Master's thesis

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Department of Circulation and Medical Imaging

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Can probiotics help to maintain weight loss in individuals with obesity - a RCT

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

Supervisor: Jessica Ann Røkenes

June 2020

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Vilde O. Jordahl

Content

Abstract

Introduction: Weight loss maintenance remains the main challenge in the obesity management. Probiotics (living bacteria) can alter gut microbiota to some extent and can potentially modulate the body weight (BW) of the host. As few studies have investigated the potential role of probiotics in preventing weight regain, the main aim of this study was to evaluate the impact of probiotics on weight loss maintenance (WLM) in individuals with obesity. Secondary aims were to evaluate the impact of probiotic on body composition and on gastrointestinal symptoms.

Methods: This study was a double-blinded randomised control trial with repeated measurements. 76 adults (37 men) with obesity (mean±SD; 29.8±3.0 kg/m^2 BMI) were randomised to receive either multi-strain probiotic capsules (NYCOPRO Ferie) or placebo capsules twice a day for nine months after an initial weight loss (WL) phase. BW, body composition (fat mass (FM), fat free mass (FFM)) (measured with air displacement plethysmography), energy intake, physical activity and gastrointestinal symptoms (measured with gastrointestinal symptoms rating scale (GSRS)) were measured at baseline (BL), week 9 (W9), week 13 (W13), and at 1 year (1Y). Linear mixed model analyses were performed to look at changes over time within groups and between groups. The results are reported as mean±SEM, significance was set at $P \leq 0.05$.

Results: At 1Y, the probiotic group increased more BW over time (1Y-W13) than the placebo group (7.6±1.5 kg, $P<0.001$ vs. 3.1±1 kg, $P=0.017$, respectively), and the differences in BW changes between groups were significant (4.5±1.5 kg, $P=0.016$). At 1Y, the probiotic group had a larger weight regain of their initial WL than the placebo group (54.9±7.2 % vs. 19.0±7.6 %, respectively), and the differences between groups were significant (35.9±10.5 %, $P=0.001$). The probiotic group maintained a WL of 6.1 % of the initial BW compared to 11.3 % in the placebo group at 1Y. Five (18.5 %) of the twenty-seven participants in the probiotic group and seventeen (60.7 %) of the twenty-eight participants in the placebo group maintained a WL of ≥ 10 % of their initial BW at 1Y. At 1Y, there were significant differences in changes over time (1Y-W13) between groups in FM (kg) (4.1±1.4 kg, $P=0.014$), FM (%) (2.4±0.8 %, $P=0.008$), and FFM (%) (2.5±0.8 %, $P=0.007$). No significant mean differences between groups were found in the changes over time (1Y-W13) in gastrointestinal symptoms 1Y.

Conclusion: This study could not find a beneficial effect of probiotics compared to placebo in terms of WLM. Our results show that the placebo group maintained their WL better than the probiotic group at 1 year. The exact mechanisms behind these findings remain to be uncovered. Probiotics were not found to have a more positive effect compared to placebo for gastrointestinal symptoms. More research is needed regarding the different types of bacterial strains and their effect on BW to recommend probiotics as a strategy in the obesity management.

Keywords: Gut microbiota, probiotics, weight loss maintenance, obesity.

Abstrakt (norsk)

Introduksjon: Vedlikeholdelse av vekttap forblir hovedutfordringene i håndteringen av fedme. Probiotika (levende bakterier) kan potensielt være gunstig for å modulere tarm-mikrobiota til en viss grad, noe som også kan påvirke kroppsvekten (BW) hos en vert. Det er få studier som har undersøkt om probiotika kan forhindre vektoppgang. Målet med denne studien var å undersøke effekten av probiotika på vedlikeholdelse av vekttap hos individer med fedme. Sekundære mål var å evaluere effekten av probiotika på kroppssammensetningen og på gastrointestinale symptomer.

Metode: Denne studien var en dobbelt blindet randomisert kontrollert studie med gjentatte målinger. 76 voksne (37 menn) med fedme (gj.snitt±SD; 29.8±3.0 kg/m^2 BMI) ble randomisert til å ta to kapsler med flerstammede probiotika (NYCOPRO Ferie) eller to kapsler med placebo per dag i ni måneder etter en vektreduksjonsfase. BW, kroppssammensetning (fettmasse (FM) og fettfri masse (FFM) (målt med air displacement plethysmography), energiinntak, fysisk aktivitet og gastrointestinale symptomer (målt med gastrointestinal symptoms rating scale (GSRS)) ble målt ved oppstart, uke 9 (W9), uke 13 (W13) og 1 år (1Y). Analysen linear mixed model ble utført for å se på endringer over tid innad i og mellom gruppene. Resultatet er rapportert som gjennomsnitt±SEM, signifikansnivået ble satt til P≤0.05.

Resultat: Ved 1Y hadde probiotikagruppen en større økning i BW over tid (1Y-W13) sammenlignet med placebogruppen (7.6±1.5 kg, P<0.001 vs. 3.1±1 kg, P=0.017), og forskjellen mellom gruppene var signifikant (4.5±1.5 kg, P=0.016). Probiotikagruppen hadde gått opp mer av deres opprinnelige vekttap sammenlignet med placebogruppen ved 1Y måling (54.9±7.2 % vs. 19.0±7.6 % av deres opprinnelige vekttap) og forskjellen mellom gruppene var signifikante (35.9±10.5 %, P=0.001). Probiotikagruppen hadde samlet opprettholdt et vekttap på 6.1 % av deres opprinnelige BW sammenlignet med 11.3% i placebogruppen ved 1Y. Fem (18.5 %) av de tjuesyv deltakere i probiotika gruppen og sytten (60.7 %) av de tjueåtte deltakerne i placebogruppen hadde suksessfullt vedlikeholdt et vekttap på ≥10 % av deres opprinnelige BW etter 1Y. Ved 1Y var det en signifikant forskjell i endring over tid (1Y-W13) mellom gruppene i FM (kg) (4.1±1.4 kg; P=0.014), FM (%) (2.4±0.8 %, P=0.008) og FFM (%) (2.5±0.8 %, P=0.007). Vedrørende gastrointestinale symptomer var det ingen signifikant forskjell i endring over tid (1Y-W13) mellom gruppene ved 1Y målingen.

Konklusjon: Denne studien fant ikke en gunstig effekt av probiotika på vedlikeholdelse av vekttap sammenlignet med placebo. Resultatene våre viser at placebogruppen opprettholdt vekttap bedre enn probiotikagruppen gjorde etter 1Y. De faktiske mekanismene som ligger til grunne for disse funnene gjenstår å avdekkes. Ytterligere forskning trengs vedrørende de ulike bakteriestammene og deres effekt på BW for å kunne anbefale probiotika i behandlingen av fedme.

Nøkkelord: Tarm-mikrobiota, probiotika, vedlikehold av vekttap, fedme.

1.0 Background

1.1 Introduction

Obesity and overweight have become a health concern worldwide. In 2016, the number of overweight people in the world had exceeded 1.9 billion adults (≥18 years old), and 650 million of them were obese (1). In Norway, data from a large longitudinal study (the HUNT-study) showed that between 1984 and 2008, the proportion of men and women with obesity increased from 7.7 % to 22.1 % and from 13.3 % to 23.1 %, respectively (2). Furthermore, Kelly and colleagues estimated that 57.8 % of the world population will be overweight or obese by 2030 if the trend continues (3).

Obesity is classified as body mass index (BMI) ≥30 kg/m^2 and overweight as BMI ≥25 kg/m^2 (1). BMI is calculated from body weight (BW) in kilograms divided by the square of height in meters. Obesity is the result of a chronic positive energy imbalance, which can result from too much energy intake (EI), or reduced physical activity (PA), or a combination of both (4). Obesity is a complex multifactorial disease where genes, environmental, physiological mechanisms, and behavioural factors are contributors to its development (5–7). The obesogenic environment characterized by a more sedentary lifestyle and an increase in the consumption of high density foods, is identified as the main common cause leading to increased obesity (8,9).

The risk of developing numerous of non-communicable diseases such as type 2 diabetes mellitus, cardiovascular diseases, musculoskeletal disorder, some types of cancer, metabolic syndrome, sleep disorder, high blood pressure, and increased risk of mortality is higher in individuals with obesity compared to normal-weight individuals (4,10). Obesity can also have a great impact on the individual's quality of life (11). Studies show that individuals with obesity have, usually, lower self-esteem, and are more likely to suffer from depression, anxiety, and body dissatisfaction (4,11). Obesity, with or without comorbidities, can potentially be a major economic burden to society because of higher need of health care, and possibly affect work productivity (12,13). Therefore, it is crucial to find methods that can help prevent and treat overweight and obesity.

Several studies have been conducted in order to find methods that can lead to weight loss. Methods ranging from various energy-restricted diets, educational programs on lifestyle (e.g., meal replacement products, increased physical activity, behavioural techniques) (14) to bariatric surgery (15). All of these methods have shown that they can help with weight reduction (14). Further, studies have found that 5-10 % weight loss is sufficient to improve health-related

risk factors (16,17), and, therefore, weight loss has been one of the main targets of obesity management.

However, further research has shown that the biggest challenge of obesity management is how individuals can maintain the new and reduced body weight (BW) over time (18–21). A study of 249 subjects with severe obesity who underwent a lifestyle intervention and found that after 4 years , only 28% of the 99 participants who completed the study maintained a weight loss of 10 % of their initial BW (18). Other studies have shown that, in the long term, individuals that have gone through diets or bariatric surgery regained a large amount of the initially lost weight, and some even regained all of the weight they have lost (22–24). Wing and Hill reported that among individuals with obesity, approximately only 21 % were successful in achieving long-term weight loss maintenance (WLM) (25). A review investigating the effect of weight regain and metabolic risk factors found that even a small weight regain (2-6 %) could reverse the positive effects seen after WL (26). This indicates that long-term WLM is difficult to achieve, and, therefore, strategies to effectively help with WLM are needed.

Previously, studies have tried to find common determinants among successful weight loss maintainers. The National Weight Control Registry (NWCR) is one of the largest observational studies that was conducted over a 10-year period (27). The study investigates the presence of common determinants among individuals with successful WLM. They found that low energy and low fat diets, frequent self-weighing (to catch slip ups), a consistent eating pattern (week and weekend days), eating breakfast every day, performing high levels of PA (1 h/day) are common determinants of successful weight loss maintainers (20,27). Similar results were found in a recent review of WLM, in addition to showing that portion control, less sugar, and increased intake of fruit and vegetables were positive predictors of successful WLM (28). Moreover, motivational, behavioural and cognitive determinants, and the amount of support or follow-up after WL are factors that can also affect the success of WLM (29–33).

Further research has investigated the underlying physiological mechanisms that may impact in WLM success, and found that bacteria in the gut microbiota (GM) can play an important role when it comes to the body's energy metabolism and fat metabolism (34–36). Although the overall impact of the bacteria in the GM has not been fully uncovered, an imbalance in the GM has been associated with several diseases, such as gastrointestinal diseases (37,38), obesity, and related comorbidities (39–45). For example, some studies have shown that individuals with obesity can have a different composition and a reduced bacterial diversity in their GM compared to normal-weight individuals (39,44). This indicates that the GM could play a role in obesity.

Probiotics (living bacteria) have been found to be able to prevent an imbalance in the GM, and has been suggested as strategy in treatment of obesity (46–49). There is growing evidence suggesting that the supplementation of probiotics can influence WL. Two systematic reviews and meta-analyses have shown that overall supplementation with probiotics (mainly strains with Lactobacillus and Bifidobacterium genera) give significant WL and fat mass losses compared to controls (48,50).

However, in relation to the probiotics effect on WLM, little research has been conducted. To our knowledge, there is only one study conducted by Sanchez and colleagues, which was a randomized control trial (RCT), that investigated the effect of *Lactobacillus rhamnosus* CGMCC1.3724 on WL and WLM in 125 individuals with obesity (in men and women) (51). They reported larger BW and fat mass losses in the WLM phase in the group consuming probiotics versus the placebo group among women only. However, this study was limited due to short WLM phase (only 12 weeks). Nevertheless, this implies that there may be a positive effect of probiotics in relation to WLM, but more research is needed. Thus, the aim of this study was to investigate if probiotics could potentially be beneficial for WLM.

1.2 Theoretical background

1.2.1 Definition of weight loss maintenance

There are different definitions of what successful WLM is. Wing and Hill (25) proposed a weight loss of ≥10 % of their initial BW and maintenance for ≥1 year as a definition, and has been widely used in other studies (18,27,52,53). The definition is based upon the proposition that 10 % weight loss provides several health benefits because it reduces the risk factors of developing health-related diseases, such as type 2 diabetes mellitus and heart diseases (20). Other definitions have also been used, such as, maintaining all of the initial WL for a period of two years; achieving no more than 5 % (or 2.3 kg) of weight regain over period of 4 years; or maintaining a weight change of no more than ±2.3 kg at the end of the study (52,54,55). A recent study has tried to find an appropriate definition of successful WLM from published data from the Look AHEAD trial. They suggested that, ≤25 % weight regain should be used because this allows some weight regain and there are few individuals that manage to maintain 100 % of their initial WL (56).

1.2.2 Gut microbiota and probiotics

The GM can be considered as our greatest ecosystem and is largely represented in our gastrointestinal tract (57,58). The human GM consists of trillions of different microorganisms, such as bacteria, archaea and eukarya (58,59). The development of new techniques, based on the sequencing of the 16S ribosomal RNA gene, have allowed us to explore the different types of microorganisms and to start to better understand the human GM (60).

The colonization of the human GM begins at birth, where maternal bacterial flora is transferred to the infant during vaginal birth (61,62). Genetics, type of birth, breast-feeding or formula, introduction to solid food and later diet, environmental, and use of antibiotics are factors that influence the further development of the human GM (61–65). From around the age of three, humans start to develop a more complex and stable GM like an adult human being. Adults have a complex and diverse GM that has been shown to be stable over time (44). The GM are mainly populated by bacteria from Bacteroidetes (includes Lactobacillus), Firmicutes, Proteobacteria, and Actinobacteria (includes Bifidobacterium) (47,66,67). During adulthood, the food intake, macronutrient composition and the environment have an impact on the GM composition and diversity, and, hence, on inter-individual differences in the GM among subjects (40,68,69).

One of the main mechanisms that links GM to obesity is the involvement in the fermentation of non-digestible carbohydrates, which increases the amount of short-chain fatty acids (SCFAs) (mainly acetate, propionate, and butyrate) (70,71). The production of SCFAs can have an impact on peptide YY (PYY) and glucagon-like-peptide one (GLP-1), which can promote satiety, and, potentially, supress EI (70,72). SCFAs can have an impact on insulin signalling, which is associated with fat accumulation (73). SCFAs are also a source of energy and can account for 10 % of EI (74). It has been found that individuals with obesity have a higher amount of SCFAs in their stool compared to lean individuals (75), and therefore, it is believed that they can harvest more energy from their diet than individuals with normal weight (35,75).

Ley and colleagues (44) were one of the first to link GM to obesity in humans. They first found that obese mice had lower levels of Bacteroidetes and a higher proportion of Firmicutes than lean mice (76). Later, they found similar findings in humans (44). Additionally, they also found that WL can change the Bacteroidetes/Firmicutes ratio towards what is seen in non-obese. However, the findings are inconsistent as other studies have not found differences between the GM of individuals with obesity and normal weight, or even found the opposite - a higher level in Bacteroidetes and lower levels of Firmicutes (75,77). Overall, these findings may indicate

that there are some differences in the composition of the GM between individuals with obesity and normal weight individuals. It is therefore thought that the alteration of the GM composition could be used as a strategy in the obesity management.

Probiotics are defined as living microorganisms (living bacteria) that can give health benefits to its host (78). There are various types of products containing probiotics, such as dairy products, fermented products, and supplements. Probiotics can alter and affect GM and hinder an imbalance in the GM, which in turn, can potentially modulate body weight (48,50,79,80). Moreover, obesity has been associated with gastrointestinal symptoms, such as diarrhoea (81), constipation (82), reflux (83) and abdominal pain (84), and some findings indicate that probiotics could be beneficial for improving the outcomes related to these symptoms (85). In addition, probiotics have no contraindication for its use in the long-term (80). Lactic acid bacteria, belonging to Lactobacillus and Bifidobacterium, is most commonly used as it appears to have the most beneficial effect on human GM (79,86–88). As mentioned, the literature on probiotics and WL seems promising but more research is needed to determine its effectiveness in relation to WLM.

1.3 Objectives and hypothesis

The aim of this study is to evaluate the impact of probiotics on weight loss maintenance in individuals with obesity. The primary outcomes of interest are the mean differences in body weight and weight regain. The secondary outcomes of interest are the mean differences in body composition (fat mass (FM) and fat free mass (FFM)) between groups, and to evaluate the impact of probiotics on gastrointestinal symptoms (e.g., diarrhoea, constipation).

The hypothesis is that those who received probiotics have maintained their WL better than the placebo group, and that probiotics have a positive effect on body composition and on gastrointestinal symptoms.

2.0 Method

2.1 Study design

This study was a randomised double-blinded control trial with repeated measurements. Participants were randomized to take probiotics or placebo capsule twice a day for 9 months. This study is a continuation of a larger study, aimed at weight loss (ASKED study, see clinical trial.gov, number NCT02944253). Before the randomization phase, participants underwent an 8 weeks powder based low-energy diet (LED), then 4 weeks of refeeding, and finally a weight

stabilization phase. The study was conducted at the obesity clinic at St. Olav hospital in Trondheim, Norway.

2.2 Study population

Seventy-six healthy adults (men and women, 18-65 years old) with obesity class I or II (30 kg/m^2 < BMI > 40 kg/m$^{2)}$) were recruited through advertisement, posters and flyers placed in Trondheim and through St. Olavs and NTNU's intranet. Participants had to be weight stable (<2 kg variation) for the last 3 months and not dieting to lose weight prior to the study enrolment. They could not have consumed probiotics for the last 6 months and not have used antibiotics for the last 3 months to meet the inclusion criteria. Participants were excluded if they were pregnant, breast-feeding, dealing with drug or alcohol abuse within the last 2 years, took medication known to affect appetite or induce weight loss, or were enrolled in another obesity treatment program. In addition to the criteria listed above, those with a history of psychological disorders, bariatric surgery, metabolic diseases (such as hypo/hyperthyroidism and type 1 or 2 diabetes mellitus), eating disorders, lactose intolerance, gastrointestinal disorders (particularly cholelithiasis), kidney, liver, lung and cardiovascular disorders, rheumatoid arthritis, Crohn's disease, or malignancies were also excluded for this study.

2.3 Ethics

This study was conducted according to the Helsinki declaration and accepted by the Norwegian Regional Ethics Committee (REK) (Ref.,2016/1297). The protocol was registered at Clinical trials.gov (number NCT03287726). Participation in this study was voluntary, and a written informed consent was obtained from all participants before they enrolled in this study (details in Appendix VI). They could withdraw from the study at any time.

2.4 Detailed protocol

2.4.1 Weight loss phase and weight stabilization phase

Participants went through 8 weeks of LED (1000 kcal/day), with different amounts of carbohydrate (CHO) (70, 100 or 130g CHO/day). The diet was powder based in the form of milkshakes (strawberry and chocolate flavoured) and soup (chicken and tomato flavoured flavoured) and participants received five portions (200 kcal/portion) per day. At week 9 (W9), they were gradually reintroduced to normal food while withdrawing from the powder based low carbohydrate diets. At week 13 (W13), study personnel gave an individualised prescription to the participants for a diet that matched their energy needs. The prescribed diet was designed to

achieve weight stabilization and had a content of 50-60 % CHO, 15-20 % protein and 20-30 % fat. Energy needs were estimated from resting metabolic rate (RMR) x PA level (PAL) factor measured with activity armbands at W9.

2.4.2 Probiotics intervention

At W13, the participants were randomized into two groups (simple randomization using the program webCRF3 (NTNU) (89)), to receive either capsules containing probiotics or placebo. They were instructed to take two capsules per day, one at breakfast and other at dinner. Capsules with probiotics contained multi-strain probiotics manufactured from NYCOPRO (Takeda AS) called NYCOPRO Ferie and are commercially available. The multi-strain probiotics included seven different bacterial strains (*Bifidobacterium bifidum W23, Lactobacillus acidophilus W37, Lactobacillus casei W56, Lactobacillus plantarum W21, Lactobacillus rhamnosus W71, Lactobacillus salivarius W24, and Lactococcus lactis W58*), and the dose had a concentration of $2{,}5*10^9$ CFU/capsule (the prescribed daily dose in this study was 2 capsules ($5*10^9$ CFU/day). The placebo capsules were NYCOPRO placebo and contained a Capsugel Coni-Snap transparent size zero (0.3 g), consisting of 100% bovine gelatin (microcrystalline cellulose), and was approved by European farmacope. The placebo capsules were manufactured by Kragerø Tabelettproduksjon AS.

The participants were given a new dietary prescription at W13 based on new measurements with the aim of long-term weight loss maintenance. Participants were advised to increase their consumption on fish, lean meat, poultry, vegetables, fruits, and limit the intake of dietary fats, fatty meat, sweets, pastries, and dessert. In addition, they were advised to increase their levels of exercise (i.e., walking, skiing, jogging, or swimming) and overall daily PA levels. The advice is based on the Norwegian Directory of Health's national guidelines for nutrition and PA (90,91).

Under the intervention, participants were followed-up on a monthly basis by two research nurses. Weight was measured, and the participants answered questions about nutrition, PA and daily life in general. The participants were supplied with one-month's worth of capsules, or enough to last until the next visit. They were instructed to bring back the capsules that they did not consume to measure compliance.

2.5 Data collection

The participants were measured at baseline (BL), W9, W13 and 1 year (1Y) (see Figure 2: study design, Appendix I). Each participant came for test day in the morning in a fasted state (at least 10 hours), having had no consumption of nicotine, alcohol, caffeine, or participation in strenuous activity over the last 12 hours. Anthropometric measurements, body composition, RMR and PA were measured each time points. Gastrointestinal symptoms rating scale (GSRS) were measured at W13 and 1Y. Stool samples were collected each time points. Further details regarding measurements are described below.

2.5.1 Anthropometric measurements

Body weight was measured with BW scale (SECA Hamburg, Germany). All participants were measured in underwear only. Height was measured with stadiometer (SECA Hamburg, Germany) to the nearest 0.1 cm. All participants were measured without shoes and where told to look straight forward. Waist and hip circumferences were measured with tape-measure three times to the nearest 0.1 cm, and the average of the three measurements was used further. Waist circumference was performed by placing a finger above the cristia iliaca. The hip circumference was measured at the fullest part of the hip, right above the middle of the buttocks. Both measurements were performed with the participant in standing position. Weight and height were used to calculate BMI (equation: kg/m^2).

2.5.2 Body composition and body weight

Body composition (FM and FFM (kg and %)) were measured with air displacement plethysmography method, (this study used a BOD POD, COSMED, Italy (92)). This method is a validated method that is based on the same principle as multi-compartment methods, like under water weighing (93). During each test day, the BOD POD and its weight scale were calibrated. The scale is calibrated with a known weight (20 kg). The BOD POD calibration is a two-step calibration. First, the volume is calibrated with a known volume (50 l cylinder). Then a second calibration with the participant details entered (height, ethnicity, gender, and birth date) is performed prior to each test. The BOD POD uses the relationship between pressure and volume to determine whole body density (D_b) (93). The measurement of the participant is performed twice to verify consistency and must be performed within 150 ml of each other to pass (the average of those two measurements is then used). If the second measurement fails to meet the criterion, a third measurements is performed. The computer system determines D_b from the measured BW (mass (in kg)) from the weight scale and body volume (V_b) using the

following equation: D_b = mass/V_b (94). V_b is corrected for the thoracic gas volume (V_{TG}) by either measuring it or by using predicted V_{TG} (93). This study used predicted V_{TG}. Once the D_b is determined, a standard formula is used to calculate FM %. This study used the Brozek formula (FM % = (4.57/D_b - 4.142) $*$ 100) for individuals with obesity (95). This formula is designed to estimate fat content in individuals with excess fat. The FM % is then used to calculate FFM %, FM (kg), and FFM (kg) by the following formulas:

$$(\text{FFM \%} = 100\text{-FM \%}),$$

$$\text{FM (kg)} = \frac{(FM\ \%)(\text{mass})}{100\ \%}$$

$$\text{FFM (kg)} = \text{mass-FM (kg)}$$

All participants were tested wearing non-metallic undergarments and were given a swimming cap in order to reduce air volume produced from hair (96). They had to remove all types of metallic objects, like jewellery. They were told to sit still in one position, be quiet and breathe normally throughout the whole measurement.

2.5.3 Resting metabolic rate

RMR was measured with indirect calorimetry (Vmax Encore 29N, Care Fusion, Germany) using a standardized procedure. The flow sensor was calibrated each test day (with a 3 l pump), prior to the test. The participants were asked to sit still for 10 minutes prior to the test and were measured lying down on a bed. A canopy was placed over their head to capture the oxygen uptake (VO_2) and the carbon dioxide production (VCO_2). Under the test, they were instructed to relax, but not to fall asleep, to breathe normally and not to change their position throughout the test. The participants were measured over 15 minutes or longer if needed to achieve 10 minutes of a steady state (97). The first 5 minutes were excluded from the data. The RMR was calculated by taking the average of the measurement taken in steady state.

2.5.4 Food diary

A food diary was used to collect information about how much and what types of food were eaten (see Appendix VII). The food diary was filled out over a period of 3 days (two weekdays and one weekend day) and contained detailed information about the type of food, quantity, and preparation method. Food diaries were collected at W13 and 1Y. The total energy intake and amount of macronutrients (carbohydrate, protein and fat) were calculated using a computer-based food planner, Kostholdsplanleggeren (Helsedirektoratet and Mattilsynet) (98). The

averages of the 3 collected days of EI (kcal/day) (2 weekdays and 1 weekend day) were used in the statistical analysis.

2.5.5 Physical activity

Accelerometers were used to collect data regarding the PA levels of the participants. This study used the SenseWear armband from BodyMedia, Pittsburgh, PA, USA. Participants were asked to have it on for seven continuous days. They wore it on their non-dominant hand, over the midpoint of the triceps muscle. Participants were instructed to always have it on (also at night-time), except for when they showered or when they participated in other activities that would expose the armband to water (see user manual, Appendix VIII). All data from the PA armband was carefully checked for missing data. Only days with complete data (measured uninterrupted over 24 h) were used, and they had to have ≥ 3 completed days of data to be included. The armband gives data on the total number of minutes spent in PA, how many minutes in each PA levels (from sedentary to very vigorous), and average PAL per day. The average of the total number of minutes spent in PA from the completed days (min/day) were used in the statistical analysis.

2.5.6. GSRS

Gastrointestinal symptoms were evaluated using the GSRS questionnaire. ASKED study used a Norwegian version of the GSRS questionnaire with a set of 24 questions (see Appendix X). For this thesis, only 15 of the questions (in bold in the GSRS questionnaire, Appendix X) were used in the analysis as these questions are the original questions that are used in existing research and are validated (99). GSRS questionnaire uses a seven-graded Likert scale. The scale ranges from 1-7, where one represents no symptoms and seven represents the greatest amount of symptoms. The questionnaire is divided into five different sub-dimensions (i.e., diarrhoea, indigestion, constipation, abdominal pain, and reflux). To summarize, a score for each dimension's mean value was calculated, as well as for all the 15 questions for an overall total score of the GSRS (see Appendix IX for description of the GSRS questionnaire):

- Diarrhoea (questions 13, 14, and 16)
- Indigestion (questions 8, 9, 10, and 11)
- Constipation (questions 12, 15, and 17)
- Abdominal pain (questions 1, 6, and 7)
- Reflux (questions 3 and 4)

If the participant had missing data containing less than 50% within a dimension, the mean score of the non-missing questions would be imputed. If the missing data were more than 50% within a dimension, the dimension would be excluded from the analysis.

2.5.7 Stool samples

The participants received tubes so they could collect the stool samples at home. They were instructed to collect it as close to test day as possible and store it in a freezer (-20°C). On test day, the stool samples were collected first in the morning and stored in a freezer (-80°C) until they were analysed.

2.6 Power calculation

A sample size of 74 participants would be needed to detect a difference of 2.0±3.0 kg body weight between the groups, at a power of 80 % and a significant level of P≤0.05. To compensate for potential dropout of around 20 %, the total sample size of 89 participants was needed.

2.7 Statistical analysis

All data was analysed using IMB SPSS statistics 25. Statistically significance level was set to P≤0.05. Only participants that completed the study (i.e., with data at 1Y) were used in the final analysis (called completers) given that no significant differences were found between the completers and the non-completers in the main characteristics analysed at week 13. All variables were tested for normality using Shapiro Wilk-test QQ-plots and histogram. Characteristics of the participants are presented as mean±standard deviation (SD).

Independent sample t-test or Mann Whitney U test analysis, depending on the normality of the data, were performed to look at differences between groups and genders at BL and W13. Paired samples t-test or Wilcoxon Signed Ranks Test analysis, depending on the normality of the data, were performed to assess changes over time between BL and W13 within groups. The results are presented as mean±SD.

Linear mixed model (LMM) analyses were used for the main outcome (BW) and body composition (FM (kg, %) and FFM (kg, %)) to compare differences between the groups in changes over time (1Y-W13). The LMM was adjusted for Bonferroni post hoc pairwise comparisons. The residuals were also tested for normality. Due to the small sample size of the study, three separate LMM models were performed. One without adjustments, one adjusting for EI (kcal/day) and one adjusted for total PA (min/day). Results are given in mean±standard error of the mean (SEM).

BW regain from W13 to 1Y was calculated as a percentage of the initial WL (see equation below). The differences between the groups in weight regain were tested with an independent samples t-test. The results are given in mean±SEM.

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

We used the definition by Wing and Hill to define successful WLM (maintaining a weight loss of ≥10 % of their initial BW) (25), and which was calculated as follows:

$$\text{Percentage WL of initial BW: } \frac{WL}{Initial\ BW} * 100.$$

Paired samples t-test or Wilcoxon Signed Ranks Test analyses, depending on the normality of the data, were used to look at differences over time (1Y-W13) for EI (kcal/day) and total PA (min/day). Pearson correlations were performed for normally distributed data and Spearman correlations were performed for the non-normally distributed data to analyse the correlation between the variables BW and body composition with EI (kcal/day) and total PA (min/day) at 1Y.

GSRS was analysed with a Mann-Whitney U test, due to no normal distributed data, to look at differences between group at W13, 1Y and changes over time (1Y-W13). Wilcoxon Signed Ranks Test analysis were used, due to non-normality distributed data, to evaluate differences over time (1Y- W13) within groups. The results are given in mean±SEM.

3.0 Results

3.1 Study population

Of the 101 participants that were recruited for the ASKED study, 76 participants started the probiotic intervention. Overall, 46 participants did not complete the study (see flowchart in Figure 1). At W13, there were 37 participants in the probiotic group and 27 of them completed the study. In the placebo group, there were 39 participants at W13 and 28 of them completed the study. Overall, 55 participants completed the study and were included in the final analysis.

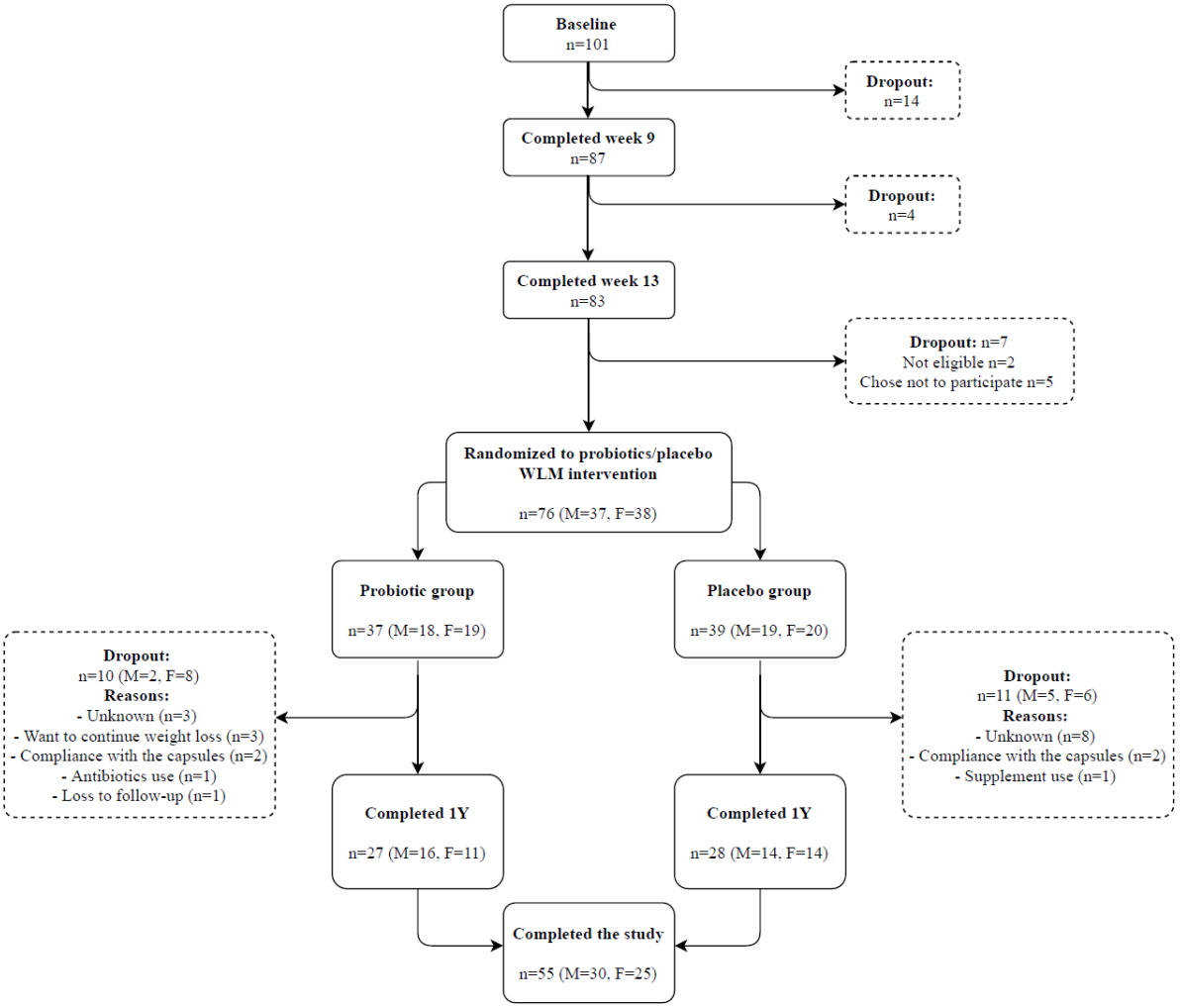

Figure 1. *Flowchart of the study population. n, number; M, Males; F, Females; WLM, weight loss maintenance; 1Y, 1 year.*

At BL, the participants were 46.3±9.3 years old on average with a BW of 105.7±14.8 kg and a BMI of 34.6±3.4 kg/m^2 (see Table 1). Males were significantly taller, had a higher BW, waist circumference, and FFM (kg) (P≤0.001) than females. Females had a significantly higher hip circumference (P=0.003) and FM (%) (P≤0.001) than males. There was a significant decrease in BW, BMI, hip and waist circumferences, FM (kg and %) and FFM (kg), and a significant increase in FFM (%) (P≤0.001) over time (W13-BL) (details in Table 1). There were no significant differences between the groups at BL.

At W13 participants had an average BW of 91.0±12.1 kg and a BMI of 29.8±3.0 kg/m^2 (see Table 1). Participants had a significant mean body weight loss of -14.6±3.8 kg (P<0.001) from BL to W13. Males had a significantly higher BW and FFM (kg and %) (P<0.001) than females. Females had a significantly higher hip circumference (P=0.001), FM (kg) (P=0.004), and FM (%) (P<0.001) than males.

Table 1. Characteristics of completers at baseline and at week 13, and changes between time points (week13-baseline).

	Completers baseline (n=55)	**Completers week 13** (n=55)	**ΔWeek 13-baseline** (n=55)
Gender M/F (%)	30/25 (54.5/45.5)	--	--
Age (years)	46.3±9.3	--	--
Height (cm)	175.0±8.9[c]	--	--
BW (kg)	105.7±14.8[c]	91.0±12.1[c]	-14.6±3.8***[c]
BMI (kg/m^2)	34.6±3.4	29.8±3.0	-4.7±0.9***[c]
Hip circumference (cm)	115.7±8.6[b]	106.5±7.5[c]	-9.2±4.6***
Waist circumference (cm)	112.5±11.2[c]	100.3±9.2	-12.2±6.5***[c]
FM (kg)	42.9±8.8	30.5±8.0[b]	-12.2±2.4***[c]
FM (%)	40.9±6.5[c]	33.6±7.7[c]	-7.2±2.4***[c]
FFM (kg)	62.9±10.9[c]	60.2±10.3[c]	-2.5±1.8***[a]
FFM (%)	59.3±6.2[c]	66.4±7.7[c]	7.0±2.3***[c]

*Data presented as mean±SD. M, Males; F, Females; BW, body weight; BMI, body mass index; FM, fat mass; FFM, fat free mass. ***, P≤0.001 over time.*
[a] Differences between genders at significance level P≤0.05.
[b] Differences between genders at significance level P≤0.01.
[c] Differences between genders at significance level P≤0.001.

At W13, there were no significant differences between the groups (probiotic and placebo) in any of the variables studied (see Table 2). Both groups had a significant weight loss from baseline to W13 (P<0.001). The probiotic group had lost 13.3 % of their initial BW, while the placebo group had lost 14.0 % of their initial BW between BL and W13. There was a significant decrease in BMI, body composition, waist, and hip circumferences in both groups (P<0.001). There were no significant differences between groups in the changes over time (W13-BL) in any of the variables studied (see Table 2).

Table 2. Differences between groups (probiotic and placebo) in completers at week 13 and changes over time (week 13-baseline).

	Week 13		ΔWeek 13-baseline	
	Probiotic (n=27)	**Placebo** (n=28)	**Probiotic** (n=27)	**Placebo** (n=28)
Gender M/F (%)	16/11 (59.3/40.3)	14/14 (50.0/50.0)	--	--
Age (years)	48.1±8.9	44.4±9.4	--	--
BW (kg)	90.8±11.4	91.2±12.9	-14.3±3.8***	-15.0±3.7***
BMI (kg/m^2)	29.8±2.9	29.8±3.2	-4.7±1.0***	-4.8±0.9***
Hip circumference (cm)	105.9±7.5	107.1±7.5	-9.9±4.5***	-8.6±5.0***
Waist circumference (cm)	101.4±8.8	99.4±9.6	-11.6±5.3***	-12.7±7.6***
FM (kg)	29.9±6.7	31.1±9.1	-11.8±3.5***	-12.5±3.2***
FM (%)	33.2±6.8	34.0±8.5	-6.8±2.3***	-7.7±2.4***
FFM (kg)	60.2±9.6	60.2±11.2	-2.8±1.9***	-2.3±1.7***
FFM (%)	66.8±6.8	66.0±8.5	6.8±2.3***	7.3±2.2***

*Data presented as mean ±SD. M, Males; F, Females; BW: body weight. BMI: body mass index. FM: fat mass. FFM: fat free mass. ***, P≤0.001 within groups.*

3.2 Body weight

At 1Y, both groups had significant mean changes in BW (kg) over time (1Y-W13) (see Table 3). The probiotic group had an increase of 7.6±1.5 kg (P<0.001) and the placebo group an increase of 3.1±1 kg (P=0.017) of the weight they had lost at W13. The mean difference in BW change between groups (4.5±1.5 kg) was significant (P=0.016). After adjusting for EI (kcal/day), the differences between the groups in the changes over time were no longer significant (P=0.202). After adjusting for total PA (min/day), the change over time (1Y-W13) in BW was no longer significant within the placebo group only (P=0.107).

Moreover, there was a significant mean difference in weight regain of their initial WL between the groups of 35.9±10.5 % (P=0.001) at 1Y. The individuals in the probiotic group had a mean weight regain of 54.9±7.2 % of their initial WL (W13-BL) while the placebo group had a mean weight regain of 19.0±7.6 % of their initial WL (W13-BL).

At 1Y, the probiotic group maintained a WL of 6.1 % of initial BW and the placebo group maintained a WL of 11.3 % of their initial BW. Five (18.5 %) of the twenty-seven participants in the probiotic group and seventeen (60.7 %) of the twenty-eight participants in the placebo group maintained a WL of ≥ 10 % of their initial BW at 1Y.

3.3 Body composition

Significant mean changes over time (1Y-W13) in FM (kg) were found within groups (see Table 3). The probiotic group increased their FM by 7.1±1.0 kg (P<0.001) and the placebo group increased their FM by 3.1±1.0 kg (P=0.007) from W13 to 1Y, and the differences in the changes between groups were significant (4.1±1.4 kg; P=0.014). After adjusting for EI (kcal/day), there were no longer significant differences between the groups in FM (kg) (P=0.202). After adjusting for total PA (min/day), the changes in FM (kg) in the placebo group over time were no longer significant (P=0.107), and the differences in the changes between groups remained significant (P=0.043).

The percentage of FM increased significantly over time (1Y-W13) within groups (see Table 3). The probiotic group had an increase of 4.4±0.6 of FM (%) (P<0.001) while the placebo group had an increase of 1.9±0.5 of FM (%) (P=0.002) from W13 to 1Y. The differences in the changes in FM (%) between groups were significant (2.4±0.8 of FM (%), P=0.008). After adjusting for EI (kcal/day), the differences in the changes between groups in FM (%) were no longer significant (P=0.080). After adjusting for total PA (min/day), the changes in FM (%) in the placebo group over time (1Y-W13) were no longer significant changes (P=0.092), and the differences in the changes between groups remain significant (P=0.012).

No significant changes over time (1Y-W13) within or between groups were found in FFM (kg) (see Table 3).

Both groups had significant mean changes over time (1Y-W13) in percentage of FFM (see Table 3). The probiotic group had a decrease of 4.4±0.6 of FFM (%) (P<0.001) whereas the placebo group had a decrease of 1.8±0.6 of FFM (%) (P=0.005) from W13 to 1Y. The difference in the changes between groups were significant (2.5±0.8 of FFM (%), P=0.007). After adjusting for EI (kcal/day), there were no longer significant differences between groups (P=0.109). After adjusting for total PA (min/day), the changes in FFM (%) in the placebo group over time (1Y-W13) were no longer significant (P=0.092), and the differences in the changes between groups remained significant (P=0.012).

Table 3. Changes over time (from week 13 to 1 year) in completers split by genders assessed within and between groups (probiotic and placebo).

	Completers (n=55)			**Males** (n=30)			**Females** (n=25)		
	Probiotic (n=27)	**Placebo** (n=28)	**Diff. groups** (n=55)	**Probiotic** (n=16)	**Placebo** (n=14)	**Diff. groups** (n=30)	**Probiotic** (n=11)	**Placebo** (n=14)	**Diff. groups** (n=25)
Without adjustments									
BW (kg)	7.6±1.1***	3.1±1.1*	4.5±1.5*	9.6±1.4***	4.1±1.5*	5.5±2.1*	5.6±1.7**	2.2±1.5	3.4±2.3
FM (kg)	7.1±1.0***	3.1±1.0**	4.1±1.4*	8.3±1.1***	3.4±1.4*	4.9±1.8*	5.9±1.5***	2.8±1.4	3.2±2.0
FM (%)	4.4±0.6***	1.9±0.5**	2.4±0.8**	4.8±0.8***	1.9±0.8*	2.8±1.1*	4.0±0.9***	1.9±0.8*	2.1±1.1
FFM kg	0.5±0.3	0.0±0.3	0.5±0.5	1.3±0.5*	0.6±0.5	0.7±0.7	-0.3±0.6	-0.6±0.5	0.3±0.7
FFM (%)	-4.4±0.6***	-1.8±0.6**	-2.5±0.8**	-4.8±0.8***	-1.9±0.8*	-2.8±1.1*	-4.0±0.9***	-1.8±0.8	-2.2±1.2
Adjusted for energy intake (kcal/day)									
BW (kg)	6.9±1.2***	3.9±1.1**	2.9±1.6	7.9±1.7***	5.0±1.6*	2.9±2.3	5.9±1.6**	2.9±1.5	3.0±2.1
FM (kg)	6.9±1.2***	3.9±1.1**	2.9±1.8	7.9±1.7***	5.0±1.6	2.9±2.3	5.7±1.6**	2.7±1.5	3.0±2.1
FM (%)	4.4±07***	2.4±0.6**	2.1±0.9	4.9±1.0***	2.9±0.9**	2.0±1.3	4.0±09***	1.9±0.9	2.1±1.2
FFM (kg)	0.3±0.4	0.1±0.4	0.2±0.6	0.9±0.7	0.7±0.6	0.2±0.9	0.4±0.6	-0.51±0.6	0.1±0.8
FFM (%)	-4.5±0.7***	-2.5±0.6***	-2.0±0.9	-4.9±1.0***	-2.9±1.0*	-2.0±1.4	-4.0±0.9***	-2.0±0.9	-2.0±1.2
Adjusted for total PA (min/day)									
BW (kg)	6.3±1.2***	2.3±1.1	4.0±1.6*	7.1±1.6***	2.9±1.5	4.2±2.1	5.5±1.8*	1.7±1.5	3.8±2.3
FM (kg)	6.3±1.2***	2.3±1.1	4.0±1.6*	7.1±1.6***	2.9±1.5	4.2±2.1	5.5±1.8*	1.7±1.5	3.8±2.3
FM (%)	4.0±0.7***	1.3±0.6	2.7±0.9*	4.0±0.9***	1.6±0.8	2.4±1.2	4.0±1.0***	1.1±0.9	2.9±1.3
FFM (kg)	-0.0±0.4	0.1±0.4	-0.1±0.6	0.7±0.6	0.6±0.5	0.1±0.8	-0.8±0.6	-0.4±0.5	-0.3±0.8
FFM (%)	-4.0±0.7***	-1.3±0.6	-2.7±0.9*	-4.0±0.9***	1.6±0.8	-2.4±1.2	-4.0±1.0***	-1.1±0.9	-2.9±1.3

*Linear mixed model with Bonferroni correction. Data presented as estimated marginal mean±SEM. n, number; diff, differences; BW, body weight; FM, fat mass; FFM, fat free mass; PA, physical activity. *P≤0.05, **P≤0.01, ***P≤0.001.*

3.4 Body weight and body composition among males and females gender

Males in the probiotic group had significant mean changes over time (1Y-W13) in BW (9.6±1.4 kg, P<0.001), FM (kg) (8.3±1.1 kg, P<0.001), FM (%) (4.8±0.8 %, P<0.001), FFM (kg) (1.3±0.5 kg, P=0.038) and FFM (%) (-4.8±0.8 %, P<0.001), (see Table 3). After adjusting for EI (kcal/day) and total PA (min/day), there were no longer significant differences in changes over time in FFM (kg). Males in the placebo group had significant changes over time (1Y-W13) in BW (4.1±1.5 kg, P=0.031), FM (kg) (3.4±1.4 kg, P=0.046), FM (%) (1.9±0.8 %, P=0.041) and FFM (%) (-1.9±0.8 %, P=0.049). After adjusting for EI (kcal/day), there were no longer significant mean differences in FM (kg) over time (1Y-W13). After adjusting for total PA (min/day), there were no longer differences over time in BW, FM (kg, %) and FFM (%). At 1Y, there were significant differences in the changes between groups among males in BW (5.5±2.1, P=0.031), FM (kg) (4.9±1.8 kg, P=0.032), FM (%) (2.8±1.1 %, P=0.030), and FFM (%) (-2.8±1.1 %, P=0.033). After adjusting for EI (kcal/day) and total PA (min/day), no significant differences between groups among males were seen in the changes of all the aforementioned variables.

Females in the probiotic group had significant mean changes over time (1Y-W13) in BW (5.6±1.7, P=0.006), FM (kg) (5.9±1.5 kg, P=0.001), FM (%) (4.0±0.9 %, P<0.001) and FFM (%) (-4.0±0.9, P<0.001), (see Table 3). The variables were still significant after adjusting for EI (kcal/day) and total PA (min/day). Females in the placebo group had significant mean changes over time (1Y-W13) only in FM (%) (1.9±0.8 %, P=0.047). There were no longer significant changes over time after adjusting for EI (kcal/day) or total PA (min/day) in FM (%). No significant differences were found in changes in BW or in body composition between groups among females at 1Y.

3.5 Energy intake and physical activity

No significant differences between the groups at W13 and at 1Y were seen in EI (kcal/day) or in total PA (min/day). There were also no significant differences between groups in EI in the mean changes over time (1Y-W13) (probiotic group: mean increase of 193±490 kcal/day and placebo group: mean increase of 67±470 kcal/day) or in total PA (probiotic group: mean decrease of -15.9±57.6 min/day and placebo group: mean decrease of -1.2±40.2 min/day), see Table 5, Appendix II and Table 6, Appendix III.

However, in the probiotic group, there was a significant negative correlation between FM (%) and total PA (min/day) (rho= -0.438, P=0.047) and a significant positive correlation between

the FFM (%) and total PA (min/day) (rho= 0.438, P=0.047) (see Table 7, Appendix IV). While, in the placebo group, there was a significant positive correlation between FFM (kg) and EI (kcal/day) (rho= 0.393, P=0.042) and a significant positive correlation between FFM (%) and total PA (min/day) (rho= 0.684, P≤0.01). In addition, there was a significant negative correlation between FM (kg) and total PA (min/day) (r= -0.593, P=0.002), and a significant a negative correlation between FM (%) and total PA (min/day) (rho= -0.684, P≤0.01) in the placebo group.

3.6 GSRS questionnaire

At W13, there was one missing person in the placebo group in the dimension indigestion. At 1Y, there was one missing person in both groups in the dimensions diarrhoea and constipation. This was due to the fact that there were more than 50 % missing values within the dimension.

No significant differences between the groups were found at W13 and at 1Y in total score, diarrhoea, indigestion, constipation, abdominal pain, or reflux symptoms (see Table 4). However, the placebo group had a significant decrease in constipation symptoms (P=0.020) and a tendency towards an increase in diarrhoea symptoms (P=0.061) over time (1Y-W13). There were no significant differences in the changes over time (1Y-W13) between the groups in any of the variables of the GSRS questionnaire analysed.

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

	Score at week 13		**Score at 1 year**		**Δ1 year-week13**		
	Probiotic (n=27)	**Placebo** (n=28)	**Probiotic** (n=27)	**Placebo** (n=28)	**Probiotic** (n=27)	**Placebo** (n=28)	**Diff. groups** (n=27)
Total score	1.59±0.96	1.55±0.09	1.57±0.11	1.50±0.08	-0.02±0.09	-0.05±0.08	-0.03±0.12
Diarrhoea	1.33±0.11	1.25±0.07	1.28±0.16	1.41±0.12	-0.05±0.19	0.17±0.12	0.20±0.22
Indigestion	1.87±0.16	1.85±0.14	1.83±0.17	1.73±0.13	-0.04±0.14	-0.18±0.15	-0.12±0.20
Constipation	1.74±0.20	1.69±0.19	1.72±0.26	1.40±0.16	-0.02±0.14	-0.31±0.13*	-0.30±0.19
Abdominal pain	1.69±0.10	1.61±0.11	1.58±0.95	1.66±0.12	-0.11±0.11	0.05±0.12	0.16±0.16
Reflux	1.09 ±0.05	1.11±0.07	1.22±0.11	1.11±0.06	0.13±0.10	0.00±0.7	-0.13±0.12

*Data presented as mean±SEM. GSRS, Gastrointestinal Symptom Rating Scale; diff, differences; n, number; *P≤0.05.*

3.7 Differences in follow-up

There were no significant differences between the groups based on the nurse that followed-up the participants, or in the frequency of the follow-ups throughout the study.

4.0 Discussion

The present study aimed to investigate the potential impact of probiotics on long-term WLM for individuals with obesity. The secondary aims were to see the impact of probiotics on body composition and on gastrointestinal symptoms. The hypothesis was that, those who received probiotics would maintain their WL better than the placebo group, and that probiotics would have positive effect on body composition and on gastrointestinal symptoms.

This study is one of the few studies that investigated the effect of probiotics versus placebo in the WLM, among individuals with obesity. The results showed that, both groups had significantly increased their BW over time (1Y-W13), and there were significant mean differences over time (1Y-W13) in BW and in body composition changes (with exception for FFM in kg) between groups at 1Y. However, after adjusting for EI, the differences in BW and in body composition between groups were no longer significant.

Among males, there were a significant differences in changes over time (1Y-W13) between the groups in BW and body composition (with the exception of FFM in kg). However, after adjusting for EI and total PA, the differences were no longer significant. Among females, there were no significant differences in the changes over time (1Y-W13) between the groups in BW or in body composition.

Regarding the BW regain, the probiotic group regained more BW compared with placebo group and the difference between groups was significant (35.9±10.5 %, P=0.001). Moreover, the probiotic group maintained a WL of 6.1 % of the initial BW compared to 11.3 % in the placebo group. Five (18.5 %) of the twenty-seven participants in the probiotic group and seventeen (60.7 %) of the twenty-eight participants in the placebo group maintained a WL of ≥ 10 % of their initial BW at 1Y. Concerning the gastrointestinal symptoms, there were no significant differences in the changes over time (1Y-W13) between the groups in any of the gastrointestinal symptoms analysed. However, the placebo group had a significant decrease in constipation symptoms at 1Y.

Previous studies, conducted in animals and humans, have indicated that probiotics could be a tool in the obesity management (51,80,100–104). Borgeraas and colleagues (2017) (50) have

conducted a systematic review and meta-analysis on the effect of probiotics and WL among individuals with overweight and obesity. They found that overall, the consumption of probiotics significantly reduced BW compared to placebo (-0.60 95% CI: -1.19 – 0.01 kg), but the effect size was small and 6 of the total 15 studies included in the review could not find a significant change in BW. However, a recent systematic review and meta-analysis conducted by Wang and colleagues (2019) (48) found similar results and reported that, overall, the probiotic group experienced a significant WL compared to control group. To our knowledge, there is only one study that has investigated the association between probiotics and WLM, a study conducted by Sanchez and colleagues (51). They conducted a randomized controlled trial comparing probiotics (two capsules per day with one strain of *Lactobacillus rhamnosus* CGMCC1.3724) and placebo in 125 participants with obesity (both genders). The study had a 12-week WL phase and a 12-week WLM phase. They reported no significant differences between the probiotic group and the placebo group in BW in the WLM phase. This result is not in line with the results of the present study, which found a significant difference between the two groups in BW in the long-term. Sanchez and colleagues (51) had used the supplementation of probiotics already in the WL phase and the study had a shorter duration compared to our study and these may be considered two of the reasons why we got different results. However, further analysis showed in this study that, after adjusting for EI the differences between groups were no longer significant.

Despite no significant differences in weight loss over time between groups, Sanchez and colleagues (51) reported that, their probiotic group had lost more weight during the WLM phase compared to the placebo group (-5.3±4.3 vs. -3.9±4.2 kg, respectively). Our results showed that the probiotic group gained significantly more BW over time than the placebo group, which suggests that placebo group had a better outcome in relation to WLM. When comparing our results to those reported by Sanchez and colleagues (51), there appear to be differences in the development of weight change among the placebo and probiotic groups.

Moreover, when the data was analysed according to gender, Sanchez and colleagues (51) found a significant difference between the probiotic group and the placebo group in BW among females. The females in their probiotic group had lost more weight (-0.8 kg) in the WLM phase, while the females in their placebo group had a small weight gain (0.1 kg). They did not find significant differences between groups among males. Our results show that among females, there were no significant differences between groups in BW, while there was a significant difference in BW between groups among males. However, after adjusting for EI and total PA,

our results showed that there were no longer significant differences between the groups among males. The differences between our findings and those reported by Sanchez and colleagues indicate conflicting results.

Studies on mice have found that probiotics seem to be beneficial for weight regain compared to controls or placebo (105,106). In the present study (using sample of humans) showed that the probiotic group had a larger BW regain than the placebo group at 1Y, and that this difference was significant between the groups (35.9±10.5 %, P=0.001). This indicate that in the present study, the consumption of probiotics was not more beneficial than placebo in preventing weight regain and assisting in WLM.

Wing and Hill reported that, using their definition of successful WLM keeping a WL of ≥10 % of the participant's initial BW for ≥1 year, approximately 21 % of the individuals who attempt to lose their body weight are successful in long-term WLM (25). Applying the same definition in the current study, our results show that, the probiotic group maintained a WL of 6.1 % of their initial BW, while the placebo group maintained an 11.3 % of their initial BW. Thus, following Wing and Hill's definition of successful WLM, the participants in the probiotic group did not succeed, while the participants in the placebo group succeeded. Furthermore, only five of the twenty-seven participants (18.5 %) in the probiotic group managed to have successful WLM and were within the range of what is considered as successful WLM (≥10 % WL of their initial BW at 1 year). Whereas, among the twenty-eight participants in the placebo group, seventeen of them (60.7 %) managed to succeed with WLM, which indicates that the placebo group had a very high success rate. Hence, the results of the study were able to convey that there is a significant difference in success between the two groups.

A secondary aim was to see if probiotic consumption had a positive impact on body composition. Sanchez and colleagues (51) reported no differences in the WLM phase between the groups in body composition after consuming probiotics or placebo, but similar with the BW, they found a significant difference between groups among females. The females in the probiotic group continued to lose FM in the WLM phase, while the females in the placebo group gained more FM in the WLM phase and the differences between groups were significant. Moreover, studies investigating probiotics in relation to WL have found a significant reduction in FM with probiotics supplementation (48,107,108), but other studies have not found the same effect (109,110). The systematic review and meta-analysis by Wang and colleague (48) found among nine studies included in the analysis, that overall, there was a significant reduction in FM in the probiotic group compared with the control group (mean -0.91, 95% CI: -1.19, -0.63 kg).

Szulinska and colleagues (107) are one of the first to report a beneficial effect on FM with the use of probiotics with multiple strains. They included two groups with different dosages of probiotics and a placebo group. Although the two probiotic groups had significant reductions in FM, and the placebo group did not have a significant reduction, and the differences between the groups were not significant. However, the study was only conducted in postmenopausal females with obesity. In the results of the present study, both groups had increased their FM and the differences between groups were significant, which does not correspond with the findings reported by Sanchez and colleagues (51). However, after controlling for EI, our results demonstrated that there were no longer significant differences between the groups. Moreover, our results showed that, there were significant differences in FM between groups among males, and no significant differences in FM between groups among females, which is the opposite of what Sanchez and colleagues reported (51). However, after controlling for EI and total PA, no significant differences could be found in FM between groups among males. These results seem to provide some evidence that probiotics have an effect on body composition, but there are some conflicting findings, especially between males and females, which needs to be investigated further.

The results in the present study showed that, there were some significant differences between the groups, and that the placebo group was the most successful group in terms of WLM. This does not correspond with what we had hypothesized beforehand. Due to these findings, attempts were made to find explanatory reasons for the differences seen between groups and why the placebo group had better outcome than the probiotic group.

EI and energy expenditure are two components that can have a big impact on BW and body composition (111–113). If EI exceeds energy expenditure, a positive energy balance occurs, which can lead to an increase in BW. Furthermore, it has been found that the amount of protein consumed can have an effect on body composition and prevent weight regain (114,115). Therefore, we investigated whether EI, protein intake, or energy expenditure could have an impact on the present study's results. Total PA (min/day) was used as a measure of energy expenditure. We were unable to find significant differences between the groups in the amount of protein consumption. As a result, protein intake was not considered further in the statistical analysis of BW and body composition.

Regarding EI and PA, the probiotic group had a larger increase over time (1Y-W13) in EI compared to the placebo group (193±490 kcal/day and 67±470 kcal/day, respectively), and had a larger reduction in their total PA (-15.9±57.6 (min/day) and -1.2±40.2 (min/day),

respectively) compared to the placebo groups, but the differences between the groups were not significant. However, given the significant correlation between the BW and body composition and EI and total PA, the results were adjusted for EI and total PA. The results showed that, when the EI was controlled for in the statistical analysis in both groups, there were no longer significant differences between the groups in BW and body composition at 1Y. When the total PA was adjusted for, the significance level in FM (%) and FFM (%) differences between groups decreased at 1Y (from $P \leq 0.01$ to $P \leq 0.05$). This suggests that EI and total PA may have accounted for some of the significant differences seen between groups.

Moreover, it is also known that underreporting is an issue in terms of self-reported EI, and studies have found that individuals with overweight and obesity may underreport their EI around by 20 % (116,117). Upon further investigation in the comparison of the participants energy needs to maintain their weight at 1Y (calculated using RMR and PAL values) against the self-reported EI revealed possible underreporting of EI in both groups. The average energy needs estimated for the probiotic group were 2201±423 kcal/day at W13 and 2413±398 kcal/day at 1Y. The probiotic group reported their EI to be 1749±314 kcal/day at W13 and 1911±619 kcal/day at 1Y (an underreporting of 20.5 % and 20.8 %, respectively). Similarly, the average energy needs estimated for the placebo group were 2261±495 kcal/day at W13 and 2323±452 kcal/day at 1Y. The placebo group reported their EI to be 1704±371 kcal/day at W13 and 1742±498 kcal/day at 1Y (an underreporting of 24.6 % and 25.0 %, respectively). Despite some missing data for reported EI which may influence the comparison between reported EI, the apparent differences between reported EI and what is needed can lead to suggesting that underreporting has occurred.

Furthermore, it should also be noted that both groups did not follow the amount of PA that is associated with successful WLM. The NWCR reported that high levels of PA (over 150 min/day) is associated with successful WLM (20,27). However, a sub-group of the participants in the NWCR have managed to be successful in WLM with lower levels of PA (118). Both groups in this study had less than 100 min/day of total PA, with no significant differences between the groups. However, the groups had different outcomes regarding successful WLM, where the placebo group managed to be more successful than the probiotic group in WLM, despite lower levels of PA than what is associated with successful WLM.

There is also some evidence that the dose and number of strains used is of relevance in relation to weight management, but the results presented are conflicting. The systematic review and meta-analysis by Wang and colleagues (48) did some sub-analysis comparing studies using

higher ($\geq 10^{10}$ CFU) and lower ($<10^{10}$ CFU) doses of probiotics, and comparing those using probiotics with a single strain and multiple strains. They found a smaller reduction in BW among those studies that had a higher dose versus a lower dose and single strain versus multiple strains. Szulinska and colleagues (107), which had two probiotic groups compared to placebo group. They used probiotics with multiple strains in their study that are similar to ours. The probiotics contained nine different bacterial strains (*Bifidobacterium bifidum W23, Bifidobacterium lactis W51, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, Lactococcus lactis W19,* and *Lactococcus lactis W58*), where five of the strains were the same as in our probiotic capsules (*Bifidobacterium bifidum W23, Lactobacillus acidophilus W37, Lactobacillus casei W56, Lactobacillus salivarius W24, and Lactococcus lactis W58)*. also Moreover, the two probiotics groups had different dosages of probiotics, one group with a high dose ($1*10^{10}$ CFU) and the other group with low dose ($2.5*10^{9}$ CFU). Despite differences in dosages of probiotics, Szulinska and colleagues were unable to find a significant difference in BW between the three groups after 12 weeks. However, they found a significant reduction in FM within the probiotic groups and not within in the placebo group, but the differences between the groups were not significant. The present study, which used multiple strains with a dose of $5*10^{9}$ CFU/day, found significant differences between groups, but it was the placebo group that managed to maintain more of the initial WL and FM losses than the probiotic group. This indicates that the dose and number of strains are of importance and the plausibility that the probiotics chosen in this study may not have been beneficial for WLM. More research is needed to determine the right dose and number of strains that are the most beneficial.

There is insufficient knowledge on which types of bacterial strains produce a positive effect in modulating BW. A systematic review and meta-analysis of 14 studies concluded that probiotics with *Lactobacillus gasseri, Lactobacillus rhamnosus, Lactobacillus amylovorus, Lactobacillus plantarum* in combination with *Lactobacillus curvatus, Lactobacillus acidophilus* in combination with *Lactobacillus casei* could help with WL in individuals with overweight and obesity (103). Another meta-analysis investigated the strain specific effect of Lactobacillus on BW (119). They included 17 RCT's in humans, 51 studies on farm animals and 14 experimental models, and they found that, the supplementation with *Lactobacillius acidophilus* resulted in a significant weight gain in human and animals, while the supplementation with *Lactobacillus plantarum* and *Lactobacillus gasseri* was associated with weight loss in animals and humans, respectively. This suggests that not all types of strains produce the same effect, and elucidates

the possibility that our study had not used an effective mixture of bacteria to prevent weight regain (this study used *Bifidobacterium bifidum W23, Lactobacillus acidophilus W37, Lactobacillus casei W56, Lactobacillus plantarum W21, Lactobacillus rhamnosus W71, Lactobacillus salivarius W24, and Lactococcus lactis W58*).

It has also been shown that keeping the new weight for as long as possible increases the chances to succeed in long-term WLM (27). Based upon the data illustrated in Figure 3 (Appendix V), the placebo group managed to maintain their initial WL twice as long compared to the probiotic group, and could be one of the reasons why they had a better WLM in the end. Another interesting finding from Figure 3 is that, the placebo group decreased their weight in the last month before 1Y measurements, while the probiotic group continued to increase their BW. The amount of time the placebo group was able to maintain their initial weight loss could account for some of the differences seen between the probiotic and the placebo groups. What happened with the placebo group to cause the weight loss during that period is unclear.

Studies have found that individuals who frequently have support and follow-ups after WL have a 1.37 times higher chance to succeed with their WLM compared to those not receiving any form of support (120). Since there were two different nurses conducting the follow-ups, and since all participants did not attend all the follow-up visits, we investigated whether there were any significant differences between the groups in their follow-ups. We could not find any significant differences between the groups which had follow-ups with different nurses, or with regards to the frequency of the follow-ups throughout the study.

Research has shown that antibiotics can alter the composition and diversity of the GM in a negative way (65,121). Therefore, the study assessed if any of the participants used antibiotics during the WLM phase. Five participants used antibiotics under the intervention, and all of them belonged to the placebo group. These participants had used antibiotics for a period of three to ten days, between the follow-up visits two to eight. Statistical analysis was performed with and without the participants that used antibiotics to see if antibiotic use during the study could have impacted our results. There were no significant differences in our results when the analysis was performed with or without the participants that used antibiotics. Therefore, those five participants were not excluded from the analysis.

Another secondary aim was to see if the supplementation of probiotic had a positive effect on gastrointestinal symptoms. Studies have shown that a higher proportion of individuals with obesity have chronic diarrhoea compared to normal weight individuals (8.5 % and 4.5 %,

respectively) (81). Obesity has also been associated with abdominal pain (84), constipation (82), and reflux (83). There are some evidence showing that supplementation of probiotics could be beneficial for gastrointestinal symptoms, such as diarrhoea, constipation and abdominal pain (85). Our results showed no significant differences in changes over time (1Y-W13) between the groups in gastrointestinal symptoms. Furthermore, there were no significant changes over time within the probiotic group. However, the placebo groups had a significant decrease in constipation symptoms and a tendency towards an increase in diarrhoea symptoms (P=0.061) over time (1Y-W13). One explanation could be that the placebo capsules contains microcrystalline cellulose, which is a form of fibre. Fibre is one dietary component that can impact the composition of the GM (122). However, since the placebo capsules contained such a small amount of microcrystalline cellulose (0.3g), which is lesser than what was reported by another study (123), the amount used in this study should not have had an effect. However, we cannot be certain. The present study could not find that probiotics had a greater effect compared to placebo in gastrointestinal symptoms.

Strength and limitations

One of this study's strengths was that it was a double-blind randomised controlled trial, which is a study design that is preferred because of its ability to minimise potential bias. Furthermore, the measurements were performed using standardised protocols, which also minimises potential bias. We used air displacement plethysmography to measure body composition, which is seen as the gold standard, since it is based on the same principle as hydrostatic weighing (underwater weighing) and is validated (93). Our study also gave the participants updated information on their energy needs throughout the study, based on their measurement of RMR and PA. We followed-up the participants on a monthly basis, and measured BW frequently (at each follow-up visit), which is found to be associated with long-term WLM (27). Moreover, the close follow-up gives the opportunity to see the developmental changes in BW over time, making it possible to see where the two groups started to deviate from each other. In addition, the support provided during follow ups has been shown to be a key determinant for successful WLM (120).

This study was a 9-month intervention with the consumption of probiotics or placebo, which can be considered a long study duration and a strength of the study. Other studies typically have a duration of 12 weeks (48,50,51,107)**.** It is conceivable that we could have obtained a different result if this study only lasted for 12 weeks, which can be seen to some extent in Figure 3 (Appendix V). A 12 weeks length duration of supplementation of probiotics seems to be sufficient to produce changes in BW and FM in studies conducted in WL interventions. Sanchez

and colleagues (51) had only 12-week WLM phase, which is a short time compared to what the timeframe used in the definition by Wing and Hill (25) and also compared to the length of time used by our study.

Wang and colleagues (48), did a sub-analysis of studies using supplementation of probiotics with capsules/powder or food. They found that BW was significantly reduced in studies using capsules/powder compared to food, thus, suggesting that capsules/powder are the most beneficial form of probiotics used in the weight loss interventions. However, the number of studies that were compared were not equal (seven studies used capsules/powder and 3 studies used food). Our study gave the supplementation of probiotics in capsules, which may be more beneficial than supplementation though food.

The small sample size can be seen as a limitation of this study. Our study did not have the number of participants that was calculated to be necessary to have sufficient statistical power for our results. Hence, the results may have been different with a larger sample size.

Dietary intake has been shown to influence the GM composition and diversity (124,125). This study has taken into account the participants energy and protein intake given its association with BW. However, although the W13 measurements were taken after a wash-out period of the LED and all participants had consumed normal foods for 4 weeks prior to the probiotic/placebo intervention, the differences in the macronutrient composition of the diets under the WLM phase may have had an impact on the GM. This, in turn, may have also affected our results.

Attempts were made to control for the compliance of the capsules. Unfortunately, as participants did not report sufficient data on the number of capsules they did not consume, and the study did not establish strict protocols to help the participant remember to report back, and to provide instructions on what to do when capsules were not consumed, we were unable to address the compliance of capsule consumption in a satisfactory manner. Stool samples taken would be able to provide a measure of compliance with probiotic consumption. Additionally, it could have given the opportunity to investigate if there were differences between the groups in their GM composition at W13 and explore the effect of probiotics consumption on the participants GM. However, the analysis of the stool samples was not completed in time for the purposes of this master project.

Further studies

There are few studies investigating the effect of probiotics in relation to WLM. Therefore, overall, more research with a larger sample size is needed to be able to establish stronger conclusions on the impact of probiotics on WLM. As most studies only have a duration of 12 weeks, future studies should look into the effect of probiotics in a longer term and include faecal analysis of the GM composition and diversity, to see the effect of the probiotics used. Future studies should also investigate if other strains and combination of strains could have a better effect on WLM than ours. In addition, studies should take the participants' dietary composition into consideration when considering the effect of probiotics.

5.0 Conclusion

Strategies for successful WLM are important aspects for reversing the current increase in obesity and overweight seen today. This study provides new insight with respect to probiotics and WLM. This study could not find a beneficial effect of probiotics compared to placebo in terms of WLM. Our results show that the placebo group maintained their WL better than the probiotic group in the long-term. The exact mechanisms behind these findings remain to be uncovered. Probiotics were not found to have a more positive effect compared to placebo for gastrointestinal symptoms. More research is needed on the different types of bacterial strains and their effect on BW to recommend probiotics as strategy in obesity management.

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Appendix I. Study design.

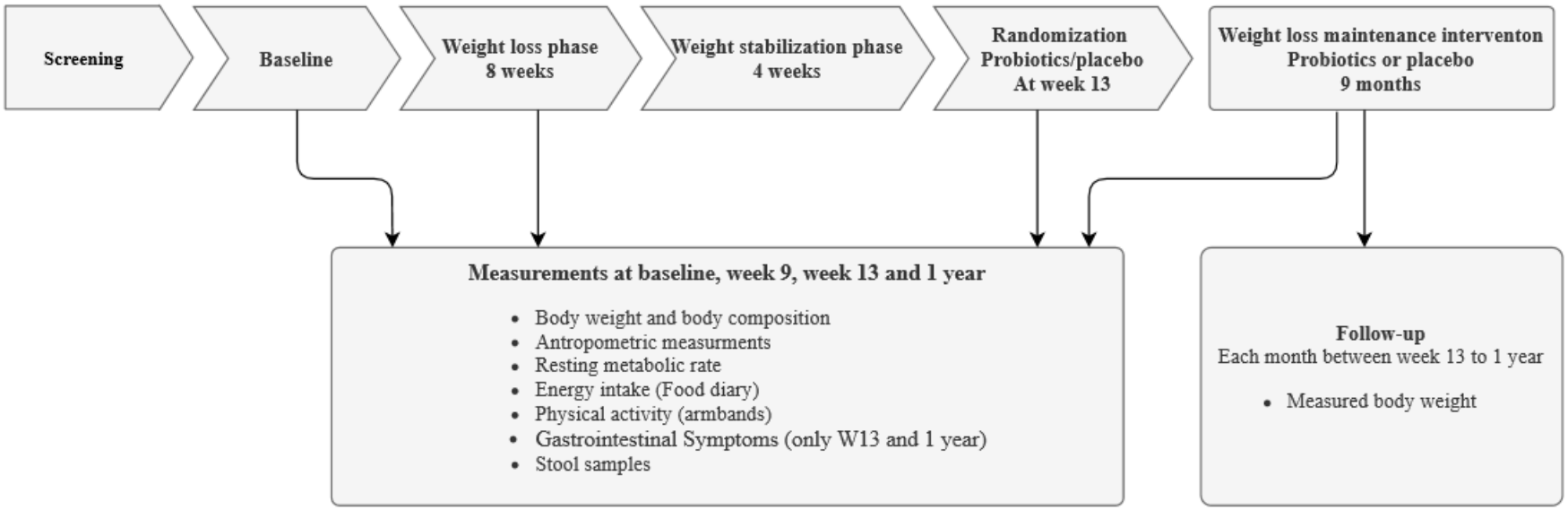


Figure 2. *Study design.*

Appendix II. Energy intake.

Table 5. Energy intake at week 13 and 1 year, and differences between groups (probiotic and placebo) over time (1 year-week 13).

	Probiotic (mean±SD)	**Placebo** (mean±SD)	**Diff. groups** (mean±SEM)
Week 13	**(n=20)**	**(n=23)**	**(n=20)**
Energy intake (kcal/day)	1749±314	1704±371	45±106
1 year	(n=27)	(n=27)	**(n=27)**
Energy intake (kcal/day)	1911±619	1742±498	169±153
Δ 1 year- week 13	**(n=20)**	**(n=22)**	**(n=20)**
Energy intake (kcal/day)	193±490	67±470	126±148

Diff, differences; n, number. Energy intake from self-reported food diary (average kcal intake per day).

Appendix III. Physical activity levels.

Table 6. Physical activity at week 13 and 1 year and differences between groups (probiotic and placebo) over time (1 year-week 13).

	Probiotic (mean±SD)	**Placebo** (mean±SD)	**Diff. groups** (mean±SEM)
Week 13	**(n=23)**	**(n=26)**	**(n=23)**
Total PA (min/day)	70.3±54.2	66.2±46.8	4.1±14.4
Total steps/day	7298.3±2300.1	7624.2±2312.0	325.9±666.2
PA sedentary (min/day)	1146.7±201.5	1102.4±94.9	44.3±44.2
PA light (min/day)	232.3±59.8	242.5±62.0	10.2±17.5
PA moderate (min/day)	68.4±53.6	64.5±44.9	3.9±14.1
PA vigorous (min/day)	2.1±5.5	1.3±3.6	0.9±1.3
PA very vigorous (min/day)	0.0±0.0	0.5±2.7	0.5±0.6
1 year	**(n=22)**	**(n=25)**	**(n=22)**
Total PA (min/day)	62.2±32.3	71.2±40.6	8.9±10.8
Total steps/day	7759.6±2242.8	7592.1±2452.7	167.5±689.0
PA sedentary (min/day)	1106.8±90.5	1138.1±86.8	31.3±25.9
PA light (min/day)	246.5±82.4	216.1±64.8	30.4±21.5
PA moderate (min/day)	59.8±29.5	67.8±38.6	8.0±10.1
PA vigorous (min/day)	2.3±4.7	2.6±5.7	0.3±1.5
PA very vigorous (min/day)	0.1±0.6	0.8±2.7	0.6±0.6
Δ 1 year - week13	**(n=20)**	**(n=23)**	**(n=20)**
Total PA (min/day)	-15.9±57.6	-1.2±40.2	14.6±15.0
Total steps/day	279.2±3210.1	262.2±2343.7	16.9±849.6
PA sedentary (min/day)	-46.2±254.0	28.0±94.9	74.2±57.0
PA light (min/day)	21.7±85.3	-16.5±60.6	38.2±22.3

Table 6. Continuing	**Probiotic** (mean±SD)	**Placebo** (mean±SD)	**Diff. groups** (mean±SEM)
PA moderate (min/day)	-15.7±57.0	-2.9±40.7	12.8±15.0
PA vigorous (min/day)	-0.7±4.6	1.4±4.1	2.1±1.3
PA very vigorous (min/day)	0.2±0.7	0.2±1.0	0.1±0.3

Data presented as mean±SD or mean±SEM. PA, physical activity; n, number; diff, differences.

Appendix IV. Correlation analysis of energy intake and physical activity.

Table 7. Correlation analysis between body weight and composition with energy intake (kcal/day) and total PA (min/day) at 1 year.

	Probiotic (n=27)		**Placebo** (n=27)	
	Energy intake r/rho	Total PA r/rho	Energy intake r/rho	Total PA r/rho
BW (kg)	0.269	0.153	0.373	-0.088
FM (kg)	0.338	-0.225	0.090	-0.593**
FM (%)	0.345	-0.438*	-0.166	-0.684**
FFM (kg)	0.143	0.338	0.393*	0.388
FFM (%)	-0.345	0.438*	0.166	0.684**

PA, physical activity; BW, body weight; FM, fat mass; FFM, fat free mass; r, Pearson or rho, spearman correlation; *P≤0.05; **P≤0.01.

Appendix V. Body weight over time.

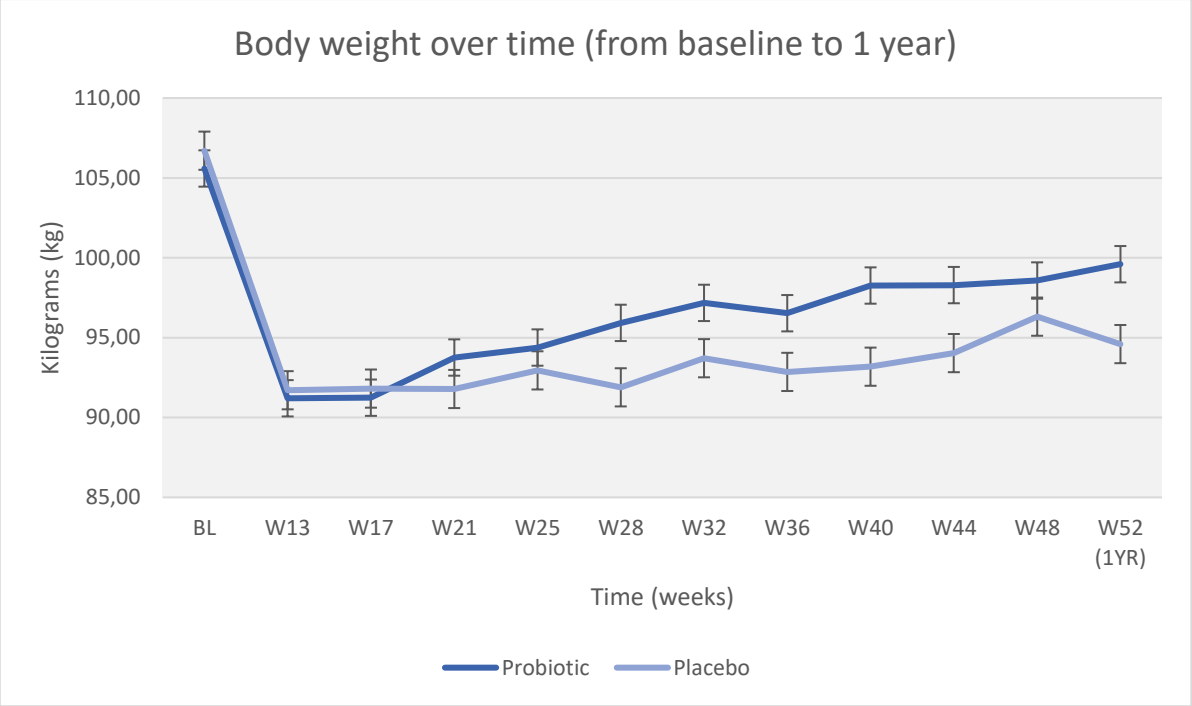


Figure 3*. Changes in body weight over time (from baseline to 1 year) for probiotic and placebo group. Result are presented as mean±SEM, BL, baseline; W#, week#; 1YR, 1 year.*

Appendix VI. Consent form.

Forespørsel om deltakelse i et forskningsprosjekt
Hvilken mengde karbohydrat kan man spise og samtidig redusere sult, men øke metthetsfølelse?

Bakgrunn og hensikt

Dette er en forespørsel til deg om å delta i en forskningsstudiet med utgangspunkt i en 8-ukers lavkaloridiett hvor karbohydrat inntaket vil variere mellom deltakerne etterfulgt av en 4 ukers fase hvor måleter 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 46770240.
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 hormonellprevensjonsmetoder.

Bakgrunn for studien
Lavkalori dietter er en relativt sikker metode for å gå ned i vekt og gir også et raskt vekttap. Slike dietter kan gi vekttap på 8-10% i løpet av 8 uker. Dette kan også gi bedring i overvekts relaterte sykdommer og risiko faktorer. Vi vet at lavkalori dietter som er lav på karbohydrater kan indusere ketose, en tilstand som antas å forårsake undertrykkelse av appetitt. Det antas at ketose oppstår når forbruket av karbohydrater er lavt. Det lave forbruket av karbohydrater fører ofte til en begrensning av matvarer som frukt, grønnsaker, melkeprodukter, helkorn/fullkorn og belgfrukter som er gunstig for en persons helse. Den maksimale mengden karbohydrater i en lavkalori diett som er forbundet med ketose er derimot ukjent. Mengden karbohydrater man kan

spise før man trigger appetittfølelsen, når man er i ketose, er også midlertidig usikkert. Det er behov for mer kunnskap om hvordan ketose fungerer, og hvordan vi kan innlemme mer karbohydrater i en lavkalori diett må undersøkes videre. Dessuten vet vi at probiotika kan hjelpe med vekttap, men få studier har sett på vekttap vedlikehold.

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

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

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

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

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

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

Tidsskjema for intervensjonsperioden (12 uker) - felles for alle
Du vil få utdelt et variert utvalg av mat/diett produkter (milkshakes, supper) tilsvarende et daglig energiinntak på 1000 kcal med forskjellige makro-næringsstoff fordeling. Du skal utelukkende spise disse produktene imens du er i diettens aktive fase (8 uker) (standardisert for alle), men du oppfordres til å drikke rikelig vann (minst 2,5 liter) og eventuelt kalorifri drikke i tillegg. Du vil så få time hos en forsker hver uke for ukentlig oppfølging. Gjennomgang av kostdagbok, veiing og urin-og avføringsprøver er en del av diettfasen. Overgangen fra diett-produkter til normal-kost vil skje gradvis i løpet av studieuke 9 og 10.

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

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

Kapittel B – Personvern, biobank, økonomi og forsikring

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

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

NTNU ved administrerende direktør er databehandlingsansvarlig.

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

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

Økonomi
Studien finansieres av midler fra NTNU.

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

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

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)
Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix VII. Food diary.

Matdagbok

A. Instruksjoner

Vennligst noter alt du spiser og drikker i løpet av dagen. Skriv ned så mange detaljer du klarer og hvis mulig bruk merkenavn. Ikke glem å notere tilberedningsmetode og bruk husholdningsmål (eks: dl, liten tallerken, teskje osv.) eller pakningsstørrelse for å beskrive mengden av mat/drikke du spiser/drikker (bruk kjøkkenvekt hvis du har tilgang til det). Dette blir du nødt til å gjøre i tre dager, inkludert minst én helgedag. Velg dager som representerer ditt vanlige mat- og drikkeinntak.

B. Eksempel

Dato	Tid	Mat/drikke	Tilberedning s-metode	Mengde (omtrentlig)
torsdag 23/01/2008	Frokost 7.30	Lettmelk Sukker Hvitt brød		1 glass 2 teskje 2 mellomstore skiver
	Snack 10.00	Banan		1 stor
	Lunsj 11.30	Grovt brød Smør Hvitost		4 skiver 1 spiseskje 4 tynne skiver
	Snack 15.00	Kaffe (filter) Sukker Kit Kat (sjokolade)		1 kopp 1 teskje 1 plate
	Middag 18.00	Laks Broccoli Poteter	Grillet Kokt Kokt	1 stort stykke 1 kopp 2 middels store
	Snack 21.00	Fruktyoghurt Digestivekjeks		1 liten boks 3 middels store

C. Din matdagbok

Dato	Tid	Mat/drikke	Tilberedningss-metode	Mengde (omtrentlig)
__/__/__				

Appendix VIII. User manual for activity armband.

Brukermanual for SenseWare armband

1. Armbånd & sensor tåler ikke vann, ta den av når du dusjer, bader (etc).
2. Elektromagnetiske forstyrrelser: skal du i CT-scan eller lignende må armbåndet tas av. Dette informerer som regel helsepersonell om.
3. Armbåndet skal være på minst 7 dager, ta det av etter den 8. dagen.
4. Armbåndet må være på hele døgnet - også når du sover. Tas kun av maks 1 time per dag (f.eks. når du dusjer).
5. Armbåndet skrur seg på når du tar det på, og skrur seg av når du tar det av. Du trenger hverken å trykke på sensoren eller lade den i den perioden du skal bruke båndet.
6. Tørk av synlig skitt eller svette i det tidsrommet du tar av armbåndet (max 1 time av per 24 timer).
7. Ta med deg båndet tilbake til oss neste gang du skal innom, men sørg for at du har brukt det sammenhengende i minst 7 dager før.
8. Bruk armbåndet på din *ikke-dominante* arm, dvs. er du høyrehendt, bruk den på venstre overarm.

Appendix IX. Description of the Gastrointestinal Symptom Rating Scale (GSRS).

The Gastrointestinal Symptom Rating Scale (GSRS)

The Gastrointestinal Symptom Rating Scale (GSRS) was originally constructed for measuring changes in psychopathology. On the basis of clinical experience and reports in the literature on gastrointestinal symptoms of patients with IBS (Irritable Bowel Syndrome) and PUD (Peptic Ulcer Disease), a selection of relevant items was made. The original questionnaire is an interview-based rating scale but has been modified to become a self-administered questionnaire.

Scoring The questionnaire, which contains 15 items, uses a seven-graded Likert scale, where 1 represents the most positive option and 7 the most negative one.

A mean value for the items in each dimension should be calculated:

Diarrhoea syndrome:

- 11. Increased passage of stools,
- 12. Loose stools,
- 14. Urgent need for defecation

Indigestion syndrome:

- 6. Borborygmus
- 7. Abdominal distension
- 8. Eructation
- 9. Increased flatus

Constipation syndrome:

- 10. Decreased passage of stools
- 13. Hard stools
- 15. Feeling of incomplete evacuation

Abdominal pain syndrome:

- Abdominal pain
- 4. Sucking sensations
- 5. Nausea and vomiting

Reflux syndrome:

- 2. Heartburn
- 3. Acid regurgitation

References

Svedlund J, Sjödin I, Dotevall G. GSRS - A clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci 1988;33:129-134.

Dimenäs E, Glise H, Hallerbäck B, Hernqvist H, Svedlund J, Wiklund I. Quality of Life in Patients with Upper Gastrointestinal Symptoms. An Improved Evaluation of Treatment Regimens? Scand J Gastroenterol 1993;28:681-687.

Dimenäs E, Glise H, Hallerbäck B, Hernqvist H, Svedlund J, Wiklund I. WellBeing and Gastrointestinal Symptoms among Patients Referred to Endoscopy Owing to Suspected Duodenal Ulcer. Scand J Gastroenterol 1995;30:10461052.

Missing values

If there is missing data for a patient in the GSRS questionnaire at an occasion and the missing data is less than 50 % of the item scores within a dimension, the missing items will be imputed using the mean score of the non-missing item scores. If more than 50 % of the item scores are missing, no imputation will be performed and the dimension score will be excluded from the analysis.

If a patient's participation in the study is discontinued, the value at the last available observation will be carried forward in the analysis.

Appendix X. Gastrointestinal symptom rating scale (GSRS)

GSRS – Ventrikkelmobilitet og diabetes

Les dette først:
Undersøkelsen inneholder spørsmål om forskjellige symptomer og om du har hatt slike symptomer i løpet av siste uken.
Sett kryss ved det alternativet som best passer for deg og din situasjon.

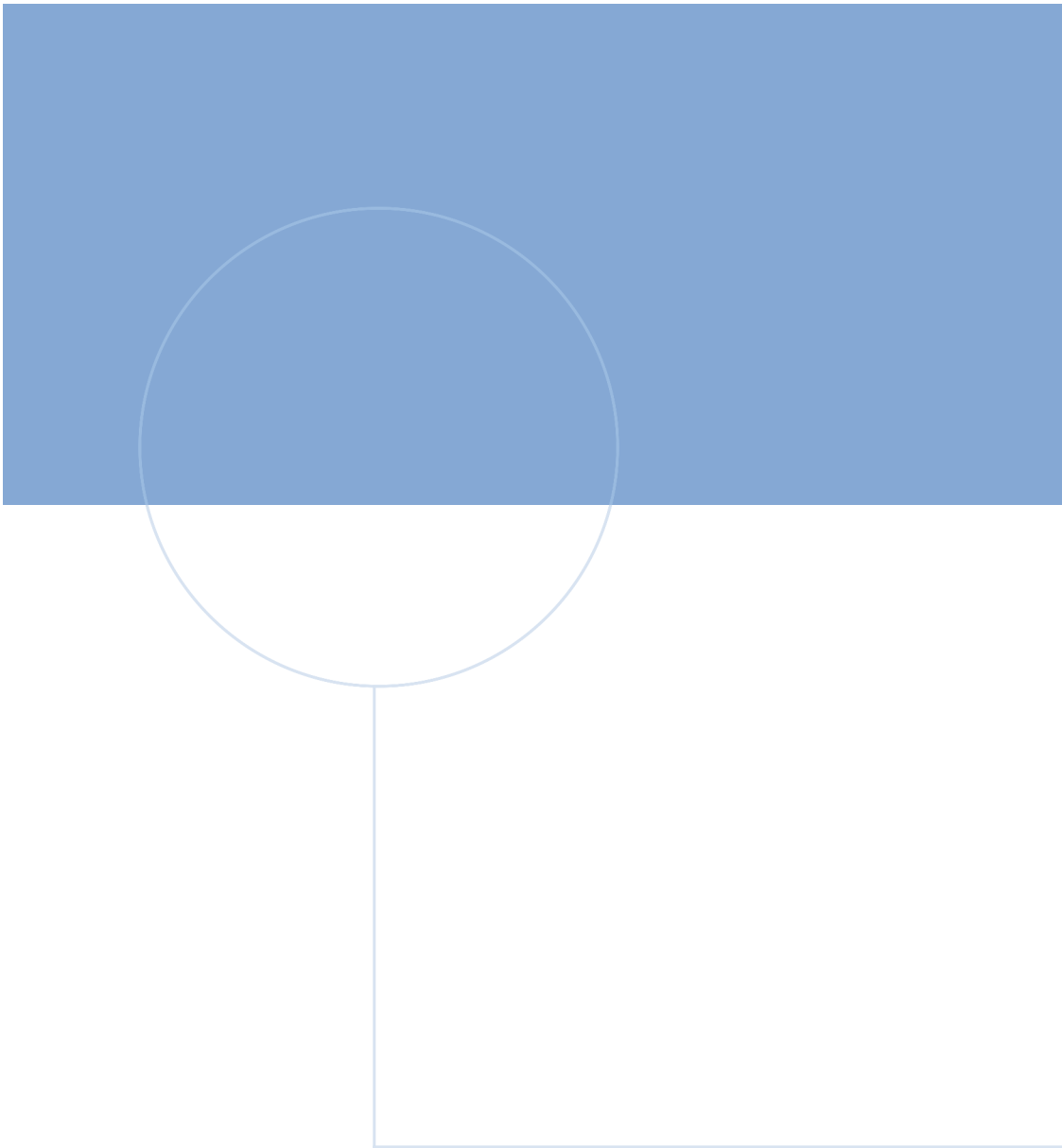
	Ingen plager i det hele tatt	Ubetydelige plager	Milde plager	Moderate plager	Ganske alvorlige plager	Alvorlige plager	Meget alvorlige plager
1. Har du i løpet av siste uken vært plaget med magesmerter?	☐	☐	☐	☐	☐	☐	☐
2. Har du i løpet av den siste uken vært plaget av *smerter eller ubehag i magen som ble lindret etter avføring?*	☐	☐	☐	☐	☐	☐	☐
3. Har du i løpet av den siste uken vært plaget av *halsbrann*? (Med halsbrann menes en sviende eller brennende følelse bak brystbeinet.)	☐	☐	☐	☐	☐	☐	☐
4. Har du i løpet av den siste uken vært plaget av *sure oppstøt*? (Med sure oppstøt menes plutselige oppstøt av surt mageinnhold.)	☐	☐	☐	☐	☐	☐	☐

	Ingen plager i det hele tatt	Ubetydelige plager	Milde plager	Moderate plager	Ganske alvorlige plager	Alvorlige plager	Meget alvorlige plager
5. Har du i løpet av den siste uken vært plaget av *vedvarende syresmak i munnen*?	☐	☐	☐	☐	☐	☐	☐
6. Har du i løpet av den siste uken vært plaget av *sug i magen*? Med sug i magen menes her en følelse av behov for å spise mellom måltidene.)	☐	☐	☐	☐	☐	☐	☐
7. Har du i løpet av den siste uken vært plaget av *kvalme*? (Med kvalme mener vi at du føler deg uvel.)	☐	☐	☐	☐	☐	☐	☐
8. Har du i løpet av den siste uken vært plaget av *rumling i magen*? (Med rumling i magen menes vibrasjoner eller "buldring" i magen.)	☐	☐	☐	☐	☐	☐	☐
9. Har du i løpet av siste uken vært plaget av *oppblåsthet*? (Med oppblåsthet menes utspiling, ofte forbundet med luft i magen.)	☐	☐	☐	☐	☐	☐	☐
10. Har du i løpet av siste uken vært plaget av *raping*? (Med raping menes behov for "utlufting", ofte forbundet med lindring av følelse av oppblåsthet.)	☐	☐	☐	☐	☐	☐	☐
11. Har du i løpet av siste uken vært plaget av *luftavgang*? (Med luftavgang menes her behov for å "slippe seg", ofte forbundet med lindring av følelse av oppblåsthet.)	☐	☐	☐	☐	☐	☐	☐

	Ingen plager i det hele tatt	Ubetydelige plager	Milde plager	Moderate plager	Ganske alvorlige plager	Alvorlige plager	Meget alvorlige plager
12. Har du i løpet av siste uken vært plaget av *forstoppelse*? (Med forstoppelse menes minsket avføringshyppighet.)	☐	☐	☐	☐	☐	☐	☐
13. Har du i løpet av siste uken vært plaget av *diaré*? (Med diaré menes økt avføringshyppighet.)	☐	☐	☐	☐	☐	☐	☐
14. Har du i løpet av siste uken vært plaget av *løs avføring*? (Hvis du har hatt vekslende hard og løs avføring, gjelder dette spørsmålet bare i hvilken utstrekning du har følt deg plaget av at avføringen har vært løs.)	☐	☐	☐	☐	☐	☐	☐
15. Har du i løpet av siste uken vært plaget av *hard avføring*? (Hvis du har hatt vekslende hard og løs avføring, gjelder dette spørsmålet bare i hvilken utstrekning du har følt deg plaget av at avføringen har vært hard.)	☐	☐	☐	☐	☐	☐	☐
16. Har du i løpet av siste uken vært plaget av *tvingende avføringsbehov*? (Med tvingende avføringsbehov menes raskt oppståtte behov for å gå på toalettet, ofte forbundet med en følelse av mangelfull kontroll.)	☐	☐	☐	☐	☐	☐	☐

	Ingen plager i det hele tatt	Ubetydelige plager	Milde plager	Moderate plager	Ganske alvorlige plager	Alvorlige plager	Meget alvorlige plager
17. Har du i løpet av den siste uken i forbindelse med *avføring hatt en følelse av ufullstendig tømming i tarmen*? (Med ufullstendig tømming av tarmen menes at det trass i anstrengelser i forbindelse med avføring gjenstår en følelse av ufullstendig tømming?)	☐	☐	☐	☐	☐	☐	☐
18. Har du i løpet av den siste uken vært plaget av *slim i avføringen*?	☐	☐	☐	☐	☐	☐	☐
19. Har du i løpet av den siste uken vært plaget av *at du føler deg mett kort tid etter at du har begynt å spise*?	☐	☐	☐	☐	☐	☐	☐
20. Har du i løpet av den siste uken vært plaget av *at du føler deg mett lenge etter at du har avsluttet måltidet*?	☐	☐	☐	☐	☐	☐	☐
21. Har du i løpet av den siste uken vært plaget av *brekninger* (mislykkede forsøk på å kaste opp)?	☐	☐	☐	☐	☐	☐	☐
22. Har du i løpet av siste uken vært plaget av *oppkast*?	☐	☐	☐	☐	☐	☐	☐

	Ingen plager i det hele tatt	Ubetydelige plager	Milde plager	Moderate plager	Ganske alvorlige plager	Alvorlige plager	Meget alvorlige plager
23. Har du i løpet av den siste uken vært plaget av *synlig utspiling*? (Med synlig utspiling menes økning i livvidden, slik at du noen ganger må løsne på beltet, buksen eller skjorten.)	☐	☐	☐	☐	☐	☐	☐
24. Har du i løpet av siste uken vært plaget av *overfølsomhet overfor enkelte typer matvarer*?	☐	☐	☐	☐	☐	☐	☐



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