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How does the use of probiotics for weight loss maintenance affect appetite?

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Abstract

Introduction: An increasing number of studies has shown that probiotic supplementation can have beneficial effects on appetite with regards to maintain body weight loss in the long term. This study evaluated the impact of probiotic supplementation with a multistrain probiotic (NYKOPRO Ferie, Takeda AS) on subjective and hedonic feelings of appetite, and on objective markers of appetite, in the context of a weight loss maintenance (WLM) program. The potential role of probiotics in the development of obesity led us to investigate the effects of probiotic consumption on appetite during WLM.

Methods: Males (n=30) and females (n=25) with obesity (BMI: 29.8±3.2 kg/m²) participated in a double blinded, randomized, placebo-controlled trial, that included a nine-month WLM phase (from week 13 to one year). During the WLM phase, each subject consumed two capsules per day of either a placebo or a probiotic formulation. Subjective feelings of appetite (hunger, fullness, desire to eat, and prospective food consumption) were measured using a visual analogue scale, food hedonics were measured using the Leeds food preference questionnaire, and plasma concentrations of appetite-related hormones (active ghrelin, total peptide YY (PYY), total glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK)) were measured to evaluate changes in appetite from week 13 to one year between and within the two intervention groups.

Results: The probiotic supplementation was not associated with improvements in WLM, no significant differences were found between groups in changes over time (from week 13 to one year) in subjective feelings of appetite, in food hedonics, or in appetite related hormones. No significant differences over time were observed within groups in any of the subjective feelings of appetite. At week 13, the placebo group, in the pre-meal, had significant higher values in the explicit liking and wanting for high fat and sweet foods (P<0.05). At 1 year, no significant differences were found between or within groups. There was a significant decrease over time in both groups for all appetite-related hormones in both basal and incremental area under the curve (iAUC) (with the exceptions of iAUC total GLP-1 in the probiotic group, and basal and iAUC CCK that did not change significantly in both groups).

Conclusions: Taken together, these observations do not support the hypothesis of probiotic supplementation having beneficial effects on appetite in the obesity management compared to placebo. More research to confirm the effect of probiotic supplementation on appetite in long-term WLM is needed. More studies with fewer/different types of probiotic are needed to know more about how probiotics, possibly, can help with obesity management.

Relevance: Finding strategies to prevent weight regain is a great challenge in obesity management. Changes in appetite towards increased hunger play an important role in why individuals are unsuccessful in maintaining their body weight loss in the long-term. Probiotics are found to have beneficial effects on appetite. The findings in this study did not support our hypothesis, but it contributes to better understand the action of the NYKOPRO Ferie probiotics on appetite and WLM, and will therefore contribute to help with the selection of probiotics as a tool for benefiting appetite in WLM in future research.
Keywords: Probiotic, placebo, weight loss maintenance, RCT, obesity, feelings of appetite, food hedonics, appetite-related hormones.

Sammendrag

Introduksjon: Flere studier viser at probiotika kan ha en fordelaktig innvirkning på appetitt når det kommer til å stabilisere kroppsvekt på lang sikt etter vektnedgag (WLM). Denne studien evaluerte effekten av en multistrain probiotika (NYKOPRO Ferie, Takeda AS) på subjektive og hedoniske følelser av appetitt, og objektive markører av appetitt, under en intervensjon med intensjon om WLM. Den potensielle rollen til probiotika i fedmeutvikling fikk oss til å undersøke effektene av probiotika på appetitt gjennom en fase med WLM.

Metode: Menn (n=30) og kvinner (n=25) med fedme (BMI: 29.8±3.2 kg/m²) deltok i en dobbel-blindet, randomisert, placebo-kontrollert studie, som inkluderte ni måneder med WLM (fra uke 13 til 1 år). Gjennom perioden med WLM fikk alle deltakerne beskjed om å innta to kapsler med enten placebo eller probiotika daglig. Subjektive følelser av appetitt (sult, metthet, ønske om å spise, og fremtidig matinntak) ble målt ved bruk av visuell, analog skala, hedonisk appetitt («liking» og «wanting» av mat) ble målt ved Leeds food preference questionnaire og plasma konsentrasjon av appetitt relaterte hormoner (aktiv ghrelin, total peptide YY (PYY), total glucagon-lik peptide-1 (GLP-1), og cholecystokinin (CCK)) ble målt for å evaluere endringene i appetitt fra uke 13 til ett år i mellom og innen de to intervensjons gruppene.

Resultater: I denne studien var ikke inntak av probiotika assosiert med fordelaktig innvirkning på appetitt og WLM. Det ble ikke funnet noen signifikante forskjeller mellom grupper i endring over tid (fra uke 13 til ett år) i subjektive følelser av appetitt. I uke 13 hadde placebo gruppen signifikant høyere verdier før test-måltidet i «liking» og «wanting» av fet og søt mat (P<0.05). Etter ett år ble det ikke funnet noen signifikante forskjeller mellom gruppene i hedonisk appetitt. Det ble funnet en signifikant nedgang over tid av alle appetitt-relaterte hormoner i begge gruppene, i både basal og inkrementelt areal under kurven (iAUC) (med unntak av iAUC total GLP-1 i probiotika gruppen, der det ikke var signifikant forskjell, og i basal og iAUC CCK der det ikke ble funnet signifikante endringer i noen av gruppene).

Konklusjon: Funnene i denne studien støtter ikke hypotesen om at probiotika har fordelaktig innvirkning på appetitt ved WLM sammenliknet med placebo. Det er behov for videre forskning for å bekrefte funnene av effektene probiotika kan ha i langvarig vedlikehold av vektnedgang. Flere studier med færre eller andre typer probiotika må til for å vite mer om hvordan probiotika mulig kan være til hjelp i fedme behandling.

Relevans: En stor utfordring innen fedmebehandling er å finne gode strategier for å forebygge vektoppgang ved vedlikehold av vektnedgang over tid. Endringer i appetitt spiller en viktig rolle i hvorfor så mange feiler på sikt med å WLM. Forskning viser at probiotika har fordelaktig virkning på appetitt. Funnene i denne studien støttet ikke hypotesen, men bidrar med å gi bedre forståelse av hvordan NYKOPRO Ferie probiotika virker på appetitt og vedlikehold av vektnedgang, og vil dermed bidra til å videre velge hvilke probiotika som kan ha en fordelaktig effekt på appetitt i fedmebehandling i videre forskning.

Nøkkelord: Probiotika, placebo, stabilisering av kroppsvekt etter vekttap, RCT, fedme, subjektive følelser av appetitt, hedonisk appetitt, appetitt-relaterte hormoner.

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Abbreviations

1YR	One year
ARC	Arcuate nucleus
AUC	Area under the curve
BL	Baseline
BMI	Body-mass index
BW	Body weight
CCK	Cholecystokinin
CFU	Colony-forming unit
CNS	Central nervous system
CV	Coefficient of variation
DTE	Desire to eat
GI	Gastrointestinal
GLP-1	Glucagon-like peptide 1
HFNS	High fat non-sweet
HFS	High fat and sweet
iAUC	Incremental area under the curve
LED	Low energy diet
LFNS	Low-fat non-sweet
LFPQ	Leeds food preference questionnaire
LFS	Low fat and sweet
LMM	Linear mixed model
PA	Physical activity
PFC	Prospective food consumption
PYY	Peptide YY
RCT	Randomized controlled trial
REK	Regional ethics committee
RMR	Resting metabolic rate
SCFA	Short chain fatty acid
VAS	Visual analogue scale
W13	Week 13
WL	Weight loss
WLM	Weight loss maintenance

1.0 Background

1.1 Introduction

The prevalence of overweight and obesity is increasing globally. In 2016, the global prevalence of overweight [BMI: 25.0-29.9 kg/m^2] was 39 % in adults aged 18 years and older. The prevalence of obesity [BMI > 30.0 kg/m^2] was 15 % in women and 11 % in men (1). In Norway, the same trend of increasing rates of overweight and obesity is seen. Data from the HUNT study (2006-2008) showed that 25 % of the Norwegian population had obesity (2).

Obesity is classified as a disease and is characterized as being chronic, progressive, and relapsing (3). It is also a risk factor for chronic diseases such as cardiovascular diseases, type 2 diabetes, and some types of cancer (4). The World Health Organization (WHO) estimates that, worldwide, 2.8 million individuals die each year as a consequence of overweight/obesity (5).

There are multifactorial and complex reasons for why individuals develop obesity. For the majority of individuals with obesity, excess weight gain is a result of a continuous higher energy intake compared with the energy expenditure, creating a chronic positive energy balance (6).

The most effective treatment for obesity is to reduce body weight. Even a modest reduction in weight can reduce the morbidity and mortality associated with obesity (7). To lose weight, individuals need to reduce their energy intake to a level below their energy expenditure. A variety of different diets and lifestyle interventions can be used as tools to create an energy deficit. Overall, energy restricted diets can be an effective way to induce clinically significant weight loss if patients are able to adhere to them (8)(9). However, the biggest challenge in obesity management is to maintain the weight loss in the long-term (10)(11)(12)(13). The majority of adults with obesity experience a significant weight regain, and some relapse to their original weight or even above baseline body weight (12)(15).

Successful weight loss maintenance can be defined as "individuals who have intentionally lost at least 10 % of their body weight and kept it off at least for 1 year" (14). It is estimated that only 10-20 % of individuals who experience at least a 10 % weight loss are able to maintain it over an one-year period (15). Weight loss maintenance in the long-term is challenging due to the interactions between the obesogenic environment, biology, and behavior (16). Another factor that makes weight loss maintenance challenging is the fact that weight loss is accompanied by persistent endocrine adaptations (such as an increase in ghrelin) that increase appetite (namely an increase in hunger feelings) (17). Finding possible solutions that effects appetite feelings in a positive manner would make weight loss maintenance more sustainable, and is something that needs to be researched further.

Losing weight and keeping it off reduces the risk of developing type 2 diabetes and reduces other risk factors associated with obesity, as heart diseases, sleep apnea, high blood pressure among others. Moreover, maintaining a modest weight loss also improves psychological functioning, mood, self-body image, and binge eating (18). In the "Keep It

Off" behavioral study, they identified several common determinants among individuals who managed to maintain their weight loss over a two-year period (19). These successful maintainers follow 60-90 minutes of daily physical activity, do regular weighing, follow a diet moderate in calories, have daily breakfast, also have social support, and do not let "lapses" turn into full "relapses" (17). Even all these common determinants are not enough to help more than 10-20 % of the ones trying to maintain their body weight reduced. Thus, more strategies are needed to get a higher success rate of weight loss maintenance.

There are different mechanisms contributing to weight relapse, such as increased hunger and reduced motivation in the long-term (20). A common reported side effect of dieting is increased hunger. As a result of caloric restriction, the increased hunger is most likely driven by an upregulation in the basal secretion of the orexigenic (appetite stimulating) hormone ghrelin observed after weight loss (21)(22).

Ghrelin, which also is known as the "hunger hormone", stimulates appetite, and is the only known appetite stimulating hormone (21). In contrast to ghrelin, there are several gut peptides that are associated with satiety (anorexigenic peptides), namely peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and CCK (23), that are released after a meal, and reduce appetite (24). Ghrelin and the satiety peptides are part of the homeostatic appetite control system that is responsible for the regulation of the energy needs of the body, and, to ultimately, maintain the energy balance (24).

There is another appetite control process known as the hedonic appetite system (or non-homeostatic process of eating) where "liking" and "wanting of food" (reward-based eating) play an important role in controlling the appetite of the individual due to its ability in overriding the homeostatic appetite regulation (eating beyond the physiological energy needs). The hedonic appetite system regulates food consumption via neuronal reward-based pathways, and encourages repeated consumption of palatable, salient foods (25)(26). The reward-based eating has the capability of overruling the homeostatic mechanisms of satiety and hunger and, thus, can lead to overeating, and, lastly, weight gain (27). Finding strategies that affect both the homeostatic and the hedonic appetite systems in a positive manner would be a helpful tool in the obesity management.

Lately, some research has been done regarding the potential effect of using probiotics on appetite to prevent relapse. A recent randomized control trial (RCT) found that, probiotics (e.g., *Lactobacillus rhamnosus* CGMCC1.3724) can have beneficial effects on appetite, mood, and on eating behavior (28), particularly in women. The supplementation of *Lactobacillus rhamnosus* showed significant benefits on fasting fullness, decreased food cravings as well as increased weight loss (28). However, few studies have looked at whether the gut microbiota and probiotics can have an impact on the maintenance of weight loss in individuals with obesity. In particular, more research needs to be done to assess if probiotics have an effect on appetite with regards to maintaining weight loss in the long-term.

1.2 Theoretical background

1.2.1 Appetite and weight loss maintenance

Humans eat in episodes, for example meals and snacks (29). With meals, humans usually eat until satiated (comfortably full) and then do not eat for a while when in satiety. After a meal, the drive to eat is generally low, and it builds up until the next eating episode (30). The food intake is regulated by two complementary drives, the homeostatic and the hedonic pathways. The homeostatic pathway controls the energy balance by the depletion of energy stores (25), and there are several hormones that play a significant role in the food regulation, namely ghrelin (orexigenic hormone), and the anorexigenic gut hormones, including PYY, GLP-1, and CCK.

The secretion of ghrelin in normal-weight individuals is increased during the fasting state and reduced during distension of the stomach (31)(32). Several studies have reported that ghrelin levels are negatively associated with BMI, whereas individuals with obesity have been shown to have low basal ghrelin levels, that seem to be related to insulin resistance and high BMI (33).

The stomach and small intestines secrete a number of satiety gut hormones in response to nutrients. This release of satiety factors from the gastrointestinal (GI) tract are influenced by the macronutrient composition of the meal (34)(35)(36)(37). PYY is a short peptide released from cells in the ileum and colon in response to food consumption. It is synthesized by the L cell in the distal gut (38). It is secreted following a meal where proteins exert a more potent effect than lipids and carbohydrates (17)(39). PYY works directly through the vagal afferent and arcuate nucleus (ARC) receptors (17). It has been shown to have an inhibitory effect on gastric motility and on neuropeptide Y (NPY) resulting in reduced appetite (40).

GLP-1 is a gut peptide derived from pre-proglucagon. It is produced in the body as a response to glucose ingestion (4). GLP-1 acts through the vagal brainstem, signaling pathway and centrally through hypothalamic actions. It reduces food intake in a dose-dependent manner (41).

CCK, which is also a peptide hormone of the GI system, is responsible for stimulating the digestion of protein and fat. It acts as a short-term satiation signal. It is produced in the proximal small intestine and is released, postprandially, in response to fatty acids, small peptides, and amino acids. It is primarily acting through vagal afferent fibers and is gradually reduced upon fasting (42)(20)(11). CCK plays an important role in secretion of pancreatic enzymes, gastric acid, and gastrointestinal motility (43). Individuals with obesity are found to be less sensitive to release of CCK. This might promote overeating and weight gain (44).

However, the human appetite is not only dependent on internal factors to meet physiological and homeostatic needs, it is also related to rewards. The reward-related drive to eat (the hedonic pathway) is thought to be mainly influenced by the corticolimbic pathways in the central nervous system (CNS) (45). The hedonic pathways, or reward-based regulation, is to a large extent determined by environmental and external factors, as "liking" and "wanting" of food, food cues, availability, and emotions (30)(46)(47). Hedonically driven motivation to eat has been associated with individuals with obesity, with

higher hedonic hunger feelings, as well as selective attention to food cues, and food cravings compared to normal weight individuals (48). In individuals with obesity, hedonic hunger is increased by enhanced "wanting" which reflects appetite, and enhanced "liking" for palatable foods (49). However, it is still unknown if the differences in hedonic hunger feelings seen in individuals with obesity compared to normal weight individuals are sustained after individuals with obesity have experienced a weight loss followed by a period of weight loss maintenance.

The most challenging part within obesity management is weight loss maintenance (14). The definition of weight loss maintenance used in the present study, is the recommendation from Wing and Hill that defines successful weight loss maintenance as the weight loss of equal to or greater than 10 % of the initial body weight that is maintained for at least a period of one year (14). This definition focuses on overall health, and even if individuals still are classified as having obesity after maintaining a weight loss of greater than 10 % of initial body weight, they still have an overall improved health. Moreover, the weight loss must also be intentional to be considered "successful" (14). Overall, only 10-20 % of individuals who experience a body weight loss of 10 % or more of initial body weight, are able to maintain it in long-term (15). There are several reasons to why individuals are unable to sustain their body weight loss in the long-term, and changes in appetite is one of the major challenges. Studies have shown that after a weight loss, appetite markers change (namely an increase in basal ghrelin) (50). Several studies have measured feelings of appetite using visual analogue scales, and demonstrated that a diet-induced weight loss is associated with increased hunger feelings in the fasted state as well (51)(52). However, it is still unknown if the changes in appetite that occur during weight loss are sustained with prolonged weight loss maintenance (53).

1.2.2 Gut microbiota and probiotics

Gut microbiota (GM) is involved in several mechanisms in humans and can be considered as our largest endocrine system (54). The GM is composed of trillions bacteria belonging to mainly two bacterial divisions: *Firmicutes* and *Bacteroidetes* (28). The *Firmicutes* accounts for about 90 % of the gut microbiota and include *Bacillus, Lactobacillus, Mycoplasma*, and *Clostridium.* The *Bacteroidetes* accounts for the remaining 10 %, and includes *Bacteriodes* which is the most abundant in humans (55)*.*

Gut microbiota has a function in metabolite production, vitamin production, immune system, and influences the epithelial homeostasis (56)(54). It plays an important role in storage, absorption, and in the energy flux obtained from dietary intake (40). Food intake and the macronutrient composition of the diet have an impact on the composition of the gut microbiota and is one of the reasons for inter-individual differences in microbiota (54). Several studies have been done, both in animals and humans, to try to understand how the gut microbiota work and how to improve it in order to bring health benefits to its host.

Compared to individuals with normal weight, individuals with obesity have alterations in the diversity and composition of gut microbiota (40). This disruption in the microbial composition is associated with fat storage and altered body weight (40). Although reports on the composition of the gut microbiota in individuals with obesity are not uniform, the reduced microbial diversity seems to be a recurrent finding (40). Studies have shown that dietary intake can modulate gut microbiota's function and structure (57). This has led to

question if probiotic supplementation can be used in prevention and treating obesity by altering the gut microbiota towards the one found in normal weight individuals.

Probiotics are defined as living microorganisms, bacteria, that can give health benefits to its host (58). Probiotics can alter and affect gut microbiota, which can potentially regulate body weight (59)(60). Furthermore. it is thought that probiotics can influence appetite (e.g., subjective feelings of appetite and appetite-related hormones), mood, and eating behavior traits (59)(61).

There are various types of products containing probiotics, such as dairy products, fermented products, and supplements. The most commonly used lactic acid bacteria that seems to have beneficial effects on human microbiota, belongs to *Lactobacilli* and *Bifidobacteria* (56)(62)(63). A study by Sanchez et al. (59) showed that supplementation of *Lactobacillus rhamnosus* displayed concordant changes in appetite, with a decrease in food cravings, increased fasting fullness, and less hunger and desire to eat, as well as increased weight loss (in females only) compared to placebo controls. Clinical studies have shown satiety-inducing effects of probiotics in normal weight individuals (64). It seems that probiotic supplementation have the ability to help individuals feel more full and decrease their desire to eat. Weight loss maintenance is a big challenge in obesity management, due to relapse and weight regain. Appetite plays a large role in why individuals have difficulties maintaining their weight loss. Finding ways to try to solve the problem of relapse is of importance. The potential effects on appetite control and appetite-related behaviors with probiotic supplementation that ultimately could contribute to body weight loss maintenance led us to investigate the effects of probiotic supplementation on appetite.

1.3 Aim and hypothesis

The aim of this study is to evaluate how the use of probiotics for weight loss maintenance affects appetite. The primary outcomes are the differences in subjective feelings of appetite, and the secondary outcomes are the changes in hedonic feelings of appetite and in appetite-related hormones, during the weight loss maintenance.

The hypothesis in this study is that participants receiving probiotics will experience less hunger and more protective changes in their appetite profile compared to the participants receiving placebo, during the weight loss maintenance.

2.0 Methods

2.1 Study design

This study is a randomized double-blinded controlled trial with repeated measurements. The participants were referred to this project after the completion of ASKED study, where participants underwent an 8-week low-energy diet (LED), followed by a 4-week refeeding- and weight stabilization phase. Following the completion of this weight stabilization phase (at week 13), these participants were randomized to take probiotic or placebo capsules twice a day for a period of 9 months. During the 9 months on placebo/probiotic capsules,

participants were asked to maintain their body weight, and were followed-up monthly by staff at the Hospital clinic to assist them with maintaining their weight. This study has been registered on clinicaltrials.gov (NCT02944253), approved by the Regional Ethics Committee in Norway (Ref. 2016/1297), and is conducted in accordance with the guidelines stated on the Helsinki Declaration. Participants anonymity were ensured throughout the study, and the consent form that all participants had to read and sign in order to participate in the study is attached in Appendix I.

2.2 Participants

Inclusion criteria defined in the ASKED study
Healthy volunteer adults (18-65 years old) both men and women, with class I or II obesity (30 kg/m^2 < BMI > 40 kg/m^2), weight stable (<2 kg variation in body weight within the last 3 months), and not currently dieting to lose weight where included in this study.

Exclusion criteria defined in the ASKED study
Participants who were pregnant, breast-feeding, dealing with drug or alcohol abuse within the last two years, taking medication known to affect appetite or induce weight loss, and enrolled in another obesity treatment program where excluded from the study. In addition to the criteria listed above, those with a history of psychological disorders, those who have 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, cardiovascular, rheumatoid arthritis, Crohn's disease, and malignancies were also not able to participate in this study. Moreover, consumption of probiotics over the last 6 months and use of antibiotics over the last 3 months of the study were also exclusion criteria.

2.3 Recruitment

Participants who have concluded ASKED study at week 13 were asked to participate in the current study. Those who agreed to participate in this study were randomized to consume probiotic capsules or placebo for a period of 9 months aimed at achieving weight loss maintenance from week 13 (W13) to 1 year (1YR). The study design is attached in Appendix II.

2.4 Randomization and blinding

Participants were randomized using a simple computer-based randomization (WEBCRF3 50/50) where participants were allocated to either placebo or probiotic weight loss maintenance interventions. As this study is double blinded, a St. Olavs Hospital staff member not affiliated with the study was responsible for assigning placebo or probiotics to the participants.

2.5 Detailed protocol

2.5.1 Weight loss maintenance interventions

At week 13, all participants received a dietary prescription aimed at weight loss maintenance (based on measurement of resting metabolic rate x physical activity level (Appendix III)), and were randomized into two groups, to get either placebo or probiotic capsules. The participants were instructed to take one capsule twice a day with a meal, and were asked to maintain a stable body weight (weight loss ≥ 10 % of initial body weight sustained for one year (14)).

At each follow-up, the nurses measured the body weight of the participants. They were given a monthly prescription of either placebo or probiotics capsules. With the first prescription of placebo/probiotics, each participant received 66 capsules. During the consecutive follow-up appointments, the participants received 62 capsules. The participants were asked to bring back/report how many capsules they had left, and to report any side effects or issues with adherence associated with capsule intake.

The probiotic capsules contained a formula consisting of seven strains, including *Lactobacillus acidophilus, Lactobacillus rhamnosus* W71, *Lactobacillus plantarum* W21, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Bifidiobacterium bifidum* W23, and *Lactococcus lactis* W58. The probiotics capsules were manufactured by NYCOPRO (Takeda AS) and are commercially available as "NYCOPRO Ferie". The concentration of probiotics in each capsule were $2,5*10^9$ CFU (colony-forming unit) with an advised daily dose of two capsules a day ($5*10^9$ CFU/day).

The placebo capsules were created using Capsugel Coni-Snap transparent size zero, and were made up of 100 % bovine gelatin (microcrystalline celluloses). The capsules were approved by European pharmacopeia (Ph.Eur). The placebo capsules were a NYCOPRO placebo, manufactured by Kragerø Tablettproduksjon AS. The total weight of the placebo capsule was 0.3 grams.

2.5.2 Data collection

Participants came in one week prior to the test day to pick up a food diary (Appendix IV) to log their food consumption, and an accelerometer to monitor physical activity (Appendix V) during the week of test day.

The participants were assessed at week 13 (W13) and 1 year (1YR) time points. They were scheduled to meet between 7.30 am and 8.00 am after an overnight fast (at least 10 hours). They were asked not to do any moderate to high intensity activities and to not consume any alcohol, caffeine, or nicotine. Only the consumption of water was allowed, after 8.00 pm the night before the test day. They were asked to bring a stool sample, the physical activity monitor, and to take a urine sample upon arrival.

2.5.3 Body weight and body composition

Body weight and body composition were measured using air-displacement plethysmography (Bod Pod, COSMED, Rome, Italy) (65). The measurements were done after an overnight fast and were done immediately upon the participants arrival.

Each morning before participants came in to testing, the Bod Pod was calibrated. The equipment was calibrated using a 5-step calibration. The participants were asked to remove all metals and jewelry, to only wear close-to-body underwear, and a Lycra swim cap to cover all of their hair. The participants were then asked to be quiet, sit as still as possible, and try to relax during the test. Two repeated measurements were performed for each participant to verify consistency. If the measurements were not consistent, a third measurement was performed.

2.5.4 Resting metabolic rate

The resting metabolic rate (RMR) of the participants was measured. The equipment used to measure RMR was indirect calorimetry (Vmax Encore 29N, Care Fusion, Germany). The equipment was calibrated every morning before participants came in for their test day. Flow sensor calibration was done using a 3 l pump. Acceptation of the calibration was valid when the results were within ± 3 % of 3 l.

Before performing the RMR test, participants were asked to sit still for at least 10 minutes. They were asked to lay down on a hospital bed and asked to relax, and breathe as normal as possible without falling asleep. Talking or disruptions were not allowed. A canopy was placed around the head of the participants. The canopy captured oxygen uptake (VO_2) and carbon dioxide production (VCO_2).

The measurement was done continuously for at least 15 minutes or longer. The first five minutes of the test was used for stabilization and adjusting of the equipment, and was always removed from the test data before calculation of RMR. To complete the test, a measurement of a minimum of ten minutes of steady state data was needed. RMR was determined by calculating the average of the measurement taken in steady state (66).

2.5.5 Physical activity

Throughout the study, participants were asked to maintain their physical activity (PA) levels. The PA levels were assessed by asking participants to wear physical activity monitors (BodyMedia, SenseWear, Pittsburgh, PA, USA) for a 7-day period in advance of the test day. Data from the monitors were considered valid and included in the results, when participants had worn the devices for three or more complete days, including at least one weekend day, and they had to be worn for more than 95 % (22.8 hours per day) of the time (Appendix V) (67).

2.5.6 Subjective feelings of appetite

Subjective feelings of appetite were measured using an electronic 100-mm visual analogue scale (VAS)(Appendix VI). This is a validated method for measuring appetite (68). VAS was used to measure feelings of hunger, fullness, desire to eat (DTE), and prospective food consumption (PFC). Measurements of VAS were collected in fasting, immediately after a standardized breakfast (15 minutes after participants started to consume food), and then every 30 minutes after the meal for a duration up to 2.5 hours. The questions assessed using VAS are shown in Table 1.

Questions assessed using 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

Table 1: Questions assessing subjective feelings of appetite (hunger as an example).

The standardized breakfast contained ~600 kcal, of which 49 % of energy from carbohydrates, 35 % from fat, and 16 % from protein. The meal consisted of oatbread, butter, cheese, strawberry jam, orange juice, and a choice of either milk or cherry-yoghurt. Macronutrient composition of the breakfast meal can be seen in Table 6, Appendix VII.

2.5.7 Food hedonics

The participants completed a computer-based food hedonic assessment in fasting and immediately after breakfast. The questionnaire used was the Leeds Food Preference Questionnaire (LFPQ) from University of Leeds, UK. The questionnaire provides measures of different components of food preference and food reward (Appendix VIII). Participants chose what they would prefer to consume at the time the questionnaire was administered when looking at pictures of food, and to which extent they like each food, and their responses were recorded and used to compute mean scores of the outcome measures for the four food categories presented below (69):
- High fat and sweet
- Low fat and sweet
- High fat and non-sweet
- Low fat and non-sweet

From this, the outcome measures explicit liking and explicit wanting were calculated.

2.5.8 Appetite-related hormones

Blood samples for the analysis of appetite-related hormones (active ghrelin, total GLP-1, total PYY, and CCK) were collected in fasting and every 30 minutes up to 150 minutes, after a standardized breakfast meal (Table 6, Appendix VII).

A St. Olavs nurse inserted the venous catheter and collected the blood samples in fasting, and the master students had the responsibility to draw blood at all other time points. The nurses were available to help if blood clots or other issues with the blood drawing were to happen.

Blood was collected in 3x 4 ml EDTA tubes at each time point (0, 30, 60, 90, 120, and 150 minutes). A premixed 20 µl inhibitor (consisting of 10 µl of DPP-IV (Merck Millipore, Germany) and 10 µl of Pefabloc (Roche Diagnostic, Germany) was added immediately to a 1 ml whole blood vial. Blood samples were centrifuged (1000 G for 10 min at 18°C), and plasma was then stored at -80°C. Plasma samples were analyzed for active ghrelin, total GLP-1, and total PYY in duplicate with Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Merck Millipore, USA). CCK was analyzed using an "in-house" RIA method (70). The intra-assay coefficient of variation (CV) was <10 %, and the inter-assay CV was 20 %.

2.5.9 Weight maintenance and weight regain

The method for calculating body weight (BW)- maintenance and regain were done by a different master student that I collaborated with during the collection and plotting of the data. The body weight regain was calculated as a percentage of participants initial weight loss (from baseline (BL) to week 13 in the ASKED study):

$$\text{Weight regain:} \frac{BW(kg)\ 1Y - BW(kg)\ W13}{BW(kg)\ W13 - BW(kg)\ BL} * 100$$

The equation is a measure of successful weight loss maintenance, using the previously mentioned Wing and Hill definition of what to consider as a successful weight loss maintenance (14).

2.6 Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 26 (SPSS In., Chicago IL). Statistical significance was set at $P<0.05$. Only participants that completed the study (i.e., with data at 1YR) were used in the final analysis (called completers).

The data is presented as estimated marginal means ± standard error of the mean (SEM) and mean ± standard error of the mean (SEM) for the baseline (W13) characteristics of the participants. The Shapiro test and assessment of normal Q-Q plots were used to test for normality. The Mann-Whitney U-test was used when the data was not normally distributed.

Repeated measurements were analyzed using linear mixed-effects models (LMM), with restricted maximum-likelihood estimation, including fixed effects for time, group, and their interaction. Bonferroni correction was used for post hoc pairwise comparisons.

All the analysis was done for completers, except an Independent t-test or Mann Whitney U-test performed on completers and dropouts to check for significant differences between them.

Total area under the curve (tAUC) to quantify the total postprandial rise in subjective feelings of appetite and hormones was calculated from 0 to 150 minutes using the trapezoidal rule. Positive incremental area under the curve (iAUC) was calculated as tAUC-fasting value x time.

Power calculation of a total sample size of 54 participants will be needed to detect a difference in appetite in this study, calculated with power of 80 %, and significance level set at P<0.05.

3.0 Results

3.1 Study population

Eighty-three participants completed the W13 test day. Eight of these participants did not continue into the current study. A total of twenty-eight out of seventy-six participants, or 36.8 % of the participants, did not complete this study. The reasons for dropouts are shown in the Figure 1, and includes compliance with the capsules, use of other supplements containing probiotics, use of antibiotics, participants wanting to lose more weight, lost to follow-up, and unknown reasons. Fifty-five participants completed the intervention and were included in the final analysis (completers).
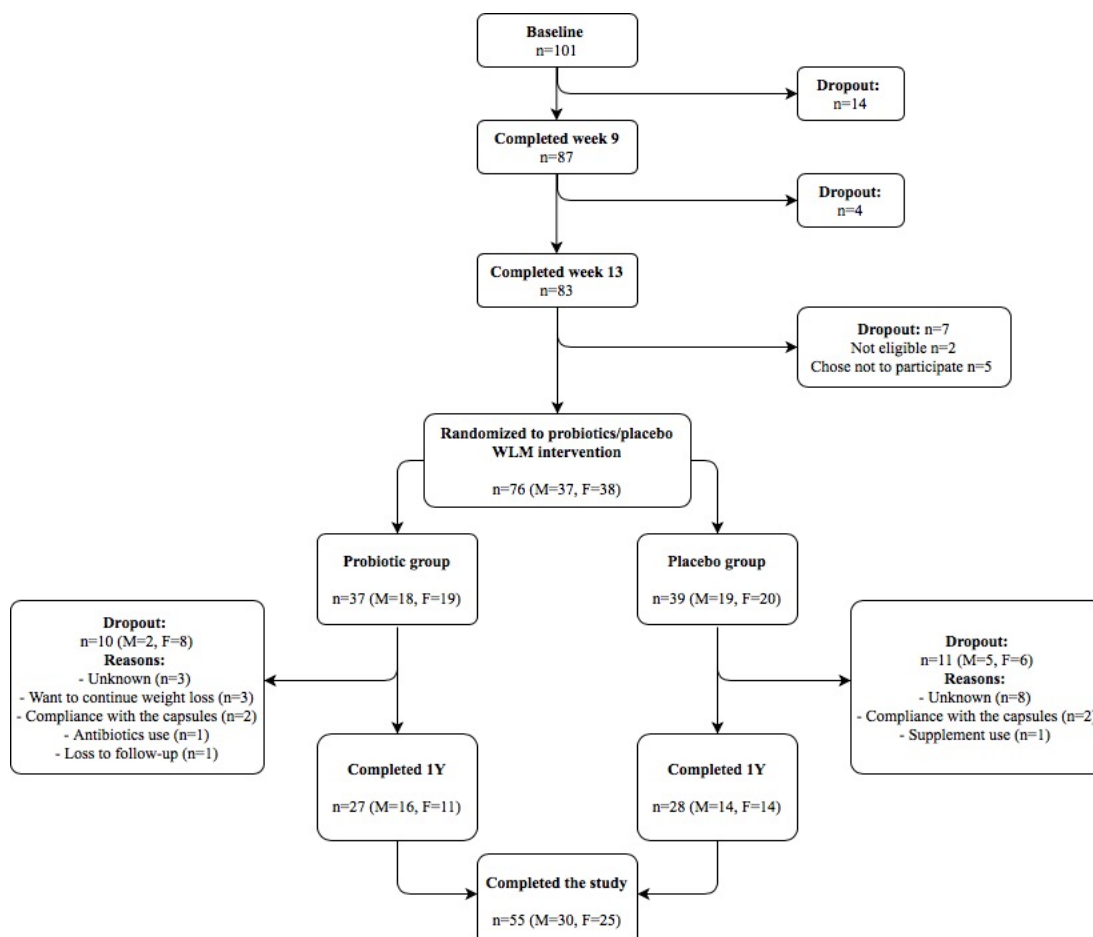

Figure 1: Flowchart showing completers, dropouts, and placebo/probiotic groups. n; number of participants. M; males. F; females.

Baseline characteristics of the completers are shown in Table 2.

	Week 13		
Group	Placebo (n=28)	Probiotic (n=27)	Participants together (n=55)
Gender M/F (%)	14/14 (50.0/50.0)	16/11 (59.3/40.3)	30/25 (54.5/45.5)
Age (years)	44.4±9.4	48.1±8.9	46.6±9.3
Height (cm)	175.2±8.7	174.8±9.2	175.0±8.9
BW (kg)	91.2±12.9	90.8±11.4	91.0±12.1
BMI (kg/m^2)	29.8±3.2	29.8±2.9	29.8±3.0
FM (%)	34.0±8.5	33.2±6.8	33.6±7.7
FM (kg)	31.1±9.1	29.9±6.7	30.5±8.0
FFM (%)	66.0±8.5	66.8±6.8	66.4±7.7
FFM (kg)	60.2±11.2	60.2±9.6	60.2±10.3
RMR (kcal/day)	1500±266	1497±213	1499±239
TEE (kcal/day)	2692±524	2720±515	2705±515
PA (minutes/day)	66.2±46.8	70.3±54.2	68.1±49.9
Energy intake (kcal/day)	1704±371	1749±314	1725±342

Data presented as mean ±SD. M, Males; F, Females; BW; body weight. BMI; body mass index. FM; fat mass. FFM; fat free mass. RMR; resting metabolic rate. TEE; total energy expenditure. PA; physical activity.

Table 2: Baseline characteristics in completers only. Differences between groups (probiotic and placebo) at week 13.

At week 13, the participants who completed the study (n=55) were on average 46.6±9.3 years old and had a mean BMI of 29.8±3.0 kg/m². There were no significant differences in any of the characteristics analyzed between those who dropped out and the participants that completed the study. Further, there were no significant differences in any of the characteristics studied between the placebo and probiotic groups at week 13.

At one year, both the placebo and probiotic groups had significant mean changes in body weight (kg) over time (1YR-W13). The placebo group had an increase in BW from W13 to 1YR of 3.1±1 kg (P=0.017), and the probiotic group had an increase of 7.6±1.5 kg (P<0.001) from W13 to 1YR. There was a significant mean difference between groups in body weight change of 4.5±1.5 kg. (P=0.016)

The results for body weight regain and body weight maintenance were performed by a different master student that I collaborated with during the collection and plotting of the data. The student have consented to the use of her results in the present study.

There was a significant mean difference in weight regain of the participants´ initial weight loss (from baseline to week 13 in the ASKED study) between groups of 35.9±10.5 % (P=0.001) at 1YR. Participants in the placebo group had a mean weight regain of 19.0±7.6 % of their initial weight loss (W13-BL), while the participants in the probiotic had a mean weight regain of 54.9±7.2 % of their initial weight loss (W13-BL).

The number of participants that succeeded with weight loss (WL) maintenance (WL of ≥10 % of initial BW at one year), was seventeen out of twenty-eight participants in the placebo group, which equals 60.7 %. While in the probiotic group, there were five out of twenty-seven participants who successfully maintained their body weight, which equals a percentage of 18.5 %.

3.2 Subjective feelings of appetite

The average values in subjective feelings of appetite at week 13, at one year, and the changes over time (1YR-W13) are shown in Table 3. The differences over time and within groups for all subjective feelings of appetite variables (hunger, fullness, desire to eat, and prospective food consumption) were not significant.

	Week 13		1 year		Δ 1 year-week13	
Group	Placebo (n=28)	Probiotic (n=27)	Placebo (n=28)	Probiotic (n=27)	Placebo (n=28)	Probiotic (n=27)
Fasting hunger (mm)	31.1±4.9	30.4±5.0	30.1±4.9	38.8±5.0	-0.9±5.7	8.4±5.9
iAUC hunger (mm)	6885.5±679.5	6730.5±692.0	6497.1±679.5	6966.1±692.0	-388.4±899.0	235.5±915.0
iAUC fullness (mm)	10905.5±770.0	11060.1±770.4	11358.9±756.5	10764.7±770.5	453.4±918.5	-295.4±978.3
iAUC DTE (mm)	8298.8±803.0	9215.8±817.8	7611.9±803.0	8588.6±817.8	-686.8±1044.4	-627.2±1063.6
iAUC PFC (mm)	8397.8±735.2	8411.1±748.7	9202.8±735.2	9321.1±748.7	805.0±829.2	910.0±844.4

Data presented as mean±SEM. iAUC; incremental area under the curve. DTE; desire to eat. PFC; prospective food consumption.

Table 3: Subjective feelings of appetite for both groups (probiotic and placebo) at week 13 and 1 year, changes over time (1 year-week 13) within and between groups.
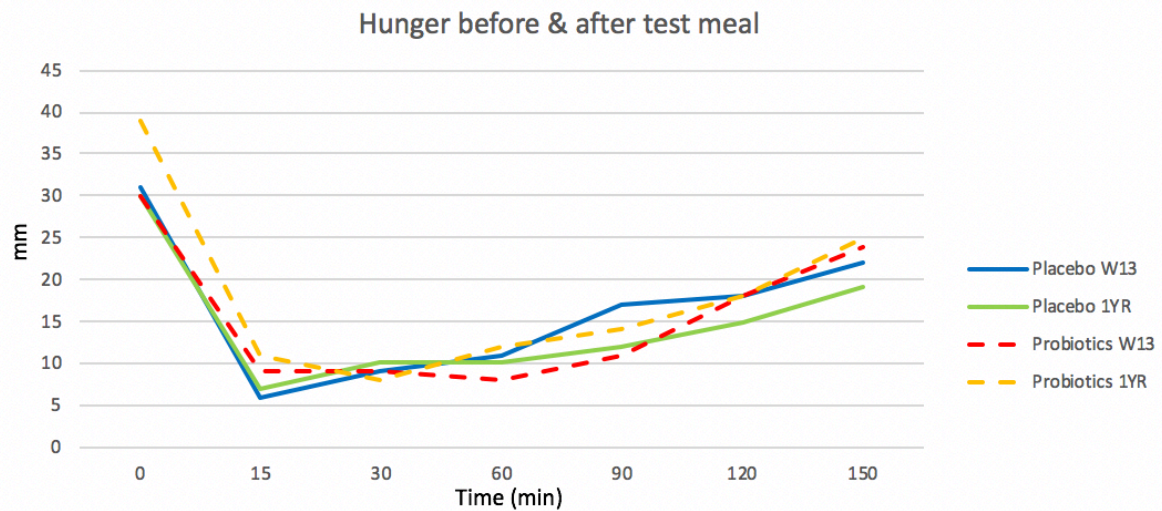
Figure 2: Mean changes in hunger feelings over time (from W13 to 1YR) between groups.

Figure 2 shows the progression in feelings of hunger measured in fasting (0 minutes), immediately after the consumption of the test meal (15 minutes), and every 30 minutes for a period of 2.5 hours (150 minutes) between groups at week 13 and at one year. Both probiotic and placebo groups followed a similar pattern where immediately after the food consumption of the test meal, there was a decrease in feelings of hunger with gradual increases over the 2.5-hour period. Despite the increases over time (over the 2.5-hour period), hunger levels did not return to levels reported in fasting. There was a non-significant decrease in the placebo group and a non-significant increase in the probiotic group for changes in fasting huger over time (1YR-W13) (placebo P=0.873, probiotic P=0.160).

Moreover, the placebo group experienced non-significant decrease in iAUC hunger over time (1YR-W13), while the probiotic experienced a non-significant increase in iAUC hunger over time (1YR-W13). Despite that (the groups went in opposite directions), there were no significant differences between groups.
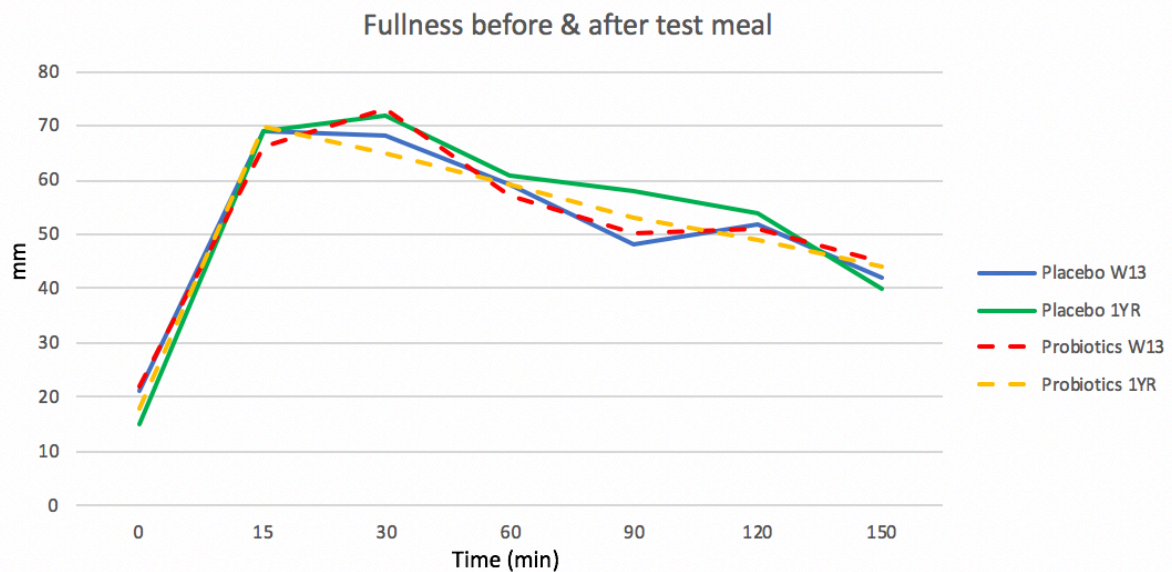

Figure 3: Mean changes in fullness over time (from W13 to 1YR) between groups.

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Like Figure 2, Figure 3 shows the progression in feelings of fullness. Both of the groups followed a similar pattern where they in fasting had low levels of fullness, and immediately after the consumption of the test meal, there was an increase in feelings of fullness. Between 15 and 30 minutes after the test meal, the feelings of fullness gradually decreased over the 2.5 hour period. Despite the decrease over time (over the 2.5-hour period), fullness levels did not return to levels reported in fasting.

In the placebo group, a non-significant increase (P=0.490) in iAUC fullness over time (1YR-W13) was observed, whereas, the probiotic group had a non-significant decrease (P=0.656) in iAUC fullness. Despite the opposite direction on iAUC fullness over time (1YR-W13), there were no significant differences between groups (P=0.424)
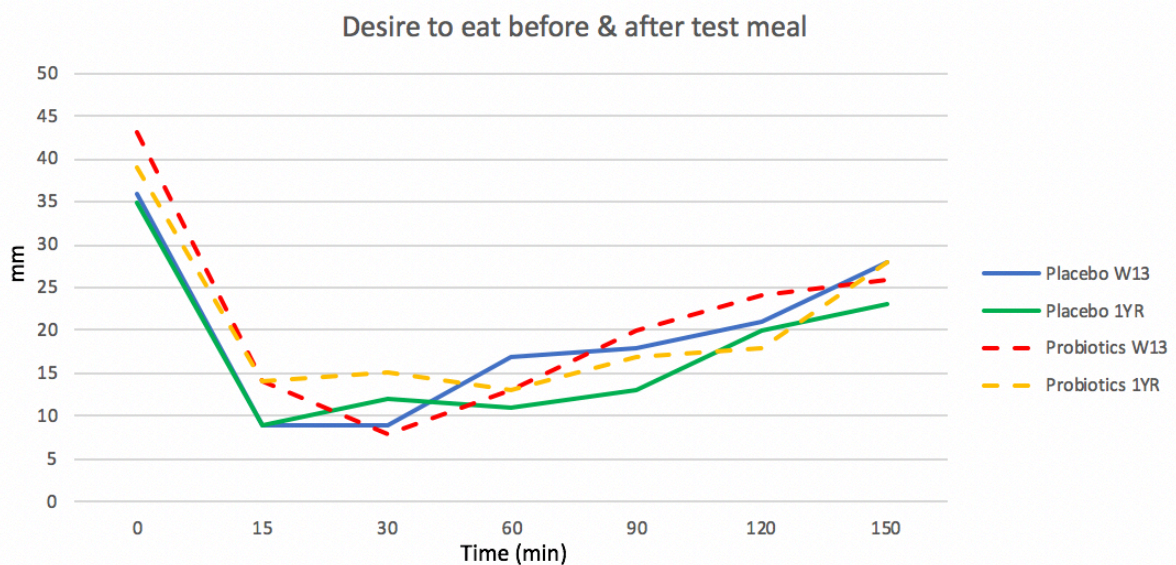
Figure 4: Mean changes in desire to eat over time (from W13 to 1YR) between groups.

Similar to hunger, the progression in desire to eat, shown in Figure 4, shows a pattern where immediately after the consumption of the test meal, there was a decrease in desire to eat, with gradual increases over the 2-5 hour period. Similarly, to hunger, the desire to eat levels did not return to levels reported in fasting.

Both in the placebo and the probiotic groups, there was a non-significant decrease over time (1YR-W13) in iAUC desire to eat. The placebo group had a greater decrease in iAUC desire to eat compared to the probiotic group, but without significant level (P=0.464). Moreover, there were no significant differences over time (1YR-W13) within any of the groups (P=0.581 and P=0.616, respectively placebo and probiotic groups).
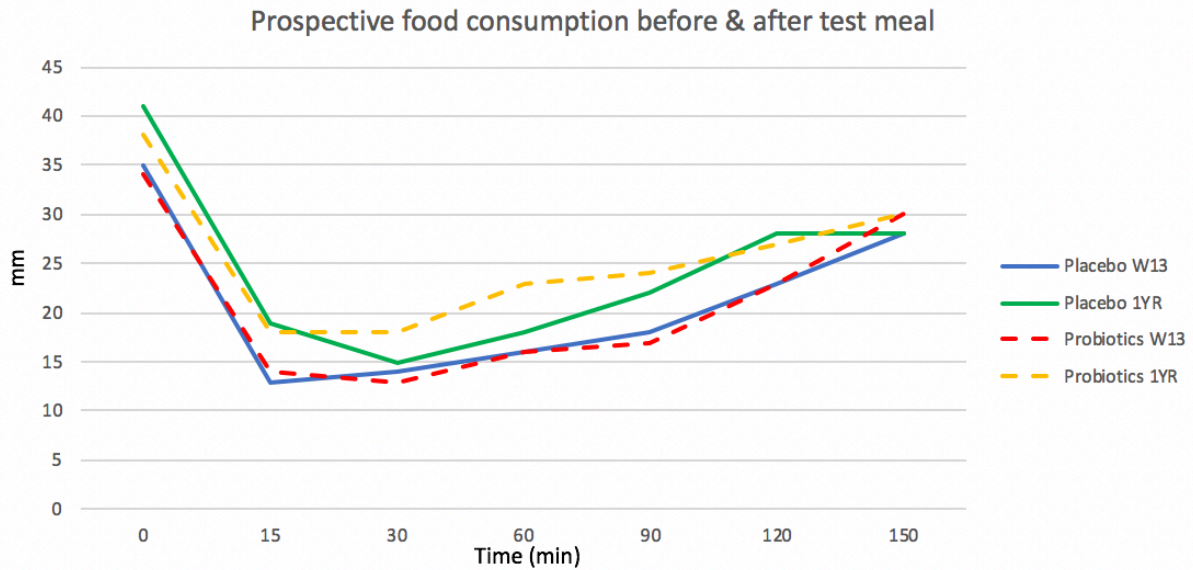
Figure 5: Mean changes in prospective food consumption over time (from W13 to 1YR) between groups.

Figure 5 shows the progression in feelings of prospective food consumption. Similar to both hunger and desire to eat, the progression of feelings of prospective food consumption decreased immediately after consumption of the test meal, and gradually increased over the 2.5 hour period. Despite the increases over time (over the 2.5-hour period), the prospective food consumption levels did not return to levels reported in fasting.

Both the placebo and the probiotic group experienced non-significant increases in iAUC prospective food consumption over time (1YR-W13). Tough it appears that the probiotic group had a greater increase in feelings of prospective food consumption at one year compared to week 13, this change over time was not significantly different between groups (P=0.273), neither the changes over time (1YR-W13) in this group (P=0.408). Similarly, no significant change in iAUC prospective food consumption over time (1YR-W13) was found in the placebo group (P=0.775).

3.3 Food hedonics

Table 4 shows mean pre- and post- meal values for placebo and probiotic groups at week 13 and one year, and the changes over time (1YR-W13) in food hedonic assessments.

Group	Placebo						Probiotic						Diff. groups			
	Week 13		1 year		Δ 1 year-week13		Week 13		1 year		Δ 1 year-week13		Week 13		1 year	
Food hedonics	Pre-meal	Post-meal	Pre-meal	Post-meal	Pre-meal	Post-meal	Pre-meal	Post-meal	Pre-meal	Post-meal	Pre-meal	Post-meal	Pre-meal	Post-meal	Pre-meal	Post-meal
Explicit liking HFS	45.3±4.0	22.3±4.0	45.7±4.8	21.3±4.1	0.4±3.7	-1.0±3.7	30.9±4.8	17.6±4.1	34.3±4.0	17.6±4.1	3.4±3.8	0.0±3.8	14.4±5.7*	4.7±5.9	11.4±5.9	3.7±5.7
Explicit liking LFS	52.2±3.7	21.3±3.8	43.6±3.8	16.0±3.7	-8.6±4.2	-5.3±4.2	41.4±3.8	14.7±3.8	41.5±3.8	16.7±3.8	0.1±4.2	2.1±4.2	10.7±5.3	6.7±5.4	2.1±5.4	-0.7±5.3
Explicit liking HFNS	33.2±4.2	17.3±4.2	30.4±4.2	17.8±4.2	-2.9±3.8	0.5±3.8	21.5±4.3	16.4±4.3	25.6±4.3	17.7±4.3	4.2±3.8	1.3±3.8	11.8±6.0	0.9±6.1	4.8±6.2	0.1±6.0
Explicit liking LFNS	41.7±3.6	24.0±3.6	39.7±3.6	21.2±3.6	-2.0±3.8	-2.8±3.8	37.8±3.6	20.2±3.6	40.8±3.6	23.9±3.6	2.9±3.8	3.7±3.8	3.9±5.1	3.8±5.1	-1.1±5.1	-2.7±5.1
Explicit wanting HFS	42.7±4.0	21.0±4.0	43.2±4.0	20.0±4.0	0.5±3.9	-1.0±3.9	31.2±4.0	16.4±4.0	33.4±4.0	17.0±4.0	2.2±3.9	0.6±3.9	11.5±5.6*	4.6±5.7	9.8±5.7	3.0±5.6
Explicit wanting LFS	46.8±3.9	22.0±4.0	42.1±4.0	16.0±4.0	-4.8±4.2	-6.0±4.2	41.5±4.0	15.8±4.0	40.4±4.0	16.4±4.0	-1.1±4.3	0.7±4.3	5.3±5.6	6.3±5.6	1.7±5.6	-0.4±5.6
Explicit wanting HFNS	30.8±4.2	16.5±4.2	29.1±4.2	17.0±4.2	-1.7±4.0	0.5±4.0	22.2±4.3	16.1±4.3	25.1±4.3	16.1±4.3	2.9±4.0	0.0±4.0	8.6±6.0	0.4±6.0	4.0±6.0	0.9±6.0
Explicit wanting LFNS	40.1±3.5	22.1±3.6	38.3±3.6	19.7±3.5	-2.4±3.8	-2.4±3.8	36.4±3.6	18.5±3.6	41.1±3.6	22.2±3.6	4.7±3.8	3.8±3.8	4.4±5.0	3.6±5.1	-2.7±5.1	-2.5±5.0

*Data presented as mean±SEM.; *P≤0.05*
HFS; high fat and sweet. LFS; low fat and sweet. HFNS; high fat non-sweet. LFNS; low fat non-sweet.

Table 4: Food hedonics for both groups (probiotic and placebo) at week 13 and 1 year, changes over time (1 year-week 13) within and between groups.

3.3.1 Explicit liking

For the explicit liking of high fat and sweet foods, there was a significant mean difference between groups in pre-meal assessment at week 13 of 14.4±5.7 mm (P=0.013) with the placebo group having higher levels of explicit liking for high fat and sweet foods than the probiotic group (placebo: 45.3±4.0 mm, probiotic: 30.9±4.8 mm). At one year, there was a tendency towards a higher value in the pre-meal assessment for explicit liking in the placebo group, with a mean difference of 11.4±5.9 mm (P=0.050). However, no significant differences were found between or within groups in the post-meal at week 13 and one year, or in the changes over time (1YR-W13) in both pre-meal and post-meal assessments between groups in their explicit liking of high fat and sweet foods.

Regarding the explicit liking of low fat and sweet foods, no significant differences within groups were found either in pre-meal or post-meal assessments for the placebo or probiotic group at any time points (W13 or 1YR), nor significant differences were found between or within groups in the changes over time (1YR-W13).

Looking at the explicit liking of high fat and non-sweet foods, there was a tendency towards a higher value in the pre-meal assessment for explicit liking in the placebo group when compared with probiotic group, with a mean difference of 11.8±6.0 mm (P=0.054), but no significant differences in the post-meal assessment between groups, at week 13. However, there were no significant differences between groups in both pre-meal and post-meal assessments at one year. Moreover, no significant differences were found between or within groups in the changes over time (1YR-W13), in both pre-meal and post-meal assessments, in their explicit liking for high fat and non-sweet foods.

For the explicit liking of low fat and non-sweet foods, no significant differences were found in both pre-meal and post-meal assessments, at any time points (W13 or 1YR), between groups, nor significant differences were found between or within groups in the changes over time (1YR-W13).

3.3.2 Explicit wanting

Similar to the explicit liking for high fat and sweet foods, a significant mean difference of 11.5±5.6 mm (P=0.044) was found in the explicit wanting for high fat and sweet foods between groups in pre-meal assessment, at week 13. The placebo group had a higher explicit wanting for high fat and sweet foods in pre-meal assessment compared to the probiotic group at week 13 (placebo: 42.7±4.0 mm, probiotic: 31.2±4.0 mm). However, no significant differences were found between groups in the post-meal assessment at week 13. At one year, there were no significant differences between groups in both pre-meal and post-meal assessments, nor significant differences within groups when compared with week 13 (pre and post-meal assessments). Moreover, no significant differences were found between groups in the changes over time (1YR-W13), in both pre-meal and post-meal assessments, in their explicit wanting for high fat and sweet foods.

For the explicit wanting of low fat and sweet foods, no significant differences between or within groups were found in both pre-meal or post-meal assessments, at any time points

(W13 or 1YR), nor significant differences were found between or within groups in the changes over time (1YR-W13).

Regarding the explicit wanting of high fat and non-sweet foods and the explicit wanting of low fat and non-sweet foods, no significant differences between or within groups were found in both pre-meal or post-meal assessments, at any time points (W13 or 1YR), nor significant differences were found between or within groups in the changes over time (1YR-W13).

3.4 Appetite-related hormones

Table 5 shows mean values of plasma concentration of appetite-related hormones for placebo and probiotic groups at W13 and 1YR, and changes over time (1YR-W13).

Variable	Week 13		1 year		Δ 1 year-week13		Diff. group (sig. level)
	Placebo (n=28)	Probiotic (n=27)	Placebo (n=28)	Probiotic (n=27)	Placebo (n=28)	Probiotic (n=27)	
Basal active ghrelin (pg/mL)	305.2±82.7	510.2±82.7	86.2±77.6	83.2±77.6	-218.9±110 *	-427.0±109.9 **	0.227
iAUC active ghrelin (pg/mL)	64149.3±4995.1	69459.2±4791.3	19602.3±4611.3	18218.7±4699.7	-44547.0±6411.6 *	-51240.5±6608.4 *	0.512
Basal total GLP-1 (pg/mL)	119.0±8.5	113.8±8.4	52.5±8.2	57.9±8.1	-66.5±9.4 **	-55.9±9.4 **	0.993
iAUC total GLP-1 (pg/mL)	43844.1±1633.9	42666.6±1633.9	21229.4±1633.9	21855.8±1618.7	-22614.7±1211.9 **	-20810.8±1189.9 **	0.929
Basal total PYY (pg/mL)	85.2±12.7	110.4±12.9	31.0±13.2	42.2±12.7	-54.2±10.7 **	-68.2±10.5 **	0.281
iAUC total PYY (pg/mL)	26740.9±546.2	28865.5±564.7	9751.7±564.7	9268.0±564.2	-16989.2±591.7 **	-19597.5±591.7 **	0.753
Basal CCK (pg/mL)	0.7±0.2	0.8±0.2	0.9±0.2	0.7±0.2	0.2±0.2	-0.1±0.2	0.719
iAUC CCK (pg/mL)	253.2±38.2	260.9±38.9	199.8±38.2	286.8±38.9	-53.4±45.9	25.8±46.8	0.282

*Data presented as mean±SEM. iAUC; incremental area under the curve. n, number;*P≤0.01 **P≤0.001*

Table 5: Plasma concentration of appetite related hormones for both groups (probiotic and placebo) at week 13 and 1 year, changes over time (1 year-week 13) within and between groups.

There were a significant decrease over time in both groups for all appetite-related hormones in both basal and incremental area under the curve, with the exceptions of iAUC total GLP-1 in the probiotic group, and basal and iAUC CCK that did not change significantly in both groups. Moreover, no significant differences between the placebo and probiotic groups were found for any of the appetite-related hormones analyzed at week 13, at one year, nor significant differences were found between or within groups in the changes over time (1YR-W13).
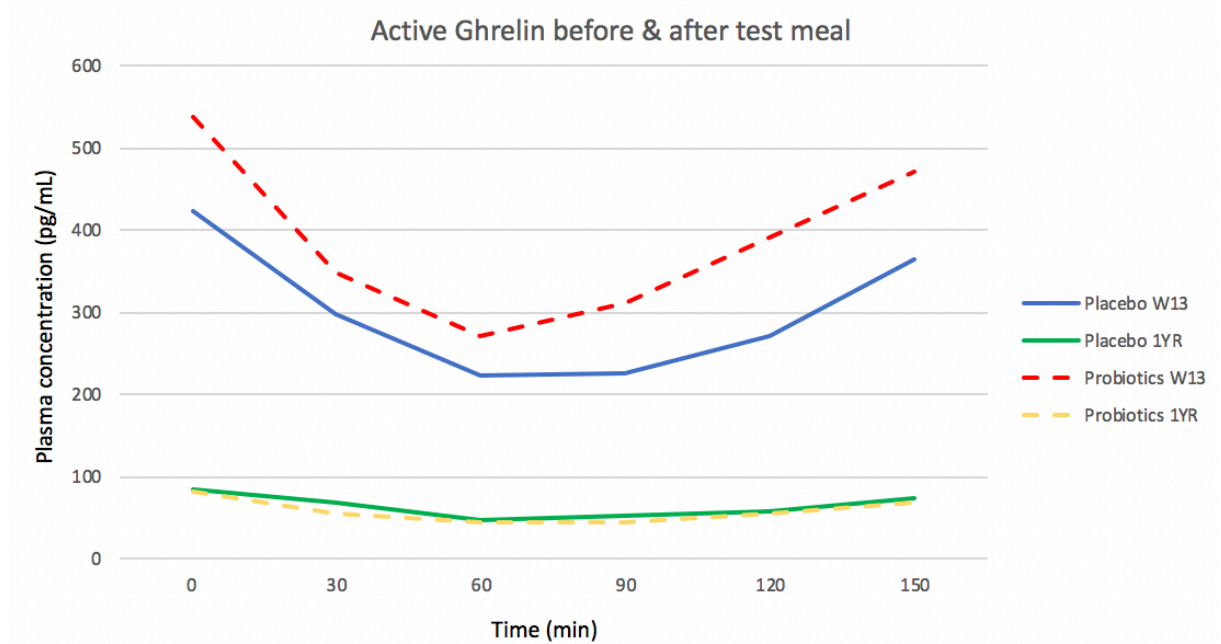
Figure 6: Mean changes in plasma concentration of active ghrelin over time (from W13 to 1YR) between groups.

Figure 6 shows the progression in active ghrelin measured in fasting (0 minutes), and every 30 minutes for a period of 2.5 hours (150 minutes), between groups at week 13 and one year. Both the placebo and probiotic groups followed a similar pattern. At week 13, both groups had a similar pattern where immediately after consumption of the test meal and up to 60 minutes, there was a decrease in active ghrelin plasma concentrations. After 60 minutes of the test meal, both groups gradually increase active ghrelin over the 2.5-hour period. Despite the increases over time (over the 2.5-hour period), active ghrelin levels did not return to levels reported in fasting.

Interestingly, both groups followed a completely different pattern at one year when compared with week 13, where both placebo and probiotic groups had stable low values of active ghrelin from fasting and up to 2.5 hours after the consumption of the test meal. In fasting, there was a significant decrease from week 13 to one year in basal ghrelin in both groups. The placebo group had a decrease of -218.9±110 (pg/mL) (P=0.005), whereas the probiotic group had a decrease of -427.0±109.9 (pg/mL) (P≤0.001). Moreover, the decrease in iAUC from week 13 to one year was significant within both placebo and probiotic groups. The mean difference of the decrease in iAUC active ghrelin was -44547.0±6411.6 (pg/mL) (P=0.007) for the placebo group, while in the probiotic group, the mean difference of the decrease in iAUC active ghrelin was -51240.5±6608.4 (pg/mL) (P=0.002).

Figure 7: Mean changes in plasma concentration of total GLP-1 over time (from W13 to 1YR) between groups.

Figure 7 shows the progression of total GLP-1 measured over time (over the 2.5-hour period), for both groups at week 13 and one year. The placebo group followed the same pattern at week 13 and at one year, where immediately after the consumption of the test meal, there was an increase in total GLP-1, with the highest concentration seen at 30 minutes after the test meal. At 60 minutes after the test meal, the concentration of total GLP-1 decreased and continued stable up to 2.5 hours. In the probiotic group, the increase in total GLP-1 was observed from 0 to 30 minutes, but from 30 minutes up to 2.5 hours, the observed pattern of the plasma concentration of total GLP-1 kept rising at week 13, but was more stable up to 2.5 hours at one year. In basal GLP-1 plasma concentrations, there was a significant decrease in total GLP-1 concentration within groups from week 13 to one year, with a mean difference of -66.5±9.4 (pg/mL) (P≤0.001) for the placebo group and -55.9±9.4 (pg/mL) (P≤0.001) for the probiotic group. However, a significant decrease over time (1YR-W13) in iAUC of total GLP-1 was observed in the placebo group only, with a mean difference of -22614.7±1211.9 (pg/mL) (P≤0.001).

Figure 8: Mean changes in plasma concentration of total PYY over time (from W13 to 1YR) between groups.

Figure 8 shows the progression of total PYY measured over time (over the 2.5-hour period), for both groups at week 13 and one year. The basal concentrations of total PYY decreased significantly from week 13 to one year in both groups, with a mean difference of -54.2±10.7 (pg/mL) (P≤0.001) in the placebo group, and with a mean difference of -68.2±10.5 (pg/mL) (P≤0.001) in the probiotic group. Moreover, there was also a significant decrease over time (1YR-W13) in the iAUC concentrations of total PYY in both groups, with a mean difference of -16989.2±591.7 (pg/mL) (P≤0.001) in the placebo group, and a mean difference of -19597.5±591.7 (pg/mL) (P≤0.001) in the probiotic group.
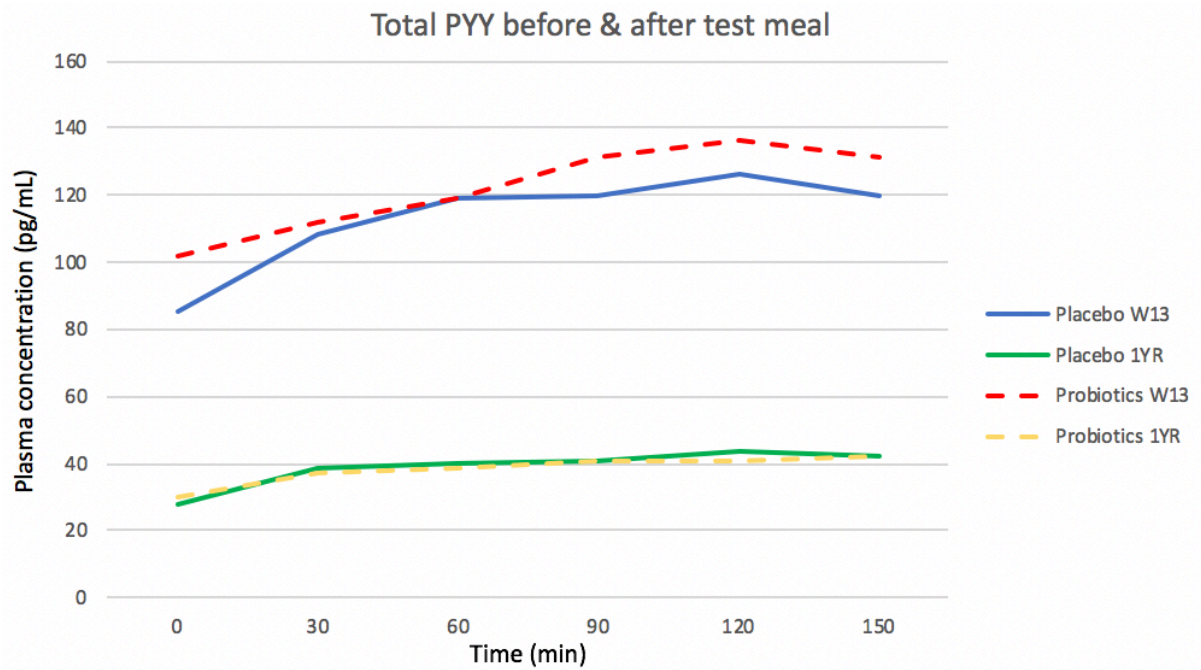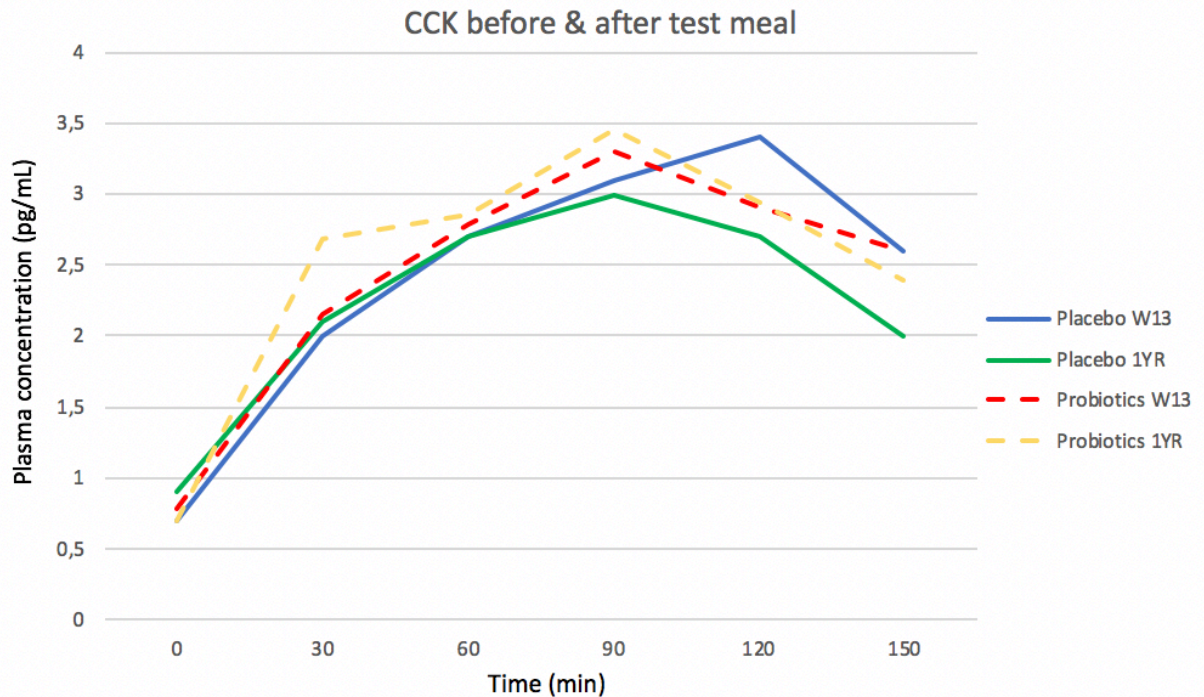
Figure 9: Mean changes in plasma concentration of CCK over time (from W13 to 1YR) between groups.

Figure 9 shows the progression in concentration of CCK measured over time (over the 2.5-hour period), for both groups at week 13 and one year. Both placebo and probiotic group followed a similar pattern at week 13 and one year, where, immediately after the consumption of the test meal, there was an increase in CCK concentration, with a gradual rise up to 90 minutes for all participants, except the placebo group at week 13, which continued to rise until 120 minutes after the consumption of the test meal. After reaching the highest concentration (at 90 and 120 minutes, respectively), the concentration of CCK gradually decreased over the 2.5-hour period. The CCK concentration did not return to levels reported in fasting for any of the groups at any of the time points. No significant differences were found in basal CCK and in the iAUC of CCK, between or within groups, at any time point, and over time (1YR-W13).

4.0 Discussion

The present study aimed to evaluate how the use of probiotics for weight loss maintenance affects subjective and hedonic appetite feelings, and objective markers of appetite measured in plasma concentrations of appetite hormones, from week 13 to one year. The hypothesis of the study was that participants receiving probiotics would experience less hunger and more protective changes in their appetite profile than participants receiving placebo.

In the present study, probiotic supplementation was not found to be associated with improvements in weight loss maintenance. No significant differences were found between probiotic and placebo groups in changes over time (from week 13 to one year) in subjective feelings of appetite, in food hedonics, or in appetite related hormones. Even though the results were not statistically significant, there were clinical relevant findings for the obesity management that are worth discussing.

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Weight loss maintenance is a great challenge in the obesity management. In the present study, 22 out of a total of 55 participants succeeded in weight loss maintenance at one year (using the definition of weight loss ≥ 10 % of initial body weight sustained for one year(14)). This equates to a total success of 40 %. Interestingly, there were greater numbers of participants who succeeded with weight loss maintenance in the placebo group, where 17 out of a total of 28 participants managed to maintain their weight, compared to the probiotic group, where only 5 out of a total of 27 participants managed to maintain their weight throughout the study. The remaining participants, 11 of the participants receiving placebo treatment and 22 of the participants receiving probiotic treatment respectively, did not succeed in maintaining their weight. Even though there was a significant mean difference between groups in body weight regain over time (4.5±1.5 kg (P=0.016)), significant differences between groups in appetite were not found after 1 year of weight loss maintenance using probiotic/placebo capsules.

4.1 Probiotic supplementation

The results regarding weight loss maintenance show that, in the present study, the consumption of probiotic capsules was not a contributing factor to weight loss maintenance. Other studies using some of the similar probiotic strains used in present study found different results. One of the probiotic strains used in the present study was *Lactobacillus rhamnosus*. Sanchez et al. (59) looked into the effects of supplementation with *Lactobacillus rhamnosus* and found that, females receiving probiotics experienced a higher mean body weight loss than the females receiving placebo. They found that females receiving probiotics had lower hunger sensations and a higher decrease in food cravings, as well as a displayed greater efficiency to suppress the desire to eat than the placebo controls. Sanchez et al. (28) also demonstrated that supplementation of *Lactobacillus rhamnosus* helps women with obesity to achieve sustainable weight loss. They had a similar study design as the present study, with a randomized, double blinded placebo-controlled trial, where participants underwent a weight loss phase followed by a weight loss maintenance phase.

However, the research conducted by Sanchez et al had a length of 24-weeks, with a 12-week weight reduction phase followed by a 12-week weight maintenance phase. The supplementation of placebo/probiotic over 12-weeks of WL maintenance only, and significant results only found among females, not in males. In the present study, analysis of differences between and within males and females were performed for subjective feelings of appetite, food hedonics and appetite-related hormones, but the results are not included as no significant differences was found. Moreover Million et al. (71) demonstrated that higher levels of *Lactobacillus reuteri* was associated with individuals with obesity, and that higher levels of *Bifidobacterium animalis, Lacbobacillus parcasei,* and *Lactobacuillus plantarum* were associated with normal weight individuals. These findings support the importance of probiotic supplementation in body weight management, and further research could possibly look into a probiotic supplement containing only bacteria that are known to be supportive of normal weight, and explore the possible effects on appetite and on body weight maintenance. The probiotic formula used in the present study contained several other strains of probiotics that possibly explain why the probiotic consumption did not prevent body weight regain and why similar changes in appetite profile was not found.

4.2 Subjective feelings of appetite

Regarding the subjective feelings of appetite, no significant results were found, between or within groups, at week 13 and one year time points, and over time, in feelings of hunger, fullness, desire to eat, and prospective food consumption. However, there were some interesting observations found worth discussing.

Looking into subjective feelings of hunger (Figure 2), the placebo group experienced a non-significant decrease in subjective feelings of fasting hunger over time. Contrastingly, the subjective feelings of fasting hunger in the placebo group went in the opposite direction, with a non-significant increase in fasting hunger feelings over time. Several studies have demonstrated that fasting hunger increase after a period of weight loss followed by a period of weight loss maintenance. A study by Sumithran et al.(53), performed a 10-week weight-loss intervention in individuals with obesity, where participants were followed-up one year after the intervention. They looked into subjective feelings of appetite using the same visual analogue scale used in the present study. Similar to our findings, they found no significant differences in fasting hunger from week 10 (weight loss phase) to week 64 (weight loss maintenance phase). However, they found a statistically significant change from baseline to week 10 and to week 62, with higher mean ratings of fasting hunger. The present study did not include the baseline measurements of appetite because week 13 was treated as a new baseline for this study given that the participants had completed the same 4-week weight stability period that should have "washed out" the differences in the macronutrient composition between diets during the weight loss phase. However, there is a possibility that there remaining effects from the different diets at week 13 were present that could account for the differences seen in week 13. The macronutrient composition of the diets used during the weight loss phase should be explored further.

Nymo et al. (72) conducted a study with a similar design as the present study, where 100 individuals with obesity underwent an 8-week very-low calorie diet, followed by a 4-week refeeding phase (week 13), and a one-year weight loss maintenance program. They looked into the long-term sustainability of changes in appetite after weight loss. Using visual-analogue scales as a tool to measure subjective feelings of appetite, they found an increase in fasting hunger from baseline to week 13 that was sustained at one-year follow-up. Moreover, Nymo et al. (72) found significantly increased mean ratings of hunger from baseline to week 13, but this was not sustained at one year. This goes along with our results, where no significant differences in subjective feelings of iAUC hunger over time were found from week 13 to one year. Since the same was seen in both Nymo et al. (72), and the present study (in both placebo and probiotic groups), with no significant differences between groups in subjective feelings of iAUC, it seems that the probiotic supplement used in the present study had no effect on subjective feelings of iAUC hunger over time.

For the subjective feelings of fullness (Figure 3), no significant differences were found within and between groups over time. In the study conducted by Sumithran et al. (53) they found a significant decrease in fullness from week 10 to week 62. Even though they did not include probiotics in their study, their participants regained almost 50 % of their

lost weight, similar to the probiotic group in the present study, where this group experienced a weight regain of 54.9 %. The probiotic group in the present study followed a similar pattern as the Sumithran study with and observed decrease in iAUC fullness over time, but it was not significant (P=0.656). Moreover, there were no significant differences in feelings of iAUC fullness found in the placebo group, indicating that the probiotic supplement used in the present study had no effect on subjective feelings of fullness.

In the present study, there was a non-significant decrease in desire to eat over time found in both the placebo and the probiotic group. This is in contrast to Sanchez et al. (59) who found a greater increase in desire to eat in the female participants receiving probiotics compared to participants receiving placebo. The differences were only found in female participants, and can possibly be a reflection of the greater weight loss found in females receiving probiotic compared to the placebo controls in their study, or the baseline characteristics and sample size, as well as the length of the intervention (24-weeks vs one year in the present study). The present study performed analyses for differences in desire to eat in males and females, but no significant difference was found in any gender, so the results are not included in the thesis.

Both the placebo and the probiotic group experienced a non-significant increase in feelings of prospective food consumption over time, in the current study. This is in agreement to Sumithran et al. (53), who found no significant differences in prospective food consumption between week 10 and week 62. As there were no differences between groups in the present study, and the results were in agreement with Sumithran et al.´s (53) study who did not use probiotic supplementation, indicating that the probiotic supplement used in the present study had no effect on subjective feelings of prospective food consumption.

4.3 Food hedonics

In the food hedonic assessment, no significant differences over time were found between or within groups, in pre-meal and post-meal assessments, except for the differences between groups, pre-meal at week 13 for explicit liking of high fat and sweet foods (W13: 14.5±5.7 (P=0.013)), and the pre-meal at week 13 for explicit wanting for high fat and sweet foods 11.5±5.6 (P=0.044)), with the placebo group having greater values in the variables mentioned. Moreover, there was a tendency towards a higher value, in the pre-meal assessment, in the explicit liking for high fat and non-sweet foods in the placebo group when compared with probiotic group (P=0.054), and there was a tendency towards higher values in the pre-meal assessment of high fat and sweet foods for explicit liking in the placebo group when compared with probiotic group (P=0.050). A possible reason to why the placebo and the probiotic group were different at week 13 could be related to the differences in amount of carbohydrate in the diets during the weight loss intervention. Both groups underwent the same 4-week weight stability period that should have "washed out the differences between the diets, but there is a possibility that there were still remaining effects from the diets at week 13, so the macronutrient composition of the diets used during the weight loss phase should be explored further.

Sanchez et al. (28) who performed a similar probiotic/placebo study did not use the same food hedonic assessment as in the present study. They performed an assessment for food cravings (State-Trait Food Craving Questionnaire-Trait) and found that a higher decrease

in food cravings was observed in participants receiving probiotics versus participants receiving placebo. It could be interesting to combine the LFPQ food hedonic assessment used in the present study and the State-Trait Food Craving Questionnaire-Trait to compare the liking and wanting of food and food cravings with the use of probiotics/placebo.

To the best of my knowledge, the present study is the only study that has used the LFPQ for evaluating changes during weight loss maintenance, and more studies are needed for comparison of the results found. Regardless, the findings support the importance of hedonic hunger in body weight management, and is something that offers a potential target for improving appetite control in body weight maintenance. More research is required to know if probiotic supplementation have a positive effect on hedonic hunger in body weight maintenance.

4.4 Appetite-related hormones

In this study, no significant differences between groups at any time points or over time were found for the basal and iAUC measurements of active ghrelin, total GLP-1, total PYY, and CCK. Nevertheless, a significant decrease over time was found within groups in active ghrelin in both basal and iAUC plasma concentrations. Also, a significant decrease over time within both groups was found for basal total GLP-1. A significant decrease in iAUC total GLP-1 over time was found in the placebo group only. Basal and iAUC plasma concentrations of total PYY were both significantly decreased over time in both groups. No significant differences were found in basal or iAUC CCK plasma concentration within groups over time.

In the longitudinal study conducted by Nymo et al. (72), they reported a sustained increase in basal and postprandial active ghrelin plasma concentrations from BL to week 13 sustained at one year follow-up. Papandreou et al. (33) found that individuals with obesity have lower concentrations of ghrelin than age-matched lean control subjects, which is an indication that ghrelin is downregulated in human obesity. Even though no significant differences were found between groups in active ghrelin over time in the present study, there could be a threshold of amount of body weight change needed to affect changes in the gut appetite hormones, and is something that could be explored in further research.

It is well known that individuals with obesity have a blunted secretion of total GLP-1 (73). In the current study, a significant decrease over time within both groups were found in basal GLP-1 and in iAUC total GLP-1, but a significant decrease in iAUC total GLP-1 was found in the placebo group only. Interestingly, the group that received probiotics did not have a significant decrease in iAUC total GLP-1 even though they had a lower percentage of successful weight loss maintenance (18.5 % in the probiotic group, vs 60.7 % in the placebo group). It is known that significant alterations in the composition of the gut result in changes in the release of satiety hormones (28). The probiotic supplementation could be the reason to why the iAUC total GLP-1 in the probiotic group did not significantly decrease over time in iAUC total GLP-1 in the placebo group. More studies are needed to confirm the possible effect of probiotic supplementation on GLP-1.

In the present study, both basal and iAUC plasma concentrations of total PYY were significantly decreased over time in both the placebo and the probiotic group. This is in

agreement to Nymo et al. (72) who found a significant decrease in mean in total PYY from baseline to one year. Le Roux et al. (74) explained that, "observed lower postprandial levels of PYY in individuals with obesity may result in an increase in food intake to achieve the same level of satiety as seen in normal-weight subjects. PYY release from the intestinal tract may be inhibited in individuals with obesity, leaving individuals with obesity with a functional deficiency in PYY-induced satiety. Low plasma PYY may therefore reinforce obesity" (74). As no significant differences were found between groups and the results agree with other studies, it is likely that the probiotic supplementation did not have an effect on total PYY in this study.

4.5 Strengths and Limitations

This study is, to the best of my knowledge, the first study to compare appetite changes in weight loss maintenance using supplementation of probiotics with placebo control. This study has both strengths and limitations. The main strength is the study design, with this study conducted as a randomized double-blinded controlled trial, considered to be the "gold standard" of experimental design. This is due to its ability to minimize different types of bias. The duration of the study is also a strength. With the longer weight loss maintenance period, we were able to see the effect of probiotic supplementation at different time points than what have been previously explored (other studies on probiotics and weight loss maintenance were shorter (total 24-weeks)). Repeated measurements were standardized and performed at week 13 and one year, which reduce the bias and variance by controlling for factors that cause variability between subjects. The Bod Pod, which is well recognized and based on the "gold standard" principle as hydrostatic weighting, was used to assess changes in body weight (65). The appetite of the participants was assessed using both subjective feelings appetite (by using a validated method (VAS), the liking and wanting of food (using LFPQ), and objective measures (appetite related hormones), in fasting and postprandially. All plasma hormone analysis was analyzed in the same assay to reduce intra-assay variability, and analysis was performed by experienced bioengineers. Bonferroni adjustments were used for the multiple time comparisons to adjust for the increase risk of finding statistically significant differences by chance alone. The participants had monthly follow-ups to help them with maintaining their weight loss in terms of support, which makes them more likely to succeed with weight loss maintenance in the long-term (19).

There are also some limitations in this study. All participants consumed the same breakfast meal, with the same amount of food. We did not adjust the meal size according to either body weight, gender, or energy needs. Females consumed a larger relative energy load compared with males, which possibly affected the variables measured for appetite to some degree. However, if we had adjusted the test meal the appetite response would be blunted because of reduced nutrient stimuli independent of gender, body weight or energy needs. The multiplex assay used for the measurements of appetite hormones (except for CCK) is likely to result in less accurate and precise measurements compared with optimized assays for each individual hormone, however these are commonly used. The sample size is also a possible limitation, with 55 completers of the study divided into placebo (n=28) and probiotic groups (n=27) this study may be underpowered to investigate the association between changes in the selected appetite variables. Collection of stool samples for compliance with the probiotic capsules were collected, but due to the deadline of delivering

this master thesis, the analysis of the stool samples was not ready to be analyzed, and, thus, was not included in present study. The probiotics used were containing seven different strains of bacteria. Other studies have found positive effects on appetite using only one strain (28).

Practical implications

Weight loss maintenance is a big challenge in obesity management, due t relapse and weight regain. Appetite plays a large role in why individuals have difficulties maintaining their weight loss. In the present study, probiotic supplementation was proposed as a strategy to help with weight loss maintenance with the potential beneficial effects that it has on appetite, on appetite sensations, and on eating behavior. However, in the present study, we did not find that probiotic supplementation had beneficial effects on appetite, nor did it help with long-term weight loss maintenance. Therefore, more studies are needed to explore the potential effect of probiotic supplementation further. The present study is also, to the best of my knowledge, the first study evaluating probiotic supplementation on appetite and WLM one year after the weight loss intervention (other studies have used 12 and 24 weeks interventions) and provides insight on the potential effect of probiotic supplementation long term. The findings in this study contributes to better understand the action of the probiotic supplementation (NYKOPRO Ferie) used, on appetite and weight loss maintenance, and will therefore contribute to help in the selection of what probiotic strains that could be used as a tool for benefiting appetite in weight loss maintenance in future research.

5.0 Conclusion

In the present study, no significant differences between the two intervention groups in any of the appetite related variables over time were found. Taken together, these observations did not support the hypothesis of probiotic supplementation having beneficial effect on subjective feelings of appetite, on hedonic appetite feelings, and on appetite-related hormones in the obesity management compared to placebo. However, the changes in appetite found in the present study in general, are in line with other weight loss maintenance studies that looks into appetite, irrespective of probiotic consumption. There are few studies on the topic of weight loss maintenance and probiotics, that found probiotic supplementation to be beneficial for appetite during weight loss maintenance. Research to confirm the effect of probiotic supplementation is needed. More studies with fewer/different types of probiotics are necessary to determine if probiotics can be used in the obesity management. Moreover, there is a possibility that there exists a threshold of body weight change that is needed to affect changes in the appetite-related hormones, and is something that could be explored further.

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Appendixes

Appendix I: Consent form
Appendix II: Study design
Appendix III: Healthy eating guidelines for weight loss maintenance
Appendix IV: Food diaries
Appendix V: User manual for SenseWear armband
Appendix VI: Visual analogue scale
Appendix VII: Macronutrient composition of the test meal
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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/m²,
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)

- o 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)
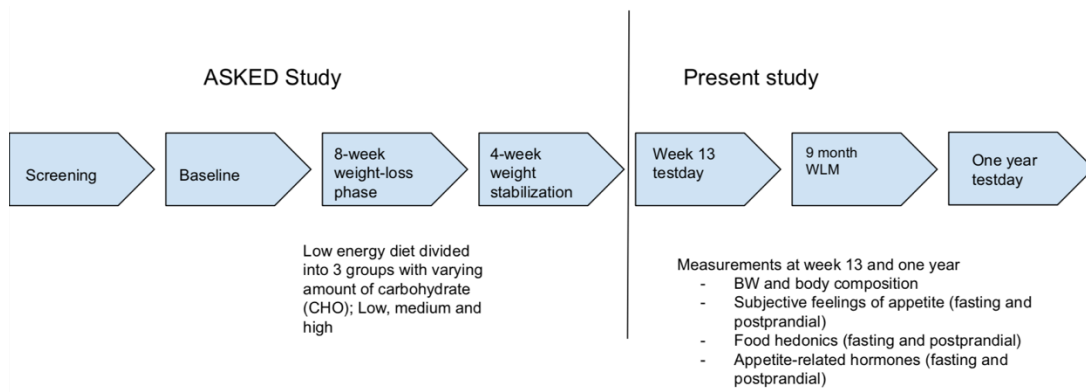
Appendix II. Study design

Figure 10: Study design.

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. Bildet er et 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.

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 karbohdrater.	• Variant: Kommentarer _____ :
Lunsj	**1** shake eller suppe * Du kan tilsette litt grønnsaker med lavt innhold av karbohdrater.	• Variant: Kommentarer _____ :
Mellommåltid, dag	**1** shake eller suppe * Du kan tilsette litt grønnsaker med lavt innhold av karbohdrater.	• Variant: Kommentarer _____ :
Middag	**1** shake eller suppe * Du kan tilsette litt grønnsaker med lavt innhold av karbohdrater.	• Variant: Kommentarer _____ :
Mellommåltid, kveld	**1** shake eller suppe * Du kan tilsette litt grønnsaker med lavt innhold av karbohdrater.	• Variant: Kommentarer _____ :

Appendix V. User manual for SenseWare armband

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

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

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

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

6. 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.

7. Tørk av synlig skitt eller svette i det tidsrommet du tar av armbåndet (max 1 time av per 24 timer).

8. 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.

9. Bruk armbåndet på din *ikke-dominante* arm, dvs. er du høyrehendt, bruk den på venstre overarm
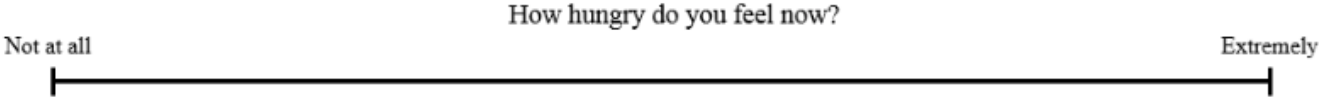
Appendix VI. Visual analogue scale

How hungry do you feel now?

Not at all |————————————————————————————————————| Extremely

Figure 11: Example of visual analogue scale

Appendix VII. Macronutrient composition of the test meal (W13, 1YR)

	W/ milk	W/yoghurt	E%
Energy kJ	2487	2564	
Energy kcal	594	613	
Fat g	23.2	24.6	35 %
Saturated fat g	13.7	15.7	
Carbohydrate g	69.2	60.2	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	22.0	16 %
Salt	1.6	1.6	

Table 6: Macronutrient composition of the breakfast meal.

Appendix VIII. Leeds food preference questionnaire

Figure 12: Typical food array used in the LFPQ.

Figure 11: Representative question for the LFPQ for assessment of explicit liking

Figure 12: Representative picture for the LFPQ assessment of explicit wanting