The effects of cytotoxicity and diet on colonic health and colorectal cancer risk after bariatric surgery

Master Thesis in Clinical Nutrition

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June 2020

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Acknowledgements

The project of which this master thesis is a part of was initiated by Prof. Dr. Kristin Verbeke, at Laboratory of digestion and absorption, through a collaboration with Prof. Dr. Bart Van Der Schueren and Dr. Matthias Lannoo at Obesity Clinic, UZ Leuven, Leuven. Principal investigator was Prof. Dr. Kristin Verbeke, and executing investigator Charlotte Evenepoel, who was also my primary supervisor.

Charlotte Evenepoel, thank you for letting me have insight in this impressive research environment, for sharing with me all your knowledge, and for countless of laboratory demonstrations.

This year abroad has taught me a lot, which I am so thankful for.

To the lab technicians and other researchers in the same laboratories, thank you for always helping me out in frustrated situations, and for answering all my questions.

To my secondary supervisor Hanna Fjeldheim Dale, thank you for stepping in, and giving me much needed guidance towards the end.

To all the participants, thank you for your time and effort.

Finally, my deepest gratitude goes to my closest supporters.

Thank you, Kristina for sharing this exchange year with me. For our good cooperation, and newfound friendship. I am so grateful.

Thank you, Heidi, for taking the time.

Thank you Oddne, for your endless support and motivational speeches when I needed it the most. You have a huge part in this.

Abstract

Background: Obesity is an increasing health problem worldwide, and is associated with severe negative health consequences such as increased risk of different types of cancer, including colorectal cancer. Bariatric surgery (BS) is one of the most used therapies to treat morbid obesity. Along with the reduced weight comes several positive health consequences, including reduced risk of obesity related cancers. However, some preliminary data are showing an increased risk of colorectal cancer after BS. Different theories connected to the metabolically changes after BS are suggested to explain this.

Aim and methods: This thesis was a part of a project where the aim was to evaluate the effect of weight loss on the gut environment. The overall aim of this thesis was to evaluate changes in faecal water (FW) cytotoxicity, diet and stool consistency in patients after BS. Three groups of patients were recruited to a 1-year follow-up study including 5 study visits. One group underwent Roux-en-Y-gastric-bypass (RYGB), the second group underwent sleeve gastrectomy (SG), and the third group were obese, but otherwise healthy individuals serving as controls. FW cytotoxicity was determined by use of Water-soluble tetrazolium salt-1 assay. Energy and macronutrient intake were registered for one week before visits by the patients using MyFitnessPal.

Results: Nine participants were included in the cytotoxicity analysis (5 BS and 4 control). Cytotoxicity as measured by the inhibitory concentration-50 levels were observed to be higher among the BS patients compared to the participants in the control group. In total sixty-seven participants (30 RYGB, 20 SG, 17 control) were included in the energy and macronutrient analysis. Energy, carbohydrate and fat intake were decreased in all three groups at 12 months compared with baseline. Protein intake in contrast, was increased between baseline and 12 months for the SG group, and decreased in the two remaining groups.

Conclusion: In conclusion, a trend of higher cytotoxicity levels was seen in BS patients compared to healthy, obese controls. The cytotoxicity gradually increased between 2 weeks and 12 months after surgery in the BS group. The energy and macronutrient analysis showed a difference in the overall energy, fat and carbohydrate intake between both RYGB and control, and SG and control.

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List of abbreviations

WHO	World Health Organization
BMI	Body mass index
CRC	Colorectal cancer
BS	Bariatric surgery
RYGB	Roux-en-Y-gastric-bypass
SG	Sleeve gastrectomy
SOS	Swedish obesity study
GI	Gastro intestinal
BA	Bile acid
FW	Faecal water
UC	Ulcerous colitis
WST	Water-soluble tetrazolium salt
CTR	Control
TARGID	Translational research centre for gastrointestinal disorders
RPM	Rounds per minute
MFP	My fitness pal
DW	Dry weight
BSS	Bristol Stool Score
IC	Inhibitory concentration

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1. Introduction

1.1 Overweight and obesity

The increasing prevalence of obesity among both adults and children has become an widespread health problem worldwide (1). Since 1975, the prevalence of obesity has nearly tripled, and in 2016 over 650 million adults were obese (2). The World Health Organization (WHO) defines overweight as a body mass index (BMI) of 25 or higher, while obesity is defined as a BMI equal to or higher than 30 (2). Excessive fat accumulation is associated with serious health consequences, increased risk of colorectal cancer (CRC) being one of them (3). Of note, overweight and obesity is largely preventable, and to some degree reversible by different types of therapy. The go-to strategy of weight loss in Europe is the multicomponent lifestyle intervention (4). This includes the simultaneously implementation of behavioural training, dietary change to reduce the energy intake, and an increase in physical activity. For patients who fail to achieve, and sustain weight loss from the latter approach, there can be indications for adding pharmacotherapy (4).

1.2 Bariatric surgery

Bariatric surgery (BS) is one of the most common methods to induce weight reduction in morbidly obese patients, and is associated with durable and effective weight loss. (5-7). Patients with morbid obesity (BMI≥40.0 kg/m_2), or with comorbidities and with BMI≥35 kg/m_2 are candidates for BS (8). In 2013, 468.609 BS were performed worldwide where Roux-en-Y-Gastric-Bypass (RYGB) was applied in the majority of these cases, followed by Sleeve Gastrectomy (SG) (9). Weight loss after BS is associated with lower mortality rates, and a reduced prevalence of multiple lifestyle-related diseases (10, 11). The latter was shown in a large study that examined whether lower mortality was associated with weight loss induced by BS in Swedish obese individuals (The Swedish Obese subjects study (SOS)) (11). The study involved 4047 obese subjects: 2010 underwent BS, and 2037 received conventional treatment.

1.2.1 Bariatric procedures

Two of the most commonly used bariatric procedures, are RYGB and SG (9). In RYGB a large part of the stomach is removed from nutrient contact, creating a small stomach pouch of approximately 30ml. The distal part of the jejunum is connected to this pouch, where the length of this can vary. In contrast to RYGB, there are no anatomical rearrangements of the intestines

when performing a SG. In this procedure, approximately 70% of the stomach is permanently removed, only the gastric sleeve remains (5). The procedures of RYGB and SG are shown in Figure 1.

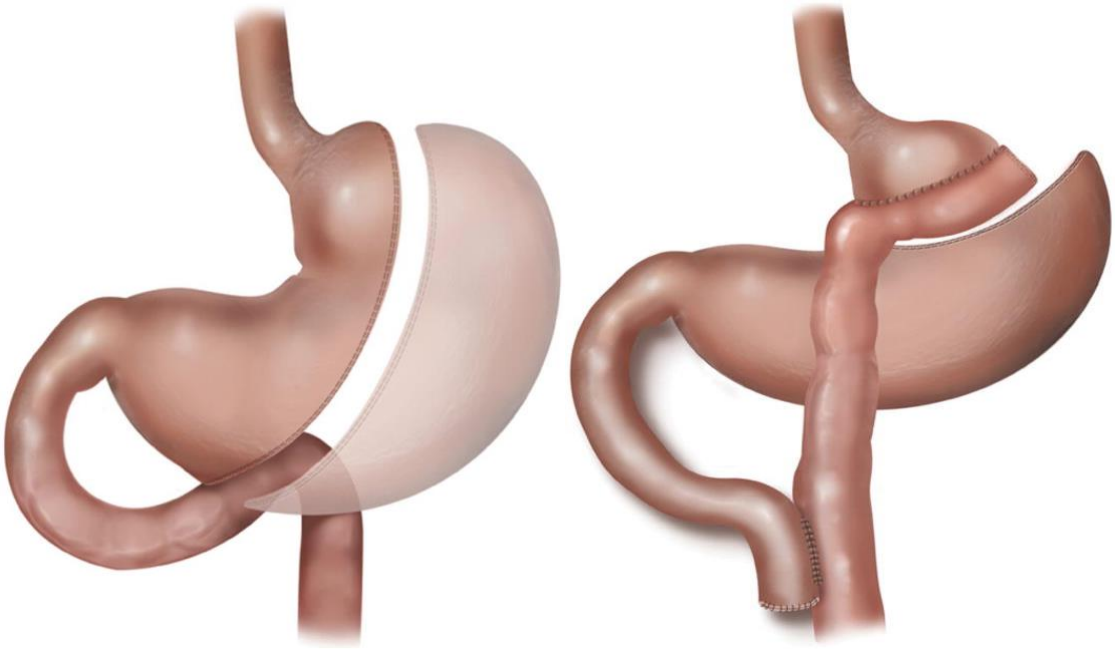


Figure 1A: Vertical sleeve gastrectomy (5). **Figure 1B:** Roux-en-Y-gastric-bypass (5).

RYGB and SG are combined restrictive and malabsorptive procedures (5). These procedures are restrictive in the way that the calorie intake must be adapted to the reduction of the stomach. Malabsorption comes from a reduced absorption area, decreased gastrointestinal (GI) secretions and diversion of nutrients from the duodenum (12). The duodenum is not effected in SG and is because of this regarded as less malabsorptive compared to RYGB. SG is also categorized as only restrictive by some (12).

1.2.2 Metabolic effects after bariatric surgery

BS is often called metabolic surgery, because of the multiple beneficial effects on the general metabolic state (13). This is thought to arise from changes in pancreatic hormones, gut hormones, bile acids (BAs) metabolism and the gut microbiome (7). For a long period, weight loss was considered as a sole reason for the beneficial health effects post BS. However, the positive health effects arising from BS can only be partly explained as a result of the induced weight loss (14). It is of note interesting that a large part of the post-operative metabolic changes take place before weight loss occurs (13). However, weight loss itself is also a contributor of

metabolically changes, as for example changes in peptide hormone secretion (15). The fact that both BS itself, and the weight loss that follows can cause metabolic changes makes it difficult to detect the cause of effect.

Some of the positive metabolic effects occurring after BS are including an increase in several peptide gut hormones involved in promoting satiety (13). The pancreatic peptide hormone insulin is suggested to be involved in the early positive metabolic effects post BS (16). Insulin sensitivity seems to improve after RYGB, and fasting insulin levels are found to decrease short time after both SG and RYGB. Improved beta cell function is proposed as a part of the explanation for the ability of BS in type 2 diabetes remission (16-18).

Altered BA metabolism and intestinal microbiota composition has frequently been suggested as contributors to the not-weight loss related metabolically effects after BS (19, 20). The results in studies looking at changes in BAs in association with BS are different in terms of the different BS procedures (5) (21). Research has shown to be more consistent in finding when it comes to changes in BAs after RYGB compared to other procedures (5). Most studies have concluded with increased fasting or postprandial total BA concentrations following RYGB (5, 22, 23). For SG, the results are more conflicting with some studies showing an increase of total BA concentration post-surgery compared with baseline (19, 24), while others are showing no increase (21, 25).

The gut microbiota are shown to change after BS (26, 27). The gastric restriction or rearrangement of the intestinal tract during a RYGB or SG can lead to changes in the intestinal microorganism pattern (28). This could be the result of reduced gastric acid production, causing an increased pH level in the colon. An incompletely digested diet as a result of the rearrangement of the intestinal tract, in addition to the change in digestive enzymes, could also possibly alter the gut environment, and effect the composition of the intestinal bacteria (28). Among other functions, microbiota plays a key role in modulating BAs. In the colon, primary BAs are deconjugated and further transformed into secondary Bas by bacteria (28).

1.2.3 Side effects and negative effects of bariatric surgery

Even though BS reduces overall mortality and morbidity, several negative consequences can occur after surgery. A Danish survey conducted in 2238 RYGB patients, examined surgical, medical and nutritional symptoms after BS, and the association with quality of life. 88.6% of

the patients reported one or more symptoms short term after surgery, where nearly one third was at some point hospitalized. The most frequently reported complaints were fatigue (54.3%), dumping (52.4%), and abdominal pain (54.4%) However, most patients reported improved wellbeing (29).

GI problems seems to be among the most frequently reported short-term side effects after different BS procedures (30). Constipation and diarrhoea are some of the most commonly GI problems reported (31). It is often difficult to determine if the complications can be directly related to the surgery, or to the change of diet that is associated with BS. Especially the liquid restrictions can off note have an effect on the constipation. It is difficult to counteract constipation when patients are only allowed to drink limited amounts, in addition to the possibility of being hindered because of discomfort, due to their restricted GI system.

1.2.4 Bariatric surgery and food intake

BS is an option for those where lifestyle- and medication-based approaches have been proven ineffective (32). To lose weight after BS and maintain weight loss its crucial to continuously restrict the energy intake. After BS, patients are asked to follow a staged progression diet starting with only liquids, and thereafter slowly adapt to solid food intake again (33). At 3 months post-surgery, patients are encouraged obtain a normal diet so that they can adapt to their new GI system. It is crucial for the patients to consume small and frequent, rather than bigger meals, to adjust to their new GI system, and avoid discomfort (33). Dumping syndrome can be a consequence, if these recommendations are not followed (34). Early dumping occurs within 1 hour after eating, and is caused by rapid fluid shifts and release of GI hormones into the intestinal lumen. The result can be contractions, pain, bloating and diarrhoea (34). Other important factors that should be addressed to avoid discomfort during the first period after BS, are separation of liquids from solid food, and intake of liquids in small portions. When the patient has returned to a solid diet, its highly recommended that simple carbohydrates are avoided at best efforts. Patients are encouraged to gradually increase the intake of foods rich in dietary fibre (35).

The energy intake is normally decreased short time after BS, followed by an increase at a later point of time (33, 36). The post-operative diet generally provides a very low caloric intake (500-800 kcal/day). This is usually followed the first weeks after surgery (33). Although current

evidence is conflicting when it comes to the long-term results of BS, a lot of patients seems to stick to a diet with a lower caloric intake post-surgery compared with baseline (37). This is in accordance with the fact that BS overall is effective in terms of weight loss (11).

As for the average population, the general dietary advices are highly relevant for BS patients, with some adaptations to customize the diet after the specific procedure. It is difficult to give individual recommendations for energy intake in general, due to great variations in the degree of physical activity, body composition and resting metabolism. An estimation of daily energy consumption for an inactive female is 2150 kcal, and 2600 for an inactive male. The Norwegian recommendations for intake-distribution of macronutrient energy percentage is 45-60 for carbohydrates, 25-40 for fat, and 10-20 for protein (38). By using estimated recommended daily nutrient consumption for an inactive man (2600 kcal), one can calculate estimated macronutrient recommendations. That gives us that the estimated recommended macronutrient intake for a man will approximately be between: 293-390 grams of carbohydrates, 72-116 grams of fat, and 65-130 grams of protein.

1.3 Colorectal cancer

CRC is an umbrella term for cancer types that starting in the colon and rectum, and they are often grouped together due to many of the overlapping features. Most CRC debuts as growths on the inner lining of the colon or rectum, known as polyps (21). The most common type is adenocarcinoma, accounting for about 96% of CRC cancers. Carcinoid tumours, GI stromal tumours, lymphomas, and sarcomas are other less common types of CRC (21).

CRC is the second most commonly diagnosed cancer form in women, and the third most common in men. The risk is higher in the Western parts of the world (Europe, Australia, United states, New Zealand), compared to countries in Africa and Asia (39). Diet and lifestyle are stated as important factors for the geographical differences (40). During the past decades, CRC has become a huge public health problem, and the prevalence of CRC is expected to further increase in the following years. Today, CRC risk accounts for approximately 10% of all cancer related mortality (41). In addition, the proportion of young adults getting diagnosed with CRC are increasing, even though the CRC risk is higher with increased age (42). Indeed, 75% of the people with rectal cancer and 80% of the people with colon cancer is older than 60

years at the time of diagnosis (41). Off note, about 90% of CRC causes occurs without any familiar history or genetic predisposition.

1.3.1 CRC risk, diet and lifestyle

The risk of having CRC is mainly associated with an unhealthy lifestyle, including increased BMI, smoking, low physical activity, low vegetable and fruit consumption and increased red meat intake (39, 42). Overall 16 % of new CRC cases have been shown to be preventable if all the potentially modifiable healthy lifestyle factors had a minimum impact. This includes, healthy weight, physical activity, smoking, alcohol consumption, and diet. The association was observed to be stronger among men than among women (43).

The relationship between red meat intake and CRC is well established as the evidence for an increased risk of CRC from consuming red and processed meat is strong (44). Several possible mechanisms have been suggested for this association, but the exact mechanisms underlying is still uncertain (45). Meat intake increases protein fermentation, as well as inducing increased intake of fat, heme and heterocyclic amines, which are suggested to play a role in CRC development (46).

On the other hand, carbohydrate fermentation is generally accepted as beneficial for the host due to the generation of short chain fatty acids (46). Dietary fibre intake has been linked to reduced risk of colon cancer (47). There is also strong evidence that intake of wholegrains decreases the risk of CRC (48). Suggested mechanisms behind this includes that wholegrains have a protective effect by binding carcinogens and regulating glycaemic response (48). Specific compounds in wholegrains have also been shown to stimulate anti-oxidative activity in experimental studies (48).

1.3.2 Colorectal cancer risk and bariatric surgery

Obesity is related to an overall increased risk of cancer, and higher amounts of body fat are associated with increased risk of a number of specific types of cancers, including CRC (49). Several studies are suggesting a decreased risk of obesity related cancers in general after BS (6, 10, 50, 51).

Even though the overall cancer risk seems to be reduced after BS, this is not the case for certain specific types of cancer, meaning that the weight reduction following BS might not lead to a decrease in all obesity-related cancers. Several studies have concluded with a decreased overall cancer risk, but with an elevated risk of CRC after BS (52, 53). The risk is seen to increase over time after BS (53, 54).

So far, it can be concluded that the results in studies exploring the change in CRC risk after BS are conflicting. While some studies suggest an increase in CRC risk following BS, other studies identified a decreased risk for all obesity-related cancers specifically, including CRC in the period after BS (10, 55).

Possible mechanisms that link BS to an increased risk of CRC are associated with the metabolic changes after surgery. A suggested link between BAs and CRC is the alteration of the intestinal epithelium caused by the secondary BAs, which are implicated as colon cancer promotors that has shown to be cytotoxic to colonic epithelial cells (56). There are still a lot of unanswered questions regarding the pathways involved. One theory is that BAs alter the stability of the membrane lipid bilayer, due to their detergent properties. The structure of the membrane is more easily damaged by BAs with increased hydrophobicity (57). Additionally, the role of BA-microbiota crosstalk in GI-cancer has gotten more attention recently. The gut microbiota can transform intestinal BAs to their unconjugated forms, which are seen to be more carcinogenic (58). In this way, the change in microbiota after BS can affect the CRC risk through interaction with the BAs.

1.4 Cytotoxicity

Cytotoxicity refers to the ability of a certain compound of being toxic to cells (59). When cells are exposed to a cytotoxic compound, different responses such as ceased cell growth and/or a halt in active division of cells (a decrease in cell proliferation) can occur. In more serious cases, the cell can undergo necrosis, apoptosis or autophagy.

Diet related faecal cytotoxicity is correlated with risk of colon cancer (60). This is partly through compounds from processed and red meat (45). Lately there has also been more focus on protein fermentation and CRC risk. Protein fermentation is considered detrimental for the

host's health (46). The link between protein fermentation and CRC has been emphasized in particular due to protein being a major constituent of meat (46). In addition to this, it has been observed that protein fermentation becomes more dominant in the distal colon, which is the area most affected by disease (61).

In a study conducted on FW cytotoxicity in ulcerous colitis (UC) patients, results showed that FW from the UC patients was significantly more cytotoxic than FW from health controls. Here, higher FW cytotoxicity was associated with specific protein fermentation metabolites, as well as lower levels of medium and short chain fatty acids (62).

Measurement of cytotoxicity can play an important role when studying the potential effects of a substrate on human cells. Cytotoxicity can be measured in several different ways. The water-soluble tetrazolium salt (WST)-1 cell assay is one of the most used methods to measure cell viability. The principle is the reduction of the tetrazolium salt WST-1 to the yellow colored formazan by cellular dehydrogenases. Formazan production is quantified by measuring UV-absorption at 120-480 nm which reflects the level of mitochondrial activity. If a cell is dead, no such activity will be left. In this way the number of living cells are counted indirectly (59).

With regard to the risk of CRC, faecal content examination is a non-invasive way of studying exposures to the colorectal mucosa, which can give us a lot of information. Feces represents the bacterial metabolism in the colon (60). Feces is composed of water, protein, undigested fats, polysaccharides, bacterial biomass, ash and undigested food residues (63).

As of today, there has only been performed animal studies looking at FW cytotoxicity after BS, and knowledge on FW cytotoxicity after BS in human subjects is limited. In a study that measured the toxicity in the FW of RYGB-operated rats, the results showed an obvious increase in the cytotoxicity of the FW post-surgery. The study included faecal samples from 12 rats at 2 and 8 weeks post-surgery (64).

2. Aim of the study

2.1 Aim

This thesis was written as a part of a larger research project, where the overall aim was to study the effect of BS on the colonic health and gut environment. The hypothesis was that possible changes in the colonic environment after BS can affect colonic health after surgery. These changes are thought to be different for RYGB than for SG, as proximally to the colon, distinct anatomical changes are induced after these surgery types. Further on, to underpin the overall aim of the research project, the objective of this thesis was to evaluate changes in faecal water cytotoxicity, diet and stool consistency in patients who underwent bariatric surgery.

2.2 Research questions

This master project had 4 specific research questions:

1. How does the FW cytotoxicity change from before BS to one year after?
2. How does the macronutrient composition change in the BS patients from before surgery to one year after surgery?
3. How is the stool consistency changed in the one year period after surgery?
4. Can the cytotoxicity analysis protocol (WST-1 assay) be more effective by changing the samples from triplicate to duplicate, without losing the accuracy?

3. Material and methods

Collection and analysis of the data in this thesis were completed and obtained in the period between the 12th of August 2019 and the 13th Of March 2020.

3.1 Study design

The project was a prospective 1-year follow-up study, including 2 groups of BS patients and 1 group of obese controls, with a goal to include in total 195 participants, 65 per group. The BS patients had prior to surgery their first of in total 5 visits for the study. The other 4 clinical visits were planned after 2 weeks, 3 months, 6 months, and 1 year after surgery. The control (CTR) group followed the same visit schedule, with baseline (when included), 2 weeks, 3 months, 6 months and 1 year (**Figure 2**).

The patient's visits were, if possible, synchronized with consultations at the doctor/dietician in the hospital. All participants received a stool collection kit for each visit (**Appendix 2**). During the patient visit, 4 blood samples of 25 ml were drawn, of which one were immediately placed on ice. In addition, a faecal sample, a general health questionnaire, a 7-days dietary record and a 7-days stool diary (rating of consistency) were brought to the visit (**Appendix 1**). Medication and pre- and probiotics use were questioned at each visit.

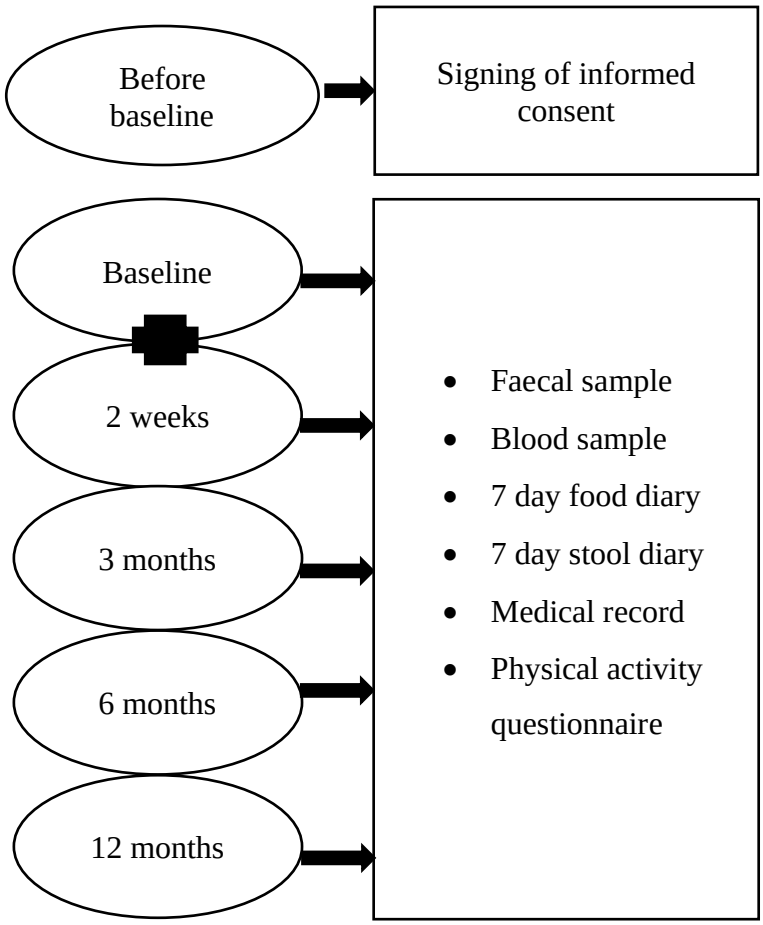


Figure 2: Overview study visits

3.2 Study population

The study included 3 groups. The BS participants were recruited into 2 groups, one group with patients that should undergo RYGB, and one with patients referred to SG. A multidisciplinary team consisting of a surgeon, endocrinologist, psychologist and dietician from the obesity clinic (UZ Leuven) decided what procedure (SG or RYGB) to perform on individual patients. The third group of participants was the CTR group. This group included obese, but otherwise healthy participants on a weight loss diet, through a group program at the Obesity clinic of UZ Leuven. The program consisted of 15 sessions of behavioural therapy and diet counselling in a period of 12 months. The cytotoxicity analysis included 2 groups. One group consisting of RYGB and SG patients, and one group consisting of CTR participants.

3.2.1 Recruitment

The BS surgery participants were recruited at the obesity Clinic of UZ Leuven (Leuven, Belgium) by Charlotte Evenepoel (PhD-candidate) in collaboration with Dr. Lannoo and Prof. Dr. Van Der Schueren. In addition, BS participants were recruited at a second hospital i.e AZ Diest (Diest, Belgium). Here the stool samples were delivered at the lab, and blood samples from patients were taken by the clinical biologist at site. This subset participants were also operated by DR. Lannoo. The group of obese but otherwise healthy participants were recruited at the Obesity Clinic of UZ Leuven on their introduction session, also by Charlotte Evenepoel (PhD-candidate).

3.2.2 Inclusion criteria

For the BS participants, the standard criteria for BS should be fulfilled: BMI≥40 kg/m_2 or 35 kg/m_2 in combination with either obstructive sleep apnoea syndrome, severe high blood pressure not treatable with 3 types of medication, or type 2 diabetes. In addition, other underlying causes of obesity were to be excluded by an endocrinologist. Furthermore, the patient had to have tried loosing weight in a non-surgical way for at least 1 year without positive results. People under the age of 18 years were not regarded as suitable for surgery. Additional inclusion criteria were no previous major abdominal surgery in the past, and no GI problems. During the last month, and the 2 weeks before the study, all participants were required to not antibiotics or pre-and probiotics, respectively. For the control group, criteria for inclusion were a BMI≥30 kg/ m_2. Subjects with a history of GI problems or abdominal surgery could not participate.

3.2.3 Subgroups for specific analysis

Different numbers of participants were included in each analysis performed for this thesis. This was due to the timeframe of the project, missing values and unpredicted challenges in the experiments. The number of included participants is clarified at the beginning of the current subchapter in the results for each specific analysis.

3.3 Sample processing

After the participant's visits, collected samples and documents were brought back to the laboratory at translational research centre for gastrointestinal disorders (TARGID). All samples from all participants were labelled with the necessary information: Patient ID, visit, name of

study, date, and type of sample. Information about the processing of the blood and stool samples for each patient were registered and saved both on paper, and in digital format.

3.3.1 Stool samples

3.3.1.1 Handling of faecal samples

At each visit, the participants brought a stool sample, collected in a pot, and one divimat with a aliquote from the same sample. The divimat is a soft plastic mat for collecting small pellets of faeces (**Appendix 2**) In their stool diary, the participants had to rate their stool consistency for 7 days with a score from 1-7 (where 1 equals hard, and 7 is liquid) using the validated Bristol Stool Form Scale.

Stool sample pot

The plastic pot with the faecal sample was stored in the fridge at 4°C until processed. Further processing had to be done within 7 days after the sample was collected by the participant. Upon processing, the faecal sample was distributed into tubes and ultra-centrifuged at 22000 rounds per minute (RPM), for 2 hours, at 4°C. After the centrifugation, the FW was collected into a plastic tube (Sarstedt AG & Co) and vortexed. This was to ensure that the content was homogenized. Finally, the FW was aliquoted into the 4*2mL (in some cases less) Eppendorf tubes (Sarstedt AG & Co) and placed on -80°C until further analysis.

Faecal dry weight

Before aliquoting the divimats, Eppendorf tubes (Sarstedt AG & Co) for measuring dry substance were weighted when empty. After the participants visit, the divimat was immediately stored in a freezer. The frozen pellets were aliquoted separated into 4 different plastic tubes, used for different measures. After aliquoting, the filled tubes were again weighted. Further on, after freeze-drying, the Eppendorf tubes were weighted for the third, and last time. To calculate the amount of dry substance (faecal dry weight) in the stool samples, a lyophilizer (CHRIST) was used to freeze-dry the samples. Samples were placed in small hard-plastic cups with 4 filled Eppendorf tubes in each cup. All the tubes were opened, and the hard-plastic cup was then covered by a cotton pad held in place by a rubber band. Thereafter, samples were frozen before placed in the lyophilizer for 72 hours.

Filtering faecal water

The FW was filtered before performing the cytotoxicity test to remove solid material, bacteria and other living microorganisms. Filtration was done through a two-step process; first step including using a 0.8µl, followed by a 0.2µl filter (Sarstedt AG & Co). After defrosting, the samples were centrifuged for 5 minutes, at 5000 RPM. In the next step, the FW was poured into a new Eppendorf tube (Sarstedt AG & Co) to obtain supernatans and remove the pellet collected at the bottom of the tube. Filtering was done by pushing all the FW through the filter by using a 10mL syringe (Henke Sass Wolf). One filter of each size, and one syringe per patient sample were used. The samples with a higher viscosity gave more resistance when going through the filter, thus more time consuming. Some of the samples with the highest viscosity needed to be partitioned into 2 filters. After filtering through both filters sizes, FW was stored in at -80°C until further analysis.

3.4 Cytotoxicity measurement of faecal water

3.4.1 Cell culture

Human colonic adenocarcinoma HT-29 cells used were obtained from ECACC (European Collection of Cell Cultures), grown in RPMI-1640 medium (ThermoFisher), with fetal bovine serum (50mL per RPMI medium flask, (in-house)), and antibiotics (gentamicin sulphate, 250µl per RPMI medium flask, (Gentauer)), at 37 °C and with 5% CO_2. Cells were defrosted at passage 147. The cells were counted using countess cell counter (Invitrogen). The cells were split, and the medium changed twice a week throughout the whole experiment.

3.4.2 WST-1 Assay

Cytotoxicity of the FW was measured using a WST-1 assay. Before incubating the cells with FW, they were loosened from the culture bottle, counted, pipetted into a 96-well plate (10_4 cells per plate) with flat bottom, and kept in the incubator for 24 hours. Afterwards, the cells were exposed to serial dilutions (1/4-1/508) of FW samples in fresh medium, and then placed in the incubator (37 °C, 5% CO_2) for 72 hours. At 71 hours of incubation, Trition X-100 was added as positive control and medium was used as negative control. At 72 hours, the reaction was stopped by splashing the plates as much as needed. Next, 10-mL (per plate), tetrazolium salt 4-[3-[4-iodophenyl]-2-4-(4-nitrophenyl)-2H-5-tetrazolio-1,3-benzene disulphonate (WST-1) dilution (1/10) was added. As WST-1 is light sensitive, the light was switched off when

preparing, and adding the dilution to the cells. In addition, the 96-well plates were wrapped in aluminium foil before placing back into the incubator (37 °C, 5% CO_2). The plates were taken out for measurement of the absorbance at 2 and 4 hours after adding WST-1 dilution. The measurements were done with a spectrophotometer, at 450nm wavelength (2103 Envision Multilabel Reader, Perkin Elmer, Waltham, MA). The viability of the negative control was set as 100%. The results are expressed as dilution at which 50% of the cells died.

Six different pools of FW were used as test samples. These were all a mix of different samples from the entire study population. The two reasons for starting the experiment with only pools were 1) To test the protocol without spilling samples, using pools as "dummy samples", and 2) To decide which pool to use in all the sessions with patient samples. In each session with participant's samples, we added minimum one pool as a control sample. This allowed us checking the quality of that experiment such that the variability between the samples is mostly biological and not due to technical variability.

3.5 Food diaries

An anonymous MyFitnessPal (MFP) account was created for each participant to fill in the 7-day dietary record before each visit. A manual of use was provided, to ensure correct reporting. MFP is an online, free application where the participants could register everything they drink and eat. The data could be extracted automatically by the researcher, to see the macronutrient intake per day. The participants could search an extensive database (MyFitnessPal food database) for food items, in addition to scanning barcodes. If it for some reason was difficult to fill the diary in digitally, it was also an option to note the intake on paper and bring this to the visit.

3.6 Data analysis

3.6.1 Stool consistency and percentage faecal dry weight

The analysis of Bristol Stool Scores (BSS) from the 7 days stool diary were used to determine the stool consistency. Faecal DW (dry weight) percentage were used for further evaluations of faecal dryness. To calculate the amount of dry substance (in g), first weight of an empty tube was subtracted from the filled tube (tube + stool sample). Secondly, the percentage DW was calculated from the total weight (both water and DW).

3.6.2 Nutrient intake

After individual participants had registered their dietary intake at MFP, PDFs with their estimated daily intake were first extracted from their user accounts. Secondly, the conversion program (Zamzar) was used to transform the PDF files to TXT documents. At this stage, all the diaries were double checked for errors manually, by a nutrition bachelor student. Thirdly, all the files were merged into one Excel file with an in-house script in R language. This script contained an additional quality control that removed single nutrient values that went above a defined cut-off value. These cut-off values were: 1500 kcal, 95 g carbohydrates, 92 g fat, and 52 g protein per food item. Finally, the food diaries were sorted by patient and time point.

3.6.3 Cytotoxicity

Inhibitory concentration (IC)-50

Results from measurements after 4 hours were used for further analysis. The cell survival was calculated as: Survival (%) = (Asample – Apos.control) / (Aneg.control - Apos.control) x 100. IC-50 is a ratio that expresses the dilution, and there is no unit used for this. All the FW samples were done in triplicate.

3.6.4 Methodological validation: Comparison of duplicate vs. triplicate analysis of cytotoxicity

After preforming the samples in triplicate for the first period of the experiment, there was a chance to evaluate if it was possible to make the protocol more efficient for further implementation. Specifically, the intention was to explore changes in accuracy of doing the samples in duplicate compared to doing it in triplicate. The purpose of doing this was to consider doing one extra patient sample per plate,

The first step in comparing the IC-50 results of duplicates vs. triplicates was to split up all the results into duplicates as an addition to the already available triplicate values, using the same template. At this point we had two sets of IC-50 values ready for statistical comparison. For this analysis, a smaller share of the results was removed, compared to the cytotoxicity analysis. The reason for this was that even if the final IC-50 were unreliable, the information about the difference in the raw data triplicates were useful for this purpose.

The second step was to perform a paired t-test for comparison between the two sets of IC-50 values.

3.7 Ethics

The study was approved by the Committee for Medical Ethics of UZ KU Leuven (s59836).
All the participants signed a written consent. The study was carried out according to the Declaration of Helsinki, and according to the guidelines for good clinical practise. The participants did not receive any compensation for the participation in this study.

3.8 Statistical analysis

IBM SPSS statistics version 25 was used to perform statistical analysis. Data from the energy and macronutrient intake, stool consistency (BSS) and percentage faecal DW were compared using a mixed model ANOVA regression analysis. The statistical differences at different time points are not included in this thesis. The three different groups (RYGB, SG and CTR) were compared to each other. Multiple comparison between the groups were performed using Turkey's honest significant difference post-hoc test. A P-value <0.05 was considered statistically significant. A paired t-test was used to compare the two data sets (duplicate IC-50 values vs triplicate IC-50 values) for the methodological validation part.

4. Results

4.1 Faecal water cytotoxicity by WST-1 assay

Results of FW cytotoxicity measured 4 hours after applying WST solution were included in the cytotoxicity analysis. FW cytotoxicity results were included regardless of missing time points, because of the limited number of samples. One of the participants had delivered a stool sample at 14 months because of missing samples, this was included in this analysis. Energy, macronutrient, BSS and percentage DW data from the 9 participants included in cytotoxicity analysis were also included in this section. The BS participants (RYGB and SG) were merged into one group in this section, and the two groups (BS and CTR) were compared for the 5 different time points. No statistics were performed for this data.

4.1.1. Sample selection cytotoxicity analysis

Samples from 9 participants were included in the analysis (**Figure 3).** These participants were the first ones recruited. All samples from 2 participants (the same WST-1 assay session) were excluded because the results were defined as unreliable, the living cell numbers in the plate were too low. Five of the included participants underwent BS, while 4 were CTRs.

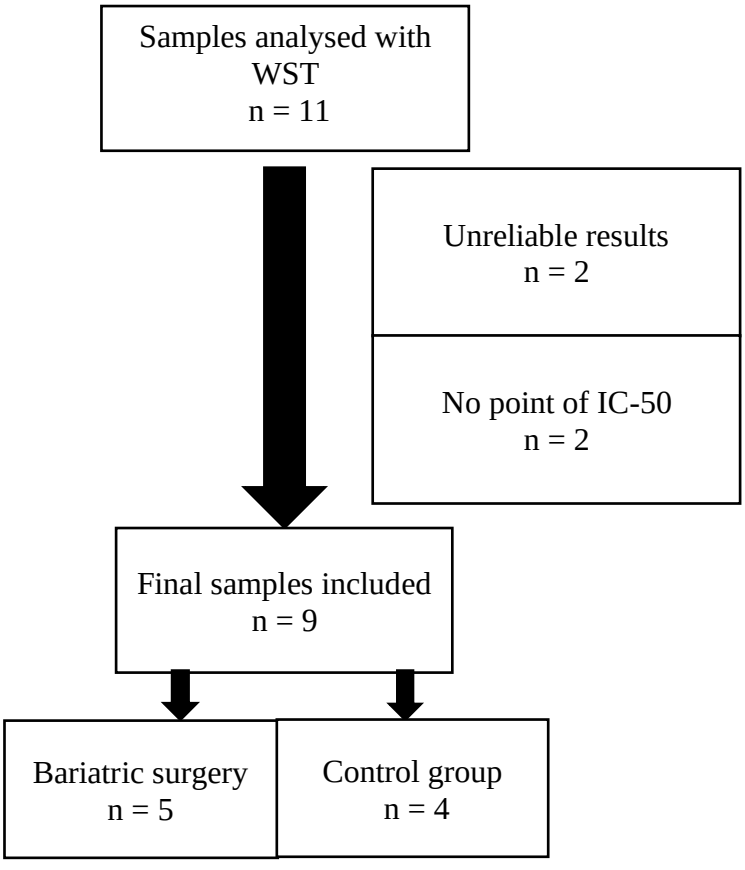


Figure 3: Flow chart sample selection cytotoxicity

4.1.2 Baseline characteristics of the participants from the faecal water cytotoxicity analysis

Baseline characteristics of the participants included in the FW cytotoxicity analysis are presented in **Table 1**. All missing values can be seen in **Appendix 3**. The baseline FW cytotoxicity levels were higher in the BS group compared with in the CTR group, with a difference of 50.9. Faecal DW percentage and BSS were similar in both groups, with a difference of 0.08 percentage points for faecal DW and 0.33 points for BSS. The baseline energy intake was 146 kcal/day higher in the BS group compared to the CTR group. The differences were more distinct in the macronutrient intake, with the CTR group consuming on average 19.12 g/day more carbohydrates and 7 g/day more protein than the BS group at baseline, whereas the BS group consumed on average 18.5 g/d more fat than the CTR group.

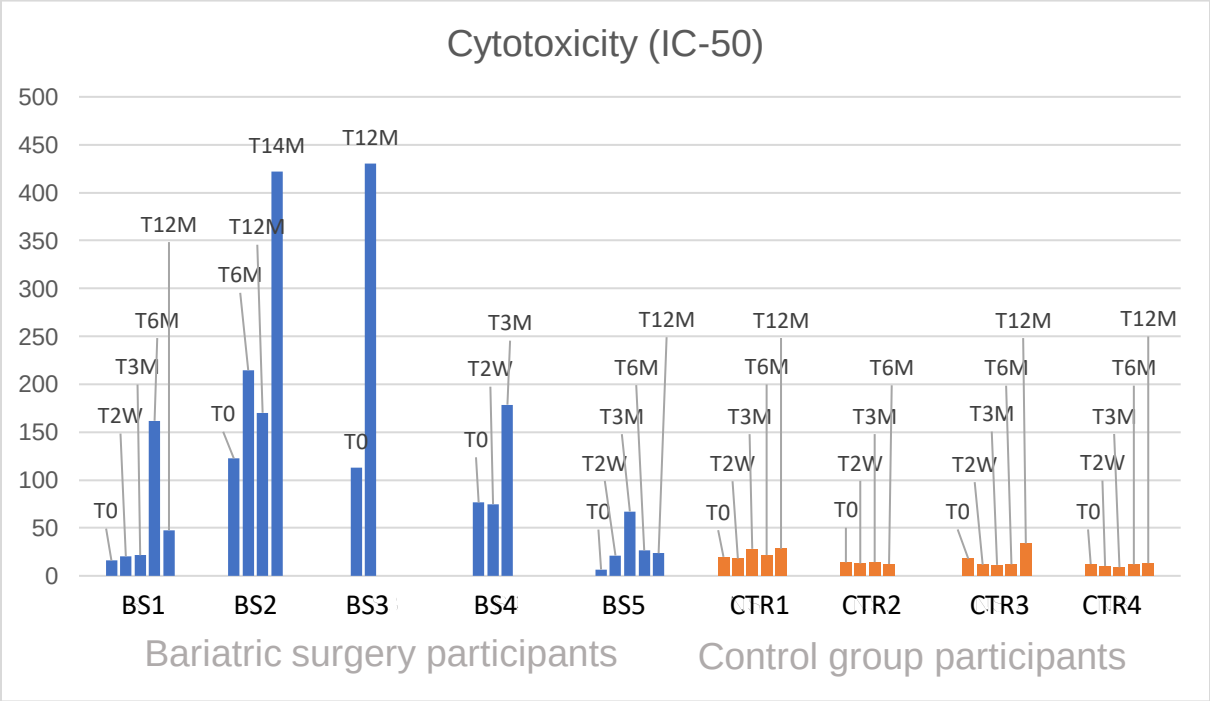
Table 1: Baseline characteristics of participants included in faecal water cytotoxicity analysis

Variable	Bariatric surgery participants N=5	Control group participants N=4
	Mean (min-max)	**Mean (min-max)**
Age (years)	46.2 (33-69)	50.5 (24-63)
BMI	35.7 (34.14-37.37)	36.6 (34.6-38.3)
Cytotoxicity FW (IC-50)	66.87 (6.57-122.54)	15.97 (12.24-19.81)
Faecal DW (percentage)	26.59 (16.72-30.57)	26.67 (20.19-35.44)
BSS	3.67 (3-4)	4 (3-5)
Energy intake (kcal)	1486 (845-2199)	1340 (1257-1716)
Carbohydrates intake (g)	148.7 (131-845)	167.82 (144-222)
Fat intake (g)	63.5 (22-107)	45 (44-52)
Protein intake (g)	54.11 (33-76)	61.1 (48-78)

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from MyFitnessPal registration (4-7 days)
2. BMI=Body mass index, FW=Faecal water, DW=Dry weight, BSS=Bristol Stool Score

4.1.3 Cytotoxicity level (IC-50)

FW cytotoxicity level for all participants, at all time-points measured are presented in **Figure 4.** The overall trend is higher cytotoxicity values in the surgery groups than in the CTR group. Three of the BS group participants also have markedly higher levels compared to the CTR group at their first measurement.

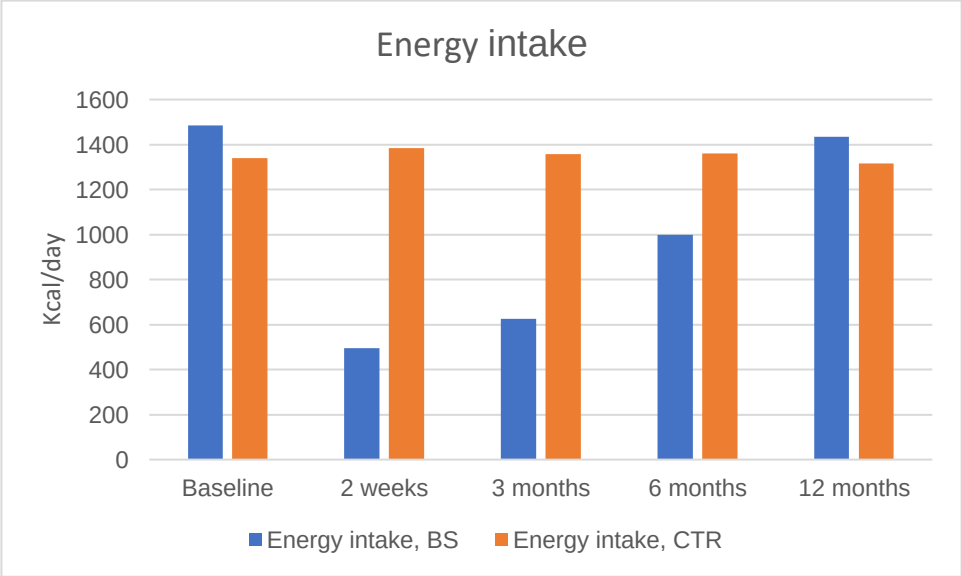


BS=Bariatric Surgery group (both RYBG and SG), CTR=Control group
T0=baseline, T2W=Two weeks, T3M=Three months, T6M=Six months, T12M=Twelve months, T14=Fourteen months
1-5=participants

Figure 4: Overview of cytotoxicity level (IC-50), from baseline to one year after surgery

4.1.4 Energy and macronutrient intake

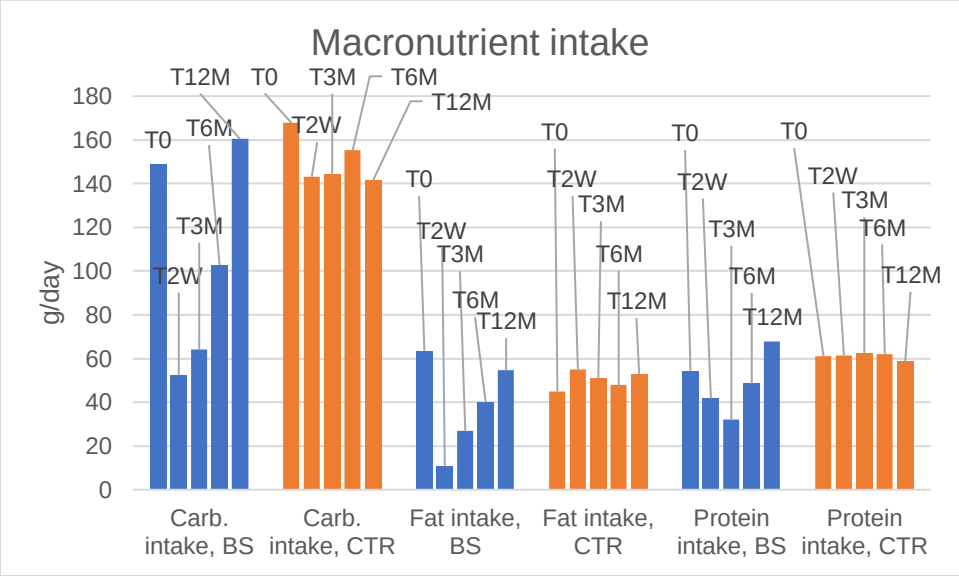
Energy intake for all visits are presented per group in **Figure 5**. There was seen a trend of more stable energy intake over the 5 visits in the CTR group compared to the BS group. The BS group showed a decrease in energy intake between baseline and 2 weeks, before increasing towards 12 months.



BS=Bariatric Surgery group (both RYBG and SG), CTR=Control group
Energy intake presented as mean group intake at each visit.
Intake is presented as average kilocalories/day estimated from MyFitnessPal food diaries (4-7 days)

Figure 5: Overview of energy intake from participants included in cytotoxicity analysis, from baseline to one year after surgery

Macronutrient intake (carbohydrates, fat and protein) for all visits is presented in **Figure 6**. The trend of a more stable intake in the CTR group compared to in the BS group is clearly shown here. The difference between the two groups were greater for the carbohydrate intake compared to fat and protein. At two weeks after surgery, the difference between the groups were more visible for fat compared to carbohydrates and protein.



BS=Bariatric Surgery group (both Roux-en-Y-gastric-bypass and Sleeve gastrectomy), CTR=Control group, Carb=Carbohydrates
T0=baseline, T2W=Two weeks, T3M=Three months, T6M=Six months, T12M=Twelve months
Macronutrient intake estimated as mean group intake at each visit.
Intake is presented as average grams per day estimated from MyFitnessPal food diaries (4-7 days).

Figure 6: Overview of macronutrient intake from participants included in cytotoxicity analysis, from baseline to one year after surgery

4.1.5 Change in cytotoxicity, faecal dry weights, BSS, energy intake and macronutrient intake, from baseline to one year after surgery

Mean values for cytotoxicity, DW, BSS and energy and macronutrient intake from all visits are presented in **Table 2**. Overview of missing values can be found in **Appendix 3**. The trend among participants in the BS group for cytotoxicity seems to be a decrease from baseline and to two weeks after surgery. From two weeks, the cytotoxicity increases between each of the remaining visits. The difference between baseline and 12 months was 101.02.

For cytotoxicity, there was observed a smaller difference between the visits in the CTR group compared to between the visits in the BS group. In the CTR group, there was a decrease between baseline and 2 weeks of 2.8. In addition, it was a decrease between 3 and 6 months of 1.24. The difference between baseline and 12 months in the CTR group was 9.11.

For faecal DW percentage, there was seen a decrease of 2.8 percentage points between baseline and two weeks post-surgery in the BS group. Further on, an increase is seen between two weeks and 3 months, before values stabilizes throughout the remaining time of the study. In comparison, there was less variation in faecal DW percentage in the CTR group compared to

in the BS group. For the CTR group there was an increase in the faecal DW percentage between 6 and 12 months of 7.78 percentage points.

In terms of energy and macronutrient data, it was a more distinct difference between the two groups compared to for the other variables. It was a decrease in intake of energy, carbohydrates and fat between baseline and two weeks post-surgery in the BS group, reflecting a decreased intake after the surgery. As expected, this trend was not seen in the CTR group. The general intake seems to be relatively stable for all visits in the CTR group, while there were bigger variations between visits in the BS group.

Table 2: Change in cytotoxicity, faecal dry weights, BSS, energy and macronutrient intake, from baseline to one year after surgery

		Bariatric surgery participants					Control group participants				
		Baseline	**2 weeks**	**3 months**	**6 months**	**12 months**	**Baseline**	**2 weeks**	**3 months**	**6 months**	**12 months**
FW Cytotoxicity (IC-50)	Mean (SD)	66.87 (53.73)	38.82 (31.29)	89.10 (80.88)	134.34 (97.09)	167.89 (186.22)	15.97 (3.45)	13.17 (3.41)	15.70 (8.61)	14.46 (4.75)	25.08 (10.69)
Faecal DW %	Mean (SD)	25.70 (5.67)	19.92 (6.18)	34.75 (15.83)	33.74 (6.70)	33.23 (9.01)	26.67 (7.46)	26.96 (7.98)	26.28 (9.23)	27.00 (5.90)	34.45 (9.66)
BSS	Mean (SD)	3.67 (0.58)	5.00 (1.41)	4.00 (1.63)	4.25 (1.26)	4.25 (1.26)	4.00 (0.82)	3.75 (2.22)	3.67 (0.58)	3.50 (1.00)	3.00 (1.41)
Energy intake (kcal)	Mean (SD)	1486 (574.20)	493 (131)	623 (554.46)	1000 (173.84)	1434 (434.41)	1340 (346.90)	1384 (319.06)	1355 (474.23)	1358(521.78)	1315 (502.69)
Carbohydrates intake (g)	Mean (SD)	148.7 (60.60)	52.29 (17.02)	64.14 (62.48)	102.72 (16.57)	160.37 (61.49)	167.82 (49.69)	143.09 (49.86)	144.49 (50.13)	155.29 (67.36)	141.43 (63.20)
Fat intake (g)	Mean (SD)	63.5 (35.88)	10.67 (10.81)	26.79 (22.78)	39.88 (6.68)	54.73 (15.49)	45 (7.35)	55.05 (5.70)	50.79 (17.72)	47.86 (17.62)	52.86 (16.50)
Protein intake (g)	Mean (SD)	54.11 (18.99)	41.67 (4.91)	32.22 (16.22)	48.54 (10.35)	67.83 (15.47)	61.1 (16.30)	61.53 (18.65)	62.38 (12.26)	62.14 (19.39)	58.79 (22.39)

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from My fitness Pal registration (4-7 days)
2. FW=Faecal water, DW=Dry weight, BSS=Bristol Stool Score

4.2 Energy and macronutrient intake

Energy and macronutrient intake (carbohydrates, fat and proteins) during the follow-up year were included in this analysis. The results are including three groups (RYGB, SG and CTR). Energy, carbohydrates, fat and protein intake were compared statistically between groups across all 5 time points. The change over time for each component is visualised through figures, and were not analysed statistically.

4.2.1 Sample selection food diaries for energy and macronutrient intake analysis

Food diaries from 67 participants were included in energy and macronutrient analysis. The selection is presented in **Figure 7**. The cut off point for removing participants from the analysis were set at minimum 4 visits. For removing diaries (one visit) the threshold was set at minimum three days, which led to an exclusion of eight diaries, while an additional 20 diaries were removed because of missing time points.

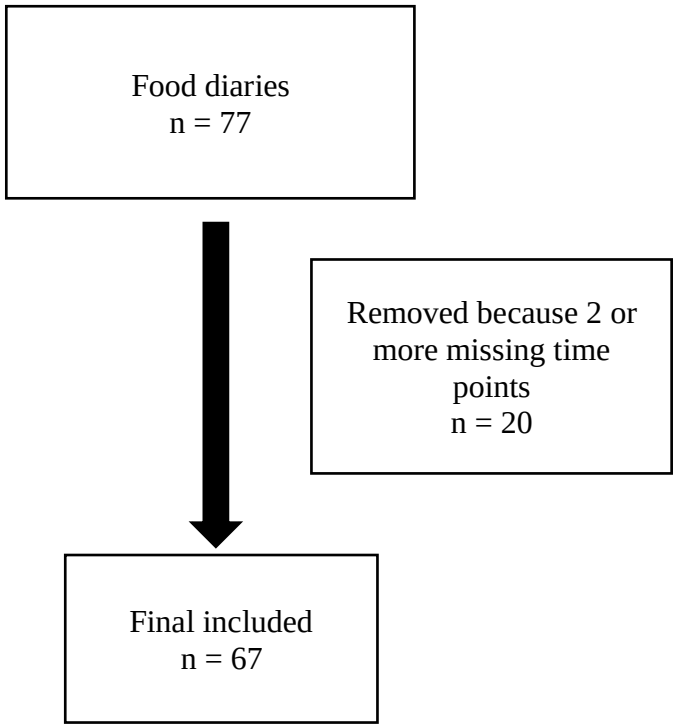


Figure 7: Flow chart sample selection food diaries

4.2.2 Baseline characteristics energy and macronutrients intake analysis

Baseline characteristics of the participants included in energy and macronutrient intake analysis are presented in **Table 3**. All missing values are specified in **Appendix 3**. Mean age was similar between the groups. Mean BMI was higher in the RYBG and SG groups compared to the CTR group.

The mean baseline energy intake was 171 kcal/day higher in the CTR group compared with in the SG group. For carbohydrates, the difference between CTR and SG was 2.04 g/day, while between CTR and RYGB the difference was 6.04 g/day. A more substantial difference in fat intake (13.3 g/day CTR vs. SG) and (7.15g/day CTR vs. RYGB) was detected, compared to carbohydrates. Protein showed greater difference for CTR vs. SG (3.93 g/day) compared with CTR vs. RYGB (1.69g/day).

Table 3: Baseline characteristics of participants included in energy and macronutrient intake analysis

Variable	RYGB N=30	SG N=20	CTR N=17
	Mean (min-max)	Mean (min-max)	Mean (min-max)
Age (years)	46.36 (26-69)	44.22 (21-64)	47.59 (28-63)
BMI	39.92 (34.14-49.48)	40.20 (35.01-52.57)	35.79 (29.75-45.17)
Energy intake (kcal)	1644 (846-2630)	1768 (839-3370)	1597 (943-2347)
Carbohydrates intake (g)	175.13 (99.43-332.43)	181.19 (85.57-304.33)	179.15 (123.86-302.29)
Fat intake (g)	66.51 (25.79-107.14)	72.55 (18-154.83)	59.35 (30.33-111.43)
Protein intake (g)	68.33 (33.50-109.14)	66.09 (4.83-158.83)	70.02 (41.29-122.86)

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from MyFitnessPal registration (4-7 days)
2. BMI=Body mass index, RYGB= Roux-en-Y-gastric-bypass, SG= Sleeve gastrectomy, CTR=Control

4.2.3 Change in energy and macronutrient intake

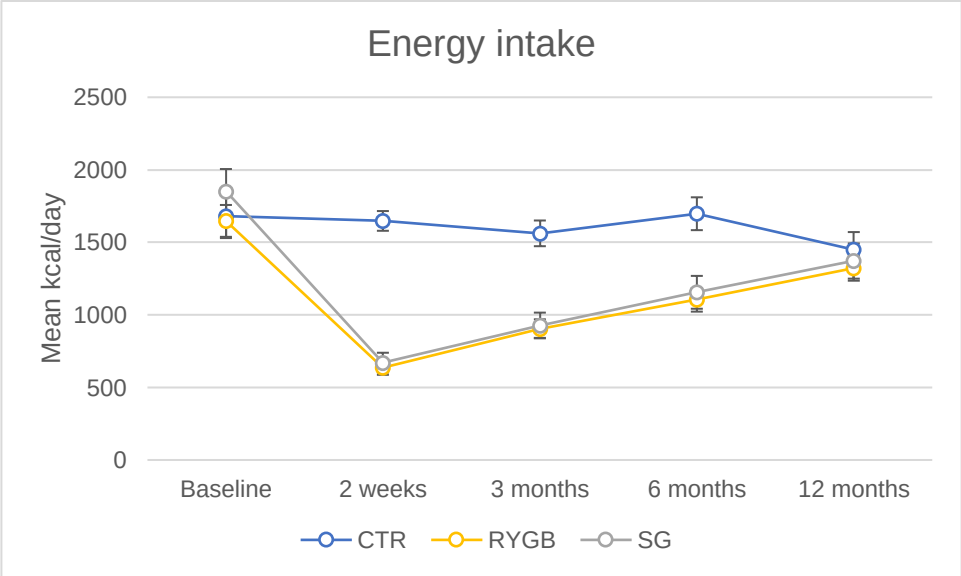
For the multiple comparison between the groups the results for energy, carbohydrates and fat were similar. There was a statistical significant difference when comparing the CTR group with both surgery groups for energy, carbohydrates and fat, while there was no statistical significant difference between the two surgery groups (**Table 5-7**). The protein intake did not differ between any of the groups. (**Table 8**)

There was a clear difference in trends between the surgery groups and the CTR group (**Table 4**). For the surgery groups the energy, carbohydrates and fat intake decreased markedly between baseline and two weeks after surgery, before gradually increasing towards the 12 months follow-up. The baseline level was higher compared to 12 months for all variables besides protein. In the RYGB group, the trend for protein was similar as for other macronutrients. In the SG group on the other hand, the protein intake at 12 months was higher than at baseline. For the CTR group there was less variation for both energy and macronutrient intake compared to the surgery groups. There was a small decrease for all variables between baseline and 12 months in the CTR group. The change from baseline to 12 months for energy, carbohydrates, fat and protein are presented in **Figure 8-11**.

Table 4: Change in energy and macronutrient intake from baseline to one year after surgery

	Baseline	2 weeks	3 months	6 months	12 months
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
RYGB n=30					
Energy (kcal)	1644 (421)	636 (173)	935 (244)	1088 (296)	1322 (345)
Carbohydrates (g)	175.13 (52.92)	62.09 (22.26)	100.66 (34.28)	116.80 (37.53)	141.69 (40.20)
Fat (g)	66.51 (19.61)	19.93 (9.27)	35.50 (9.33)	38.87 (11.82)	50.62 (15.93)
Protein (g)	68.33 (16.83)	51.34 (16.33)	51.18 (17.13)	58.77 (18.98)	65.62 (23.61)
SG n=20					
Energy (kcal)	1767 (676)	649 (173)	953 (250)	1123 (410)	1370 (509)
Carbohydrates (g)	181.19 (53.62)	66.22 (23.28)	97.96 (32.12)	114.97 (32.38)	137.33 (56.14)
Fat (g)	72.55 (34.77)	19.28 (9.36)	34.39 (9.89)	41.23 (16.54)	48.66 (14.74)
Protein (g)	66.09 (32.05)	51.95 (15.41)	59.03 (28.88)	69.20 (35.72)	82.30 (48.02)
CTR n=17					
Energy (kcal)	1597 (435)	1591 (392)	1517 (451)	1683 (529)	1450 (514)
Carbohydrates (g)	179.15 (47.97)	180.39 (66.89)	162.38 (51.91)	178.77 (58.53)	158.78 (58.08)
Fat (g)	59.35 (21.92)	59.86 (14.84)	59.72 (21.04)	66.92 (23.66)	53.40 (21.23)
Protein (g)	70.02 (21.92)	67.93 (16.43)	69.58 (14.86)	69.37 (21.26)	64.32 (21.91)

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from MyFitnessPal registration (4-7 days)
2. RYGB=Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy, CTR=Control



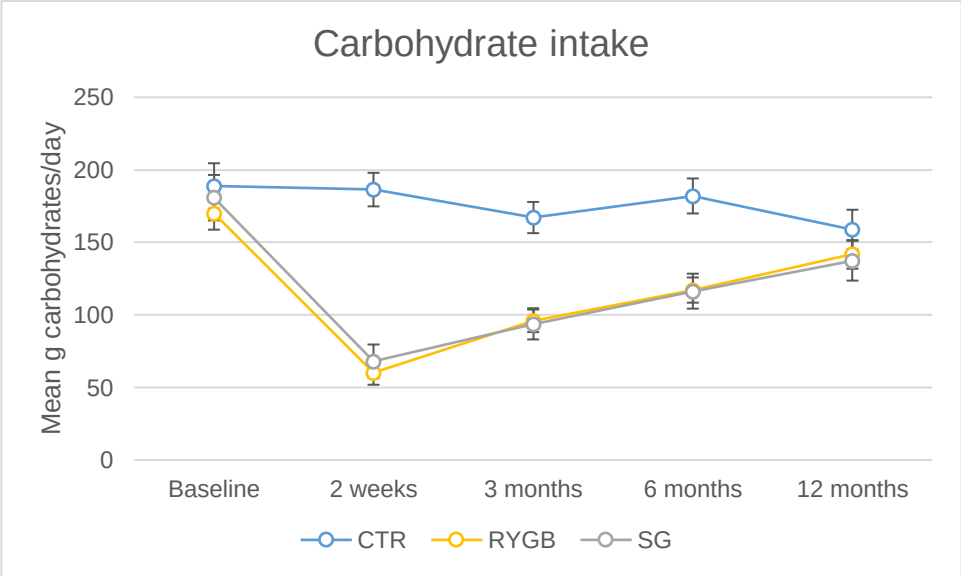
Error bars are showing standard error for all time points
CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

Figure 8: Change in energy intake, from baseline to one year after surgery

Table 5: Multiple comparison of energy intake, across all 5 time points

Comparison		Mean difference kcal/day	P-value	95% Confidence Interval	
				Lower bound	Upper bound
CTR	**RYGB**	483	0.00	241	727
CTR	**SG**	411	0.02	133	692
SG	**RYGB**	71	0.76	-170	316

1. A mixed model ANOVA regression analysis were used
2. P-value from Turkey's honest significant difference post-hoc test
3. CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy



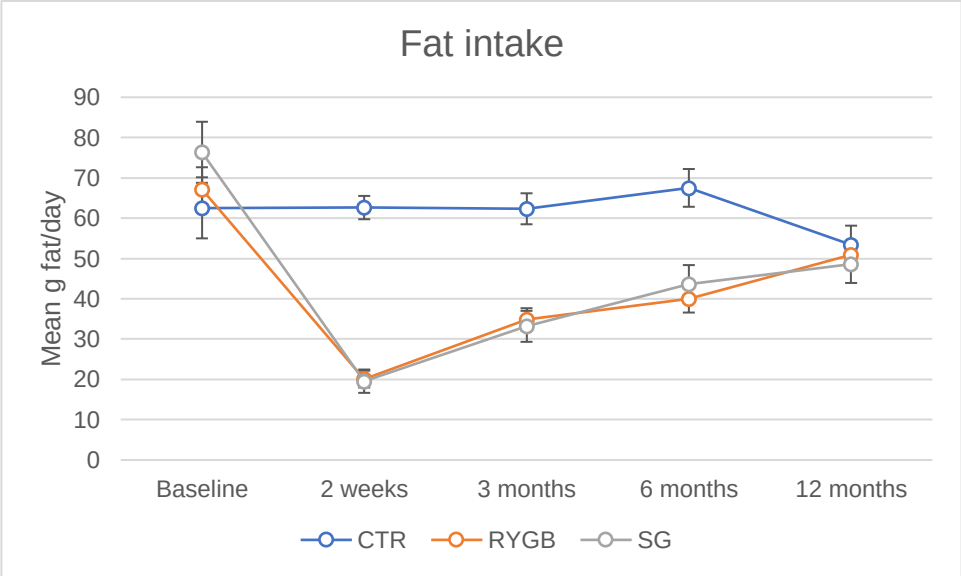
Error bars are showing standard error for all time points
CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

Figure 9: Change in carbohydrate intake, from baseline to one year after surgery

Table 6: Multiple comparison of carbohydrate intake, across all 5 time points

Comparison		Mean difference g carbohydrates/day	P-value	95% Confidence Interval	
				Lower bound	Upper bound
CTR	**RYGB**	59.62	0.00	33.13	86.12
CTR	**SG**	57.34	0.00	26.95	87.73
SG	**RYGB**	2.29	0.98	-24.20	28.78

1. A mixed model ANOVA regression analysis were used
2. P-value from Turkey's honest significant difference post-hoc test
3. CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy



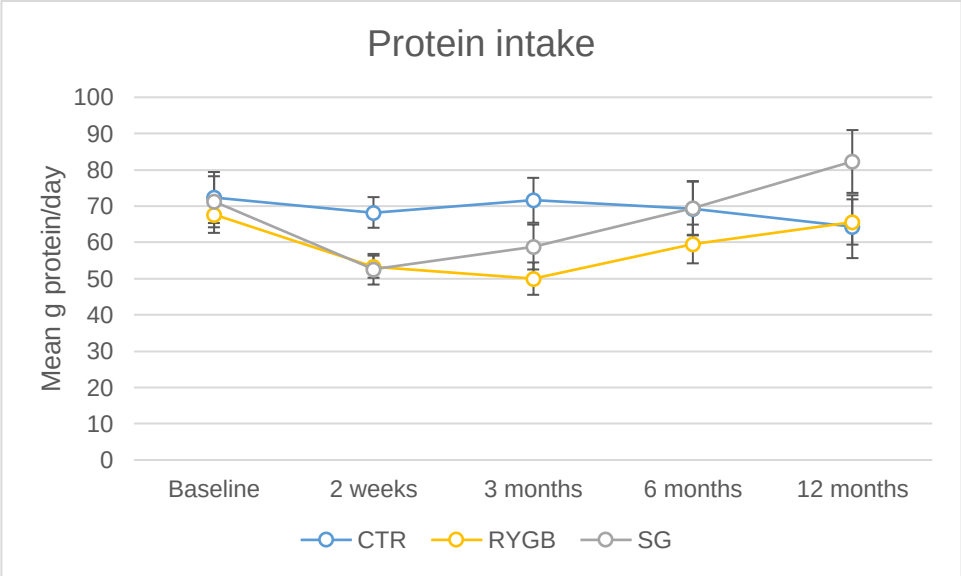
Error bars are showing standard error for all time points
CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

Figure 10: Change in fat intake, from baseline to one year after surgery

Table 7: Multiple comparison of fat intake, across all 5 time points

Comparison		Mean difference g fat/day	P-value	95% Confidence Interval	
				Lower bound	Upper bound
CTR	**RYGB**	19.07	0.00	9.93	28.22
CTR	**SG**	17.41	0.01	6.92	27.90
SG	**RYGB**	1.66	0.90	-7.48	10.80

1. A mixed model ANOVA regression analysis were used
2. P-value from Turkey's honest significant difference post-hoc test
3. CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy



Error bars are showing standard error for all time points
CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

Figure 11: Change in protein intake, from baseline to one year after surgery

Table 8: Multiple comparison of protein intake, across all 5 time points

Comparison		Mean difference g protein/day	P-value	95% Confidence Interval	
				Lower bound	Upper bound
CTR	**RYGB**	9.94	0.33	-6.89	26.76
CTR	**SG**	2.31	0.96	-16.98	21.61
SG	**RYGB**	7.63	0.52	-9.20	24.45

1. A mixed model ANOVA regression analysis were used
2. P-value from Turkey's honest significant difference post-hoc test
3. CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

4.3 Stool consistency (BSS), and faecal dry weight analysis

Faecal DW percentage and BSS results are presented in this section. Faecal DW percentage was obtained from freeze drying a small part of the stool sample delivered at each visit. The BSS was filled in for 7 days in the stool diary delivered at each visit. The results are including three groups (RYGB, SG and CTR). Faecal DW and BSS were compared statistically between groups across all 5 time points. The change over time for each of the 2 components is visualised through figures, and were not analysed statistical.

4.3.1 Sample selection faecal dry weight and Bristol Stool Score analysis

The final number of participants included in faecal DW analysis were 82, as presented in **Figure 12**. The cut-off point for selection was set at a minimum of 4 visits data available. As a result of this, 35 participants were removed.

Four BSS values were missing among these 82 participants, hence the final number of participants included in BSS analysis were 78, as presented in **Figure 13**. These 78 participants were selected from the 82 participants included in faecal DW analysis to be able to compare the results.

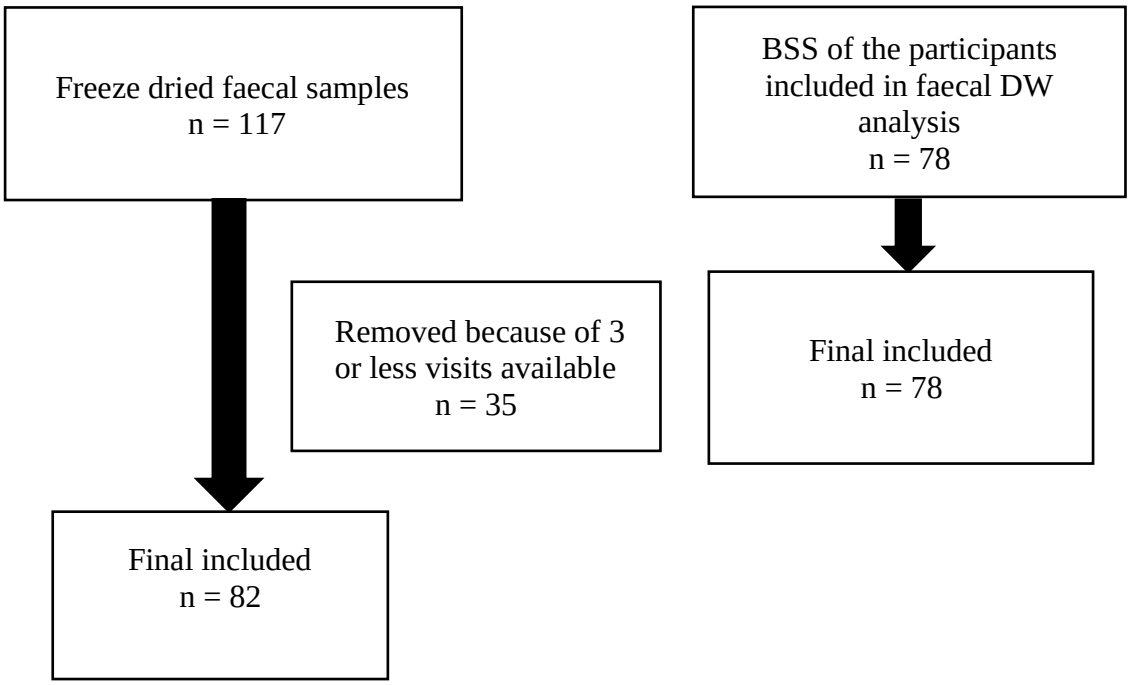


Figure 12: Flow chart faecal dry weight **Figure 13**: Flow chart Bristol Stool Score

4.3.2 Baseline characteristics faecal dry weight and Bristol Stool Score

Baseline characteristics of the participants included in faecal dry weight and Bristol Stool Score analysis are presented in **Table 9 and 10**. All missing values are specified in **Appendix 3.** For the participants in faecal dry weight analysis the age difference was greatest between CTR and SG (5.38 years) compared with CTR and RYGB (4.6 years). For BMI, the group differences for CTR vs. SG showed 3.52 kg/m2, and CTR vs. RYGB showed 3.18 kg/m2. Faecal DW

percentage was only differing 1.33 percentage points between the 2 surgery groups. Additionally, there was little variation between the groups for baseline levels of BSS, the greatest difference was 0.45 points (SG and CTR).

Table 9: Baseline characteristics of participants included in dry weight analysis

Variable	RYGB n=32	SG n=19	CTR n=31
	Mean (min-max)	Mean (min-max)	Mean (min-max)
Age (years)	45.43 (26-69)	44.65 (20-61)	50.03 (24-72)
BMI	39.96 (34.14-52.05)	40.30 (32.6-52.57)	36.78 (29.75-49.08)
Faecal DW (percentage)	25.84 (11.72-44.70)	24.51 (11.35-40.74)	27.49 (15.89-44.15)

1 The Bristol Stool Form Scale is from 1-7
2 BMI=Body mass index, DW=Dry weight, RYGB=Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy, CTR=Control

Table 10: Baseline characteristics of participants included in Bristol Stool Score analysis

Variable	RYGB n=31	SG n=18	CTR n=29
	Mean (min-max)	Mean (min-max)	Mean (min-max)
Age (years)	46.66 (26-69)	43.86 (20-60)	49.93 (24-77)
BMI	40.09 (34.14-52.05)	40.33 (32.6-52.57)	36.85 (29.75-49.08)
BSS	4.26 (3-7)	4.52 (3-7)	4.07 (1-6)

1 The Bristol Stool Form Scale range is from 1-7
2 BMI=Body mass index, BSS=Bristol Stool Score, RYGB=Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy, CTR=Control

4.3.3 Change in faecal dry weight and BSS from baseline to one year after surgery

It was no significantly overall group difference for neither faecal dry weight nor BSS (**Table 12 and 13**). The change over time differed between the 3 groups, as presented in **Table 11**. The faecal dry weight was for both surgery groups higher at 12 months compared to baseline. The change in faecal dry weight by groups over time are shown in **Figure 14.**

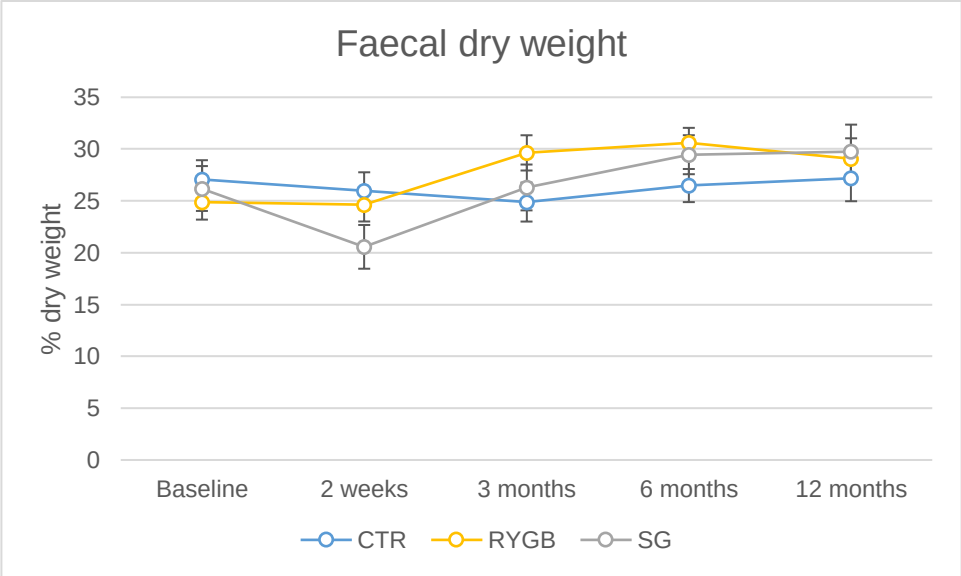
For BSS, the same trend as for faecal dry weight were observed in the RYGB group, except for the change between baseline and 2 weeks. Here, both faecal DW and BSS was decreasing. In the SG group, there were opposite trends for faecal dry weight and BSS between baseline and

two weeks, and between three and 6 months. In terms of the CTR group the trends were also opposite for faecal dry weight and BSS between all the different visits. The change in BSS by groups over time are shown in **Figure 15.**

Table 11: Change in stool consistency, from baseline to one year after surgery

	Baseline	**2 weeks**	**3 months**	**6 months**	**12 months**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
RYGB					
Faecal DW (percentage) N=32	25.84 (6.93)	25.73 (5.80)	29.04 (7.48)	30.83 (6.15)	29.04 (8.18)
BSS N=31	4.26 (1.16)	4.02 (1.32)	3.99 (0.98)	3.90 (0.98)	4.24 (1.09)
SG					
Faecal DW (percentage) N=19	24.51 (7.96)	21.53 (8.20)	25.59 (7.38)	27.83 (5.96)	29.76 (6.43)
BSS N=18	4.52 (1.38)	4.20 (1.72)	4.00 (1.56)	4.22 (1.06)	3.75 (1.42)
CTR					
Faecal DW (percentage) N=31	27.49 (7.39)	25.74 (8.90)	26.34 (7.96)	25.95 (7.30)	27.55 (11.93)
BSS N=29	4.07 (1.25)	4.00 (1.44)	4.00 (1.06)	3.87 (1.44)	3.95 (1.35)

1. The Bristol Stool Form Scale range is from 1-7
2. DW=Dry weight, BSS=Bristol Stool Score, RYGB=Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy, CTR=Control



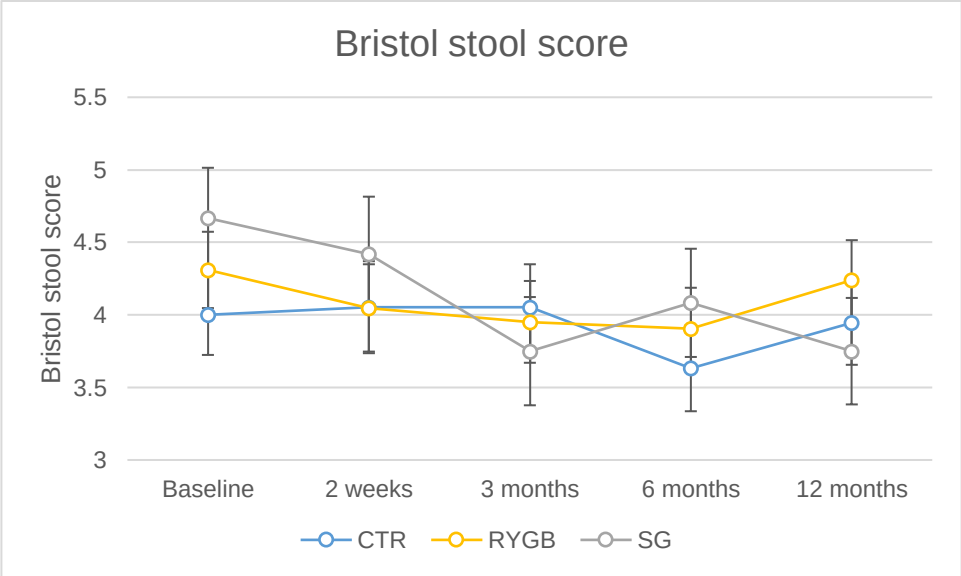
Error bars are showing standard error for all time point
CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

Figure 14: Change in faecal dry weight, from baseline to one year after surgery

Table 12: Multiple comparison of faecal dry weight, across all 5 time points

Comparison		Mean difference % dry weight	P-value	95% Confidence Interval	
				Lower bound	Upper bound
CTR	**RYGB**	-1.43	0.66	-5.36	2.49
CTR	**SG**	-0.13	0.99	-4.63	4.36
SG	**RYGB**	-1.30	0.75	-5.62	3.02

1. A mixed model ANOVA regression analysis were used
2. P-value from Turkey's honest significant difference post-hoc test
3. CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy



Error bars are showing standard error for all time points
CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

Figure 15: Change in Bristol Stool Score, from baseline to one year after surgery

Table 13: Multiple comparison of Bristol Stool Score, across all 5 time points

Comparison		Mean difference BSS	P-value	95% Confidence Interval	
				Lower bound	Upper bound
CTR	**RYGB**	-0.15	0.75	-0.67	-0.36
CTR	**SG**	-0.20	0.70	-0.79	-0.40
SG	**RYGB**	0.04	0.98	-0.54	0.63

1. A mixed model ANOVA regression analysis were used
2. P-value from Turkey's honest significant difference post-hoc test
3. CTR=Control, RYBG= Roux-en-Y-gastric-bypass, SG=Sleeve gastrectomy

4.4 Methodological validation: Comparison of duplicate vs. triplicate analysis of cytotoxicity

The FW cytotoxicity samples included in the methodological validation results are selected from the same WST-1 assay analysis as the samples in the FW cytotoxicity results. The samples were from RYGB, SG and CTR group participants, separated into to datasets (duplicate and triplicate) for statistically comparison.

4.4.1 Sample selection methodological validation: comparison of duplicate vs. triplicate analysis of cytotoxicity

Seventy-seven IC-50 results were included in the methodological validation (**Figure 16**). To be able to do the comparison between duplicate and triplicate the original sample had to be performed in triplicate, because of this two samples were excluded.

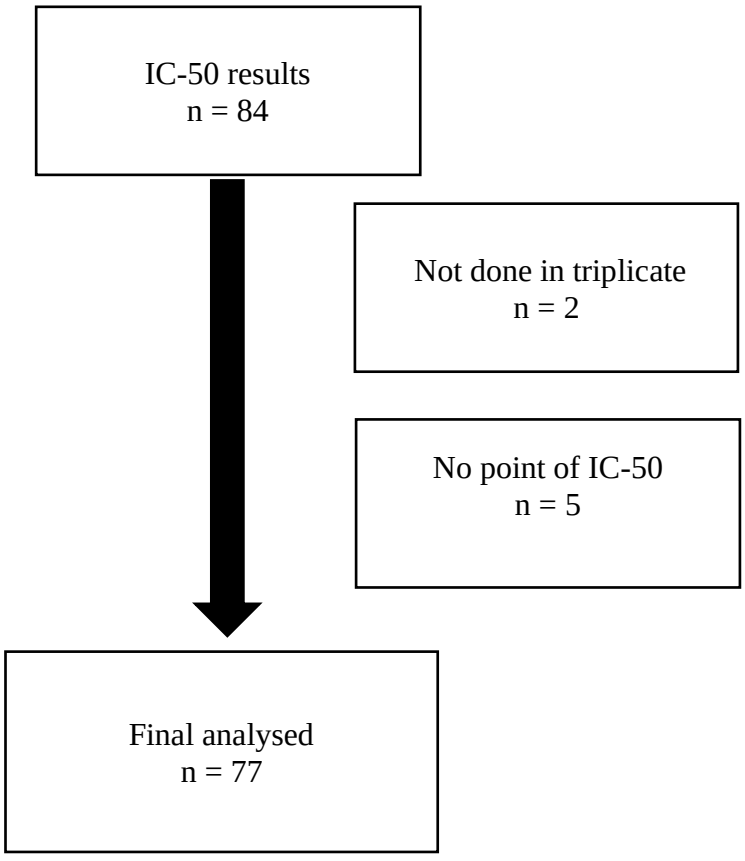


Figure 16: Flow chart IC-50 faecal water in duplicate and triplicate

4.4.2 Comparison duplicate and triplicate analysis of toxicity

The mean IC-50 value in the duplicate data did not differ significantly from the triplicate data. The mean difference was 1.09 with a p-value of 0.34.

5. Discussion

5.1 Main findings

The main findings corresponding to the 4 main research questions will be discussed in the same order as presented in the results. As the study populations are different in the 4 subchapters, the results will not be compared directly.

5.1.1 Cytotoxicity

The WST-1 assay results for cytotoxicity showed a trend of more cytotoxic faecal water among the BS group compared to the CTR group. However, it is difficult to say if this trend is due to the procedure itself, especially since the baseline levels also are higher in the surgery group (IC-50: 66.87 in surgery group vs 15.97 in CTR group), or simply due to the low number of participants. The lowest level of cytotoxicity in the BS group was found at 2 weeks. This is also the visit with the lowest faecal DW and highest BSS (more towards liquid consistency of stool). This is interesting due to the assumption of higher amounts of water in the stool contributing to diluting possible toxic compounds.

In addition to the general trend of higher cytotoxicity in the BS surgery group, it was a trend of increasingly higher levels of cytotoxicity between two weeks and 12 months was detected in the same group. Interestingly, this trend was not seen in the CTR group, which provides incentive for exploring the reason for differences further.

The visit two weeks after surgery, which had the lowest cytotoxicity level in the BS group, was also the visit with the lowest energy intake in the same group. It is naturally to think that the energy intake can influence the level of cytotoxicity, because of a smaller amount of possible cytotoxic compounds coming through the food intake. Two weeks after intervention had in addition the lowest cytotoxicity level in the CTR group, but the decrease compared to the other visits was smaller in this group vs the BS group. The caloric intake was not lower at two weeks compared to the other visits in the CTR group. Even though it´s not possible to say that this proves correlation, based on data attained for this thesis, it unveils a knowledge gap and incentive for further research on this subject.

The FW used for cytotoxicity analysis were filtered through 2 different filters. The last one with a 0.2µm pore size, which means sterile filtration (65). This gives some extra precautions to

prevent contamination in the process. Faeces consists largely of bacteria, which is removed in this stage of filtering (63). The possible sources of cytotoxicity were not analysed in this thesis. For possible further, and more thorough cytotoxicity research, an analysis of protein fermentation metabolites, short chain fatty acids, and secondary BAs could give deeper insight in the source of increased cytotoxicity (46, 62, 66).

To this date, no previous publications reporting FW cytotoxicity in BS patients have been identified. There has been performed a study on FW cytotoxicity (measured in IC-50 with WST-1 assay) in patients with ulcerative colitis (UC) (62). Data were obtained from healthy CTRs, UC patients with active disease and UC patients in remission. The results showed significantly more cytotoxic FW from the UC patients compared with healthy CTRs. The range of IC-50 values in the healthy CTR group were 11.9-22.5 compared with 12.9-34.5 in the UC patients. The IC-50 results for the BS group were substantially higher in the study conducted for this thesis. The mean values from the different visits varied from 38.82-167.89. The CTR group participants from our study varied from 13.17-25.08, which are similar to the results for healthy CTRs in the UC study.

Also, an experimental BS study in rats has been performed (64). FW from the RYGB operated rats were found to be highly cytotoxic. The results here were similar to the cytotoxicity findings in the present study.

Hence, results from the study conducted for this thesis provide new knowledge to this field of research. Measures of FW cytotoxicity by WST-1 assays are of note not something commonly performed, thus it's difficult to directly compare values with different references. Off note, it is impossible to draw any conclusions from these results due to the limited number of participants.

5.1.2 Energy and macronutrient intake

We observed a significantly overall difference for energy intake, carbohydrate intake and fat intake between the RYGB group participants and the CTR group participants, and between the SG group participants and the CTR group participants (**Table 5-7**). The protein intake was not found to differ notably between the groups. It is likely to assume that the differences observed between the CTR group and the surgery group is influenced by the dietary restrictions in the time after the surgery. This assumption is drawn because the most obvious difference in the

intake between the two surgery groups and the CTR group is observed twoo weeks after surgery.

Previously performed studies have shown a tendency of weight regain in a long-term perspective after BS (11, 67). In addition, there has previously been shown an increase in energy intake between one month and 12 months post-surgery (36). These findings are similar to the results in the present study and gives premises to longer follow up studies after BS in the future. In spite, it's important to mention that weight is not taken account for in this analysis.

At 12 months, there is a 321-kcal difference for the RYGB group compared with baseline, and a 397-kcal difference for the SG group (**Table 4**). Even though the highest intake amount were reported at the baseline visit for both groups, the baseline levels were still low compared to an average recommended intake (1644 kcal/day for RYGB, 1767 kcal/day for SG and 1597 kcal/day for CTR) (38). Off note, the overall calorie intake was low where none of the three groups exceeded 1800 kcal/day at any time-point during the 1-year follow-up. The Norwegian general dietary recommendations for an inactive man is a daily intake of 2600 kcal, hence the average intake in this study is markedly lower (38). The baseline food registration was collected without any restriction, and is therefore supposed to represent the participant's regular diet. These are all obese individuals, hence the low registered caloric intake does most likely not be a long-term reflection of their diet. Set aside the factors mentioned above, it is important to acknowledge the fact that the groups consist of both genders with a wide range of age groups, hence the nutritional requirements and recommendations will naturally be of great variety.

The composition of the macronutrients changed differently in the BS groups compared to the CTR group between baseline and 12 months. Protein intake was decreasing predominantly between baseline and 12 months in the CTR group (5.7 g/day) compared to in the BS groups. The protein intake was also decreased in the RYGB group between baseline and 1 months, although not as abundantly (2.71 g/day) as in the CTR group. For SG on the other hand, the protein intake increased between baseline and 12 months (16.21 g/day). The fat intake decreased between baseline and 12 months in all three groups. The smallest decrease was found in the CTR group (5.95 g/day), compared to 15.89 g/day in the RYGB group and 23.89 g/day in the SG group. In terms of change in diet between baseline and 12 months, observed changes in protein and fat intake can give us an indication of a greater shift towards the general dietary recommendations in the surgery groups (38). However, this is solely an assumption, due to the

lack of information about the different types of carbohydrates and fats consumed by participants. Studies have showed BS to be more effective than other weight loss interventions (7, 68). Weight loss was not analysed in the present study. Nevertheless, our results were showing a greater dietary change in the BS groups compared to in the CTR group. Off note, it's not feasible making any conclusions from results in this thesis due to a short timeframe of which this study was performed, and the limited number of participants.

5.1.3 Stool consistency

There were seen no significant differences in the multiple comparison between groups across all time points for neither faecal DW nor BSS. Therefor we cannot conclude with any difference in terms of stool consistency after BS. The results from faecal DW showed a higher percentage (more towards constipation) one year after surgery, compared with baseline values in both BS groups. For the RYGB group the increase was 3.2 percentage points, for SG the increase was 5.23 percentage points. In contrast, a smaller difference between baseline and 12 months in the CTR group were observed (0.35 percentage points).

For BSS, it was hardly any changes between baseline and 12 months in the RYGB group (0.02 point). The results showed a decrease in BSS (more towards constipation) between baseline and 12 months for the SG and CTR group (0.77 and 0.12 point, respectively).

When looking at the results from BSS and faecal DW they are showing the same trend for SG, but not for RYGB and CTR. For the two BS groups, the change is more towards a solid consistency overall. This can be discussed in context with the fact that a lot off BS patients are experiencing constipation as a side-effect after surgery (31). Constipation is defined with a BSS of 1 or 2 (69). The results from BSS in this study were showing mean scores for all groups at all visits above 3. Even though the results were more towards constipation for the SG group and CTR group when comparing baseline and 12 months, there is no evidence to conclude with constipation being a problem based on these data. For faecal DW, there has not been determined cut-off points for constipation and diarrhoea.

5.1.4 Methodological validation

When comparing the IC-50 cytotoxicity results in triplicate and duplicate, no significant difference between the two methods were observed ($p=0.34$). Based on the findings in this thesis, it seems reasonable to continue future research and similar experiments in duplicate

analysis, as this will save both time and recourses. It's worth noting that the ability to remove outlying values is removed when going from triplicate to duplicate. This means that those values are automatically included due to the impossible task of determining the most correct value

5.2 Strengths and limitations

5.2.1 Participants

The main limitation regarding the participants, is the fact that the four-different main analysis, targeting the four different research questions does not include the same number of participants. This makes it difficult to compare the examined variables with each other. Regardless of this, all the included participants had similar characteristics, due to fact that they were all obese and can give us useful information about a population group that is prioritised too little. This can facilitate important direction pointers towards where future research where future research in this group should be initiated.

Due to the timeframe and methodological adversity, the main focus of the thesis (cytotoxicity) had a very low number of included participants. This leads to the fact that the results were not suitable for statistical analysis. This is naturally a limitation. The fact that the participants selected for analysis were included by inclusion number, and not by other considerations is also a limitation. There were missing FW samples because of missing visits, drop out, or due to the amount of faecal sample, which is a limiting factor to the follow up results.

The number of participants included in energy and macronutrient analysis were a lot higher than in cytotoxicity analysis, and the increased number of participants is a strength. This gives more credibility to the observed trends in addition to the fact that this made it possible to perform statistics on the obtained results. Another limitation is the unequal division of participants between the groups (30 / 20 / 17), considering the fact that groups are compared. The rate of inclusion of participants from the different groups are impossible to control, which is reflected in the unequally numbered groups. The decision to rather include more participants than to have a lower, but equal number, was made to strengthen the results.

Eighty four participants were included in faecal DW analysis and 78 were included in the BSS analysis, which is a higher number of participants than for other analyses performed in this study. The number of participants is a strengthening factor. Here, the cut-off point for exclusion

were one missing time point, which led to 35 participants being excluded. It is a strength to avoid too many missing time points when the aim is to observe change by time after surgery.

For the methodological validation, the number of participants are also limited for the same reasons as for the cytotoxicity analysis. Regardless, the fact that each of the samples were done in triplicate, which was tested in this analysis, provides a higher number of samples.

5.2.2 Analysis

Cytotoxicity

There are a number of steps in processing and preparing the faecal sample for cytotoxicity analysis. The main limitation is the representability of the faecal sample collected. The faecal sample was mostly collected from one day, in some cases two days if the amount was insufficient, especially in cases of constipation. Even though the BSS and frequency are registered for one week before each visit, there is only one stool sample collected for each visit. Only speculations can be made about how much the recent food and drink intake, physical activity etc. influence the cytotoxicity result on a short term. This is off note beyond the scope of this thesis, but absolutely something worth looking further into. Stool consistency can possibly affect the measurement of cytotoxicity, which is not considered in the results. The explanation behind this, is the fact that a high amount of water in the FW will dilute the other possible cytotoxic compounds in the sample. The steps of homogenizing are crucial in the process of obtaining FW before filtering. If this is not done properly, the final tubes of FW will only be representable for parts of the delivered stool sample.

The WST-1 assay is a commonly used, practical and safe measure of cytotoxicity. Regardless there are many critical steps in the protocol where things can go wrong and affect the results. The cells/cell counter used in this project was to some degree a limiting factor. From the very beginning of the experiment, a challenge arose in attempting to obtain the correct number of cells in the wells used. This was discovered through a low number of living cells in the wells only filled with medium. There was also used a second cell counter to double check the counted number of cells, where number of counted cells seemed to vary too much between the different culture bottles of cells used.

The main reason for obtaining the low number of results in the cytotoxicity analysis were a contamination in the cell culture used. When this was discovered the experiment was paused further on.

Energy and macronutrient intake

There are several limitations both regarding the way the data is collected, and for the choice of data collection resources. Firstly, the data is registered by the participants themselves. This leads to uncertainties, as it is impossible to control in any way if the registration is representative of the participant's intake. The risk of over and under estimating is a known challenge when recording food intake (70).

There are additional limitations of the application MyFitnessPal. Some food items are missing in the database. Although there is a function to register new food items with the full nutritional content, it can be a challenge to register information correctly, as well as it requires a lot of extra work for the participants. For some participants, the digital registration can be difficult. An attempt to avoid the digital format from being a hinder was to offer the possibility to register on paper. Even though the digital format can be a source of wrongly estimation of intake for some participants, the value of having an app always available on your phone exceeds this aspect. The chance of misunderstanding the use off the application is also limited from having a detailed guide of how to correctly register nutrient intake.

Stool consistency

The results can give us an idea of the uncertainty of using both BSS and faecal DW, since the two measures are giving slightly varying results. Additionally, it's challenging to compare the results because the BSS was representing one week (stool diary), while the faecal DW was only from one single stool sample. There is seemingly no way of correctly converting faecal DWs to BSS, since BSS is strictly based on appearance. Faecal DW percentage and BSS as a measure of stool consistency both have some limitations. The amount of DW left after extracting the water from the stool sample is an indirect measurement of the consistency, that says something about how dry a sample is.

There will always be a possibility for mistakes that may influence the results in most research. This includes both mistakes related to the machines and equipment used, and to the methods proceeded by hand. For the faecal DW, the weighing procedure is crucial, where the final

results are unreliable if incorrect weight is registered at any of the numerous steps. Even though the mistakes most likely would be noticed, there is always a risk linked to this kind of analysis. The BSS is a well-known method of stating the stool consistency. This method is based on categorization, and has been shown to be reliable (71). Regardless, there will always be a risk of different interpretations between the participants rating the stool samples.

Methodological validation

Because of the relatively low number of samples included in the methodological validation analysis (77), there is a higher chance of the results being incidental. There will be a variation in pipetting mistakes over time. This results are regardless of this, useful to have in mind for optimising the protocol, and doing the experiment as effective as possible.

6. Conclusion and future aspects

In conclusions, we detected a trend of higher cytotoxicity in BS patients compared to healthy obese controls according to the cytotoxicity analysis, and the cytotoxicity gradually increased between 2 weeks and 12 months after surgery. The energy and macronutrient analysis highlighted a observed difference in the overall energy, fat and carbohydrate intake between both RYGB and CTR, and SG and CTR. The same difference was not seen for the protein intake. Further, we observed a decrease in intake of energy and macronutrients between baseline and 2 weeks after surgery, before a gradual increase towards 12 months in the BS groups. The same trend was not observed for the control group.

The need of studies further investigating the implications of faecal cytotoxicity in BS patients is clear, as there is little existing research. The increasing prevalence of both obesity and CRC makes this highly relevant, especially because the prevalence's are increasing in the younger population. A longer and more detailed analysis of the cytotoxicity after BS can give a better picture of the mechanisms involved in CRC development in these patients. Additional detailed analysis of the dietary compounds, possibly contributing to these effects, would be of specific interest.

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8. Appendix

Appendix 1: Study documents

Appendix 2: Instructions of how to deliver the stool samples

Appendix 3: Tables with number of participants or all variables

Appendix 1: Study documents

LABEL

Week : …./…./…. --- …./…./….

STUDIEDOCUMENTEN

S59836

Alvast hartelijk bedankt voor uw inzet!

Moest u nog vragen hebben, aarzel zeker niet mij te contacteren.

U kan mij telefonisch bereiken op het nummer 016/ 32. 81. 38 of via mijn email charlotte.evenepoel@kuleuven.be

Met vriendelijke groeten,

Charlotte Evenepoel

Translationeel Onderzoek van Gastro-enterologische Aandoeningen (TARGID)
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Medicatie en voedingssupplementen

Je mag 1 week lang (in de week voor de studievisite) noteren welke medicatie en/of supplementen je neemt.
Indien je antibiotica moet nemen, probeer dan zo snel mogelijk contact met mij op te nemen (016/32.81.38 of charlotte.evenepoel@kuleuven.be)

Naam medicatie/ voedingssupplement	Indicatie	Dosis (eenheden per dag)	Ingenomen van (dd/mm/jjjj)	Ingenomen tot (dd/mm/jjjj)

Stoelgangsdagboek

Nummer het type van de stoelgang volgens de Bristol Stoelgang Score:
(Nummering gebeurt volgens het uitzicht en de hardheid van de stoelgang)

Je mag 1 week lang (in de week voor de studievisite), noteren hoeveel maal je per dag naar het toilet bent geweest met stoelgang.

Bristol Stoelgang score

Dag 1: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Dag 2: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Dag 3: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Dag 4: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Dag 5: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Dag 6: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Dag 7: Datum: … / … / …
Hoeveel keer heeft u vandaag stoelgang gehad? Omcirkel het aantal.

0	1	2	3	4	5	meer

Als u stoelgang heeft gehad, kruis het type aan volgens de Bristol Stoelgang score:

Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7

Fysieke activiteiten

Internationale Vragenlijst in verband met Fysieke Activiteiten

De vragen gaan over de fysieke activiteit die u in de **laatste zeven dagen** gedaan hebt. Er zitten vragen bij over de lichaamsbeweging op uw werk, over uw verplaatsingen, over uw werk in huis en in de tuin, en over uw vrije tijd in verband met ontspanning, lichaamsbeweging en sport.

Uw antwoorden zijn belangrijk. Probeer op alle vragen te antwoorden, zelfs als u vindt dat u niet erg actief bent.

Dank voor uw medewerking

Een toelichting bij het beantwoorden van de volgende vragen:
- **zware** fysieke activiteiten verwijzen naar activiteiten die een zware lichamelijke inspanning vereisen en waarbij u veel sneller en dieper ademt dan normaal.
- **matige** fysieke activiteiten verwijzen naar activiteiten die een matige lichamelijke inspanning vereisen en waarbij u iets sneller en dieper ademt dan normaal.

Deel 1: Fysieke activiteiten tijdens uw werk

Deel 1 gaat over uw werk. Onder werk verstaan we: betaald werk, werk op de boerderij, vrijwilligerswerk, studiewerk en ander onbetaald werk dat u buitenshuis verricht heeft. Thuiswerk zoals huishoudelijk werk, tuinieren, klusjes en gezinstaken horen hier niet bij. Dat komt aan bod in deel 3.

1a Heeft u momenteel een baan of doet u onbetaald werk buitenshuis?

☐ Ja
☐ Nee *(Ga naar Deel 2: Vervoer)*

De volgende vragen handelen over alle fysieke activiteiten die u gedaan heeft in de laatste zeven dagen als deel van uw betaald of onbetaald werk. De verplaatsing van en naar het werk hoort hier ***niet*** bij. Het gaat hier *alleen* om de fysieke activiteiten die u **gedurende minstens 10 minuten aan één stuk** gedaan heeft.

1b Op hoeveel dagen, in de laatste zeven dagen, heeft u **zware** fysieke activiteiten gedaan zoals zwaar tilwerk, spitten, bouwwerken of trappen oplopen *als deel van uw werk?*

_____ dagen per week

☐ Geen *(Ga naar vraag 1d.)*

1c Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **zware** fysieke activiteiten *als deel van uw werk?*

____ uur ___ minuten / dag

1d Op hoeveel dagen, in de laatste zeven dagen, heeft u **matige** fysieke activiteiten gedaan zoals het dragen van lichte lasten *als deel van uw werk?*

_____ dagen per week

☐ Geen *(Ga naar vraag 1f.)*

1e Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **matige** fysieke activiteiten *als deel van uw werk?*

____ uur ___ minuten / dag

1f Op hoeveel dagen, in de laatste zeven dagen, heeft u **gewandeld** gedurende minstens 10 minuten aan één stuk *als deel van uw werk*
Opgelet, de verplaatsing te voet van en naar het werk hoort hier ***niet*** bij !

_____ dagen per week
☐ Geen (Ga naar Deel 2: *Vervoer)*

1g Hoeveel tijd in totaal heeft u op zo'n dag **gewandeld** *als deel van uw werk ?*

____ uur ___ minuten / dag

1h Indien u **gewandeld** heeft *als deel van uw werk,* in welk tempo was dat dan meestal ?
Heeft u gewandeld u in :

☐ een **hoog** tempo?
☐ een **middelmatig** tempo?
☐ een **laag** tempo?

Deel 2: Fysieke activiteiten die verband houden met vervoer

Nu volgen enkele vragen over hoe u zich verplaatst heeft naar het werk, om boodschappen te doen, naar de film te gaan enzovoort.

2a Op hoeveel dagen, in de laatste zeven dagen, heeft u zich verplaatst met een motorvoertuig zoals de trein, de bus, de wagen of de tram?

_____ dagen per week

☐ Geen *(Ga naar vraag 2c)*

2b Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan verplaatsingen *met de wagen, de bus, de trein of een ander motorvoertuig?*

____ uur ___ minuten / dag

Denk nu **alleen** aan het *fietsen en het wandelen* dat u gedaan heeft om naar het werk te gaan, te winkelen of gewoon om ergens heen te gaan.

2c Op hoeveel dagen, in de laatste zeven dagen, heeft u **gefietst** gedurende minstens 10 minuten aan één stuk *om ergens heen te gaan?*

_____ dagen per week.

☐ Geen *(Ga naar vraag 2f)*

2d Hoeveel tijd in totaal heeft u op zo'n dag **gefietst** *om ergens heen te gaan* ?

____ uur ___ minuten / dag

2e Als u *zich verplaatst heeft per **fiets**,* in welk tempo was dat dan meestal ?
Heeft u gefietst in :

☐ een **hoog** tempo

☐ een **middelmatig** tempo of
☐ een **laag** tempo

2f. Op hoeveel dagen, in de laatste zeven dagen, heeft u **gewandeld** gedurende minstens 10 minuten aan één stuk *om ergens heen te gaan ?*

_____ dagen per week

☐ Geen *(Ga naar Deel 3: Huishoudelijk Werk, Klusjes en Gezinstaken)*

2g Hoeveel tijd in totaal heeft u op zo'n dag **gewandeld** *om ergens heen te gaan ?*

____ uur ___ minuten / dag

2h Als u **gewandeld** heeft *om ergens heen te gaan,* in welk tempo was dat dan meestal ?
Heeft u gewandeld in :

☐ een **hoog** tempo
☐ een **middelmatig** tempo of
☐ een **laag** tempo

Deel 3. Huishoudelijk werk, klusjes en gezinstaken

Dit deel gaat over de fysieke activiteiten die u in de laatste zeven dagen gedaan heeft *in en rond het huis,* bijvoorbeeld huishoudelijk werk, tuinieren, onderhoudswerk of voor het gezin zorgen. Nogmaals, denk *alleen* aan die fysieke activiteiten die u **gedurende minstens 10 minuten aan één stuk** verricht heeft.

3a Op hoeveel dagen, in de laatste zeven dagen, heeft u **zware** fysieke activiteiten gedaan zoals zwaar tilwerk, houthakken, sneeuwruimen of spitten ***in de tuin of moestuin*** ?

_____ dagen per week

☐ Geen *(Ga naar vraag 3c)*

3b Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **zware** fysieke activiteiten *in de tuin of moestuin ?*

____ uur ___ minuten / dag

3c Op hoeveel dagen, in de laatste zeven dagen, heeft u **matige** fysieke activiteiten gedaan zoals lichte lasten dragen, ruiten wassen, vegen of harken ***in de tuin of moestuin*** ?

_____ dagen per week

☐ Geen *(Ga naar vraag 3e)*

3d Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **matige** fysieke activiteiten *in de tuin of moestuin ?*

____ uur ___ minuten / dag

3e Op hoeveel dagen, in de laatste zeven dagen, heeft u **matige** fysieke activiteiten gedaan zoals lichte lasten dragen, ruiten wassen, vloeren schrobben of vegen ***binnenshuis*** **?**

_____ dagen per week

☐ Geen *(Ga naar Deel 4: Fysieke Activiteiten die verband houden met Sport, Ontspanning en Vrije Tijd)*

3f Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **matige** fysieke activiteiten *binnenshuis?*

____ uur ___ minuten / dag

Deel 4: Fysieke activiteiten die verband houden met sport, ontspanning en vrije tijd

Dit deel gaat over alle fysieke activiteiten die u de laatste zeven dagen gedaan heeft, maar dan uitsluitend als recreatie, sport, training of vrijetijdsbesteding. Nogmaals, denk *alleen* aan die fysieke activiteiten die u **gedurende minstens 10 minuten aan één stuk** verricht heeft. Gelieve **geen** activiteiten mee te rekenen die u reeds vermeld hebt.

4a **Zonder het wandelen dat u reeds vermeld hebt**, op hoeveel dagen, in de laatste zeven dagen, heeft u ge**wandeld** gedurende minstens 10 minuten aan één stuk *in uw vrije tijd ?*

_____ dagen per week

☐ Geen *(Ga naar vraag 4d)*

4b Hoeveel tijd in totaal heeft u op zo'n dag **gewandeld** *in uw vrije tijd ?*

____ uur ___ minuten / dag

4c Als u **gewandeld heeft** *in uw vrije tijd*, in welk tempo was dat dan meestal?
Heeft u gewandeld in :

☐ een **hoog** tempo
☐ een **middelmatig** tempo of
☐ een **laag** tempo

4d Op hoeveel dagen, in de laatste zeven dagen, heeft u **zware** fysieke activiteiten gedaan zoals bijvoorbeeld aerobics, lopen, snel fietsen, snel zwemmen of andere intense activiteiten, *in uw vrije tijd ?*

_____ dagen per week

☐ Geen *(Ga naar vraag 4f)*

4e Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **zware** fysieke activiteiten *in uw vrije tijd?*

____ uur ___ minuten / dag

4f Op hoeveel dagen, in de laatste zeven dagen, heeft u **matige** fysieke activiteiten gedaan zoals bijvoorbeeld fietsen aan een middelmatig tempo, zwemmen aan een middelmatig tempo, tennis dubbelspel of andere activiteiten aan een matige intensiteit, *in uw vrije tijd ?*

_____ dagen per week

☐ Geen *(Ga naar Deel 5: De tijd die u zittend doorbrengt)*

4g Hoeveel tijd in totaal heeft u op zo'n dag besteedt aan **matige** fysieke activiteiten *in uw vrije tijd?*

____ uur ___ minuten / dag

Deel 5: De tijd die u zittend doorbrengt

De laatste vragen gaan over de tijd die u de laatste zeven dagen zittend doorbracht op het werk, thuis, tijdens studiewerk of in uw vrije tijd. Hierbij hoort ook de tijd dat u achter een bureau zat, bezoek kreeg, zat te lezen, of naar televisie zat of lag te kijken.
De tijd die u zittend doorbracht in een motorvoertuig, die u reeds vermeld hebt, komt hier **niet** in aanmerking.

5a Hoeveel tijd heeft u gemiddeld *gezeten* op een **weekdag**, in de laatste zeven dagen?

____ uur ___ minuten / dag

5b Hoeveel tijd heeft u gemiddeld *gezeten* op een **weekenddag**, in de laatste zeven dagen?

____ uur ___ minuten / dag

Hier mag je de informatie noteren van het staal dat je genomen hebt.

Bristol Stoelgang score

Type 1		Aparte harde stukjes Moeilijk om uit te persen
Type 2		Brokkelig, maar worstvorming
Type 3		Lijkt op een worst, maar met barsten aan het oppervlak
Type 4		Een gladde worst of slang. Zacht maar stevig
Type 5		Zachte klodders maar nog met duidelijk randen
Type 6		Zachte stukjes, vrij papperig
Type 7		Waterig, geen vaste stukken. Pure vloeistof

Datum van staal:
Lichaamsgewicht:

Appendix 2: Instructions of how to deliver the stool samples

Handleiding stoelgangstaalname

1. Opvangbakje met deksel
2. ~~Plastic vel~~ *(niet van toepassing)*
3. Plastic spatel
4. Vorm met instructiesticker
5. Plastic kaart
6. Afsluitsticker met barcode
7. Groen zakje "bewaar in vriezer"
8. Blauw zakje "bewaar in frigo"


8

De bovenstaande foto toont de benodigdheden voor één stoelgangstaalname.
<u>Alle benodigdheden</u> zijn aanwezig in jouw blauwe koelzak. Gebruik ook deze zak om het staal te transporteren.

Neem je staal 1 dag tot maximaal 3 dagen voor je studiebezoek.

Lees aandachtig onderstaande handleiding voor je aan de staalname begint. De handleiding telt 4 pagina's. Indien je dat wenst, kan je de bijgeleverde witte handschoenen gebruiken tijdens de staalname.

Neem het opvangbakje (1) en het plastic vel (2).

Vang de stoelgang op in de plastic pot.

Het is belangrijk dat de stoelgang <u>niet in contact</u> komt met water of urine.

1

Neem de plastic spatel (3), de vorm (4) en de plastic kaart (5).

Gebruik de plastic spatel om een schepje stoelgang op de vorm te leggen.

Strijk je stoelgang in de gaatjes van de vorm met de plastic kaart.

Zorg dat de gaatjes volledig vol zitten.

Verwijder overtollige stoelgang van de vorm met de plastic kaart zodat er enkel in de gaatjes stoelgang zit.

Veeg de kaart af met toiletpapier.

Het papier kan je in het toilet weggooien.

Verwijder de instructiesticker van de vorm. Dit zorgt ervoor dat het oppervlak van de vorm proper achterblijft.

Neem de afsluitsticker met barcode (6).

Kleef de afsluitsticker op de propere vorm.

Zorg ervoor dat de vorm helemaal bedekt is en de gaatjes met stoelgang afgesloten zijn.

De staalname is hiermee gebeurd.

Nu nog enkele gegevens noteren en het staal bewaren. Neem hiervoor De Bristol Stoelgang score.

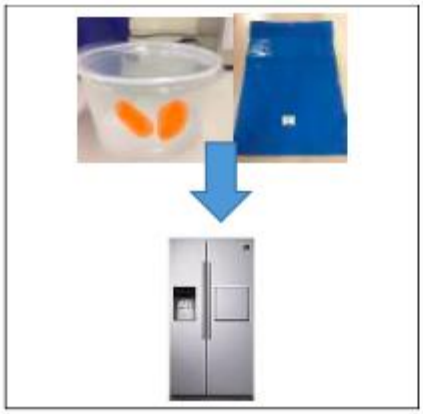
Geef de ontlasting waarvan je een staal hebt genomen een score tussen 1 en 7 aan de hand van de tekeningen en beschrijvingen op de Bristol Stoelgang scorelijst.

Omcirkel je score en noteer tevens de datum van je staalname en je lichaamsgewicht.

Nu kan je staal de diepvries in! Neem het groene zakje (7) en het blauwe plastic zakje met sticker "bewaar in de koelkast" (8).

Steek de afgesloten vorm in het groene zakje (met sticker "bewaar **in de vriezer"**).

Plaats het groene zakje meteen horizontaal in je diepvriezer.
!!**Je stoelgangstaal moet bevroren blijven tot je het inlevert bij je studiebezoek.** Om het staal gekoeld te transporteren moet je gebruik maken van het isolerend blauwe koelzakje met koelelement.



Na de staalafname bewaar je de collecte pot met uw stoelgang in de koelkast. Steek daarvoor de pot in het blauwe plastic zakje (met sticker "bewaar **in de koelkast"**).

LAATSTE STAP: Breng de gevulde pot en het gevulde matje mee naar uw studiebezoek.

! Let op gebruik hiervoor het koelzakje + de 2 koelelementen! Bindt het groene matje tussen deze 2 koelelementen met een elastiek.

De instructiesticker, de plastic kaart en de spatel kunnen mee met het huishoudelijk afval.

Heel erg bedankt!

4

Appendix 3: Tables with number of participants for all variables

Table 1: Baseline characteristics of participants included in faecal water cytotoxicity analysis

Variable	Bariatric surgery participants	Control group participants
Age (years)	n=5	n=4
BMI	n=5	n=4
Cytotoxicity FW (IC-50)	n=5	n=4
Faecal DW (percentage)	n=5	n=4
BSS	n=3	n=4
Energy intake (kcal)	n=4	n=3
Carbohydrates intake (g)	n=4	n=3
Fat intake (g)	n=4	n=3
Protein intake (g)	n=4	n=3

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from My fitness Pal registration (4-7 days)
2. BMI=Body mass index, FW=Faecal water, DW=Dry weight, BSS=Bristol stool score

Table 2: Change in cytotoxicity, faecal dry weights, BSS, energy and macronutrient intake, from baseline to one year after surgery

	Bariatric surgery participants					Control group participants				
	Baseline	**2 weeks**	**3 months**	**6 months**	**12 months**	**Baseline**	**2 weeks**	**3 months**	**6 months**	**12 months**
Cytotoxicity FW (IC-50)	n=5	n=3	n=3	n=3	n=4	n=4	n=4	n=4	n=4	n=3
Faecal DW (percentage)	n=5	n=3	n=5	n=4	n=5	n=4	n=4	n=4	n=4	n=4
BSS	n=3	n=3	n=2	n=4	n=4	n=4	n=4	n=4	n=4	n=4
Energy intake (kcal)	n=4	n=3	n=3	n=4	n=4	n=3	n=3	n=4	n=4	n=4
Carbohydrates intake (g)	n=4	n=3	n=3	n=4	n=4	n=3	n=3	n=4	n=4	n=4
Fat intake (g)	n=4	n=3	n=3	n=4	n=4	n=3	n=2	n=4	n=4	n=4
Protein intake (g)	n=4	n=3	n=3	n=4	n=4	n=3	n=3	n=4	n=4	n=4

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from My fitness Pal registration (4-7 days)
2. FW=Faecal water, DW=Dry weight, BSS=Bristol Stool Score

Table 3: Baseline characteristics of participants included in macronutrient composition analysis

Variable	RYGB	SG	CTR
Age (years)	n=30	n=19	n=17
BMI	n=30	n=20	n=17
Energy intake (kcal)	n=30	n=19	n=17
Carbohydrates intake (g)	n=30	n=19	n=17
Fat intake (g)	n=30	n=19	n=17
Protein intake (g)	n=30	n=19	n=17

1. Energy, carbohydrates, fat, and protein intake are presented as estimated average daily intake from My fitness Pal registration (4-7 days)
2. BMI=Body mass index

Table 9: Baseline characteristics of participants included in faecal dry weight analysis

Variable	RYGB	SG	CTR
Age (years)	n=30	n=17	n=31
BMI	n=32	n=19	n=30
Faecal DW (percentage)	n=32	n=19	n=31

1 The Bristol stool scale range is from 1-7
2 BMI=Body mass index, DW= Dry weight

Table 10: Baseline characteristics of participants included in Bristol stool score analysis

Variable	RYGB	SG	CTR
Age (years)	n=29	n=16	n=29
BMI	n=31	n=18	n=29
BSS	n=31	n=18	n=29

1 The Bristol stool scale range is from 1-7
2 BMI=Body mass index, BSS=Bristol stool score