC Insulin	0.251	**0.030***
iAUC PYY	0.199	0.094
iAUC GLP-1	0.067	0.574
tAUC CCK	0.178	0.127

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05*

Table 21. Correlation coefficients between changes in propionic acid and changes in appetite from BL to W9

	r	P-value
Basal Hunger	0.034	0.764
iAUC Hunger	-0.010	0.927
Basal DTE	-0.055	0.620
tAUC DTE	-0.234	**0.037***
Basal PFC	0.149	0.180
tAUC PFC	-0.144	0.200
tAUC Fullness	0.200	0.071
Basal AG	-0.203	0.108
iAUC AG	-0.122	0.325
Basal Insulin	0.173	0.138
iAUC Insulin	0.240	**0.038***
iAUC PYY	0.306	**0.009****
iAUC GLP-1	0.019	0.874
tAUC CCK	0.100	0.395

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05, and **P<0.01.*

Table 22. Correlation coefficients between changes in isobutyric acid and changes in appetite from BL to W9

	r	P-value
Basal Hunger	-0.005	0.963
iAUC Hunger	0.122	0.284
Basal DTE	-0.136	0.225
tAUC DTE	-0.280	**0.013***
tAUC PFC	-0.046	0.686
tAUC Fullness	0.147	0.189
Basal AG	0.081	0.523
iAUC AG	-0.112	0.368
Basal Insulin	0.011	0.923
iAUC Insulin	0.091	0.439
iAUC PYY	0.257	**0.030***
iAUC GLP-1	-0.107	0.374
tAUC CCK	0.009	0.942

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05.*

Table 23. Correlation coefficients between changes in butyric acid and changes in appetite from BL to W9

	r	P-value
Basal Hunger	0.010	0.932
iAUC Hunger	-0.161	0.164
Basal DTE	-0.056	0.627
tAUC DTE	-0.127	0.275
Basal PFC	0.079	0.492
tAUC PFC	-0.067	0.561
tAUC Fullness	0.085	0.461
Basal AG	-0.260	**0.043***
iAUC AG	-0.201	0.112
Basal Insulin	0.117	0.328
iAUC Insulin	0.183	0.124
iAUC PYY	0.212	0.081
iAUC GLP-1	0.145	0.233
tAUC CCK	0.014	0.907

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05.*

Table 24. Correlation coefficients between changes in isovaleric acid and changes in appetite from BL to W9

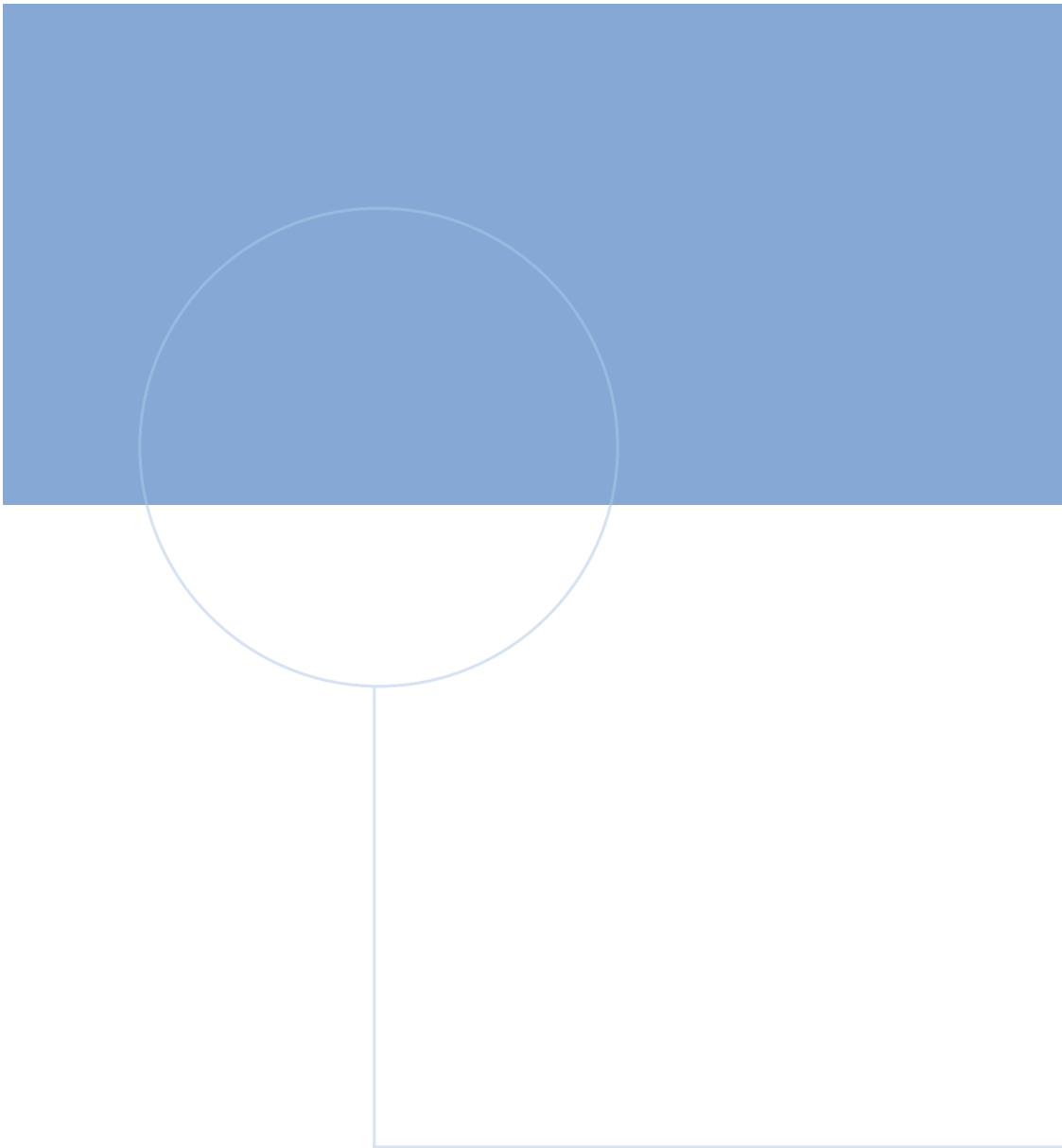
	r	P-value
Basal Hunger	0.142	0.200
iAUC Hunger	0.173	0.122
Basal DTE	-0.024	0.833
tAUC DTE	-0.298	**0.007****
Basal PFC	0.150	0.177
tAUC PFC	-0.099	0.375
tAUC Fullness	0.249	**0.023***
Basal AG	0.022	0.860
iAUC AG	-0.220	0.071
Basal Insulin	0.012	0.920
iAUC Insulin	-0.035	0.764
iAUC PYY	0.194	0.099
iAUC GLP-1	-0.107	0.370
tAUC CCK	0.000	1.000

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05, and **<0.01.*

Table 25. Correlation coefficients between changes in valeric acid and changes in appetite from BL to W9

	r	P-value
Basal Hunger	0.059	0.606
iAUC Hunger	0.047	0.682
Basal DTE	0.089	0.433
tAUC DTE	-0.239	**0.037***
Basal PFC	0.200	0.077
tAUC PFC	0.051	0.655
tAUC Fullness	0.236	**0.036***
Basal AG	-0.028	0.829
iAUC AG	-0.154	0.223
Basal Insulin	0.101	0.397
iAUC Insulin	0.024	0.845
iAUC PYY	0.241	**0.046***
iAUC GLP-1	-0.118	0.335
tAUC CCK	0.100	0.402

*iAUC: Incremental area under the curve. tAUC: Total area under the curve. DTE: Desire to eat. PFC: Prospective food consumption. AG: Acylated Ghrelin. PYY: Peptide YY. GLP-1: Glucagon-like peptide- 1. CCK: Cholecystokinin. Symbols denote significant correlation at *P<0.05.*



NTNU

Kunnskap for en bedre verden