Effect of marine peptide hydrolysate supplementation on ghrelin and insulin-like peptide 5 secretion on appetite related conditions – As tested in recovery following endurance cycling

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Master's thesis in Clinical Nutrition

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Disclaimer

The following datasets are shared with another master's student and there may therefore be some overlap; serum glucose and serum insulin, satiety and the food records. There may also occur some overlapping in regards to the method section.

Preface

This master's thesis was carried out from August 2017 to June 2018 at the Department of Clinical Science at the University of Bergen.

I would like to start by expressing my gratitude to Firmenich Bjørge Biomarine AS, Ålesund, Norway for providing financial support for this study.

Secondly, I would express my thanks to my main supervisor Prof. Trygve Hausken for listening to my master's proposal and giving me the opportunity to carry out that proposal in this study, back in the autumn of 2016. I am so grateful for your enthusiasm, knowledge, and the support I have received throughout this project. Moreover, I am very grateful for all the times you took time for my many questions and discussions.

I extend my thanks to co-supervisor: Randi Julie Tangvik for assistance and guidance in the writing stages of this thesis, and Prof. Gülen Arsland Lied for taking the time to review and discuss my work throughout the writing process.

Additionally, I would like to thank all technicians for their participation and devotion during the intervention. I would especially like to express many thanks to Ingeborg Brønstad for guidance when performing analysis measuring ghrelin and insulin-like peptide 5 in the blood. I would also give thanks to her for always having an answer to my question regarding the method.

Einar Lied provided knowledge and insight from a strategic business perspective, giving advice in the practical application of the study, and for that I am grateful. Geir Egil Eide also deserves thanks for providing statistical support.

I would like to express thanks to the participants who spent a great deal of time and effort in participation in this study.

Finally I must give many thanks to my family and friends who have been supporting me through all my studies and although not always understand what I am talking about, always took the time to listen to my enthusiastic explanation and discussion of this thesis.

Bergen, June 2018 Heidi Anett Holm Nilsen

Summary

Background: Energy homeostasis is sustained by multiple complex pathways, and appetite regulation is one of them. Malnutrition such as obesity or undernutrition, which is known to impair individual health and lower quality of life. The association between appetite regulation and gastrointestinal (GI) hormones is an attractive target for therapeutic and dietary interventions, as well as through physical activity and exercise. Ghrelin is known to increase hunger, and insulin-like peptide 5 (INSL5) is a novel hormone in appetite regulation that is suggested to also increase hunger.

Aim: This study set out to investigate if 20 mg marine peptide hydrolysate (MPH) per kg body weight would affect the secretion pattern of ghrelin and INSL5 after endurance cycling, as well as to explore the effect of the appetite regulating hormones following the endurance cycling.

Participants and method: The study population (n=14) was composed of a moderately homogenous sample population of middle-aged, well-trained, healthy males recruited in July of 2017. Anthropometric measurements, circulating GI hormones, self-reported feeling of satiety and a 4-day food intake record was measured in this study.

Results: There is significant difference in circulating ghrelin ($p = 0.014$) in treatment, compared to placebo. Additionally, there was a negative effect when adjusted for body mas index ($p = 0.03$). A positive trend in circulating INSL5 was observed, however not significant ($p = 0.12$). Correlations between ghrelin, INSL5, glucose, insulin and satiety was statistically significant ($p < 0.05$). There is a negative relationship between INSL5 and ghrelin ($p < 0.01$), and a very strong, almost linear correlation between INSL5 and insulin ($r = 0.93$, $p < 0.01$). The correlations also revealed that an increase in ghrelin secretion reduced satiation, while an increase in INSL5 increased satiation. The self-reported food intake revealed that 85 % of the participants did not meet the daily recommended intake (DRI) of energy and 62 % did not meet the DRI of protein as recommended by the Nordic Nutrition Recommendation.

Conclusion: This study discovered that a small amount of MPH had an effect on ghrelin secretion, and the absence of results for the other variables may be due to a low dosage. Interestingly, the novel INSL5 promotes satiation and are in undiscovered way involved in insulin secretion.

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Abbreviations

AG = Acylated ghrelin

AgRP = Agouti-related protein

ARC = Arcuate nucleus

BMI = Body mass index

BW = Body weight

CART = Cocaine- and amphetamine-regulated transcript

CI = Confidence intervals

E % = Percentage of total energy

EE = Energy expenditure

GI = Gastrointestinal

GLP1 = Glucagon-like peptide-1

GH = Growth hormone

GHSR-1a = Growth hormone secretagogue receptor 1a

INSL5 = Insulin-like peptide 5

LBK = Laboratory of Clinical Biochemistry

MPH = Marine peptide hydrolysate

MCT = Medium Chained Triglycerides

NPY = Neuropeptide Y

PYY = Peptide YY

PAL = Physical activity level

POMC = Pro-opiomelanocortin

PH = Protein hydrolysate

RXFP4 = Relaxin Family Peptide Receptor 4

REE = Resting Energy Expenditure

SD = Standard deviation

SEM = Standard error of mean

UAG = Unacylated Ghrelin

Chapter 1: Introduction

1.1 General background

Energy homeostasis is the vital biological process involving food intake regulation and energy expenditure. When energy intake is exceeding means of energy expenditure, it is stored as excess energy; glycogen, triglycerides or body fat (3, 4). This is known as a positive energy balance. On the other hand, there occurs a negative balance when the energy intake is lower than the energy expenditure.

Obesity is a polygenic disorder, and is associated with a state of low level of inflammation. The disorder arises through a positive energy balance over time and is commonly identified by excessive or abnormal accumulation of fat that could impair the health of the individual and is defined by a body mass index (BMI) ≥ 30 kg/m2 (5). In the later decades, incidents of obesity are fast growing and are doubled since 1980, reaching 600 million (5). This number is only expected to be continually rising unless we are able to change how we are currently combatting this issue.

The increase in portion sizes and overconsumption of energy dense foods have become a public health concern and are possibly key factors in the increasing incidents of obesity that we see worldwide (6), and especially in the western world. Obesity and its comorbidities are one of the main health challenges the world is fighting this days. The comorbidities of obesity, including a variety of metabolic disorders, cardio vascular complications, psychosocial issues and cancer have increased the risk of mortality for the affected individual (7-9)**.** To address this rising epidemic, it is important to recognise the treatment interventions that are available in targeting various short- and long-term mechanisms of energy homeostasis (10) and the effects of these treatments.

Physical activity has a great impact on human health, and it is well established that physical activity has major influence on body composition and energy homeostasis (11). Physical inactivity, similarly to obesity, increases the risk of cardiovascular disease and stroke, type 2 Diabetes Mellitus, some types of cancers such as breast cancer and colon cancer (12). It is also associated with injuries and mental health issues (13). Physical activity and exercise is recommended around the world as a strategy of weight loss and weight management, simply because it is increasing energy expenditure (14). Multiple studies have observed that exercise

suppress the feeling of hunger and alters secretion of appetite regulating hormones during and after exercise (15, 16) (also called exercise-induced anorexia (17). These responses are suggested to coincide with the fluctuations in gastrointestinal (GI) hormones which regulates appetite and energy balance due to exercise (18). By further exploring the regulation of GI hormones, especially ones that are involved in appetite regulation and energy homeostasis. This may offer a therapeutic target for obesity treatment in cooperation with exercise or in other ways. This will also further the understanding of obesity and the mechanisms of appetite and body weight regulation

1.2 The Endocrine Regulation of Appetite

Appetite and body weight control is regulated by the interactions between the GI tract, adipose tissue and the hypothalamus (7, 19). The hypothalamus is the main regulatory organ of the endocrine system in humans, and together with the brainstem receives peripheral neural and hormonal signalling regarding the availability of energy in the body at all time (7, 20). The main function of GI hormones are to optimize digestion and the absorption of nutrients in the gut (21). They are also involved in regulating blood glucose levels, exocrine secretion, growth and adipocyte function (22). The hormones which are released in the GI tract reflect the nutritional status on a meal to meal basis (22).

The arcuate nucleus (ARC) in the hypothalamus plays a significant part in the gut-brain axis, containing orexigenic (supresses satiety) and anorexigenic (increase satiety) neurons, which is stimulated by short- and long-term signals as shown in Figure 1 (1). The ARC recieves hormonal signals from the pheripheral such as the muscle, liver and adipose tissue. Circulating insulin and leptin reflects the long-term availability of energy from the body's stores either activating the orexigenic or anorexigenic neurons depending on the level of these hormones (20, 23). In contrast, short-term signals of energy availability such as peptide YY (PYY) and ghrelin are secreted in respons to meals (20). Both types of signals acts on the neurons in the ARC either promotes (orexigenic) or inhibits (anorexigenic) appetite and feeding.

Orexigenic hormones such as Ghrelin and Insulin-Like Peptide 5 (INSL5) stimulates appetite by expressing neuropeptide Y (NPY) and Agouti-related protein (AgRP), while anorexigenic hormones such as PYY and Glucagon-like peptide-1 (GLP1) inhibits appetite by expressing alpha-melanocyte-stimulating hormone (alpha-MSH) which are derived from cocaine- and

amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) (20). This is illustrated in Figure 1.

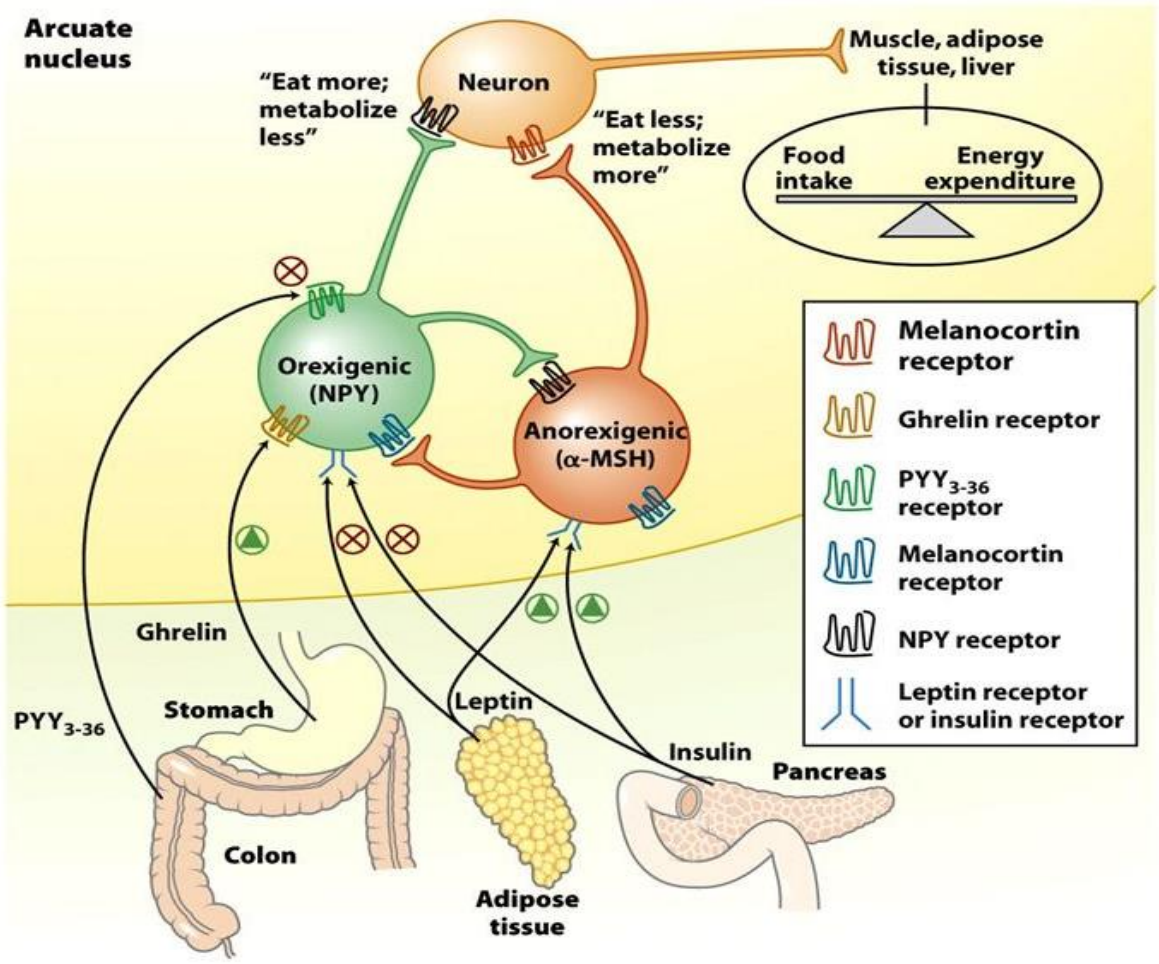


Figure 1 *The endocrine regulation of appetite. Figure from Boyle (1).*

1.2.1 Ghrelin

Ghrelin is an 28 amino acid peptide most known as an appetite-regulating hormone with orexigenic properties (24). It is mostly produces in the stomach (25) and has since discovery in 1999 (24) become a significant endocrine target in obesity treatment and appetite control. Ghrelin is also involved with and have a regulatory role in several systems and organs in the body (26-28). There are two forms of Ghrelin in circulation; acylated (AG) and unacylated Ghrelin (UAG) (24). The human Ghrelin gene has been located at chromosome 3, locus 3p25-26 (26, 29) containing 5 exons (24). Ghrelin is contributing to body weight regulation by stimulating growth hormone (GH) secretion at the pituitary in the brain (30), giving a reduction in energy expenditure and increase in adiposity (31, 32).

There are several physiological functions of Ghrelin; increasing blood glucose and decreasing insulin (33), reducing blood pressure (34), increasing cardiac output (35), it increases gastric acid secretion and gastric movement in the stomach (36). However, it is generally identified by its GH-stimulating action (37) as well as being fundamental in regulation of appetite, meal initiation and nutrient sensing (26, 27). Ghrelin is produced and secreted by ghrelineric cells mainly in the stomach (25), and can be found in the hypothalamus (38), pancreatic islets (39), heart and kidney (40).

The pathways underlying the orexigenic effects of Ghrelin are centered around the Growth Hormone Secretagogue Receptor 1a (GHSR-1a), which is found to be abundantly expressed within the ARC of the hypothalamus (41, 42). Food intake regulates and stimulates orexigenic peptide release by ghrelin-containing neurons activating NPY and AgPR neurons through the GHSR-1a. Activation of the GHSR-1a initiates the secretion of GH, which in turn stimulate appetite, adiposity, blood glucose, gastric acid secretion, gastric movement, turnover of gastric and intestinal mucosa, stimulating cardiac output and decreasing blood pressure locally (26-28, 34-36).

Ghrelin is suggested to influence blood glucose homeostasis by affecting the secretion of insulin and the signalling to the insulin receptor. Research is also starting to suggest that insulin can affect circulating ghrelin levels at some point (33, 43), however this mechanism is still being explored.

1.2.2 Insulin-Like peptide 5

Insulin-like peptide 5 (INSL5) is a part of the insulin/relaxing superfamily and have been identified as a product of the enteroendocrine L-cells in the epithelium in colon and rectum (44). INSL5, containing 135 amino acids, was first discovered in 1999 expressed in colon, rectal and uterine tissue (45). Grosse and co-workers found that INSL5 encourages feeding during situations of calorie restriction (46) and is only the second orexigenic GI hormone to be discovered after Ghrelin. It has been suggested to act on appetite and regulation of food intake by binding specifically to the Relaxin Family Peptide Receptor 4 (RXFP4), which is primarily expresses in the peripheral tissue (47, 48).

This novel peptide have also been hypothesised in regulating metabolism via either affecting insulin production and glucose tolerance (49), regulating hepatic production of glucose (50), secretion of insulin (51) or via food-intake stimulation (46). INSL5 have been shown to be

regulated by available energy in the body and the gut microbiota, as well as it has been suggested that it may be a novel hormone in hepatic glucose production, however mild effect (50). Lee and co-workers suggest that INSL5 possibly acts as a sensor of energy as well as a modulator of glucose production during energy deprivation conditions (50). INSL5 have also been reported to be stimulating secretion of insulin in vivo (51), and there have been reported a sex-dependent variance in the circulating INSL5 in lean versus obese humans (52). The exact physiological function of INSL5 have not yet been defined (48). Nor have the orexigenic properties of this peptide been fully defined.

1.3 Blood glucose homeostasis and exercise

The regulation of blood glucose involves maintain glucose levels in the blood at a constant level when facing energy intake and energy expenditure by the body. There are two main hormones involved in maintaining the targeted average range of blood glucose 4 – 5.5 mmol/l (75-110 mg/100 mL); insulin and glucagon (53). Figure 2 displays the mechanisms of blood glucose in the body. A rise in blood glucose stimulates beta cells in the pancreas which increases insulin production and circulation. Glucose uptake by cells in the body, which decreases blood glucose to stabilize blood glucose levels, and glucose is converted in to glycogen and fat by the cells. A fall in blood glucose stimulates alpha cells in the pancreas, which increases circulating glucagon. Glucagon is then converted to glucose in muscles and the liver, and then released in to the blood stream to stabilize blood glucose levels.

Preserving an adequate supply of blood glucose during exercise is crucial. During exercise, blood glucose levels are maintained or amplified by the regulation of glucose from the kidneys and liver, and from other sources of energy (54) (55). It is the autonomic nervous system, regulation of hormones and enzyme activity alterations that are involved in the coordinating the physiological response to blood glucose levels during exercise (54).

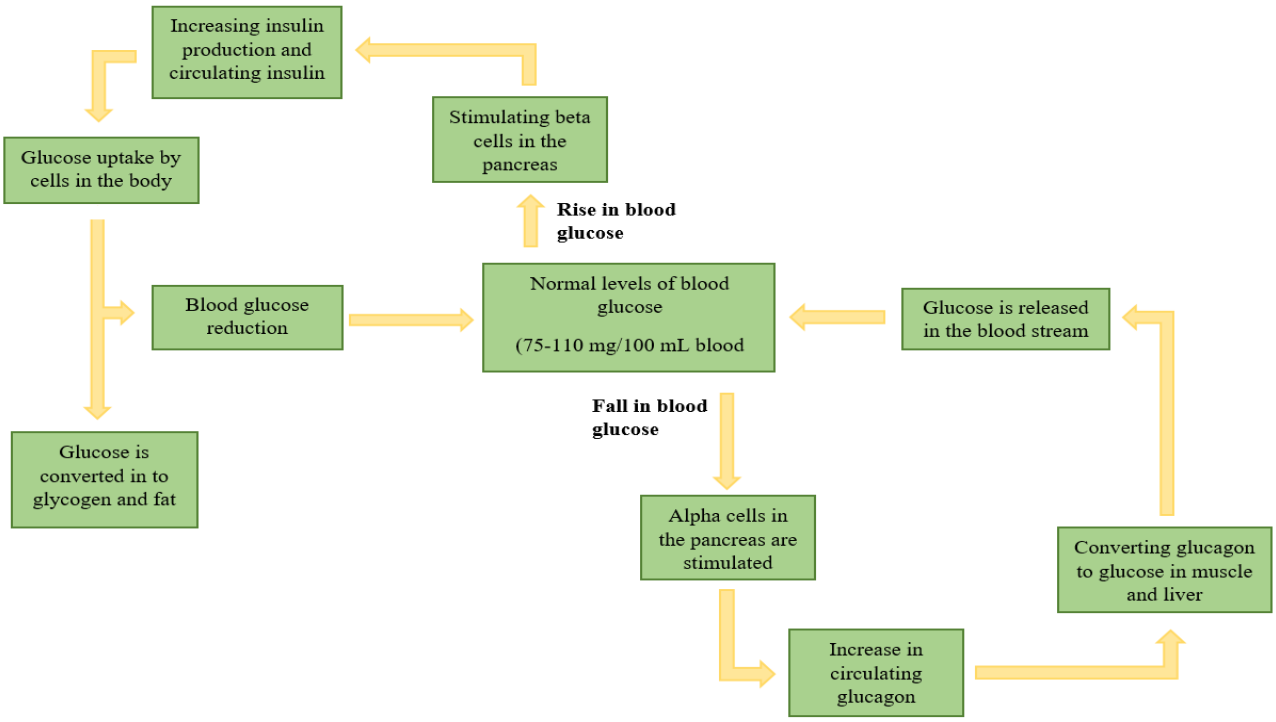


Figure 2 *Mechanisms of blood glucose homeostasis*

1.4 Proteins, satiety and weight management

Proteins are an essential part of the human diet and are the most satiating macronutrient (56-58). Dietary proteins are broken down in to shorter peptides and amino acids when in the intestine. Both peptides and amino acids have different pathways of absorption; peptides are being absorbed by a Na^+/H^+ channels, while amino acids are absorbed by specific Na^+/K ATPase channels (59, 60). Peptides act more effectively as they are rapidly absorbed and accounts for greater than 60 % of the amino acids that are absorbed by the intestine (61).

A rise in dietary protein intake have shown to have multiple positive outcomes which is thought to be due to the reduction in energy intake associated with an increase in satiety (56-58), enhancing glycaemic control (62), increased thermogenesis (63) and the positive effects on body composition, especially muscle mass (62, 64). A high protein diet seems to be beneficial in weight loss and weight management, based on the associated benefits discussed above, together with the rise in thermogenesis and for some individuals it may also have a stimulatory effect on the muscle protein anabolism (10). This suggest that an increase in protein intake, at the expense of other macronutrient, promotes satiety and thereby facilitates weight loss by reduction in energy consumption (65).

Diets high in protein, where 18-20 percentage of total energy (E%) is dietary protein have been shown to effectively manage obesity due to appetite suppression (66), however the long-term effect of a high protein diet is still not clear (67, 68).

1.4.1 Protein hydrolysates and MPH

A hydrolysate is a product of hydrolysis, and a protein hydrolysate (PH) is a product from a purified source of protein (69). It is especially attractive in exercise and sports medicine as the consumption of protein hydrolysates allows for a more rapid absorption of amino acids and therefore optimizing the delivery of nutrient to muscle tissue (70). Different protein hydrolysates vary from each other nutritionally and due to this may cause different responses (71). It generally increase the absorption kinetics and digestion, as well as it induces a greater insulin response when consumed alone (72).

Marine peptide hydrolysate (MPH) is assembled of approximately 95% short peptides, mainly dipeptides and tripeptides, which is generally largely absorbed in the mucosal cells (72). Marine peptides are especially favourable due to the vide spectra of bioactive properties; antimicrobial, antihypertensive, anticoagulant and anti-atherosclerotic, anxiolytic anti-diabetic and appetite suppression (73). Because of this, there are currently multiple marine peptide products on the market, as well as there are a few currently in clinical trials. It is a very attractive research field, especially with the bioactivity of the compound, the natural abundance and potential sustainability in production (73). MPH are through to be a very valuable nutrient resource for the pharmaceutical and nutraceutical industry as well as the food industry as a supplement that could be added into every day food items.

Siegler and co-workers examined the effects of introducing a MPH at the same time of carbohydrate and whey protein on the metabolism and performance of endurance exercise in the form of cycling (69). They found that an addition of MPH gave a significant effect on exercise metabolism, however more research is needed to investigate the performance benefits and the mechanisms behind these results and the efficacy of adding a MPH in a carbohydrate-protein training supplement (69).

1.5 Hypothesis

There is an associated between appetite and secretion of GI-hormones. When treating overweight both diet and physical activity is important.

The project leader assumed that there will be a bioactivity associated to the endocrine system with concentrations equivalent to 20 mg marine peptide hydrolysate per kg body weight.

The hypothesis is therefore that a small amount of marine peptides will affect the secretion of ghrelin and INSL5 post endurance cycling.

1.5.1 Aim

I. To explore how a marine peptide hydrolysate affect GI hormone Ghrelin and Insulin-like peptide 5 secretion
II. To explore the effect of these hormones following endurance cycling

This study may contribute to better understanding appetite regulating hormones and the mechanism of appetite and body weight regulation. This in turn offers a potential therapeutic target of treatment of underweight and obesity.

Chapter 2: Method

2.1 Study Design

This is a randomized double-blinded, placebo controlled pilot study with a crossover design investigating INSL5 and ghrelin secretion in plasma at time intervals until 120 minutes after the endurance cycling and receiving a small amount of MPH supplement. Common GI symptoms were assessed at the same time as INSL5 and ghrelin by a visual analogue scare and a categorical scale. Food intake registration was performed to investigate the diet of the study population.

This study was divided in to tree phases: baseline testing, intervention day one and intervention day tree (crossover).

2.2 Study population

The determination of the study population was estimated based on the mean changes in mean blood glucose profiles of 20%, a power of 80 %, alpha of 0.05 and with a standard deviation (SD) of 10%. These calculations estimated that 14 participants had to partake in the study.

Healthy male volunteers (n=14), between 40-50 years, medium trained with cycling as the main source of exercise, with a total amount of 8-12 hours of exercise training each week were recruited for this study. This recruitment approach attained optimal stability and low variation between the participants.

The participant recruitment started July 2017. It was conducted by publishing an informational poster (Appendix 1) on social media site of local cycling clubs in Bergen, Norway. The inclusion and exclusion criteria (sees Chapter 2.2.1) were used in screening the potential candidates per email.

2.2.1 Inclusion and exclusion

The screening of potential participants was performed per email. Inclusion and exclusion criteria is listed below.

Inclusion criteria

- Signed informed Consent
- The subject is a man between 40-50 years
- The subject has a body mass index (BMI) between 19–29
- Willing to comply with all study procedures and be available for the length of the study
- In good general health as judged by the physician at the screening visit

Exclusion criteria

- The subject has had surgery or trauma with significant blood loss or has donated blood within the last 3 months prior to the screening visit
- Diabetes type 1 or 2, or persistent high blood sugar levels
- The subject has tested positive for human immunodeficiency virus (HIV)
- Hepatitis B surface antigen (HBsAg), or hepatitis C virus antibody (anti-HCV)
- The subject has taken any investigational drugs within 1 month prior to screening
- Treated with antibiotics within 3 months prior to screening (oral, parenteral or rectal), but not spray or ointment
- Treated with steroids within 1 month prior to screening (including oral treatment)
- Treated with medication that effects the intestinal function such as, H2- protonpump inhibitors, diuretics, antiemetics, antidepressants, antacids.

2.3 Procedure

The intervention was performed at the sports lab at the Western Norway University of Applied Sciences. The tree intervention phases will be described in this chapter.

Phase one: The selected participants who was asked to partake in the study arrived for screening and baseline testing on a Thursday afternoon at the intervention facility. Participants were informed of the study aim and protocol, and signed the written consent form before start. The participants height were measured before body composition analysis was performed by using a InBody 720 (InBody) scanner. Baseline cycling test was performed and a lactate profile and a VO2 max test was conducted. After the physical cycling test, the participants received information regarding food intake registration required and a four-day food registration form was send to their email address. The participants was asked not to change their eating habits for the study period, but to adhered to some intake restrictions

mentioned in chapter 2.4. Participants received information regarding the symptom registration form so to familiarise themselves with it before the next phase.

Phase two: Participants arrived at allocated times in the morning at the intervention facility. They were immediately informed of the logistics of the day. A peripheral venous catheter was fitted before baseline fasting blood collection was conducted (see Chapter 2.3.3 for details) Next, participants received breakfast (see Chapter 2.3.1) approximately one hour before endurance cycling starts. The exercise session started with a 20 minutes warmup at 60% VO2 max subsequently followed 5 minutes cycling at 90% of VO2 max, before cyclin at 95% of VO2 max until exhaustion. The results from the cycling will not be presented as it is beyond the scope of this thesis. Immediately after the exercise session, blood collection started at 0 minutes. Participants received either the test drink A or test drink B immediately after blood collection at 0 minutes. Blood was continuously collected at the following intervals after completing the exercise session; 15, 30, 60, 90 and 120 minutes. At the time intervals of blood collection, a symptom form was filled out, see Chapter 2.3.4 for details. The participants received a standardised hot meal (see Chapter 2.3.1) immediately after blood collection at 120 minutes. Participants rested for two hours before the next session where they performed an identical exercise session as described earlier. Participants did not receive any supplementation after this exercise session and blood collection was performed as earlier; 0, 15, 30, 60, 90 and 120 minutes after completing the endurance cycling.

Phase tree: Phase tree consist of the same layout as phase two, however participants received the reverse test drink than in phase two. If a participant received test drink A in phase two, the same participant received test drink B in phase tree. Figure 3 gives a detailed account of phase two and three of the study procedure.

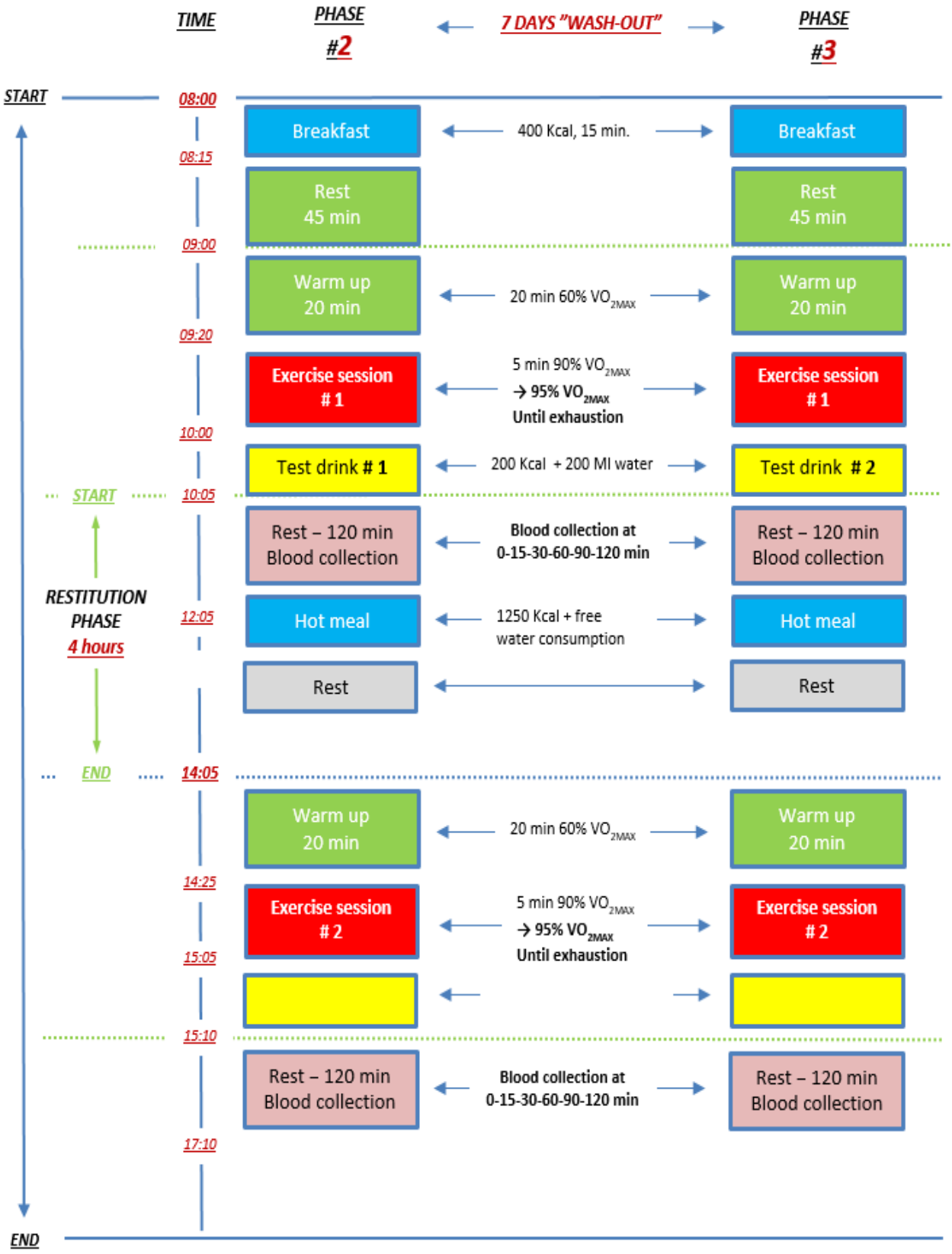


Figure 3 *Full flow-chart of phase two and three.*

2.3.1 Standardised test day foods

All participants received the same test meal, regardless of their individual needs. The breakfast was comprised of a whole wheat baguette with ham, cheese, green salad and peppers (from *'Lie-Nielsen konditori'* at Kronstad, Norway). An option of 200 ml orange juice and 200 ml coffee or tea was given to the participants. A standardized hot meal was served after 2 hours rest post exercise session 1; a readymade meal beef stroganoff (*Biff Stroganoff med ris),* or a meat free option; readymade meal of cod, carrots, potato and leek sauce (*Torsk I Purreløksaus med gulrøtter og poteter)* both from Fjordland AS. Table 1 gives an account of the nutritional value and composition of the breakfast, as well as the hot meal of beef stroganoff from Fjordland AS. Aditionally is a meat free option to the hot meal which was added during conduction of the study. Table 1 does not include the test drink, for details see Chapter 2.3.2

Table 1 *Nutritional values of test day foods, excluding test day drink.*

Meal	Quantity, grams/mL	Energy, Kcal	CHO, g	Protein, g	Fat, g
Breakfast;					
- Bread	92.6 g	228	41.9	8	2
- Ham (cooked)	24.9 g	26	0	4.5	0.9
- Norwegian cheese (Norvegia)	33.3 g	117	0	9	9
- Orange juice	200 ml	86	19.2	1.4	0.4
Total:	150.7 g	457 kcal	61.1 g	22.9 g	12.3 g
Hot meal [a)];					
Beef stroganoff, total:	460 g	423 kcal	59.8	26.7 g	8.3 g
Cod, carrot, potato and leek sauce, total:	550 g	330 kcal	37.4 g	30.8 g	5.5 g

[a)] One of the participants turned out to be pescetarian on the day of the intervention, and received a hot meal containing cod instead of beef.

Abbreviation:: CHO = carbohydrate

The participants were allowed to drink up to 2.5 to 3 litres of water during the intervention.

2.3.2 Test drink; Marine peptide hydrolysate (MPH) or placebo

The participants received test drink A or test drink B after completing exercise session 1 during phase one, and the other test drink after exercise session 1 in phase three. It was self-selected if they received test drink A or B during phase two and the other drink during phase three.

The test drinks was provided by Firmenich Bjørge Biomarin AS Aalesund/Norway, ready with the participant ID and numbered with the letter A or B. The final mixing, incorporating cold water, was conducted approximately 30 minutes before exercise completion.

The test drink was served in an opaque glass bottle. Each bottle contained a white powder of nutrients which was calculated of the individual participants weight. Test drink A contained a 20 mg MPH/BW which replaced approximately 3.2% of the whey protein. The colour, taste and smell of the powder was disguised via use of technical ingredients such as flavouring agent, acidifier and natural colouring. Each glass bottle was given a natural strawberry flavour and a pink colour to mask the difference in flavour. Both test drinks were isocaloric and isonitrogenous.

The test drinks is composed of a whey protein concentrate (WPC80 from TINE ASA, Bergen), vegetable fat (Medium Chained Triglycerides (MCT) fat powder, 70:30 from BERGAMAST) and maltodextrin from corn. The MPH powder is made from fish fillet of the Atlantic cod. The nutrient distribution is 12 % protein, 22 % fat and 66 % carbohydrates, which equals to 3,68 kcal/kg BW. The powder was mixed 1 g powder with 2 ml cold water approximately 30 minutes before completing the exercise.

Test drink A: 66 % CHO, 8,8 % WPC, 3.2 % MPH and 22 % MCT.

Test drink B: 66 % Cho, 12 % WPC, 22 % MCT.

2.3.3 Blood Collection

Acylated ghrelin (AG) is easily cleaved during sample collection and to ensure accuracy when measuring the concentration of AG in plasma. Aprotinin was added to EDTA tubes (Greiner Bio-One) prior to sample collection (26) to prevent this to happen to the samples. The plasma samples were used in measuring AG and INSL5. Serum gel tubes (Greiner Bio-One) were used in measuring blood glucose and insulin.

Participants were fitted with a peripheral venous catheter (infusjon cannula 18G, BD) by a qualified nurse on the inside of the elbow at time of arrival for phase two and three. The catheter was washed with 2.5 ml saline solution and a 3-way tap was established. Baseline (fasting) blood sample was also collected at this point. Blood sampling was then performed at 0, 15, 30, 60 90 and 120 minutes after completing the exercise session, and again at the same time intervals after exercise session two (see figure 3 for details). Prior to each blood collection, the catheter was washed with 2.5 ml saline solution.

Plasma collection

The EDTA tubes was mixed by turning the blood 8-10 times. It was put on ice until the blood sample was centrifuged for 10 minutes at 1800 x g at 4°C within 20 minutes of collection. Plasma was aliquoted into sterile cryotubes by pipetting. The cryotubes was put on ice until the sample collection was complete and during transport.

Serum collection

The serum gel tubes was mixed by turning before sitting in room temperature for a minimum of 30 minutes before centrifuging at 1800-2200 G in 10-15 minutes at room temperature. Serum was then removed by pipetting and aliquoted into designated sterile cryotubes. The cryotubes was stored on ice whilst waiting for blood sampling to be complete and during transport. All samples was stored at – 80 °C until analysed (74).

2.3.4 Symptom registration

The participants had to answer two questionnaires during blood sampling after the exercise sessions. These questions were related to GI symptoms, hunger and satiety.

The visual analogue scale (9) is a linear scale ranging from 0 till 100 mm containing the following questions and the range in which the participants marked this linear scale (see appendix 2);

I. Do you experience pain in the middle part of the stomach? Ranging from no pain to very uncomfortable.

II. Are you nauseous? Ranging from not nauseous to very nauseous.
III. Do you have a feeling of fullness in the stomach? Ranging from not at all to very full.
IV. The total discomfort in the upper part of the stomach? Ranging from not at all to very discomforting.
V. Are you satiated? Ranging from very hungry (0 mm) to fully satiated (100 mm)

2.4 Four days diet registration

The four day food intake registration was divided in to two parts; three days (one weekend day and two weekdays) between phase one and phase two, and one weekday in the washout period of one week before phase three.

The participants were informed of the concept of food intake registration and the diet registration form during phase one. The diet registration form "Kostregistrering" from Helse Bergen, Haukeland Universitetssjukehus – Section of Dietetics (see Appendix 2) was used in the introduction/walk through as well as the food registration itself. This document was sent to the participants per email and was filled out electronically in a word document. A supplementary list of commonly forgotten items to add to the food intake registration form, as well as some tips for weighing the food items was given in paper form at phase one. The participants received contact information in case if any questions or issues arises regarding the food intake registration.

During the study period there were a restriction of no more than 5 cups of coffee or tea allowed per day, and no alcohol consumption 48 hours prior to the visits.

The reported food intake in the diet registration form was added to *Kostholdsplanleggeren* (The Norwegian Directorate of Health), which is a validated database for calculating nutrients. All food items and recipes was recorded in this nutrition analysing tool.

2.4.1 Estimated Energy and Protein Requirement

Energy expenditure (EE) was calculated through the factorial method of calculating total EE from Resting Energy Expenditure (REE) times a physical activity level (PAL) coefficient for men 31-60 years of age; REE = 0.0600 W (weight, kg)) + 1,31 H (height, m) + 0.473. The

PAL was set to 1.9, based on the amount of exercise the participants was conducting each week (8-12 hours).

The protein requirement for the study population was set to 1.5 grams of protein per kg body weight (BW) per day based on the amount of exercise they do each week.

2.5 Measuring Ghrelin in human plasma

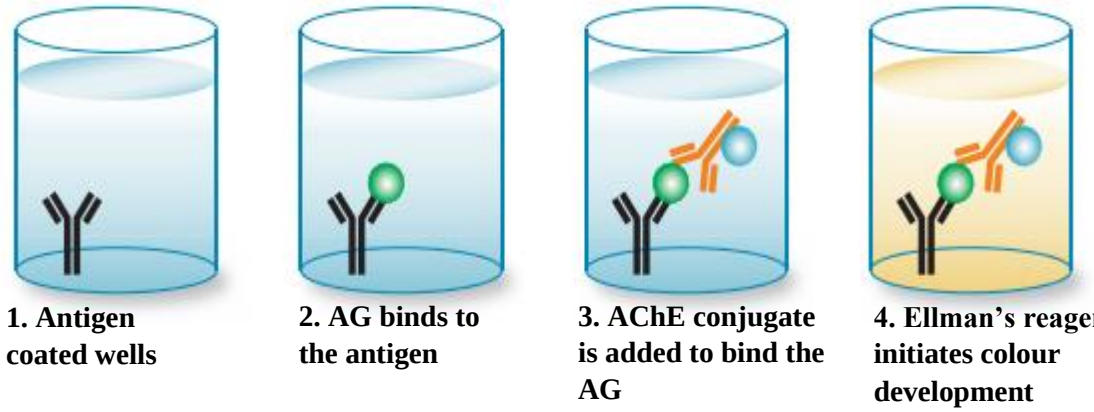
Circulating AG was measured in plasma using an Enzyme Immunoetric Assay (EIA) (Bertin Pharma, catalogue #30110797 (CMA05306) Lot #0117). The EIA kit is formatted with 96 wells and gives a quantitative measure of circulating AG in plasma. It is pre-coated with AG-specific antibodies. Sample collection is specified in chapter 2.3.3, see *plasma collection* of details. A step-by-step protocol can be found in the appendix 3.

Plate setup contained one blank, one Non Specidic Binding (NSB) and one Quality Control (QC) well. Standards one through seven was run in duplicate, and standard number eight in single due to insufficient space on the plate. Six profiles (tree individuals) was analysed per plate, see appendix 4 for example of plate setup. All samples and reagents reached room temperature before assay start.

Reagents and buffers were prepared according to protocol and samples were diluted 1+1. Samples were mixed by turning 8-10 times before pipetting out the needed amount of plasma. Plate preparation included rinsing each well with wash buffer before distributing samples and reagents in designated wells. The antibodies in the well bound to any plasma AG from the standard solutions and from the samples. The plate was placed on a the platform shaker (Heidolph Titramax 100) at room temperature for incubation for two hours. After incubation, the plate was washed with wash buffer to remove unbound residuals left. Acetylcholinesterase (AChE) conjugate was added to all wells except the blank well, before incubating for yet 2 hours on the platform shaker. The plate is washed with wash solution to remove any excess reagents before adding the Ellman's Reagent, which initiates colour developments. See Figure 4 for step-by-step illustration. Colour is developed proportional to the amount of AG bound to the wells. The plate was covered with an aluminium lid and incubated on the platform shaker for optimal colour development. Absorbance was measured periodically at 30, 45, 60 and 90 minutes of incubation at 405 nm (Spectra max plus, microplate spectrophotometer). After completion, all results was analysed to determine the time of optimal incubation, which in this

case was after 60 minutes of incubation. The sensitivity of the kit was not high enough in this EIA-kit and the results was log-log transformed to get a more complete dataset, and the SoftMax Pro software (from Molecular Devices) was used to log-log transform the results.

Figure 4 *Acylated ghrelin EIA illustration. Modified figure by (2).*



Abbreviations: AG = acylated ghrelin, AChE = Acetylcholinesterase

2.6 Measuring INSL5 in human plasma

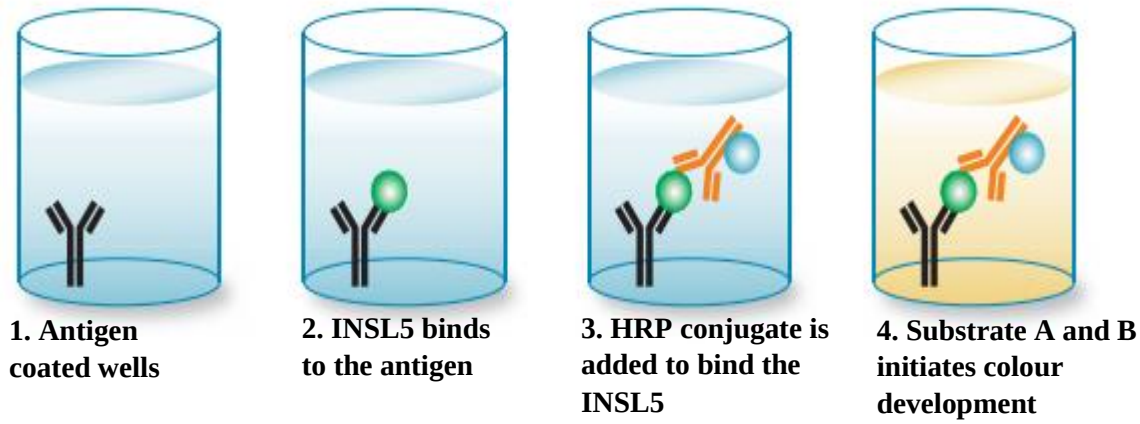
Circulating INSL5 was measured in plasma, using a sandwich Enzyme-linked immunosorbent assay (75) (MyBioScource, catalogue #MBS944278, Lot #C1402071407). The ELISA kit is formatted with 96 well, and provide a quantitative measure of circulating INSL5 in plasma. The kit is pre-coated with INSL5-specific antibodies. The sample collection is specified in Chapter 2.3.3, see *plasma collection* for details. See appendix 5 for a step-by-step protocol.

The plate setup contained one blank and five standards all in duplicates. Six profiles was analysed per plate. See appendix 6 for plate setup example. All samples and reagents reached room temperature before assay start.

Reagents and buffers were prepared according to the manufacturers protocol. Samples was mixed by turning 8-10 times before pipetting each sample. Samples and the standards were distributed to the designated wells on the plate (see plate setup). The antibodies pre-coated in the wells bound to INSL5 from the standard solutions and the samples. This was followed by Horseradish Peroxidase (HRP) conjugated antibody that binds to the antigen explicitly and

incubated for one hour at 37°C. The plate was washes with wash buffer after incubation to get rid of any unbound reagents before adding the two substrate solutions. The substrates initiated a development of colour proportional to the amount of INSL5 that is bound to the antibodies in the well. After incubating in 15 minutes at 37°C, a stop solution was added to stop the colour development and absorbance was measured. See Figure 5 for step-by-step illustration. A microplate reader (spectra max plus, microplate spectrophotometer) measured optical density of the wells within 10 minutes of adding stop solution at 450 nm. The results was log-log transformed, which was one of the options in this kit.

Figure 5 *Insulin-like peptide 5 ELISA illustration. Modified figure by (2).*



Abbreviations: INSL5 = Insulin-like peptide 5, HRP = Horseradish Peroxidase.

2.7 Blood glucose and Insulin measurement

Serum glucose and insulin were analysed at the Laboratory for Clinical Biochemistry (LKB) at Haukeland University Hospital using standardised methods for Glucose measurement (http://www.analyseoversikten.no/analyse/215) and insulin (http://www.analyseoversikten.no/analyse/22). See the links for more details.

"For statistic calculations, insulin values under the limit of determination (<2 mlE/L) were set to 1.9 mlE/L according to the recommendation from the Hormone Laboratory, Haukeland University Hospital."

2.8 Ethical considerations

This study was approved by the Western Norway Regional Committee for Medical and Health Research Ethics **(**REK 2017/56**)**. All participants received written information regarding the study before committing to the study. Participants who were asked to partake in the study were also informed that this study is completely voluntary and if they at any time could withdraw from the study without giving any reasons. The participants gave their written consent after being fully informed of the aim and the protocol of the study (Appendix 7)

The endurance exercise testing was performed on the cycle ergometer, and is a common component of exercise capacity assessment. The tests performed were strenuous, however the participants well-being was always being attended and testing would stop if the participants displayed any forms of discomfort.

Participation was anonymous and the participants received an identification number during the first phase of the study. Throughout the study period and during analysing and processing the samples, participants stayed anonymous. The data was stored on a password protected research server and the data was stored on a password protected computer during analysis of the results.

2.5 Statistics

IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL) was used to perform the statistical analyses.

Descriptive analysis was performed to characterize the participant demographics and the diet registration. Results are reported as means, percent, standard deviation (SD), mean, standard error of mean (SEM) and 95 % confidence intervals (CI). For correlations a Pearson's correlation was used.

A mixed linear regression analysis was performed for each outcome variable (ghrelin, insulin-like peptide 5, glucose and insulin) using SPSS, with respect to treatment, time, session, including adjustment for BMI. Compound symmetry correlation structure was assumed between measures from the same subject.

Pearson's and Spearman's correlation was performed between GI hormones, and measured satiety by visual analogue scale.

GraphPad Prism (GraphPad Software, Inc., San Diego, CA) was used to measure area under the curve for all variables and satiety measured by visual analogue scale.

Chapter 3: Results

3.1 Participants demographics

Fourteen healthy men participated in the study. Mean (range of) age was 46 years (40 to 58 years), body weight 80.1 kg (71.2 to 96.2 kg), and height 180.8 cm (176 to 192 cm). The mean calculated BMI (kg/m^2) was 24.5 kg/m^2, giving a range in BMI from 21.4 to 29.4 kg/m^2. For detailed descriptive statistics regarding the participant demographics see Table 2 below.

Table 2 *Descriptive statistics of participant demographics measured by the body composition analysis (n = 14).*

Characteristic	Mean (SEM)	Median (SD)	Range (Min, Max)
Age (years)	45 (1.42)	45 (5.3)	(40, 58)
Height (cm)	180 (1.1)	181 (4.1)	(176, 192)
Weight (kg)	80.1 (1.72)	79.8 (6.4)	(71.2, 96,2)
BMI (kg/m^2)	24.5 (0.6)	24.7 (2.2)	(21.4, 29.4)
MM (kg)	37.7 (0.6)	37.8 (2.3)	(33.9, 40.9)
FM (kg)	13.5 (1.2)	12.6 (4.5)	(5.8, 24.7)
FM (%)	16.6 (1.2)	15.5 (4.4)	(7.8, 25.7)
FFM (kg)	66.6 (1.0)	66.6 (3.7)	(60.4, 71.5)

Abbreviations: SEM = standard error of mean, SD = standard deviation, BMI = body mass index, MM = muscle mass, FM = fat mass, FFM = fat free mass.

3.2 Circulating active ghrelin post endurance cycling

Table 3 presents the results of a linear mixed regression analysis performed for ghrelin with respect to the independent variables: treatment, cycling session, and time (min) including tests for interactions. Statistical significant differences was found between the treatments ($p = 0.014$), between cycling session 1 and 2 ($p < 0.001$), between the time intervals of blood collection post cycling at cycling session 2 ($p < 0.001$) and for the interaction cycling session

1 and the time intervals ($p < 0.001$). There was a negative effect of endurance exercise when adjusted for BMI: -1.71 (estimate), 95 % CI: (-3.24, -0.19), $p = 0.031$.

Table 3 *Acylated ghrelin as function of treatment, session and time (n=13)* [a)].

Variable category	Estimate	95% CI	P-value [b)]
Intercept	16.00	(11.28, 20.71)	< 0.001
Treatment			**0.014**
MPH	-1.85	(-3.33, -0.37)	
Placebo	0.00	(reference)	.
Cycling session			< 0.001
1	-2.68	(-6.38, 1.03)	
2	0.00	(reference)	.
Time, min			< 0.001
0	-5.16	(-8.87, -1.45)	
15	-5.80	(-9.54, -2.06)	
30	-3.56	(-7.26, 0.15)	
60	0.78	(-2.93, 4.49)	
90	5.18	(1.47, 8.89)	
120	0.00	(reference)	.
Cycling session 1 × Time, min [c)]			< 0.001
1 × 0	2.76	(-2.39, 7.92)	
1 × 15	1.24	(-3.96, 6.45)	
1 × 30	-0.16	(-5.34, 5.02)	
1 × 60	-4.68	(-9.83, 0.48)	
1 × 90	-6.40	(-10.58, -0.22)	
1 × 120	0.00	(reference)	.

[a)] Complete dataset from participant 3 is missing. [b)] Linear mixed regression analysis. [c)] Data from 7 time points are missing total 305 samples.

Abbreviations: CI = Confidence interval, MPH = marine peptide hydrolysate.

Figure 6 show the circulating AG during session 1 compared to session 2, independent of treatment. A greater amount of circulating AG was measured in session 2 compared to session 1. This difference is statistically significant ($p < 0.001$).

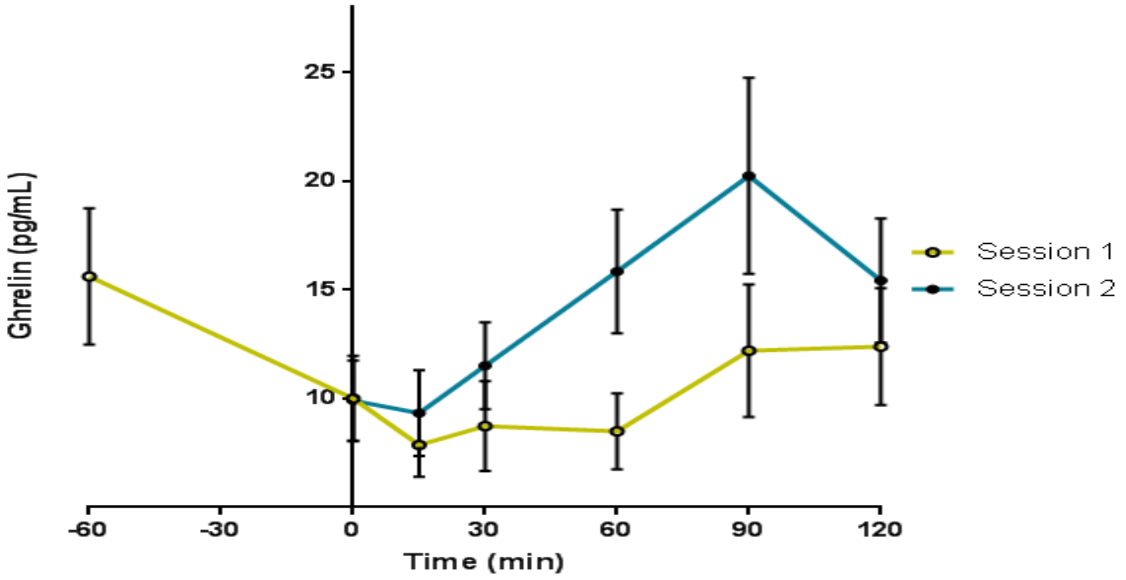
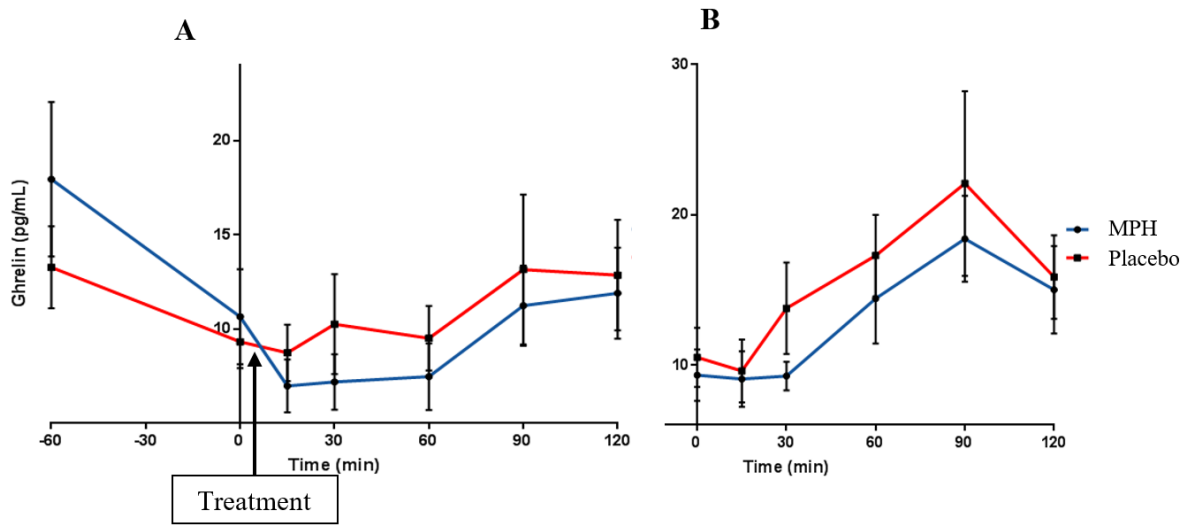


Figure 6 *Circulating acylated ghrelin measured at time points in cycling session 1 and session 2 (n=13, p < 0.001).*

Circulating AG was also calculated by an area under the curve (AUC) analysis which is shown in Figure 7, and Table 4. Figure 7A shows at what time the participants received MPH or placebo. The greatest measured circulating ghrelin in session 1 is measured at fasting (time point – 60, Figure 7A and Table 4), which was measured to be 17.96 pg/mL for MPH and 13.28 pg/mL for placebo. In session 2, the greatest measured circulating ghrelin is measured at 90 minutes after completing the endurance cycling and was measured to be 18.4 pg/mL for MPH and 22.09 pg/mL for placebo (figure 7B and Table 4).



A: cycling session 1, B: cycling session 2. P < 0.001.

Figure 7 *Circulating acylated ghrelin as function of treatment (n = 13).*

Table 4 *Acylated Ghrelin measured by area under the curve (n=13[a)]).*

	Session 1 [b)]		Session 2 [c)]	
Parameters	**MPH**	**Placebo**	**MPH**	**Placebo**
Total Area	1946	1984	1624	1951
SEM	613.1	512.2	382.1	596.9
95 % CI	(744.1, 3147)	(979.7, 2988)	(874.9, 2373)	(781.5, 3121)
First X	-60	-60	0	0
Last X	120	120	120	120
Peak X	-60	-60	90	90
Peak Y	17.96	13.28	18.4	22.09

[a)] Complete dataset missing for one participant. [b)] Data from 3 time points are missing, total 166 samples. [c)] Data from 4 time points are missing, total 152 samples.

Abbreviations: MPH = marine peptide hydrolysate, SEM = Standard Error of Mean, CI = Confidence interval.

Figure 7A display a great drop in circulating ghrelin between fasting values and the acutely effect of MPH or placebo 15 minutes after completing the exercise session and receiving the test drink. To compare this drop in ghrelin, a paired-samples t-test was conducted. There was not a significant difference in the scores for MPH (mean = 10.45, SD = 13.85) and placebo (mean = 4.48, SD = 5.78), t (11) = 2.09, p = 0.061. A non-parametric Wilcoxon Signed Ranks Test (Z) was also performed (Z = - 1.804, p = 0.077). The results from both tests suggest that treatment does not have an effect on the reduction in circulating ghrelin between fasting values and 15 minutes after exercise session ended and receiving the test drink. Treatment does not explain this drop in circulating ghrelin levels.

3.3 Insulin-Like Peptide 5 measurement

Table 5 display the results of a linear mixed regression analysis performed for INSL5 with respect to the independent variables: treatment, cycling session, and time (min including tests for interactions. There was not found a statistical difference between treatment ($p = 0.121$), however a statistical significant was founf between cyclin session 1 and 2 ($p < 0.001$), between the time intervals of blood collection post cycing session 2 ($p < 0.001$) and for the interaction between cycling session 1 and the time intervals ($p < 0.001$). There was a non significant positive effect of endurance exercise when adjusted for BMI: 0.82 (estimate), 95 % CI: (-1.78, 3.43), $p = 0.505$.

Table 5 *Insulin-like peptide 5 as function of treatment, session and time (n=14).*

Variable category	Estimate	95% CI	P-value [b)]
Intercept	5.58	(-4.89, 16.05)	< 0.001
Treatment			**0.121**
MPH	3.88	(-1.04, 8.81)	
Placebo	0.00	(reference)	.
Cycling session			< 0.001
1	10.23	(-2.26, 22.73)	
2	0.00	(reference)	.
Time, min [a)]			< 0.001
0	13.99	(1.49, 26.49)	
15	3.54	(-8.95, 16,04)	
30	0.93	(-11.57, 13.42)	
60	0.19	(-12.30, 12.69)	
90	0.40	(-12.10, 12.89)	
120	0.00	(reference)	.
Cycling session 1 × Time, min			< 0.001
1 × 0	- 2.13	(-19.44, 15.19)	
1 × 15	8.83	(-8.48, 26.15)	
1 × 30	65.13	(47.82, 82.45)	
1 × 60	71.91	(54.59, 89.22)	
1 × 90	24.64	(7.32, 41.95)	
1 × 120	0	(reference)	.

[a)] Data from 4 time points are missing total 346 samples.

Abbreviations: CI = Confidence interval, MPH = marine peptide hydrolysate.

Figure 8 display the circulating INSL5 after exercise session 1 and 2, independently of treatment. A greater amount of circulating INSL5 was measured during the first session

compared to the latter. This difference in circulating INSL5 is statistically significant ($p < 0.001$), as measured by the mixed model.

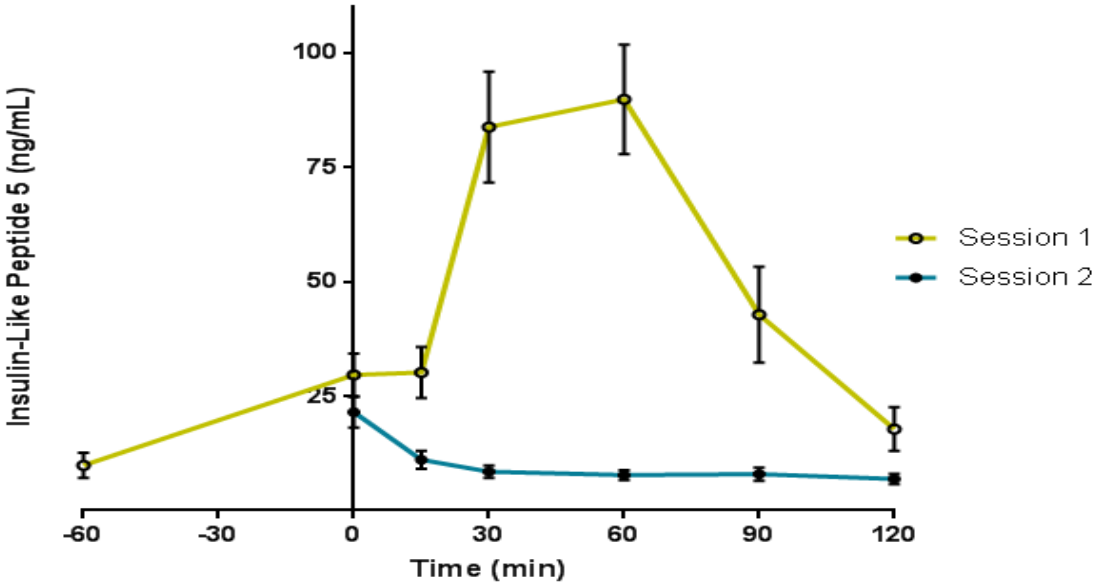
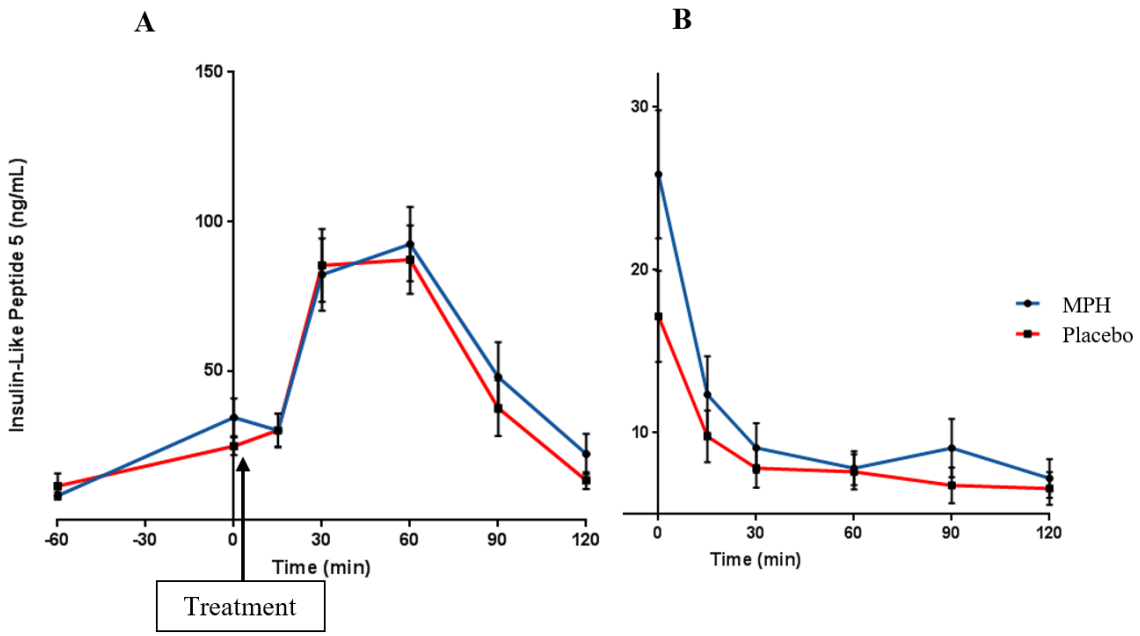


Figure 8 *Circulating insulin-like peptide 5 measured at time points in cycling session 1 and session 2 (n=14, p < 0.001).*

Circulating INSL5 calculated by AUC analysis is shown in Figure 9 and Table 6. Figure 9A show at what time the participants received MPH or placebo. The greatest concentration of INSL5 was measured at 60 minutes after completing exercise session 1. Mean INSL5 concentration reached 92.45 ng/mL after receiving the MPH and 87.27 ng/mL after receiving the placebo (Figure 9A and Table 6). The greatest consentration after session 2 was measured at 0 minues, emediately after completing the exercise session. The mean INSL5 consentration was 25.88 ng/mL after rcieving MPH and 17.15 ng/mL after receiving placebo (figure 9B and Table 6).



A: cycling session 1, B: cycling session 2. P = 0.121.

Figure 9 *Circulating insulin-like peptide 5 as function of treatment.*

Table 6 *Insulin-like peptide 5 measured by area under the curve analysis (n = 14).*

	Session 1 [a)]		Session 2 [b)]	
Parameters	**MPH**	**Placebo**	**MPH**	**Placebo**
Total Area	8386	7594	1199	981,6
SEM	476	412.5	66.16	49.1
95 % CI	7453 to 9319	6786 to 8403	1069 to 1328	885,3 to 1078
First X	-60	-60	0	0
Last X	120	120	120	120
Peak X	60	60	0	0
Peak Y	9245	87.27	25.88	17.15

[a)] Data from 2 time points are missing, total 194 samples. [b)] Data from 2 time points are missing, total 166 samples).

Abbreviations: MPH = marine peptide hydrolysate, SEM = Standard Error of Mean, CI = Confidence interval.

3.4 Glucose measurement

Table 7 present the results of a linear mixed regression analysis performed for ghrelin with respect to the independent variables: treatment, cyclin session, and time (min) including tests for interactions. Statistical significant difference was not found between treatment (p = 0.190), however there was a statistical significant difference between cycling session 1 and 2 (p < 0.001), between the time intervals of blood collection post cycling at cycling session 2 (p < 0.001) and for the interaction cycling session 1 and the time intervals (p < 0.001). There was a non-significant negative effect of endurance exercise when adjusting for BMI: -0.10 (estimate), 95 % CI: (-0.22, 0-02), p =0.092.

Table 7 *Blood glucose as function of treatment, session and time (n = 14).*

Variable category	Estimate	95% CI	P-value [a)]
Intercept	4.93	(4.50, 5.37)	< 0.001
Treatment			**0.190**
MPH	0.13	(-0.06, 0.32)	
Placebo	0.00	(reference)	.
Cycling session			< 0.001
1	-0.99	(-1.47, -0.51)	
2	0.00	(reference)	.
Time, min [b)]			< 0.001
0	1.46	(0.98, 1.94)	
15	0.24	(-0.24, 0.72)	
30	-0.07	(-0.55, 0.41)	
60	-0.06	(-0.54, 0.42)	
90	-0.03	(-0.51, 0.45)	
120	0.00	(reference)	.
Cycling session × Time, min			< 0.001
1 × 0	2.23	(1.56, 2.90)	
1 × 15	2.49	(1.83, 3.16)	
1 × 30	3.13	(2.46, 3.80)	
1 × 60	1.68	(1.01, 2.35)	
1 × 90	0.31	(-0.35, 0.98)	
1 × 120	0.00	(reference)	.

[a)] Linear mixed regression analysis. [b)] Data from 3 time points are missing, total 347 samples.

Abbreviations: MPH = marine peptide hydrolysate, SEM = Standard Error of Mean, CI = Confidence interval.

Figure 10 show blood glucose levels during session 1 compared to session 2, independent of treatment. The difference in blood glucose levels are statistically significant between sessions ($p < 0.001$), as measured by the mixed model.

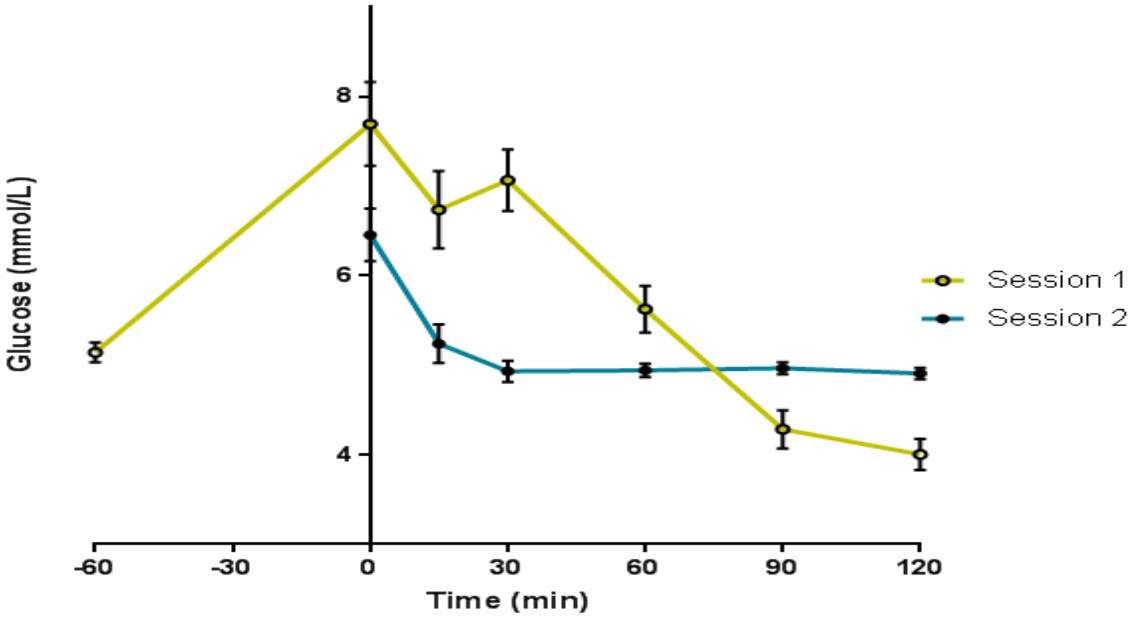
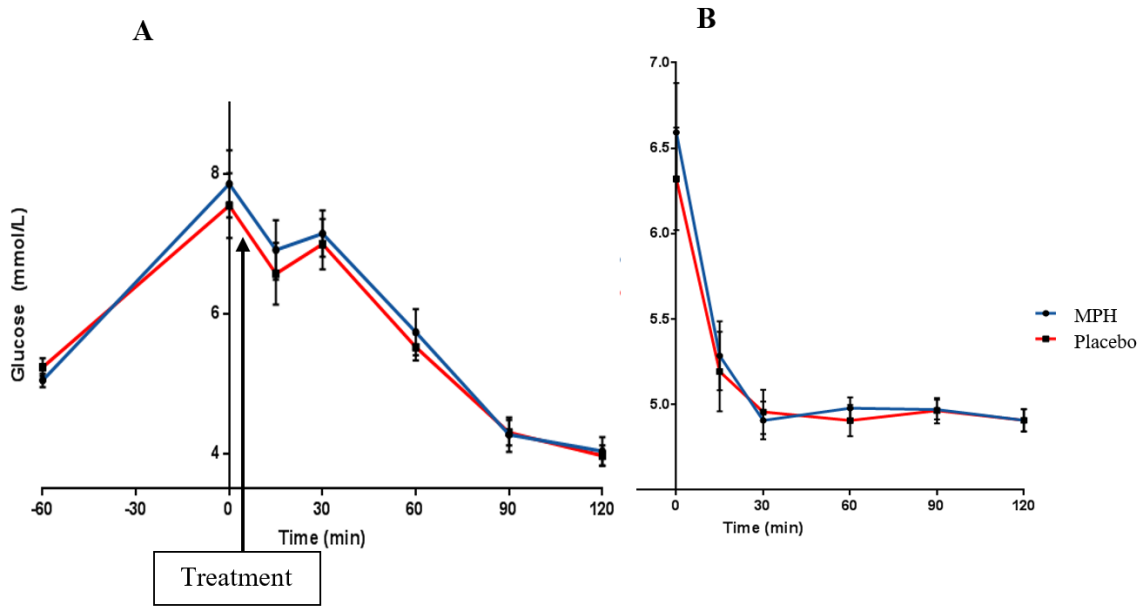


Figure 10 *Circulating blood glucose measured at time points in cycling session 1 and session 2 (n=14, p < 0.001).*

Circulating blood glucose was also calculated by an AUC analysis as shown in Figure 11 and Table 8. Figure 11A show the time participants received MPH or placebo. The greatest measured circulating glucose in session 1 was 7.85 mmol/L for MPH and 7.543 mmol/L for placebo at 0 minutes, immediately after the exercise session (Figure 11A and Table 8). The greatest measured circulating blood glucose in session 2 was measured to be 6.593 mmol/L for MPH and 6.321 mmol/L for placebo which was measured again at 0 minutes (Figure 11B and Table 8).



A: cycling session 1, B: cycling session 2. P = 0.190

Figure 11 *Area under the curve analysis of Glucose as function of treatment (n = 14).*

Table 8 *Blood glucose measured by area under the curve analysis (n = 14).*

	Session 1		Session 2 [a)]	
Parameters	**MPH**	**Placebo**	**MPH**	**Placebo**
Total Area	1071	1050	611,3	606,6
SEM	71.49	66.01	15.41	17.98
95 % CI	930,8 to 1211	920,7 to 1179	581,1 to 641,5	571,4 to 641,8
First X	-60	-60	0	0
Last X	120	120	120	120
Peak X	0	0	0	0
Peak Y	7.85	7.543	6.593	6.321

[a)] Data from 3 participants are missing, total 165 samples.

Abbreviations: MPH = marine peptide hydrolysate, SEM = Standard Error of Mean, CI = Confidence interval.

3.5 Insulin measurement

Table 9 display the results of a linear mixed regression analysis performed for insulin with respect to the independent variables: treatment, cycling session, and time (min) including tests for interactionss. No statistical significant difference was found in treatment (p = 0.572), however there was a statistical significant difference between cycling session 1 and 2 (p < 0.001), between the time intervals of blood collection post cycling at cycling session 2 (p < 0.001) and for the interaction cycling session 1 and the time intervals (p < 0.001). There was a non-significant positive effect of endurance exercise when adjustment for BMI was made: 1.05 (estimate), 95 % CI: (-0.27, 2.38), p = 0.109.

Table 9 *Serum Insulin as function of treatment[)], session and time (n = 14) [a)].*

Variable category	Estimate	95% CI	P-value [b)]
Intercept	3.44	(-2.12, 9.00)	< 0.001
Treatment			**0.572**
MPH	0.76	(-1.87, 3.39)	
Placebo	0.00	(reference)	.
Session			< 0.001
1	9.09	(2.48, 15.71)	
2	0.00	(reference)	.
Time, min [c)]			< 0.001
0	11.04	(4.42, 17.65)	
15	5.08	(-1.54, 11.70)	
30	2.69	(-3.92, 9.31)	
60	0.92	(-5.70, 7.54)	
90	0.62	(-5.99, 7.24)	
120	0	(reference)	.
Session × Time, min			< 0.001
1 × 0	-5.78	(-15.00, 3.44)	
1 × 15	0.20	(-9.02, 9.42)	
1 × 30	28.36	(19.14, 37.58)	
1 × 60	43.76	(34.54, 52.98)	
1 × 90	15.03	(5.81, 24,25)	
1 × 120	0	(reference)	.

[a)] Where serum insulin < 2 it was set to 1.9 mlE/L (37 samples). [b)] Linear mixed regression analysis.
[c)] Data from 4 time points are missing, total 332 samples.

Abbreviations: CI = Confidence interval, MPH = marine peptide hydrolysate.

Figure 12 display serum insulin during session 1 compared to session 2, independent of treatment. A greater amount of serum insulin was measured in session 1 compared to session 2. The difference is statisically significant ($p < 0.001$).

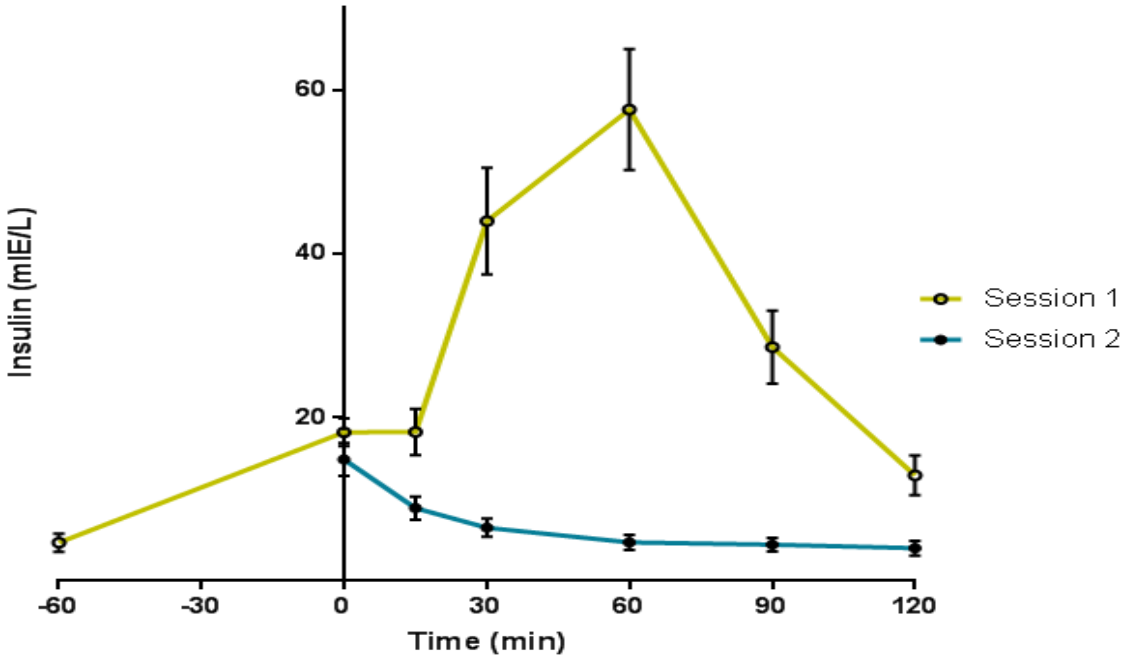
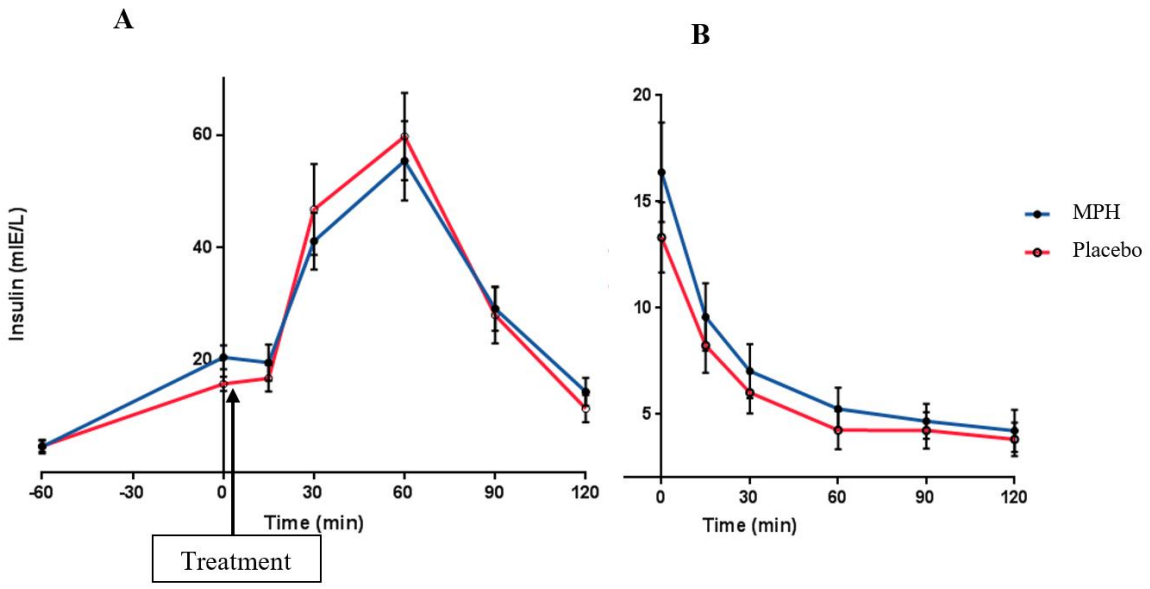


Figure 12 *Serum insulin measured at time points in cycling session 1 and session 2 (n =14, p < 0.001).*

Serum insulin was also calculated by an AUC analysis which is shown in Figure 13 and Table 10. Figure 13A show at what time the participant received MPH or placebo. The greatest measured serum insulin in session 1 was at 60 minutes and was 55.44 mlE/L after MPH and 59.76 mlE/L after placebo (Figure 13A and Table 10). In session 2, the greatest measured serum insulin was at 0 minutes, immediately after the exercise, and was measured to be 16.39 mlE/L for MPH and 13.32 mlE/L for placebo (Figure 13B and Table 10).



A: cycling session 1, B: cycling session 2. P = 0.572

Figure 13 *Circulating insulin as function of treatment.*

Table 10 *Circulating insulin measured by area under the curve analysis (n =14).*

	Session 1 [a)]		**Session 2** [b)]	
Parameters	**MPH**	**Placebo**	**MPH**	**Placebo**
Total Area	4884	4844	784,6	669,6
SEM	792.8	923.9	169.1	142.4
95 % CI	3330 to 6437	3033 to 6655	453,2 to 1116	390,4 to 948,7
First X	-60	-60	0	0
Last X	120	120	120	120
Peak X	60	60	0	0
Peak Y	55.44	59.76	16.39	13.32

[a)] Data from 1 time point is missing, total 195 samples. [b)] Data from 3 time points are missing, total 165 sample.

3.6 Association between GI hormones, and satiety

Table 11 displays a total summary of all correlations between the variables ghrelin, INSL5, glucose, insulin, and satiety measure by the Visual Analogue Scale (9) questionnaire. A Pearson's correlation coefficient (r) was calculated to evaluate the relationship between the circulating GI hormone values with glucose, insulin in the blood and satiety. A nonparametric Spearman's rank-order correlation (ρ) was also calculated. Further details are shown in the following chapters.

Table 11 *Summary of correlations [a] between GI hormones and satiety.*

	Ghrelin	INSL5	Glucose	Insulin	Satiety [b]
Ghrelin	1	r = - 0.286	r = - 0.188	r = - 0.063 [c]	r = - 0.472
INSL5	ρ = - 0.500	1	r = 0.376	r = 0.928	r = - 0.282
Glucose	ρ = - 0.212	ρ = 0.465	1	x	x
Insulin	ρ = - 0.115	ρ = 0.927	x	1	x
Satiety [b]	ρ = - 0.503	ρ = 0.233	x	x	1

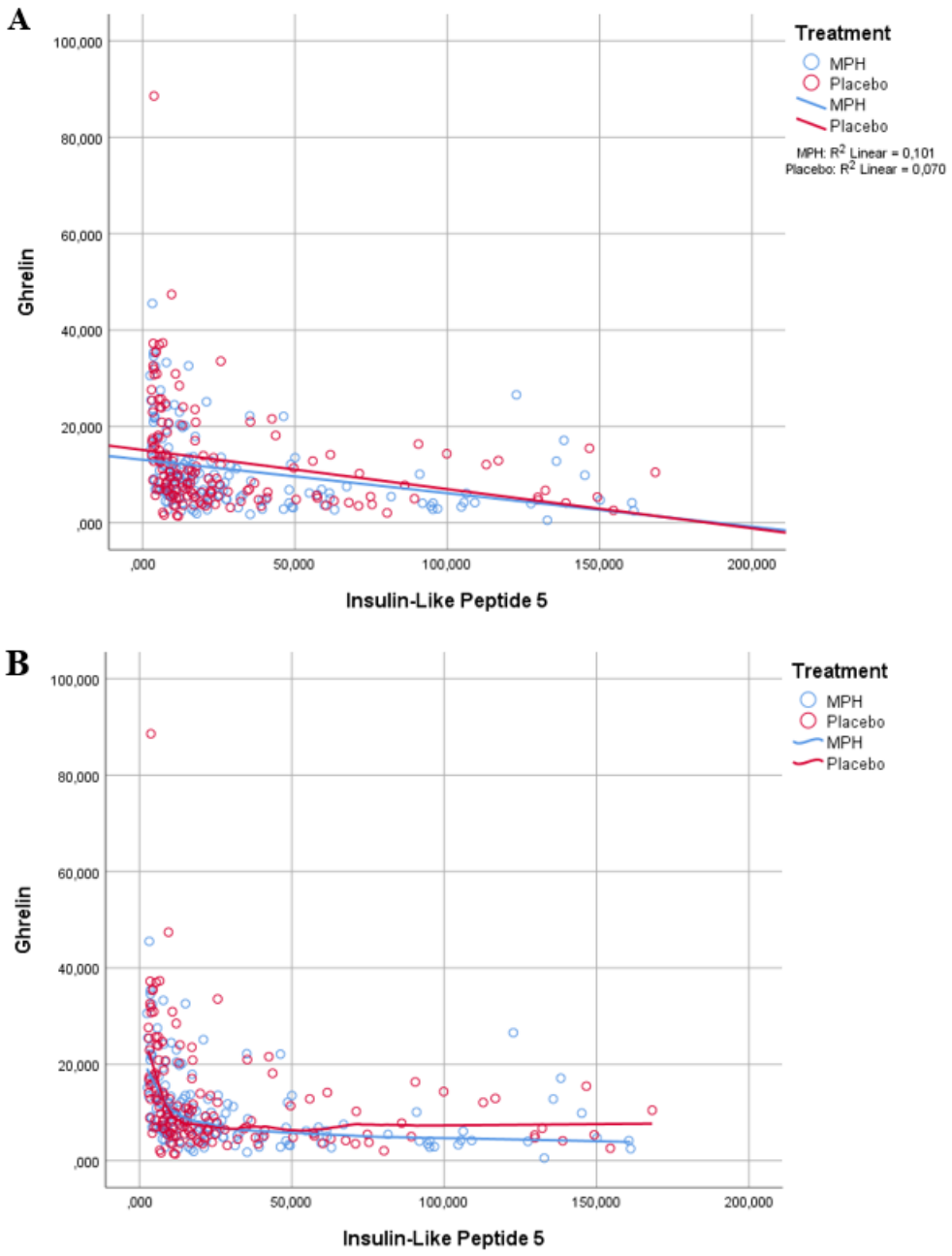
[a] Association between variables as analysed by Pearson's correlation coefficient and Spearman's rank-order correlation. [b] Satiety as measured by VAS questionnaire. All correlations are significant ($p < 0.05$) except [c].

Abbreviations: INSL5 = Insulin-Like Peptide 5.

The correlations between glucose and insulin, glucose and VAS, and insulin and VAS is beyond the scope of this thesis and will not be discussed further.

3.6.1 Association between insulin-like peptide 5 and ghrelin

The results from the correlation analysis showed a significant negative correlation between circulating INSL5 and ghrelin in the blood: $r = - 0.286$, $n = 304$, $p < 0.001$. These results are summarized in Figure 14A. A non-parametric correlation was performed and this analysis show a much stronger correlation using a non-parametric correlation coefficient ($\rho = - 0.500$, $p < 0.001$). A non-linear monotone relationship between ghrelin and INSL5, which are assumed to fit the data better, is displayed in Figure 14B. The negative correlation observed between these GI hormones suggest a novel purpose of INSL5. An increase in circulating INSL5 was correlated with a decrease in circulating ghrelin in the blood.



A: Linear relationship. B: Non-linear monotone relationship. P < 0.001

Figure 14 *Correlation between insulin-like peptide 5 and ghrelin as function of treatment (n = 304).*

3.6.2 Correlation between insulin-like peptide 5 and insulin

The results from the correlation analysis showed a very strong positive correlation between circulating INSL5 and insulin in the blood: r = 0.928, n = 332, p < 0.001. These results are

summarized in Figure 15. A non-parametric correlation was also performed, ρ = 0.927, p < 0.001. These results suggest that there is almost a linear relationship between INSL5 and insulin. This strong positive correlation showed that an increase in INSL5 are followed by an increase in serum insulin.

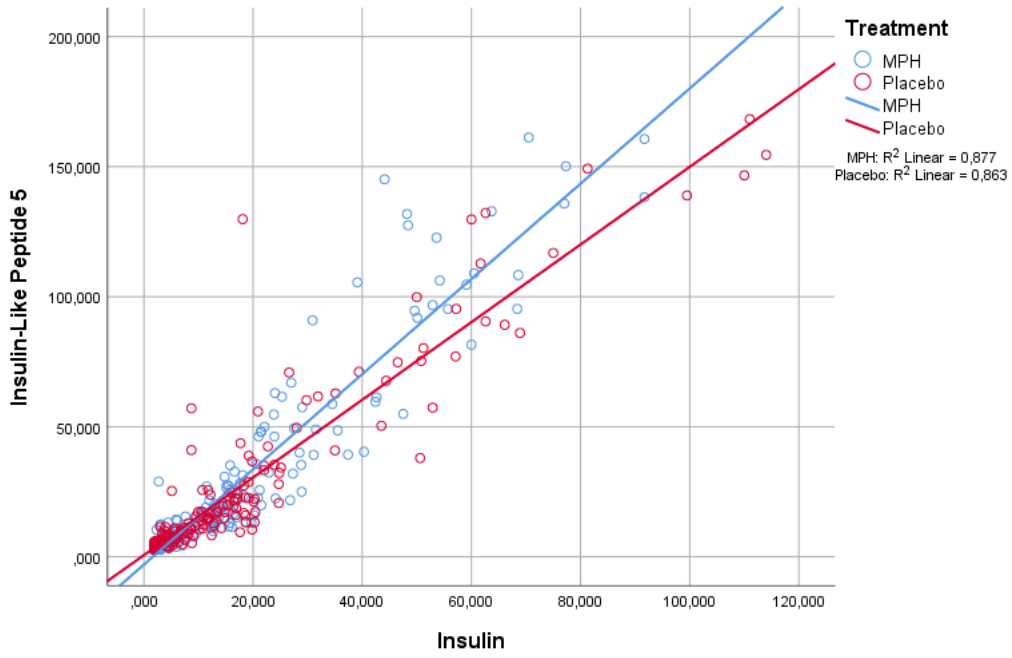


Figure 15 *Correlation between insulin-like peptide 5 and insulin as function of treatment (n =332, n < 0.001).*

3.6.3 Correlation between insulin-like peptide 5 and glucose

The results from the correlation analysis reviled a significant positive correlation between circulating INSL5 and blood glucose: r = 0.376, n = 332, p < 0.001. Figure 16 summarizes these results. A non-parametric correlation was performed: ρ = 0.465, p < 0.001. This positive correlation indicate that an increase in blood glucose follow a rise in circulating INSL5 in the blood.

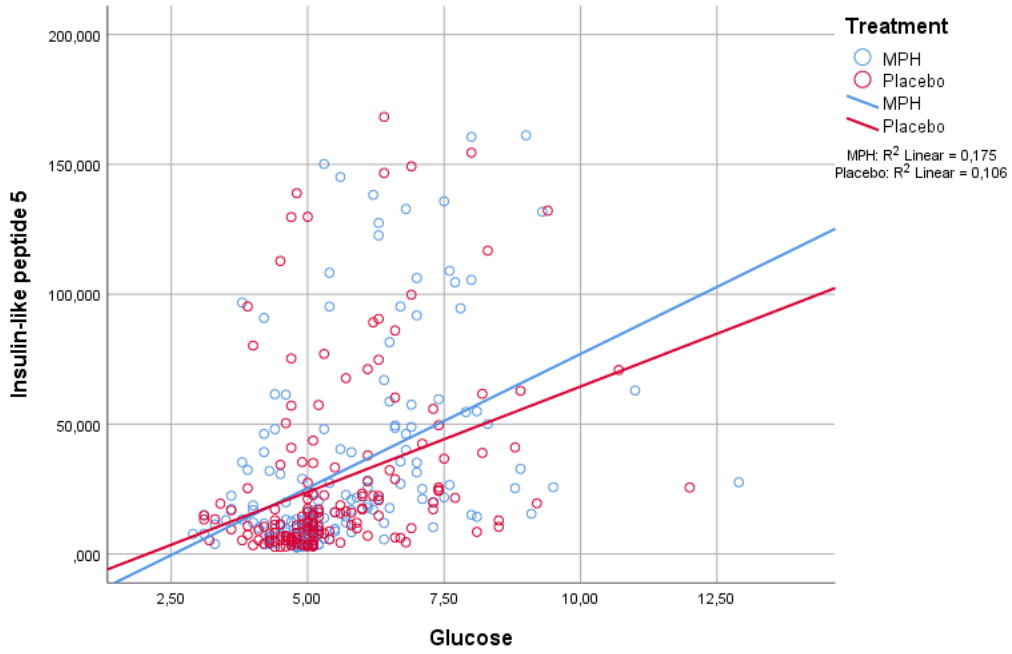


Figure 16 *Correlation between insulin-like peptide 5 and glucose as function of treatment (n = 332, p < 0.001).*

3.6.4 Correlation between ghrelin and glucose

The results from this correlation analysis have reviled a significant negative correlation between ghrelin and blood glucose: = -0.188, n = 303, p = 0.001. These results can be seen in Figure 17. A non-parametric correlation was performed: ρ = - 0.212, p < 0.001. This negative correlation reviled a reduction in circulating ghrelin when blood glucose levels increase.

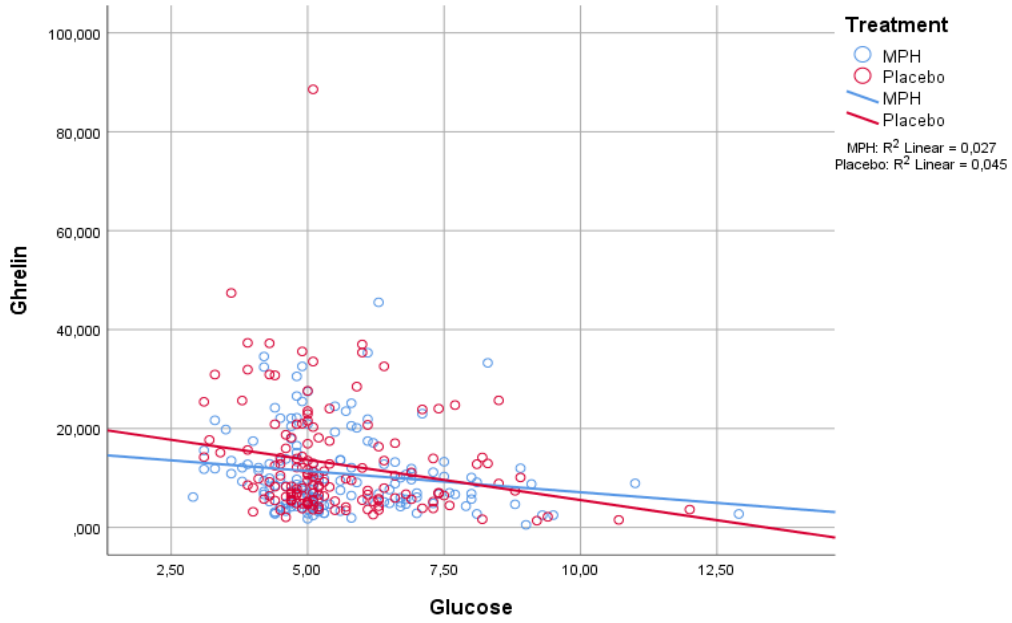


Figure 17 *Correlation between ghrelin and glucose as function of treatment (n =303, p < 0.001).*

3.6.5 Correlation between Ghrelin and Insulin

The results from the correlation analysys did not reviel a significant relationship between circulating ghrein and serum inuslin: r = - 0.063, n = 303, p = 0.274, which are summarized in Figure 18. A non-parametric correlation was also performed: ρ = - 0.115, p = 0.045, where the p-value revealed this correlation to be significant. These results show a weak negative correlation between circulating ghrelin and serum insulin.

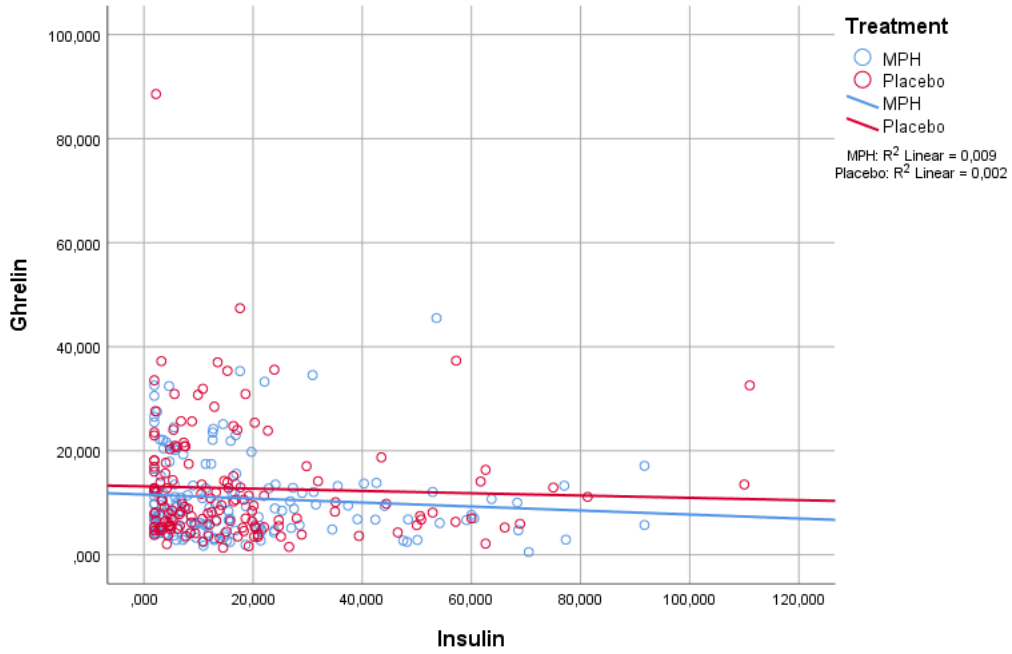


Figure 18 *Correlation between ghrelin and insulin as function of treatment (n =303, p = 0.274).*

3.7 Symptom registration

A VAS questionnaire was used to report common GI symptoms related to ingestion after receiving the test drink, after the endurance exercise at times of blood collection in session 1 and session 2. Due to administrative issues, only 43 % of the participants completed the questionnaire. Symptom I through IV (see chapter 2.3.4 for details), was equal to zero and will not be reported nor discussed in this thesis. The reported feeling of satiation, ranging

from ‘very hungry’ to ‘fully satiated’ measures satiation over time. Correlation analysis between INSL5 and satiety, ghrelin and satiety will be reported in the following chapters.

3.7.1 Association between circulating ghrelin and satiety

The results from the correlation analysis reviled a significant negative correlation between circulating ghrelin and satiety as measured by VAS: r = - 0.472, n = 140, p < 0.001, which are summarized in Figure 19. A non-parametric correlation reviled ρ = - 0.503, p < 0.001. These results shows that the greatest circulating ghrelin levels occur when in the state of ‘very hungry’ and satiation gives a rise in circulating ghrelin. This supports the research of ghrelin as an appetite suppressing hormone.

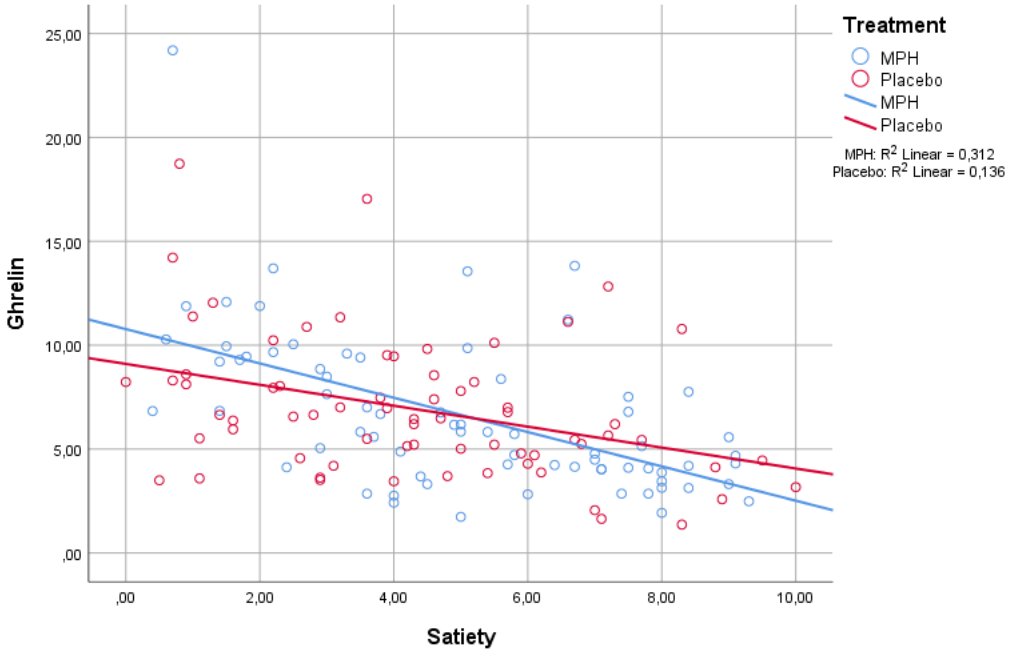


Figure 19 *Correlations analysis of ghrelin and satiety (n = 140, p < 0.001).*

3.7.2 Association between circulating INSL5 and satiety

The results from the correlation analysis showed a significant positive correlation between circulating INSL5 and satiety as measured by VAS: r = 0.282, n = 140, p = 0.001. These results are summarized in Figure 20. A non-parametric correlation revealed: ρ = 0.233, p = 0.006. These results suggest that an increase in satiety gives a rise in circulating INSL5.

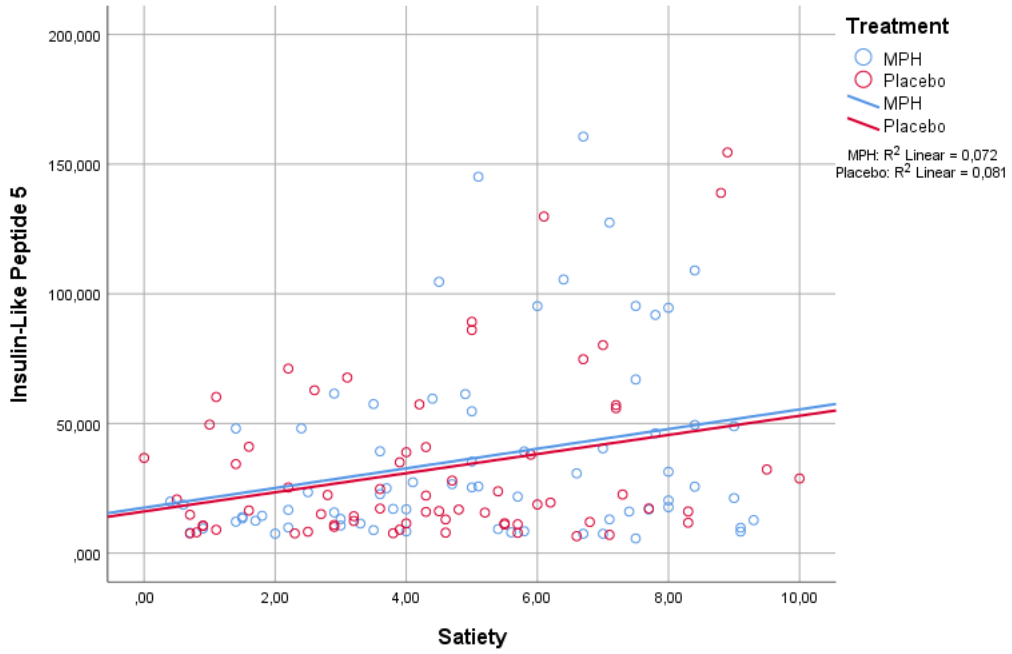


Figure 20 *Correlations analysis of insulin-like peptide 5 and satiety measured by VAS as function of treatment (n=140, p < 0.001).*

These results support previous findings in this study, where ghrelin and INSL5 seem to have opposite effect on appetite regulation and satiety.

3.7 Self-reported food intake through 4-day food registration

Self-reported food intake registration was performed to get a representative image of the diet of the study population. There was a 93 % compliance in the food intake record, and participant 12 was excluded from all analysis. For detailed descriptive statistics regarding the mean reported intake, see table 12 bellow.

The individual mean average of reported nutrient intake, compared to the daily recommended intake (DRI) of the Nordic Nutrition Recommendation (NNR) was calculated (See Appendix 9). The mean nutrient values is assumed to be representable of the diet of the study population. Based on the NNR, 85 % of the study population did not meet the DRI of energy, 62 % did not meet the DRI of carbohydrates and protein, and 54 % did not meet the DRI of vitamin D. For more details, see Appendix 9.

Table 12 *Reported mean nutrient intake (n = 13)* [a)].

Parameters	Mean (SEM)	Median (SD)	Range (Min, Max)
Energy (Kcal)	2889.9 (164.0)	2946.5 (591.3)	(2051.0, 3952.2)
Fat (g)	113.34 (8.61)	103.40 (31.06)	(70.50, 168.38)
CHO (g)	359.73 (24.67)	332.52 (88.94)	(243.17, 505.83)
Fibre (g)	27.23 (2.34)	25.80 (8.44)	(15.04, 42.70)
Protein (g)	117.21 (6.34)	112.40 (22.85)	(90.08, 159.20)
Vit. D (µg)	11.78 (2.18)	7.00 (7.85)	(3.33, 24.35)
Thiamin (76)	2.15 (0.29)	1.83 (1.07)	(0.57, 4.01)
Niacin (76)	19.26 (2.19)	18.75 (7.91)	(5.05, 33.95)
Vit. B12 (µg)	6.13 (0.86)	5.25 (3.10)	(1.85, 12.90)
Vit. C (76)	97.35 (16.40)	79.50 (59.12)	(21.50, 196.75)

[a)] One participants did not hand in the food registration form and three participants reported their food intake for two days.

Abbreviations: SEM = standard error of mean, SD = standard deviation, CHO = carbohydrate, vit D = vitamin D, vit. B12 = vitamin B12, vit. C = vitamin C.

Chapter 4: Discussion

In this study, the effect of a small amount of MPH on secretion of ghrelin, INSL5, glucose and insulin was investigated in healthy males post endurance cycling. The main findings was that MPH supplementation had a significant effect on circulating ghrelin, however not on INSL5, glucose and insulin. There was a difference, although not significant effect of a MPH supplement on circulating INSL5 after endurance cycling. Exercise have been established in supressing ghrelin, and a MPH supplement showed a further suppression of ghrelin secretion after endurance cycling. There were observed a tendency of increased INSL5 secretion after endurance cycling in MPH compared to placebo, however this was not significant. All result and the methodology are discussed in this chapter.

4.1 Discussion of results

The research field of appetite regulating hormones is expanding and the underlying mechanisms for all known hormones are not yet fully understood, especially INSL5. The aim of this study is to explore how MPH affect the GI hormone ghrelin and INSL5 secretion, and subsequently, appetite regulation. In addition, if the physical activity would influence the hormone secretion. The results from this study show that a small amount of MPH supplementation after endurance cycling gives a significant effect on circulating ghrelin secretion, not INSL5. Correlations were observed between hormone secretion, glucose and insulin. There was a negative correlation between ghrelin and INSL5, however there was a strong positive correlation between INSL5 and insulin.

4.1.1 Acylated ghrelin secretion post endurance cycling

A significant negative treatment effect was found in circulating acylated ghrelin, however independently of cycling sessions and time. This novel result suggest that the MPH supplement reduces circulating acylated ghrelin after endurance exercise. When adjusting for BMI, a negative effect was observed to be significant. A mixed model analysis revealed that there are statistical significance in circulating ghrelin between exercise sessions, time points of blood collection and between the exercise session and the time points. A potential long-term effect was observed after exercise session two, where ghrelin was consistently lower than placebo, except at 0 and 15 minutes after completing the exercise.

A negative correlation was observed between acylated ghrelin (AG) and the following parameters; INSL5, glucose and insulin. These results supports ghrelin as an orexigenic hormone. However, surprisingly there was a negative correlation between AG and INSL5. Grosse and co-workers found a significant reduction in INSL5 after feeding, and INSL5 administration stimulated food intake in mice. INSL5 administration stimulated food intake. They proposed that INSL5 were to join ghrelin as an orexigenic hormones in suppressing satiety (46).

The significant negative effect of adjusting for BMI is contradicting the general understanding of BMIs implications on ghrelin secretion. Several studies have shown that BMI is positively correlated to circulating ghrelin (33). A possible explanation of these contradicting results may be that the population in this study is over the average fit, and the level of fitness affects ghrelin secretion in some way. Another explanation may be the exercise or the type of exercise. A prolonged reduction in ghrelin secretion have been reported after exercise (77). Exercise intensity have also been found to further suppress ghrelin secretion, where high intensity training is most effective (18).

4.1.2 INSL5 secretion pattern post endurance cycling

No significant effect of treatment was observed, nor when adjusting for BMI. The linear mixed regression analysis displayed the statistical significance in circulating INSL5 between exercise sessions, time points of blood sampling and between the exercise session and the time points.

Although not significant, and a positive effect of MPH on INSL5 secretion. As mentioned, there was observed a negative correlation between circulating AG and INSL5, and additionally there was a positive correlation between INSL5 and glucose. Most surprising, there was a very strong positive, almost linear, correlation between INSL5 and insulin. INSL5 have been identified to be produced in the specialized enteroendocrine L-cells in the colon mainly (46), whilst insulin is produced in beta-cells in the pancreas (78). At this point in time, the relationship between INSL5 and insulin is not apparent and need to be further researched.

The absence of results for the majority of the variables can be explained by the low dosage, and that 20 mg per kg/body weight is not sufficient to get a significant effect. However, these results together with the negative correlation between ghrelin and INSL5 are suggesting that

INSL5, in contrast to what is previously believed, have an anorexigenic effect in appetite regulation and stimulates satiety, not hunger.

4.1.3 Blood glucose and insulin secretion post endurance cycling

There was no significant effect of treatment on either glucose or insulin secretion, nor when adjustments for BMI was conducted. These results will not be discussed further as it is beyond the scope of this thesis.

4.1.4 Symptom registration

43 % of the participants fully completed the VAS questionnaire due to administrative issues. The results are therefore only reporting trends that may be expected to occur. The VAS ranged from 'very hungry' to 'fully satiated', giving a representative measure of satiety at the time point of blood collection, which allows us to measure satiation up till 120 minutes post endurance cycling. These results were expected to negatively correlate with circulating ghrelin and INSL5 based on the orexigenic effect on appetite regulating. The participants were feeling more hungry after session 2 than session 1.

The results showed a negative correlation between circulating ghrelin and satiety measured by VAS, which supports the orexigenic effect of ghrelin (26). However, the correlation between INSL5 and satiety was found to be positive. These result are further supporting previously reported results, indicating INSL5 as an anorexigenic hormone.

4.1.5 Reported dietary intake

Results reviled that 85% of the participants did not meet their daily required energy intake which was calculated to fit the activity level of the study population. This can affect the successive results regarding macro- and micronutrients. 62 % of the study population are under the daily recommendations of carbohydrates, as well as protein intake. Results also reviled that 54% did not meet the daily requirement in vitamin D. Based on these results, the study population should increase their energy, carbohydrate and protein intake to not be in an energy deficit. Protein intake will be discussed further in Chapter 4.2.5.

Vitamin B is very important in exercise as it is involved in making red blood cells, the breakdown of protein and carbohydrates (glucose and glycogen) and in the rebuilding and the repair of tissue in the body. Low levels of vitamin B have a negative effect on performance as it reduced ATP production and oxygen delivery during exercise (79). 70 % of participants reached the DRI of thiamin, 62 % reached the DRI of niacin, and only 7 % did not reach the DRI of vitamin B12. Based on these result, the study population should increase their intake of thiamin and niacin to optimize performance in exercise.

4.2 Discussion of the methodology

In this randomized, double-blinded, placebo controlled crossover study, the following parameters was measures; anthropometrics, GI hormone ghrelin and INSL5, food intake and measurement of satiety after exercise and receiving a test drink. The strengths and limitations of the method and materials used in this study will be discussed.

4.2.1 Study population and study design

In total 14 participants contributed in the study. The participants was recruited via the social media site of local cycling clubs in Bergen. During the start-up period after the recruitment, a couple of participants withdraw from the study and changes was made to the inclusion criteria in relation to age and the age limit was extended to 58 years of age.

One of the limitations of this study is that it is a small study population because it is a pilot study. Because of this, only men was included to achieve the greatest possible degree of unambiguous results. The sample was composed of middle-aged, well-trained, healthy males. Therefore, this study is somewhat one-sided as there is reasonable cause to believe that results can alter when women are included or in a different age group. Thus, the findings might not be generalized.

Participants decided themselves on receiving treatment A or treatment B in phase two and in phase three receives the other treatment option. The treatment and placebo was prepared at the manufacturer in opaque glass flasks with white powder. Colour, taste and smell was masque.

A randomized, double-blind, placebo-controlled, crossover design is considered a desirable study design. A great benefit of a crossover design is that group A and B, in this case

treatment A and B, is compared with them-selves in a self-paired way. Because participants are their own controls and is compared against themselves, this is beneficial as it ensures for a greater biological homogeneousness in the samples. The was-out period was set to 7 days in this study based on animal-studies related to the nitrogenbalance (protein balance), that 4 days is required to adapt to a new diet. Ever though the need for a wash-out period between phases in the study is a liability, it was chosen to be 7 days to make sure that alteration in the diet in phase 2 were not affecting the diet in the next phase.

Another limitation of the study design is that with the crossover design, are assuming that there are a treatment effect of phase two have no remaining effect on the treatment effect of phase three. We also assume that participants health and stress level remains unchanged during phase two and three.

4.2.2 Anthropometric measurements

The anthropometric measurements was standardized by using exactly the same height measurer and body composition analyser for all participants.

4.2.3 GI hormone measurements

Blood samples was collected by a standardized method to insure accuracy and to get sufficient amount of blood. Glucose and insulin was measured at the Laboratory of Clinical Biochemistry (LKB) using a standardized method which is insuring high accuracy. Ghrelin and INSL5 measurement were performed in a clinical laboratory at University of Bergen with commercial EIA and ELISA kits respectively.

There are a big difference in fasting values, especially in ghrelin, independently of treatment. This can be caused my multiple individual factors; hours of sleep (80) prior to the intervention, type and amount of food consumed (81, 82) the previous day, how long the participants were awake in the morning before arriving (83) as they received individual times of arrival (every hour from 8:00 a.m. till 10:00 a.m.), general stress levels and the level of stress which arises whilst getting to the intervention location in the morning (drive, public transport, traffic jam etc.) (84, 85). These are all factors that may affect levels of ghrelin, and any test done compared to fasting values will be limited due to the variation in values.

Because there are no standardised method of measuring ghrelin or INSL5, an exact immunoreactivity for the respective ELISA needs to be furthers characterised. Gröschl and co-workers conducted and evaluation of the comparability of commercial ghrelin assays. When two different ghrelin kits were compared, it was found that the difference in the standard peptide used let to a dramatic difference in levels of ghrelin measured in the assay (86). It is therefore possible that these result are not comparable with other measured results of ghrelin or INSL5 by other researchers using kits with different immunoreactivity and from different manufacturers.

In this study, some of the values of GI hormones was missing, and the data set for participant 12 was excluded from the AG measurement due to technical difficulties. This is a limitation, and if these values were not missing or excluded, it may have affected the results of GI hormones and satiety. Potentially it could help better understand AG and INSL5 secretion after endurance exercise.

4.2.4 Symptom registration

The result of satiety measured at time points of blood collection give a representative measure of satiation during the 2-hour period after the endurance exercise.

There were observed individual differences in the VAS questionnaire results. This could be due to that some of the participants are habitual breakfast eaters, and are used to eating a larger breakfast meal, therefore scoring lower on the VAS. Others that are not habitual breakfast eaters will score higher on the VAS questionnaire, which is a limitation. To adjust for this, one can ask the participants if they are habitual breakfast eaters.

4.2.5 Food intake records

Four day diet registration was performed using a standardized diet registration form from 'Helse Bergen, Haukeland Universitetssjukehus – section of dietetics'. When the study started it was decided that a two-day diet registration was sufficient, however this was changed to four days to give a better reference of the average nutrient intake of the study population and to reflect that this is a master's thesis in dietetics. This means however that the first three participants only have a two-day food registration, one day before phase two and one in-between phase two and three. After the change was made, participants was asked to do three

day food records before phase two and record one day in-between phase two and three. 93 % of the participants complied.

One major limitation of a food intake record is that participants themselves report their intake. Humans, for different reasons are not capable of precisely accounting for what they have eaten. A meth analysis found that the participants consequently had healthies eating habits during a period of keeping a food record (87, 88). Due to subjectivity we are not able to confidently validate the results from a food intake records because it a source of systematic bias (89, 90). Because diets can change over time, a food record such as this is exposed to random errors because it only record the diet for 4 days. The greatest concerns are nonetheless systematic errors such as under- and over reporting which is suggest to be ± 20 % (88). Yang and co-workers found that when mean daily nutrient intake was estimates, a food record showed that the intake of fat, fibre, vitamin E, B_6, A and β-carotene and sodium when compared to reports intake by a food frequency questionnaire (91).

To improve compliance, communication with the participants could improve throughout the intervention, and this may lead to a low threshold for asking questions if in doubt. An option to complete and submit the food record form online or in a customized app on a smartphone at the end of the day may make it easier to log food items at work or on the go when not in the comfort of their own home. It would then be easy to monitor, and maybe ask questions when something is not clear. This method of dietary assessment is more applicable for people who frequently cook meals at home, and it is not suitable for individuals who are frequently eating outside their home (92).

One limitation in regards to the process of adding the food items and recipes in the nutrient analysation program, was that the program did not include several food items and these had to be recorded manually. Where the product declaration from the manufacturer was available, it was added, however not all micronutrients was available in that situation. This is therefore likely to affect the reported micronutrient intake.

Restriction of caffeine was set to no more than 5 cups a day, and no alcohol consumption 48 hours prior to the visits. Caffeine have been classified as having an ergogenic effect in low (3 mg/kg BW), medium (5-6 mg/kg BW) and high (> 6 mg/kg BW) doses (93, 94), and it have a half-life of approximately 5 to 6 hours with some individual variance (95). The participants got to chose if the desired one cup of coffee or tea served with the breakfast to keep caffeine intake at a low level. Some disadvantageous effects alcohol have on the human physiology is

that it influenced metabolism, myopathy of the skeletal muscle and the cardiovascular physiology (96). Alcohol should therefore not be consumed within 48 hours prior to the intervention phases.

4.2.5 Estimated protein requirement

Based on the available evidence, according to the NNR and Olympiatoppen Norway (organization that develops elite sports in Norway), a protein intake corresponding to 10–20 E% is recommended. The NNR suggest a protein intake of 0.8 g/kg BW/day, which coincides to approximately 10 E% arriving from dietary protein, provided a moderate PAL of 1.6 (97). The participants DRI of protein was set to 1.5 g/kg BW/day based on the hours of exercise the participants performs each week coinciding with the recommendations of protein in relation to exercise from the NNR and Olympiatoppen.

It is generally believed that there is an increased protein requirement related to exercise. There is an increase in demands for protein during exercise conditions that may be because on the increasing muscle mass, which is due to training. This also increases the breakdown of muscle and protein turnover in physical activity, which increases gluconeogenesis from the muscle, protein catabolism and a negative nitrogen balance if the energy needs are not met (98-100). Therefore, appropriate nutrition after exercise is important to restore homeostasis and helps the adaptive responses, and thus promotes the skeletal muscle reconditioning (101-103).

4.3 Ghrelin and INSL5 secretion in appetite regulation and body weight control

Physical activity and exercise is a common strategy in treating obesity because it increases energy expenditure. However, because energy expenditure increases the strength of hunger and is thereby driving food consumption, one can ask really how effective exercise is in weight regulation or weight loss. On the other hand are the evidence showing that appetite is suppressed and satiety is prolonged after exercise, which is further discussed below.

4.3.1 Acylated ghrelin secretion in response to exercise

Because AG is the only form of ghrelin that is able to pass the blood-brain barrier (22), it is assumed to be essential in appetite regulation. In addition, the previously measured

concentration of plasma ghrelin may disguise the vital changes in AG that arises through for example exercise. Previously it have been discovered that AG was suppressed whilst vigorously running on a treadmill (16), an increase in AG following aerobic exercise for five successive days (1 h/day) (104). Broom and co-workers confirmed that appetite suppression occurred during and after resistance and aerobic exercise, suggesting ghrelin suppresses appetite for exercise (15). They also suggest that the acylated ghrelin response to exercise may be further prolonged after higher intensity training (18).

Not only vigorous and intense training have been shown to supress AG, but also a 120 minute treadmill workout with a variety of intensity (77). This bit of research is very important as low- to moderate exercise intensity is considered to be favourable in weight loss and weight regulation. Previous research have shown the effect of physical activity and exercise on AG secretion, and this study show a further suppression after endurance cycling when MPH supplementation was taken immediately after exercising. This is valuable knowledge in the field of weight loss especially, and a MPH supplement may be a useful tool in appetite and weight regulation through suppressing hunger.

4.3.2 INSL5 an orexigenic hormone?

At this point in time, there are no available data on INSL5 secretion post exercise. These novel results suggest that INSL5 is an anorexigenic hormone which supresses hunger and promotes satiety. Self-reported satiety measured supports this claim and was shown to stimulate satiety. INSL5 is secreted mainly from L-cells in the colon, which is the site of secretion of other anorexigenic hormones; GLP-1 (105), PYY (106).

Luo and co-workers reported that INSL5 stimulated insulin secretion in vivo (51), which can explain the correlation found in this study. Because the site of secretion and the receptor for INSL5 and insulin are different. Luo proposed that INSL5 may be able to affect the secretion of GLP-1 through activating the RXFP4 receptor, and thereby affect insulin secretion (51). The mechanism of INSL5 in appetite regulation is not yet fully understood and will therefore need further research. This mechanism may offer a potential therapeutic target for appetite regulation in weight regulation as well as for type 2 Diabetes Mellitus.

Chapter 5: Conclusion and future perspective

This study have demonstrated that 20 mg marine peptide hydrolysate per kg body weight significantly reduced circulating acylated ghrelin after endurance cycling. There was a non-significant change in circulating insulin-like peptide 5, insulin and glucose. This partially support the hypothesis that suggested that a small amount of marine peptide hydrolysate supplementation would affect the secretion of ghrelin after endurance cycling.

There are definitely a greater need for more comprehensive research regarding the effect of marine peptides on the secretion of acylated ghrelin in response to exercise and how different types of exercise affect appetite regulation and meal initiation. The effect on ghrelin secretion after exercise are more established in the literature, and a greater reduction in ghrelin was found when given the marine peptide hydrolysate supplement; satiety was significantly suppressed after endurance cycling. This finding might represent improved therapeutic target for weight regulation, as physical activity and exercise is part of the recommended strategy for weight loss. This potential mechanism should be explored further to evaluate the potential in such a supplement in weight regulation.

Insulin-like peptide 5 have a similar secretion pattern to insulin, and the correlation is very strong, which suggest that there may be overlapping mechanisms in regulation of both hormones. The marine peptide hydrolysate revealed a positive trend in circulating insulin-like peptide 5, however this was not significant. Based on the novel results the author propose that insulin-like peptide 5 increase satiety. Further exploration of this hormone and its role in appetite regulation is needed to establish the mechanism and its properties fully. This mechanism may prove to be important, not only in appetite regulation, but also in treatment of type 2 Diabetes.

Due to the administrative issues, the author is not able to draw any conclusion on the measured satiety, however trends are reported; ghrelin supresses satiety while insulin-like peptide 5 promotes satiety. These results are consistent through the data sets. Satiety need to be further explored in conjunction with insulin-like 5 secretion to fully understand its appetite regulating properties.

Chapter 7: References

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Chapter 8: Appendix

Appendix 1 *Recruitment poster*.

Firmenich

HELSE BERGEN
Haukeland University Hospital

ER DU SYKLIST, OG HAR LYST TIL Å DELTA I FORSKNINGSPROSJEKT?

På Haukeland universitetssjukehus og Høgskulen på Vestlandet planlegges det et forskningsprosjekt for å undersøke effekten av et proteintilskudd på restitusjon hos syklister. Vi ønsker derfor å komme i kontakt med syklister som kan tenke seg å delta i studien. Aktuelle deltakere er: **Menn, i alderen 40-50 år, som sykler 8-12 timer i uken.**

Hva innebærer deltakelse?

Målsettingen med dette prosjektet er å undersøke effekten av et marint proteintilskudd etter en hard treningsøkt på sykkel. Vi vil studere om dette kan bedre blodsukkerprofilen, og om det kan øke restitusjonsevnen.
Deltakelse innebærer oppmøte på Høgskulen på Vestlandet 3 dager i løpet av 3 uker:

- *Testdag 1:* En ettermiddag gjøres innledende undersøkelser med testing av maksimalt oksygenopptak.
- *Testdag 2 og 3:* To påfølgende lørdager, eller to påfølgende søndager, skal det så gjennomføres:
  - Sykling på 90-95% av VO_{2max} inntil utmattelse
  - 4 timer restitusjon med inntak av ernæringsdrikke og mat
  - Ny sykkeløkt på 90-95% av VO_{2max} inntil utmattelse

Det vil være lege, fysioterapeut og sykepleier til stede under testingen. Under syklingen vil det bli målt blodsukker og laktat via et stikk i fingeren. Mellom de to sykkeløktene på testdag 2 og 3 vil det i tillegg bli tatt urin- og blodprøver.

Deltakelse vil være aktuelt fra september og utover høsten 2017.

Kunnskap om egen fysisk form – og bidrag til forskning

Dersom du deltar får du mye kunnskap om egen fysisk form, samtidig som du kan være med å bidra til viktig forskning om sykling, restitusjon og ernæring. Det er frivillig å delta i prosjektet og du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til deltakelse.

Du vil få dekket transportutgifter til og fra Høgskulen på Vestlandet, samt et gavekort på kr. 500. Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk.

Ønsker du mer informasjon, eller ønsker å melde din interesse?

Kontakt Ingunn Mjøs: ingunnmjoes@hotmail.com

Appendix 2 *VAS of GI symptoms*

Dato:	Nr:	Tilskudd:

Gradering av eventuelle magesymptomer. Sett en strek på linjen som beskriver hvordan du føler deg.

FØR INNTAK AV Drikke

1. Har du smerter i øvre midtre del av magen?

Ingen smerter	_____	Meget ubehagelig

2. Er du kvalm?

Ikke kvalm	_____	Meget kvalm

3. Føler du deg oppfylt i magen?

Ikke i det hele tatt	_____	Meget oppfylt

4. Totalt ubehag i øvre del av magen?

Ikke i det hele tatt	_____	Meget stort ubehag

5. Er du mett?

Meget sulten	_____	Helt mett

Rett ETTER INNTAK AV drikke

1. Har du smerter i øvre midtre del av magen?

Ingen smerter	_____	Meget ubehagelig

2. Er du kvalm?

Ikke kvalm	_____	Meget kvalm

3. Føler du deg oppfylt i magen?

Ikke i det hele tatt	_____	Meget oppfylt

4. Totalt ubehag i øvre del av magen?

Ikke i det hele tatt	_____	Meget stort ubehag

5. Er du mett?

Meget sulten	_____	Helt mett

Appendix 3 *"Kostregistrering" from Helse Bergen, Haukeland Universitetssjukehus – Section of Dietetics.*

Slik går du frem:

For at vi skal kunne beregne næringsstoffinntaket ditt så nøyaktig som mulig, er det nødvendig at du noterer *alt* du spiser og drikker i løpet av en 4 dagers sammenhengende periode. Perioden onsdag til lørdag (evt. søndag til onsdag) er best, for da får du med én helgedag.

Det er vesentlig at du spiser slik som du pleier i registreringsperioden.

- Angi klokkeslett for hver gang du spiser eller drikker noe.
- Beskriv mat og drikke så nøyaktig som mulig
  - *Brød*: Type, navn, grovhet, tykkelse på skiver, antall skiver. Ev. rundstykke, knekkebrød.
  - *Fett på brødet:* Type, navn, mengde, lett eller vanlig
  - *Pålegg:* Type, mengde, produktnavn, lett eller vanlig
  - *Middag:* Type kjøtt, fisk, kjøttfarse-/fiskeprodukt. Produktnavn. Fettprosent.
  - *Frukt og grønnsaker:* Rå, kokt eller hermetisk.
- Beskriv hvordan maten er tilberedt.
  - Kokt, bakt, stekt, grillet eller varmet i mikrobølgeovn
  - Er maten er renset for skinn og/eller fett?
- Hjemmelagede matretter beskrives i detalj, gjerne ved å skrive ned oppskriften bak på arket.
- Notér alt tilbehør, som saus, pickles, rømme, dressing eller krem, med navn/produsent. Oppgi også om du bruker sukker på gryn, grøt eller i te.
- Få med alle mellommåltider, samt tilfeldig spising og drikke utenom de faste måltidene.
- Kosttilskudd, som tran, vitamintabletter o.l. skal også noteres, med navn, produsent og mengde.
- Mengder kan beskrives på følgende måte:
  - aller helst skal du veie maten og føre mengden opp i gram
  - hvis du ikke kan veie, kan du angi mengder i husholdningsmål, som spiseskje, glass, desiliter eller antall, alt ettersom hva som er hensiktsmessig
  - oppgi størrelse på glassene du bruker i dl

Eksempel:

Kl	*Tirs* **dag** *14 / 1 / 11*	**Produktnavn/Produsent**	**Vekt**
0730	*1 butikkskåret skive kneip*	*Bakers*	*30g*
	m/ skrapet lag margarin	*Soft Soya*	
	3 høvelskiver hvitost, 16% fett	*Norvegia, Tine*	
	1 stor grapefrukt		*200g*
	1 stort glass lettmelk (Stort glass = 2 dl)	*Tine*	
1100	*1 beger fruktyoghurt*	*Yoplait Dobbel 0%, mango*	*125g*
	1 melkesjokolade	*Freia*	*100g*
	1 kopp svart kaffe		*150g*
1500	*kokt torsk*		*140g*
	3 små potete, kokt		*150g*
	3 toppede ss revet gulrot		
	1 ss remulade	*Idun*	
	2 store glass saft	*Lerum uten tilsatt sukker*	

Kl	..... dag ... / ... / ...	Produktnavn/Produsent	Vekt

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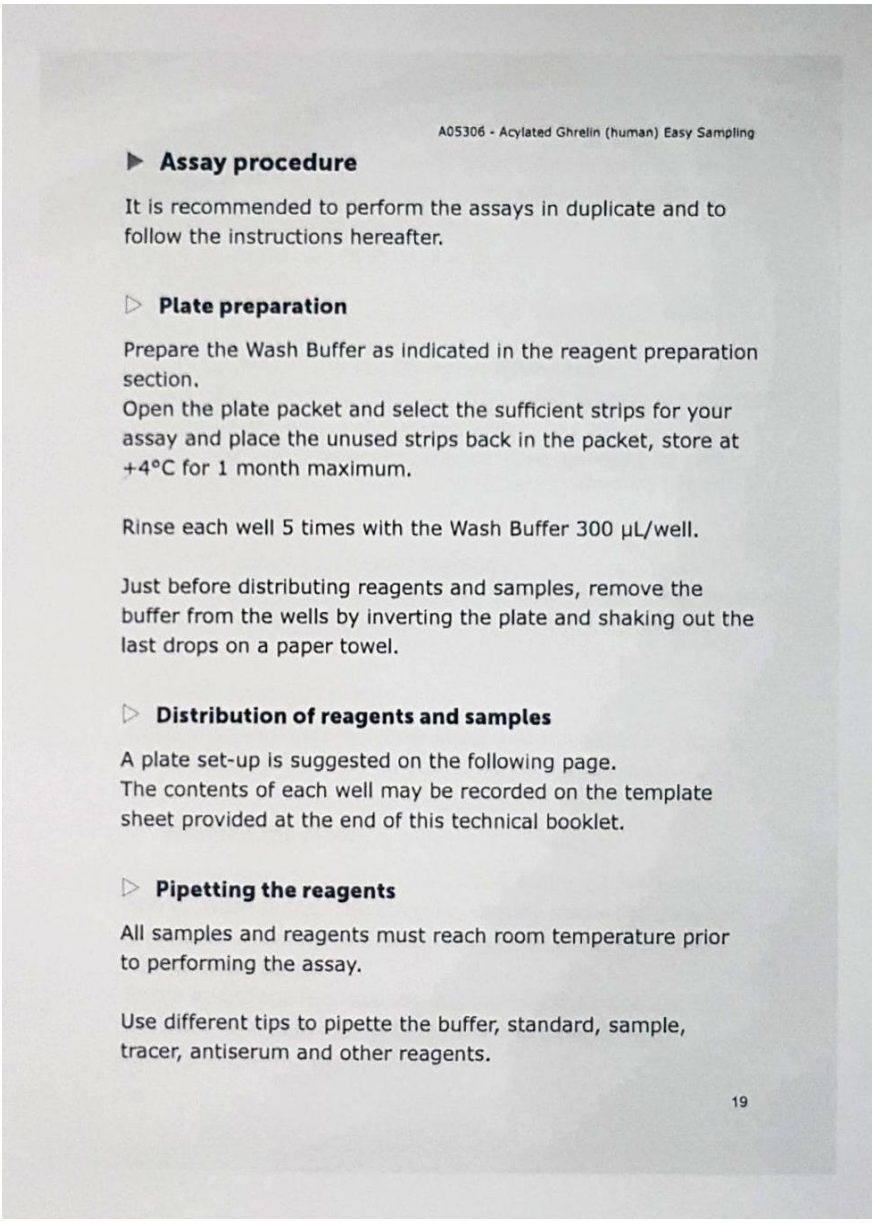
Haukeland universitetssjukehus
Avdeling for klinisk ernæring

Appendix 4 *Ghrelin EIA protocol.*



▶ Assay procedure

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

▷ Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet, store at +4°C for 1 month maximum.

Rinse each well 5 times with the Wash Buffer 300 µL/well.

Just before distributing reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

▷ Distribution of reagents and samples

A plate set-up is suggested on the following page.
The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

▷ Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipette the buffer, standard, sample, tracer, antiserum and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expeling with the pipette tip.

> **EIA Buffer**
Dispense 100 µL to Non Specific Binding NSB wells.

> **Acylated Ghrelin (human) Standards**
Dispense 100 µL of each of the eight standards S1 to S8 in duplicate to appropriate wells.
Start with the lowest concentration standard S8 and equilibrate the tip in the next higher standard before pipetting.

> **Quality Control and samples**
Dispense 100 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA Buffer.

▷ Incubating the plate

Cover the plate with the cover sheet and incubate for 2 hours at room temperature on an orbital shaker (at 600 rpm).

▷ Washing the plate

Empty the plate by turning over. Rinse each well five times with 300 µL Wash Buffer. The 5th time, slightly shake the plate for 5 minutes on an orbital shaker. Then rewash five times with 300 µL Wash Buffer.
At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

▷ Pipetting the reagents

> **Acylated Ghrelin Tracer**
Dispense 100 µL to each well, **except** blank (Bk) wells.

▷ Incubating the plate

Cover the plate with the cover sheet and incubate for 2 hours at room temperature on an orbital shaker (at 600 rpm).

▷ Developing and reading the plate

> Reconstitute Ellman's reagent as mentioned in the Reagent preparation section.

> Empty the plate by turning over. Rinse each well five times with 300 µL Wash Buffer. The 5th time, slightly shake the plate for 5 minutes on an orbital shaker. Then rewash five times with 300 µL Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

> Add 200µL of Ellman's reagent to each 96 well. Cover the plate with aluminium sheet and incubate in the dark at room temperature. Optimal development is obtained using an orbital shaker.

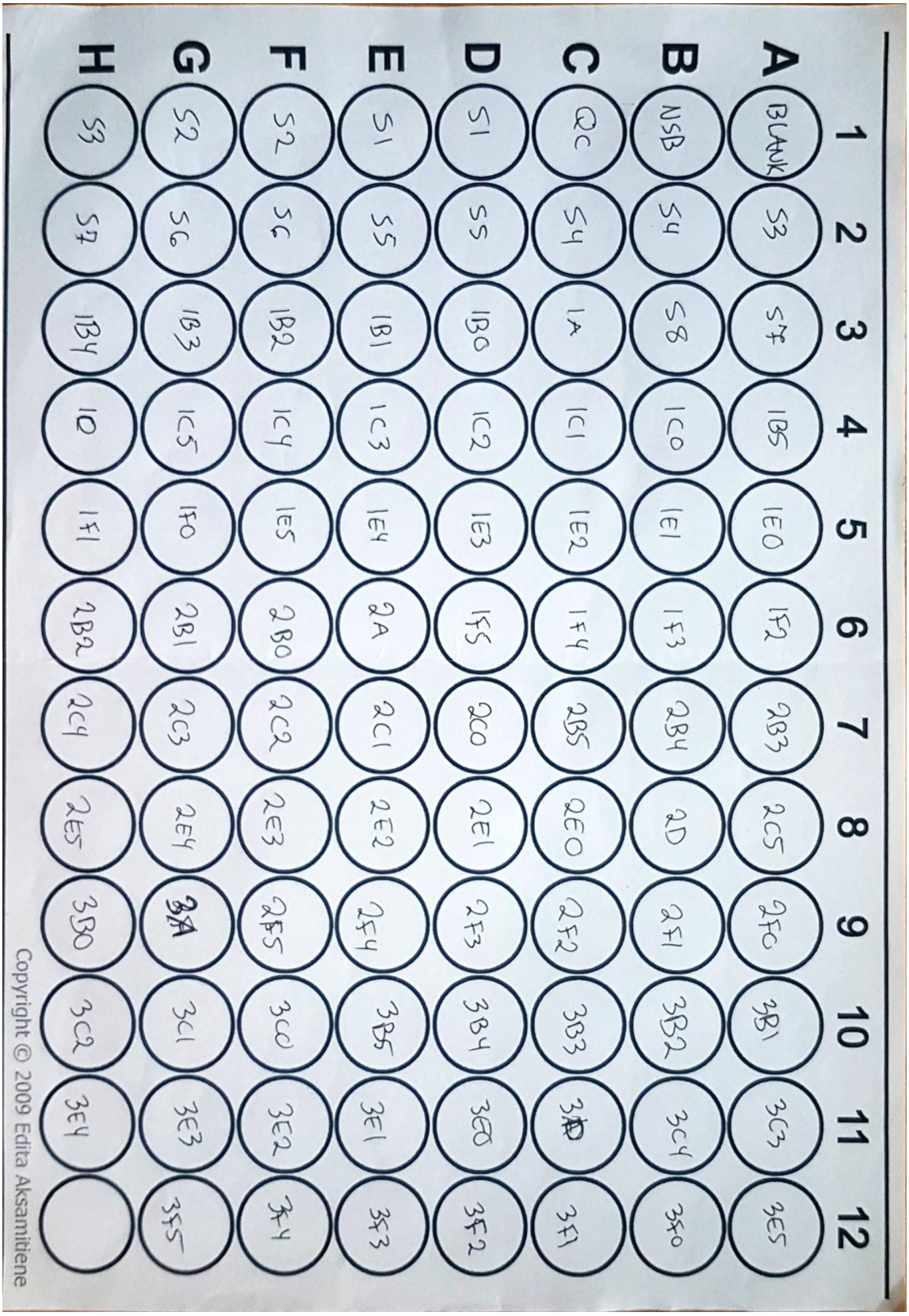
> Wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.

> Read the plate at a wavelength between 405 and 414nm (yellow colour).

After addition of Ellman's reagent, the absorbance has to be checked periodically (every 30 minutes) until the maximum absorbance has reached a minimum of 0.5 A.U. blank subtracted.

Easy Sampling Enzyme Immunoassay Protocole (volumes are in µL)				
	Blank	NSB	Standard	Sample or QC
EIA Buffer	-	100	-	-
Standard	-	-	100	-
Sample or QC	-	-	-	100
Cover plate, incubate 2 hours at 600 rpm				
Wash plate 5 times, shake 5 min, wash 5 times & discard liquid from the wells				
Tracer	-	100	100	100
Cover plate, incubate 2 hours at 600 rpm				
Wash plate 5 times, shake 5 min, wash 5 times & discard liquid from the wells				
Ellman's reagent	200			
Incubate with an orbital shaker in the dark at RT				
Read the plate between 405 and 414 nm				

Appendix 5 *Plate set-up for ghrelin.*



Appendix 6 *INSL5 ELISA protocol*

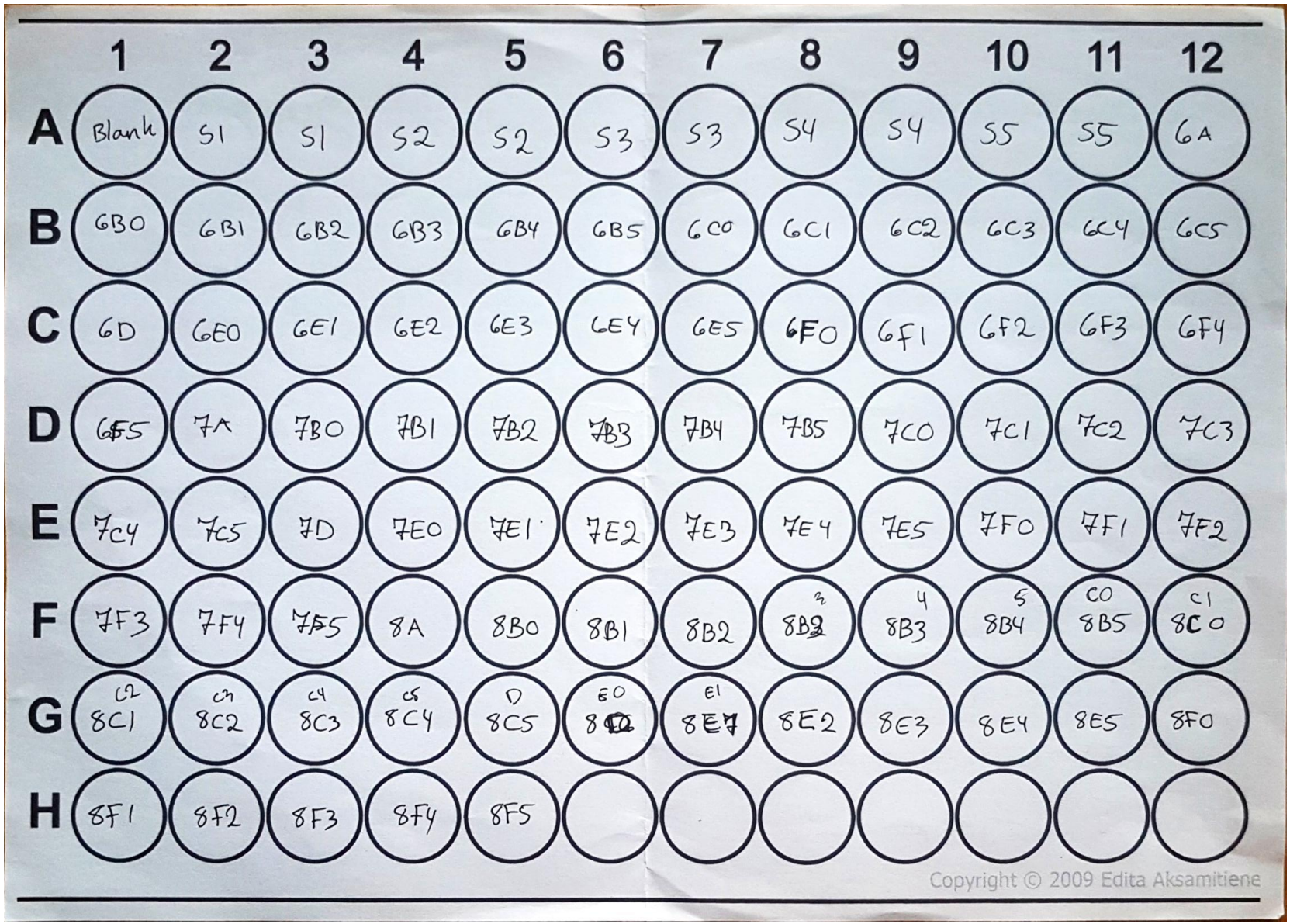
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a **Blank** well without any solution.
4. Add 50µl of **Standard** or **Sample** per well. Standard need test in duplicate.
5. Add 50µl of **HRP-conjugate** to each well(Not to **Blank** well). Mix well and then incubate for one hour at 37°C.
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer** (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
8. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

Appendix 7 *Plate set-up for INSL5*


1 2 3 4 5 6 7 8 9 10 11 12
A Blank S1 S1 S2 S2 S3 S3 S4 S4 S5 S5 6A
B 6B0 6B1 6B2 6B3 6B4 6B5 6C0 6C1 6C2 6C3 6C4 6C5
C 6D 6E0 6E1 6E2 6E3 6E4 6E5 6F0 6F1 6F2 6F3 6F4
D 6F5 7A 7B0 7B1 7B2 7B3 7B4 7B5 7C0 7C1 7C2 7C3
E 7C4 7C5 7D 7E0 7E1 7E2 7E3 7E4 7E5 7F0 7F1 7F2
F 7F3 7F4 7F5 8A 8B0 8B1 8B2 8B3 8B4 8B5 8C0
G 8C1 8C2 8C3 8C4 8C5 8E0 8E1 8E2 8E3 8E4 8E5 8F0
H 8F1 8F2 8F3 8F4 8F5
Copyright © 2009 Edita Aksamitiene

Appendix 8 *Written consent form*

Firmenich

HELSE BERGEN
Haukeland University Hospital

FORESPØRSEL OM DELTAKELSE I FORSKNINGSPROSJEKTET

PEPTID-TILSKUDD TIL SYKLISTER

Dette er et spørsmål til deg om å delta i et forskningsprosjekt for å undersøke om proteintilskudd kan bedre evnen til å restituere seg etter en hard sykkelbelastning.

HVA INNEBÆRER PROSJEKTET?

Flere studier viser at inntak av proteintilskudd i tillegg til et standardisert karbohydratmåltid etter å ha gjennomgått hard trening bedrer restitusjon, utholdenhet og yteevne. I en nylig utført studie utført i Uppsala ble det vist at tilskudd av proteiner fra torsk bedret blodsukkerprofilen med 20 % etter et standardisert måltid hos 12 friske studenter sammenlignet med en kontrollgruppe hvor det ikke ble gitt tilskudd.

Målsettingen med dette prosjektet er å undersøke effekten av proteintilskudd sammen med et standardisert måltid, servert etter en hard treningsøkt på sykkel. Vi vil studere om dette kan bedre blodsukkerprofilen, og om det kan øke restitusjonsevnen. Studien er designet som en dobbel-blindet studie, hvor du vil motta drikke henholdsvis med og uten proteintilskudd etter belastningstest på sykkel.

Fjorten friske menn i alderen 40-50 år skal inkluderes. Forsøkspersonene skal være middels trente personer fra en lokal sykkelklubb i Bergensområdet som trener 8-12 timer i uken.

I studien er det oppmøte tre dager i løpet av tre uker hvor det til sammen skal gjennomføres fem tester på ergometersykkel. Første gang (testdag 1) gjennomføres en maksimal belastningstest på ergometersykkel hvor blant annet melksyreterskel/laktatprofil og maksimalt oksygenopptak måles. Denne testen vil ta ca. 1 time.

Uken etter første test (testdag 2), vil det gjennomføres ny sykkeltest. Før testingen starter vil du få et standardisert måltid og deretter 45 min. pause før selve syklingen starter. Etter avsluttet test vil du motta restitusjonsdrikke med eller uten proteintilskudd. Dette trekkes tilfeldig. I tillegg vil du få mat, og frem til neste test 4 timer senere vil det bli tilrettelagt for hvile/restitusjon. Du skal gjøre to sykkeltester denne dagen, begge med høy intensitet som er beregnet etter resultat fra maksimaltesten som ble gjennomført på testdag 1.

En uke etter testdag 2 (testdag 3) skal du inn til ny testing og gjennomføre samme prosedyre som uken før. Denne gangen gis det proteintilskudd eller placebo i omvendt rekkefølge sammenlignet med testdag 2. Det må beregnes 9 timer både til testdag 2 og 3.
Under sykkeltestene skal du sykle med et munnstykke for å måle oksygenopptak og karbondioksidutskillelse. Det skal tas blodprøve med et stikk i fingeren for måling av

blodsukker og laktat. Testdagene skal det i tillegg måles effekt av proteintilskuddet både i blod og urin.
Blodprøver blir tatt et fra en vene i albuen tid; før, og ved tid 0, 15, 30, 60, 90 og 120 minutter etter avslutning av tester for å undersøke hormon- og blodsukkerprofil, og urin samles i en beholder.

Vi vil også registrere hva du spiser og drikker i 24 timer. Effekten av kosttilskuddet gitt sammen med det standardiserte måltidet på viktige tarmhormoner vil gi oss verdifulle opplysninger om hvordan dette kan påvirke restitusjonsevnen.

MULIGE FORDELER OG ULEMPER

Det vil være lege, fysioterapeut og sykepleier til stede under testingen, Under syklingen vil det bli målt blodsukker og melkesyre via et stikk i fingeren. Mellom de to sykkeløktene på testdag 2 og 3 vil det bli tatt blodprøver via en veneflon som blir satt inn i en vene i albuen.

Det forventes ingen ubehag av proteintilskuddet.

FRIVILLIG DELTAKELSE OG MULIGHET FOR Å TREKKE SITT SAMTYKKE

- Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte: Prosjektleder, Professor, MD, Trygve Hausken, Universitetet i Bergen: trygve.hausken@helse-bergen.no Førsteamanuensis, PhD, fysioterapeut, Bente Frisk, Haukeland universitetssjukehus, Fysioterapiavdelingen: bente.frisk@hvl.no eller MSc student/, fysioterapeut Ingunn Mjøs, Haukeland universitetssjukehus, Fysioterapiavdelingen: ingunnmjoes@hotmail.com

Du vil få dekket parkering og transportutgifter til og fra Høgskolen på Vestlandet på testdagene, samt et gavekort på kr. 500.

HVA SKJER MED INFORMASJONEN OM DEG?

Informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert.
Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste. Prosjektleder har ansvar for den daglige driften av forskningsprosjektet og at

opplysninger om deg blir behandlet på en sikker måte. Informasjon om deg vil bli anonymisert eller slettet senest fem år etter prosjektslutt.

HVA SKJER MED PRØVER SOM BLIR TATT AV DEG?

Prøvene som tas av deg skal oppbevares i en forskningsbiobank. Generell forskningsbiobank for fordøyelsessykdommer, 2012/553, Labbygget UIB, Trygve Hausken

FORSIKRING

Forsøkspersonen dekkes gjennom Haukeland universitetssjukehus sin forsikring.

GODKJENNING

Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk, REK (2017/56].

Prosjektleders navn og kontaktinfo:
Trygve Hausken, Medisinsk avdeling, Haukeland universitetssjukehus. 5021 Bergen og Klinisk institutt 1, Universitetet i Bergen
Trygve.hausken@helse-bergen.no

SAMTYKKE TIL DELTAKELSE I PROSJEKTET

JEG ER VILLIG TIL Å DELTA I PROSJEKTET

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver

Jeg bekrefter å ha gitt informasjon om prosjektet.

Sted og dato	Signatur
	Rolle i prosjektet

Table 13 *Reported nutritional intake compared to the Nordic nutrition recommendation (n = 13).*

ID	Energy (Kcal)		Fat (g)		CHO (g) [a]		Fibre (g)		Protein (g)		Vit. D (µg)		Thiamin (mg)		Niacin (mg)		Vit. B12 (µg)		Vit. C (mg)	
	RI	%	RI	%	RI	%	RI	%[a]	RI	%	RI	%	RI	%	RI	%	RI	%	RI	%
1 [b]	2359	**71 %**	93,0	25 %	297,1	**36 %**	29,2	97 %	98,0	**88 %**	24,4	244 %	3,3	239 %	24,4	135 %	3,20	160 %	169,5	226 %
2 [b]	2097	**62 %**	70,5	**19 %**	273,8	**32 %**	16,5	**55 %**	100,2	**86 %**	4,2	**42 %**	0,6	**41 %**	5,1	**28 %**	1,85	**93 %**	82,5	110 %
3 [b]	2947	**89 %**	103,4	28 %	365,8	**44 %**	28,4	95 %	151,6	138 %	20,2	202 %	1,8	131 %	34,0	189 %	9,80	490 %	79,0	105 %
4	3003	**87 %**	114,5	30 %	332,5	**39 %**	24,2	**81 %**	143,6	120 %	21,3	213 %	3,7	263 %	21,7	121 %	12,90	645 %	163,0	217 %
5	3952	111 %	154,1	39 %	497,5	56 %	30,2	101 %	159,2	133 %	14,1	141 %	4,0	286 %	29,6	164 %	9,13	457 %	196,7	262 %
6	2870	**83 %**	130,5	34 %	322,9	**38 %**	25,1	84 %	112,4	**95 %**	4,0	**40 %**	2,4	169 %	15,6	**87 %**	3,20	160 %	79,5	106 %
7	3602	111 %	160,8	**45 %**	418,6	52 %	35,8	119 %	123,2	115 %	14,1	141 %	1,6	111 %	16,5	**92 %**	8,10	405 %	112,5	150 %
8	3303	**92 %**	97,6	**24 %**	505,8	56 %	42,7	142 %	115,6	**92 %**	6,6	**66 %**	3,0	215 %	13,6	**76 %**	4,58	229 %	171,5	229 %
9	3309	**92 %**	168,4	42 %	329,6	**36 %**	24,2	**81 %**	131,4	104 %	6,9	**69 %**	2,2	154 %	25,6	142 %	6,60	330 %	56,5	**75 %**
10	2051	**58 %**	79,6	**20 %**	243,2	**28 %**	18,2	**61 %**	98,3	**79 %**	3,3	**33 %**	1,2	**87 %**	21,2	118 %	5,58	279 %	37,0	**49 %**
11	3180	**87 %**	107,3	27 %	468,0	51 %	39,8	133 %	103,8	**81 %**	21,9	219 %	1,8	128 %	12,5	69 %	5,25	263 %	21,5	**29 %**
12 [c]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	2680	**81 %**	99,2	27 %	368,9	45 %	25,8	86 %	90,1	**81 %**	7,0	**70 %**	1,3	**95 %**	12,1	**67 %**	5,08	254 %	52,2	**70 %**
14	2216	**56 %**	94,4	**22 %**	253,0	**26 %**	15,1	**50 %**	96,6	**67 %**	5,2	**52 %**	1,1	**80 %**	18,8	104 %	4,40	220 %	44,0	**59 %**

NNR: energy = REE × PAL, Fat = 25 – 40 % of E %, CHO = 45 – 60 % of E %, Fibre = 25-35 g, Protein = 1.5 g × BW, Vit D = 10 µg, Thiamin = 1.4 mg, Niacin = 18 mg, Vit. B12 = 1 µg, Vit C = 75 mg.

[a] CHO: Carbohydrates, including fibre. [b] Registration, mean out of 2 days. [c] No data available

*Abbreviations***:** *Abbreviations*: SEM = standard error of mean, SD = standard deviation, CHO = carbohydrate, vit D = vitamin D, vit. B12 = vitamin B12, vit. C = vitamin C, NNR=Nordic Nutrition Recommendation, RI = Reported intake, % = RI in percentage compared to NNR, E% = Percentage of total energy

Appendix 9 *Reported nutritional intake compared to the Nordic nutrition recommendation (n = 13).*