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The effect on subjective satiety sensation after intake of dietary fat of different quality

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Abstract

Background: The prevalence of overweight and obesity in society is increasing worldwide and it is one of the biggest public health issues today. Recent studies suggest that different dietary fatty acids can affect hunger and satiety signaling. The aim of the present study was to investigate how a test meal with different fat quality, either high in PUFA or SFA, affected subjective satiety sensation and glucose response in healthy individuals. In addition, measure the repeatability of a self-reported satiety sensation questionnaire.

Method: 21 healthy, normal weight individuals completed this double blinded postprandial crossover intervention study; 18 women and 3 men. The participants consumed two different muffins, either high in SFA or PUFA, at four visits (V1 – V4), and filled out a visual analogue scale (VAS) fasted, and at seven time points within 180 minutes at each visit. Blood glucose was measured at the same time intervals as VAS. Wilcoxon sign rank test and Bland Altman Plot were used to compare effects and repeatability between the meals.

Results: This study show no significant differences in how participants perceived satiety after intake of SFA compared to PUFA, using VAS. When studying the repeatability of VAS, we found no significant differences in subjective satiety between the two periods after intake of SFA. After intake of PUFA, we observed a significant difference in sensations for food cravings and desire to eat sugary foods. We observed no effect on postprandial glycemic response after intake of SFA compared to PUFA.

Conclusion: Fat quality in one meal did not affect subjective satiety sensation and glycemic response in healthy subjects postprandially. However, VAS had an overall good repeatability in subjective satiety sensations after intake of the same test meal at two different occasions, indicating that VAS in general could be used as a measure of subjective satiety sensation.

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Abbreviations

AUC	Area under the curve
ARC	The arcuate nucleus
BMI	Body mass index
CRF	Case report form
CVD	Cardiovascular disease
CCK	Cholecystokinin
CNS	Central nervous system
ENS	The enteric nervous system
FAO	Food and Agriculture Organization
GBA	The gut-brain axis
GI	Gastrointestinal
GLP-1	Glucagon-Like Peptide-1
HDL	High density lipoprotein
iAUC	Incremental area under the curve
LDL	Low Density Lipoprotein
MUFA	Monounsaturated fatty acids
NCDs	Non-communicable diseases
OsloMet	Oslo Metropolitan University
PYY	Peptide YY
PUFA	Polyunsaturated fatty acid
REK	Regional Committees for Medical and Health Research
SFA	Ethics
SD	Standard deviation
T2D	Type 2 diabetes
V	Visit
VAS	Visual analogue scale
WHO	World Health Organization

1.0 Theoretical background

1.1 Overweight and obesity

The prevalence of overweight and obesity is increasing globally and has nearly tripled since 1975. In 2016, 1.9 billion adults over 18 years old where overweight, and of these 650 million were obese. Overweight and obesity is a major risk factor for developing non-communicable diseases (NCD's) (Folkehelseinstituttet, 2017), which currently causes more deaths than all other causes combined, and by 2030 expected to increase to 52 million worldwide (World Health Organization, 2014). According to a study by Grover et al., (2014) overweight and obesity could shorten lifespan with up to 8 years and it is highly associated with risk factors for developing type 2 diabetes (T2D) and cardiovascular disease (CVD) (Grover et al., 2014). In Norway, the mean weight has increased over the last 40 – 50 years, and about 20% of the population is now overweight or obese. Among these, 25% of teenagers, 25% men, 20% women aged 40 – 45, as well as 15 – 20% of children, are overweight or obese (Folkehelseinstituttet, 2017).

Body-mass index (BMI kg/m^2) is an index used to classify weight, and WHO (World Health Organization) defines obesity as ≥ 30 kg/m^2, and BMI ≥ 25 kg/m^2 as overweight (World Health Organization, 2018). The increase in weight correlates with the shift in diet to more high-energy dense foods rich in sugars and fat, and a lack of vitamins and minerals, in addition to inactivity and a more sedentary lifestyle (World Health Organization, 2017). High blood pressure, certain types of cancer, T2D, nonalcoholic fatty liver disease, mental disorders and musculoskeletal disorders are all disorders linked to overweight, obesity and NCD's (Angelantonio et al., 2016).

Obesity is a complex combination of genetic, metabolic and environmental factors, and the etiology of obesity is still unclear (Moussavi, Gavino & Receveur, 2008). Assessing effects of foods with various properties can provide information on factors affecting overall appetite control, more specifically, what is leading to overconsumption (Green, Delargy, Joanes & Blundell, 1997). Different dietary fatty acids have also been shown to influence satiety (Kaviani & Cooper, 2017), and the degree of fat saturation, as well as chain length, can impact hunger and satiety signaling, fat oxidation rate, and possibly energy expenditure (Kaviani & Cooper, 2017). Of particular interest is looking at subjective sensation after intake of fat with different quality, and more specifically comparing PUFA and SFA. Following

guidelines towards a healthy diet, helps protect against NCD's, and this includes that unsaturated fat (PUFA), should be preferred instead of saturated fat (SFA) (World Health Organization, 2015).

1.2 Dietary fat

1.2.1 Recommendations for dietary fat

Nutrition recommendations are published by the Nordic Council and form the basis for the recommendations of the Directorate of Health in Norway. Nordic Nutrition Recommendations 2012 forms the basis for today's quantitative recommendations on fat (Nordic Nutrition Recommendations, 2012). In connection with the recommendations from 2012, a systematic review from February 2012 on fat and risk factors, concluded that there is convincing causal link between replacing SFA with unsaturated fat and a lower risk of cardiovascular disease (Nordic Nutrition Recommendations, 2012). Recommended intake of total fat is 25 – 40% of energy (E%) intake, and saturated fat should be limited to 10 E%. According to the National Directorate's publication on the development in Norwegian diet and food supply statistics, the diet's total fat content decreased between 1970s until the 1990s, from about 40% to 35%. Since then, the total fat content increased some between 2000 to 2016, from about 34% – 37%. The percentage of saturated fat in the diet has not changed significantly since 1975. Many people ingest more SFA than recommended, with an average intake of 15 E% (Helsedirektoratet, 2017). Monounsaturated fatty acids (MUFA) and PUFA should be at least 2/3 of the total fat intake, with 10 – 20 E% and 5 – 10 E%, respectively (Helsedirektoratet, 2016). The intake of polyunsaturated fats has declined from 6 E% in 1980, to 5 E% in 2015, which means that PUFA is in the lower range of recommendations. PUFA is well known for its health effects, and it would be positive with an increase in PUFA in replacement of SFA (Helsedirektoratet, 2016).

1.2.2 Fatty acids

Fat is the most energy dense nutrient with nine kilocalories (kcal) (37 kilojoule) per gram. Dietary fat plays a key role in the body and is an important source of energy, as well as an important source for essential fatty acids, and a carrier for fat soluble vitamins (Rustan & Drevon, 2005). In addition, fatty acids act as signaling molecules and stimulates the release of peptides in the gut inducing satiation and satiety (Rebello, O'Neil & Greenway, 2015). The energy storage accounts for most of the fat in the body, and this fat serves as a heat insulator

and shock absorber. Depot fat is stored primarily under the skin, abdomen and muscles (Rustan & Drevon, 2005). Fatty acids are carbon chains with a methyl group at one end of the molecule, and a carboxyl group at the other end (Figure 1). Fatty acids can be divided into SFA, mono unsaturated fatty acids (MUFA) and PUFA. Fatty acids with one double bond are called MUFA, for instance oleic acid (C18:1), while PUFAs have two or more double bonds, like linoleic acid (C18:2). There are two fatty acids which are produced by plants and plankton, that are essential to humans; alpha linolenic acid (ALA) (C18:3, omega-3) and linoleic acid (LA) (C18:2, omega-6). SFA are saturated or filled with hydrogen, hence, are without any double bonds (Rustan & Drevon, 2005). Examples are lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0).

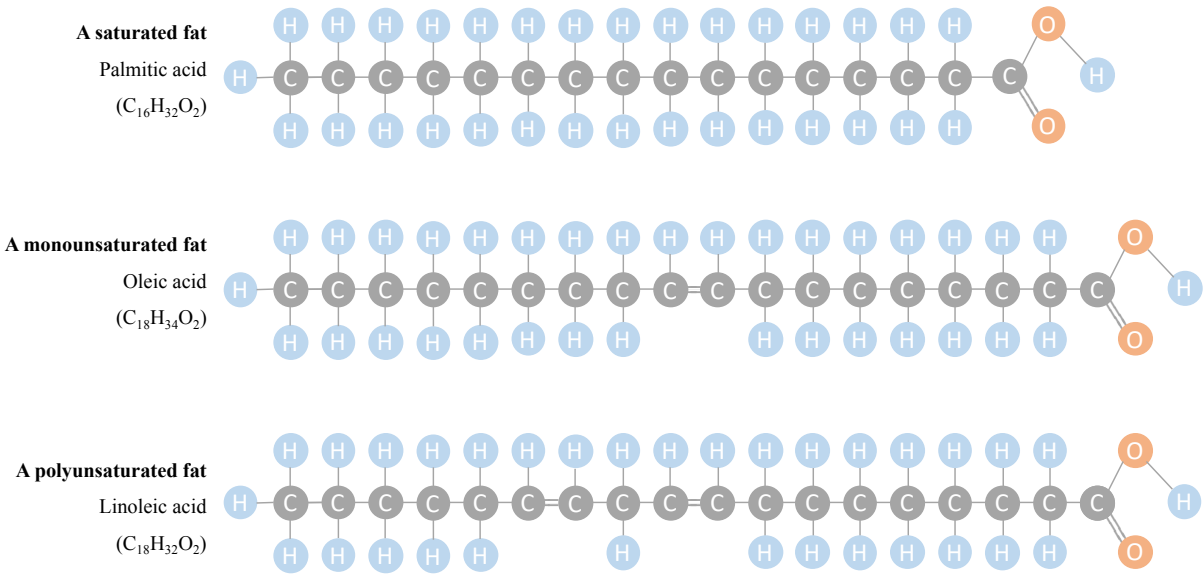

Figure 1. Schematic structure of fatty acids.

1.2.3 Fat metabolism and health effects

The risk of developing CVD as a result of changes in blood cholesterol levels is well known to be affected by fat quality. Replacement of SFA with MUFA and PUFA, especially omega-6 PUFA, decreases the plasma concentration of total and low-density lipoprotein (LDL)-cholesterol. The mechanism for these effects may be increased uptake of LDL particles from the circulation by the liver (Rustan & Drevon, 2005). LDL receptors in the liver are regulating the levels of plasma LDL, determined by the rate of LDL production and clearance. Lipoproteins are divided according to size and density: chylomicrons are large particles, but the least dense ones, and their size can vary according to the amount of fat ingested. They are made in the intestines, and these are large triglyceride rich particles, and involved in

cholesterol and triglyceride transportation to peripheral tissue and the liver. Very low-density lipoprotein (VLDL) is produced by the liver, and are smaller but denser than chylomicrons, however their size can vary depending on how much triglycerides there are in the particle. LDL is even smaller and denser, derived from VLDL and Intermediate density lipoprotein, and these particles carries most of the cholesterol into the circulation. Finally, High-density lipoprotein (HDL) is rich in cholesterol and phospholipids, and these are small, but very dense. HDL are particles important for the reverse cholesterol transport from the peripheral tissue to the liver, having anti-inflammatory, anti-oxidant, anti-thrombotic as well as anti-apoptotic properties in the body (Feingold & Grunfeld, 2015). Hypertriglyceridemia, low HDL levels, obesity, T2D and inflammation are associated with large amounts of small dense LDL particles, that has lower affinity for LDL receptors, and this can lead to a prolonged time in the circulation, giving increased risk of CVD (Feingold & Grunfeld, 2015).

SFA is shown to decrease the activity of LDL receptors, as compared to MUFA and PUFA, thereby contributing to less degradation of LDL cholesterol and to increased LDL cholesterol levels in the blood, while PUFA have the opposite effect (Fernandez & West, 2005). According to the Food and Agriculture Organization (FAO) and WHO, there is convincing evidence that there are three saturated fatty acids that increase LDL cholesterol; Lauric acid (C12:0), Myristic acid (C14:0) and Palmitic acid (C16:0). In addition to a positive correlation between fat intake and overweight and adiposity (Lissner & Heitmann, 1995), there is convincing scientific evidence that SFA may negatively affect several factors related to cardiovascular diseases and atherosclerosis, whereas PUFA may reduce the risk of CVDs and exert several beneficial effects on lipid metabolism, insulin sensitivity and satiety regulation (Helsedirektoratet, 2016).

In addition to the known effects of how different fat quality can impact cholesterol levels and the development of CVD, some studies also show that fat quality can affect glycemic regulation (insulin and blood glucose). SFA is seen to increase, and PUFA decreases blood glucose (Imamura et al., 2016). The underlying mechanisms of how intake of SFA can disrupt glycemic regulation, and increase the risk of developing T2D, are not fully understood. Diets rich in SFA are associated with increase adipose tissue inflammation and metabolic diseases, whereas diets rich in PUFA have been shown to counteract inflammation and promote a lean and metabolically healthy phenotype (Caesar, Tremaroli, Kovatcheva-Datchary, Cani & Bäckhed, 2015). Blood glucose levels have been shown to improve by replacing

carbohydrates or saturated fats with polyunsaturated fats. Notably, it is not enough to reduce intake on carbohydrates or saturated fat alone, because it is the addition of PUFA in the diet that has shown effect on blood glucose control (Imamura et al., 2016).

1.3 Satiety regulations

Dietary fat is shown to have the overall weakest effect on satiety compared to carbohydrates and protein. It is implicated in the rise of obesity as it is highly palatable and the most energy dense nutrient. Due to fat being a major contributor to overall energy intake, fat with satiating properties could possibly influence satiety by reducing overall energy intake (Maher & Clegg, 2018). Satiety is the term we use at the end of a meal, and the feeling of fullness that continues after eating. Satiety is probably suppressing further eating between meals until hunger returns. Satiation refers to a feeling of satisfaction after a meal and develops in the process of eating. Satiation determines the meal size (Amin & Mercer, 2016; Benelam, 2009; Hall et al., 2012; Green et al., 1997). Anyway, feelings of satiation can be overridden by sensory and cognitive stimuli that affect food intake, as for instance liking or wanting a food can overcome the physiologic feelings of feeling full or being hungry. Sensory-specific satiation affect satiety in a way when people might be able to eat a dessert, after being satiated from the main course (Hall et al., 2012).

The regulation of long- and short-term energy balance in healthy individuals is controlled quite well, and the energy intake and expenditure have to be relatively equal to maintain a stable body weight (Klok et al., 2006). However, for some people there is an imbalance between energy intake and expenditure (Näslund & Hellström, 2007), and internal biological factors like resting metabolic rate, and external factors like energy density of food consumed, are influencing energy intake and further the energy balance (Beaulieu, Hopkins, Blundell & Finlayson, 2017). Excess weight gain is the result of an imbalance in energy intake and expenditure, where energy intake is much higher than the energy we spend (Klok et al., 2006; Näslund & Hellström, 2007). Because obesity continues to be a growing epidemic, it is important to understand the mechanisms that regulate energy intake. The brain needs to have the ability to regulate and detect energy stores in such a way that energy intake and expenditure correlate (Ahima & Antwi, 2008). Earlier, the focus was on hypothalamic control for food intake, whereas today there is research recognizing that the pancreas, adrenals and the gastrointestinal (GI) tract also is involved in short term feeding control. In addition,

adipose tissue has been found to play an important role in long term control of food intake (Näslund & Hellström, 2007).

Behavior modification is a recommended step in obesity prevention, however evidence shows that weight loss is rarely maintained over the long term, after programs only targeting lifestyle changes (Teixeira et al., 2015). It is, indeed, therefore important to understand other factors affecting satiety, when looking into food and behavior change, to reduce the risk of obesity (Amin & Mercer, 2016). Understanding the mechanistic regulation of hunger and satiety is crucial and urgent in order to prevent obesity (Heisler & Lam, 2017). Satiation and satiety is believed to be impacted by food composition, and macronutrients are suggested to differ in their effect, with protein being more satiating than carbohydrate, and the least satiating macronutrient being fat (Hall et al., 2012). Studies have demonstrated that high-fat diets give rise to overconsumption, because fat is less potent than carbohydrate and protein in signaling satiety (Blundell & Macdiarmid, 1997; Green, Burley & Blundell 1994; Flint, Helt, Raven, Toubro & Astrup, 2003). On the other hand, research conducted on different foods, nutrients and their effect on satiety, has shown that energy density is what appears to affect satiety the most. If energy density is controlled, the macronutrient composition does not seem to matter that much, but high fat foods tend to have a higher energy density than the other macronutrients (Benelam, 2009). Since any food or drink can affect satiation and satiety with varying capacity, it is important to determine what we can do to reduce satiation or satiety, for a given amount of energy (Benelam, 2009).

Systems within the brain are sensing and integrating food intake that are influenced by emotional factors, social cues and learned behavior. Energy stores, recent energy intake and presence of specific nutrients are all signals interpreted in the brain (Badman & Flier, 2005). The neurons of the hypothalamus controlling food intake and energy expenditure, are responding to satiety signals from the circulation. Inflammation and damage on neurons controlling food intake and energy expenditure has been seen in some studies after intake of SFA. Inflammation triggered by SFA is shown to negatively impact satiety regulation and inhibit appetite regulation in the brain (Araujo, Moraes, Cintra & Velloso, 2016).

1.3.1 Fuel oxidation and satiety regulation

Some research suggest that satiety is linked to an increase in fuel oxidation, and that fluctuation and availability of some substrates, mainly glucose and fatty acids, control eating.

Thus, signals that are generated through post absorptive energy intake, control satiety (Alfenas & Mattes, 2003; Harrold, Dovey, Blundell & Halford, 2012). In between meals, satiety is controlled metabolically by the gut peptide hormones Glucagon-Like Peptide-1 (GLP-1), cholecystokinin (CCK) and Peptide YY (PYY), inhibiting food intake as it passes through the GI tract, also serving roles in meal processing. Hormones like leptin, ghrelin and adiponectin affect appetite, where ghrelin stimulates hunger, leptin promotes satiety, and adiponectin can affect the insulin response (Carlson, Turpin, Wiebke, Hunt & Adams, 2009). Long term satiety is controlled by insulin, amino acid concentration in the blood as well as glucose and oxidation of nutrients in the liver. Where fat being oxidized might be more satiating than fat being stored, and PUFA is oxidized faster than SFA and MUFA (Alfenas & Mattes, 2003). Signals from fuel oxidation are further integrated in the brain controlling homeostatic and hedonic appetite and satiety (Amin & Mercer, 2016).

1.3.2 Homeostatic appetite control

Homeostatic control of appetite can be explained in three levels of a psychobiological system. These are psychological and behavioral events, peripheral physiological and metabolic events, and neurotransmitter and metabolic interactions in the brain. All these events are incorporated in this system and termed in the satiety cascade including before, during, termination and after food is consumed (Beaulieu et al., 2017). The biological need to maintain the body's energy stores, are regulated by the homeostatic appetite control. Motivation to eat is increased as energy stores are depleted, and negative feedback signals are generated as soon as this need is met. On the contrary, this system does not work as well when there is excess energy; hence, the system is more sensitive to a deficit in energy than overconsumption. In other words, weight gain is allowed easier than weight loss by the system (Harrold et al., 2012). Homeostatic appetite control interacts with non-homeostatic appetite control, in a complex relationship affecting overall appetite. Non-homeostatic appetite is such as food hedonics and behavioral traits (Beaulieu et al., 2017).

Hedonic thoughts about food contributes to meal size and frequency, as it is the sensory system signaling the appetite for salt, sugar and fat. Weather we want or like a certain type of food are reflected by food hedonics, and influence food preference and choice (Beaulieu et al., 2017). Wanting food is thought to be slightly more important for overconsumption of food in obese individuals, because of attraction towards certain foods. Liking is the sensory pleasure from certain foods, and this tend to stay the same and is not affected by obesity

(Beaulieu et al., 2017). We can further define appetite as a motivational drive to eat a certain amount of food, regulated in a complex variety of signals within the body (Heisler & Lam, 2017). The regulation of feeding is monitored in many brain regions, cell types and other specific projections within the body.

The arcuate nucleus (ARC) is the most important site for integrating both exogenous and endocrine signals. Appetite is dependent on the feeling of whether energy levels are adequate in the body and has therefore an important homeostatic component for energy levels. The drive to eat is low when the interoceptive feeling of energy is adequate, and high when energy levels are perceived as inadequate by the interoceptive sensations (Heisler & Lam, 2017). Various peripheral organs send neural and chemical messages regarding the energy status in the body to the CNS. Several circulating hormones and factors induced by the eating sensation, like taste, smell and the feeling in the mouth, have access to regions of the CNS controlling energy homeostasis. Furthermore, from the peripheral system these regions in the CNS get signals about ingestion and utilization of nutrients, like absorption, metabolism and energy storage (Harrold et al, 2012). The CNS is integrating all the signaling information to maintain energy homeostasis (Bauer, Hamr & Duca, 2015; Harold et al., 2012). Examples of signals are firstly distension and chemoreceptors in the gut, energy status and conversion from the liver, generated via afferent vagal signals from nucleus of the solitary tract/area postrema complex in the brain stem. Secondly, receptors sending CNS signals that detect circulating levels of nutrients and their metabolites in the periphery, and lastly, glucose and neurotransmitter precursor substances having crossed the blood brain barrier and entering the brain directly altering CNS neurochemical activity in specific sites for regulation (Harrold et al., 2012).

1.3.3 Satiety cascade

The satiety cascade outlines the process of behavioral and physiological events following food intake, and the termination of eating until hunger signals return, illustrated in Figure 2 (Bellisle, 2008; Blundell, 2010). In other words, it explains events that stimulates eating (preprandial), signals triggered by food ingestion and termination (prandial), and lastly, processes following eating termination (postprandial) (Harrold et al., 2012). Eating and drinking occasions during the day are influenced by many factors, and the total number of meals and how much is consumed during the day, is therefore reflected both by satiation and satiety (Benelam, 2009). With that in mind, the satiety cascade is showing satiation and

satiety influences over time that includes taste, texture, smell and other associations of the eating associations that might occur.

After food is ingested, the brain receives signals from post digestive factors, which initially is caused by the distention of the stomach. Satiation and satiety promoting hormones are further released from the gut as digestion goes on. Specialized receptors are detecting nutrients at different sites in the body, as well as the brain (Benelam, 2009), and post digestive signals are received by the central nervous system (CNS) from the gut, in the prandial phase. Furthermore, distention of the gut is signaled by mechanoreceptors, in the presence and amount of food consumed. The presence of nutrients is detected by chemoreceptors giving information about the ingested food, while the vagus nerve is giving information from signals in the GI tract. Lastly, when circulating nutrients from the GI tract enters the peripheral circulation, prandial and postprandial signals are activated. Receptors in the CNS can be activated by circulating nutrients as they are metabolized in organs or peripheral tissue, or the nutrients can serve as post absorptive satiety signals if they enter CNS directly via the circulation (Harrold et al., 2012).

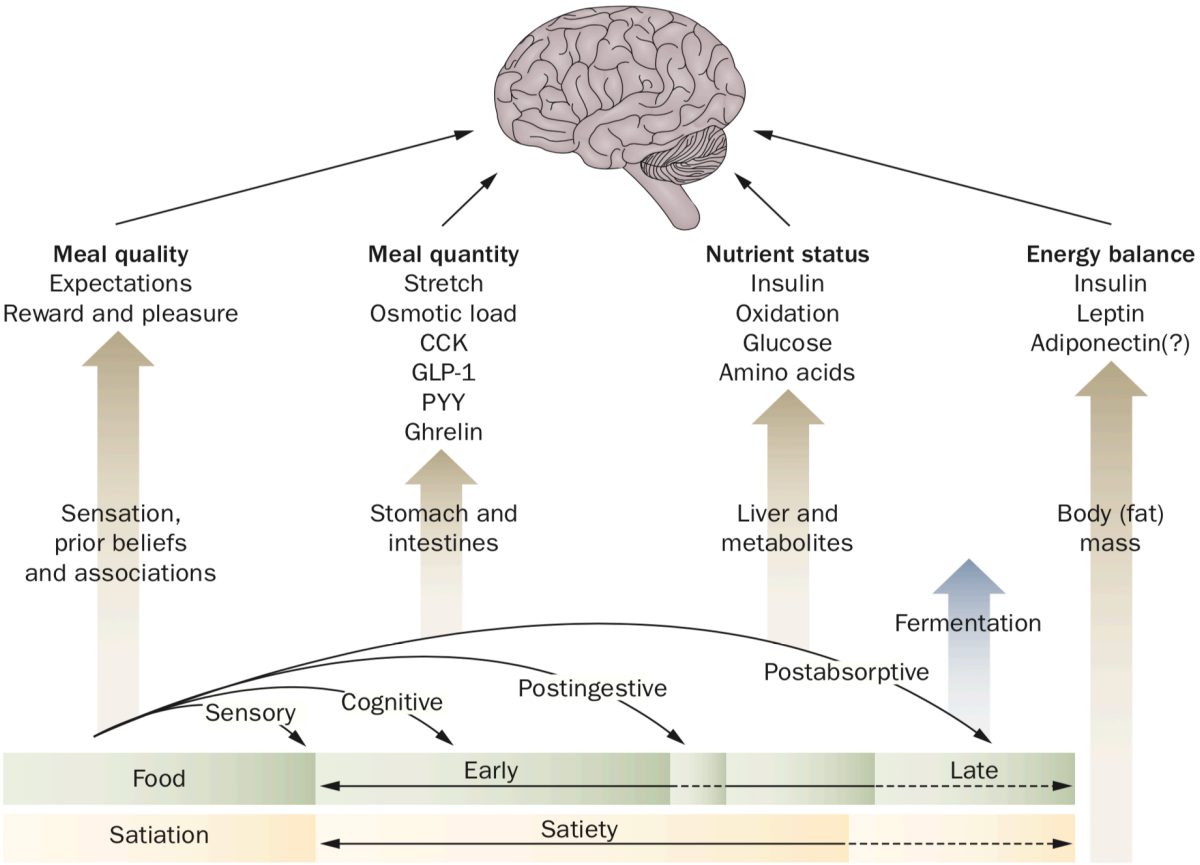

Figure 2. The Satiety Cascade shows relationship between satiation and satiety, and some mediating psychological and physiological processes (Blundell, 2010).

1.3.4 Tonic and Episodic signals

After food ingestion, peripheral influenced signals arise, which we classify as either tonic or episodic factors (Harrold et al., 2012). To reflect amount of body fat, "Tonic" or long-term adiposity signals like insulin and leptin, is released continuously. In addition, "episodic" or short-term signals fluctuating depending on food intake (Bauer et al., 2015). Tonic and episodic signals determine how much is eaten, although they differ in effect and time. A constant need for energy influenced over the long term is generated by tonic factors, while episodic signals are those triggered when food is ingested (Harrold et al., 2012). During digestion, chemicals released by the GI tract act as satiety signals. Some peripheral signals recognized as having an important role in satiety are CCK, GLP-1, PYY and amylin (Harold et al., 2012). The hormones secreted from the gut when food has been consumed, act on areas in the brain either directly or indirectly to promote satiety. Even if episodic signals are released when episodes of eating happen, and tonic signals reflect the amount of energy stored in the body, they may overlap some as they both control feeding behavior (Benelam, 2009; Harrold et al., 2012).

1.3.5 The enteric nervous system and the gastrointestinal tract

The enteric nervous system in the GI tract influences exocrine secretion, motility, blood supply and secretion of gut hormones, and is involved in every aspect of the gut function. Inside and between submucosal and myenteric plexuses local signaling take place, and afferent signaling from the gut to the brain happens in vagal and splanchnic nerve pathways (Badman & Flier, 2005). Peptides in the gut have multiple targets, including exocrine glands, smooth muscle, afferent nerve terminals and the brain (Badman & Flier, 2005). Short term control of food intake involves the CNS, however also the adrenals, pancreas and the GI tract (Näslund & Hellström, 2007).

Peptides in enteroendocrine cells of the mucosa of the GI tract and in central areas which controls and regulates food intake, can be found in the enteric nervous system and vagal afferent nerves. This could for instance be neurons from the GI submucosal and myenteric plexuses, and endocrine cells found in the intestinal mucosa and pancreatic islets displaying orexin, and orexin receptor immunoreactivity (Näslund & Hellström, 2007). Throughout the GI epithelium, there are at least 15 different types of enteroendocrine cells. Many different hormones and signaling molecules are produced and released in these cells, and this together makes the GI tract the largest endocrine organ in the body (Sam et al., 2012). The peripheral

and vagus nerve systems are sensing both mechanical distention and chemical stimulation by many nutrients, released as hormones (for example CCK and GLP-1) and affecting sensory nerves. When nutrients releasing these hormones are delivered to the intestines, CCK and GLP-1 activates the brain stem through the afferent vagus nerve from their specific receptors (Näslund & Hellström, 2007).

The GI is very important for digestion and absorption of nutrients. Before food even enters the mouth, endocrine and exocrine secretions, as well as gut motility, are stimulated by visual, olfactory and gustatory senses. In addition, hormones that control our feeding mechanisms, are secreted in our GI tract (Ahima & Antwi, 2008). In fact, more than 20 different regulatory peptides are released by the GI tract, which influences many physiological processes. Exocrine glands, smooth muscles and the peripheral nervous system are all tissues gut hormones influences and act on. Not only do they contribute to the short-term satiety feeling, they do possibly also reduce food intake as they decrease hypothalamic orexigenic signaling, and increase anorectic signaling. In addition, these gut peptides mediate the feedback mechanisms inhibiting intestinal passages, which prolong gastric distention and increases satiety between meals. The mechanisms by the gut peptides CCK, PYY, GLP-1, oxyntomodulin and the effect of the CNS combined, facilitates the control of food intake and postprandial passage through the GI tract (Sam, Troke, Tan & Bewick, 2012).

Systems within the brain that sense and integrates signal about energy stores, energy intake and also specific nutrient classes, are influenced by emotional, social and learned behavioral factors. The ARC of the hypothalamus, receives inputs from other areas of the brain and is accessible to circulating factors (Figure 3). Signals related to total energy stored in adipose tissue, immediate changes in available energy, which includes nutrients in the gut, are received in the ARC (Badman & Flier, 2005). The long and short-term signals are not very independent of each other, as long-term insulin and leptin signals can also modulate short term nutritional input.

1.3.6 Gut peptides regulating satiety

Signals influencing food intake and energy expenditure can be separated into two categories, long- and short-term signals. Mediators of intestinal satiation such as GI signals are released as nutrients passes into the stomach and intestines (Rebello et al., 2015; Wren & Bloom, 2007). Enteroendocrine cells located in the GI tract and nutrients are interacting to stimulate

the release of peptides that influences appetite regulation locally, centrally and peripherally (Rebello et al., 2015). These peptides can function as short-term satiety signals and very likely long-term regulators of body weight (Wren & Bloom, 2007). GLP-1, PYY and CCK, are released from intestinal L-cells in response to ingestion of nutrients and appear to act in part as satiety signals as well as possibly participating in long term body weight regulation (Wren & Bloom, 2007). These are known to be anorexigenic, physiologic regulators for appetite (Näslund & Hellström, 2007).

CCK is a satiety hormone, a strong mediator as a satiety gut peptide, shown to inhibit food intake (Benelam, 2009; Harrold et al., 2012; Näslund & Hellström, 2007; Wren & Bloom, 2007). When nutrients, especially from fat and protein-rich foods, enter the gut, CCK is rapidly released into the circulation postprandially. In addition to inhibit food intake, CCK is involved in delaying gastric emptying, stimulating pancreatic enzyme secretion and trigger the release of gallbladder bile salts which promotes fat and protein digestion in the duodenum. These actions are optimizing the digestion of fat and protein in the small intestine (Sam et al., 2012; Wren & Bloom, 2007). SFA, long chain fatty acids, amino acids and small peptides influences CCK to be released postprandially. The release of CCK in response to long chain fatty acids, influences PYY release which inhibits the orexigen hormone Ghrelin (Sam et al., 2012). CCK and PYY will act to inhibit food intake and suppress appetite together.

PYY is released from the GI tract and induces satiety after food intake. It probably has a longer duration compared to other satiety peptides (Näslund & Hellström, 2007). Enteroendocrine cells in the stomach can detect low levels of PYY, and alongside the small and large intestine, these levels increase. Levels of PYY in a fasting state will be low, and higher postprandially as the PYY release are secreted in proportion with energy consumed (Sam et al., 2012). The pattern of PYY secretion in response to a meal raises the possibility that it may be a physiological satiety signal, acting to terminate the meal and stimulating coordinated GI responses to aid digestion and absorption (Wren & Bloom, 2007).

GLP-1 and glucagon-like peptide-2 are released in equal amounts in the blood stream after a meal (Näslund & Hellström, 2007), and is first and foremost involved in the stimulation of GI motility. It is found in the brain, and shown to inhibit food intake when administered centrally, but physiologically, it does not influence appetite (Murphy & Bloom, 2006). GLP-1 is located and synthesized by L cells in the distal small intestine, where it is co-released with

PYY. Several studies show that in appetite regulation, GLP-1, is a hormone that can stimulate the release of insulin and inhibit glucagon and delays gastric emptying, inhibits food intake and is important in the metabolism and absorption of macronutrients (Harrold et al., 2012). Because GLP-1 slows gastric emptying after a meal, the requirement for insulin is reduced (Näslund & Hellström, 2007).

1.3.7 Gut Brain-Axis and satiety signals

The GI releases the majority of short-term satiety signals. Some key gut peptides like CCK, GLP-1, gastric inhibitory peptide and PYY, communicate important information regarding the size and composition of a meal to the brain, and the gut-brain axis (GBA) represents a bidirectional signaling axis that is vital for metabolic regulation (Figure 3). Several studies have investigated the effect of different nutrients, including fatty acids, on satiety regulation and gut peptide release (Araujo et al., 2016). GBA is the bidirectional communication between the enteric nervous system and the CNS, and both cognitive and emotional centers of the brain is influenced by the GI tract and vice versa (Carabotti, Scirocco, Maselli, & Severi, 2015; Mayer, Tillisch & Gupta, 2015). The GBA is further a modulator in the regulation of blood glucose levels, adipocyte function and energy expenditure, thus influencing to proper maintenance of energy homeostasis after food intake (Hussain & Bloom, 2013).

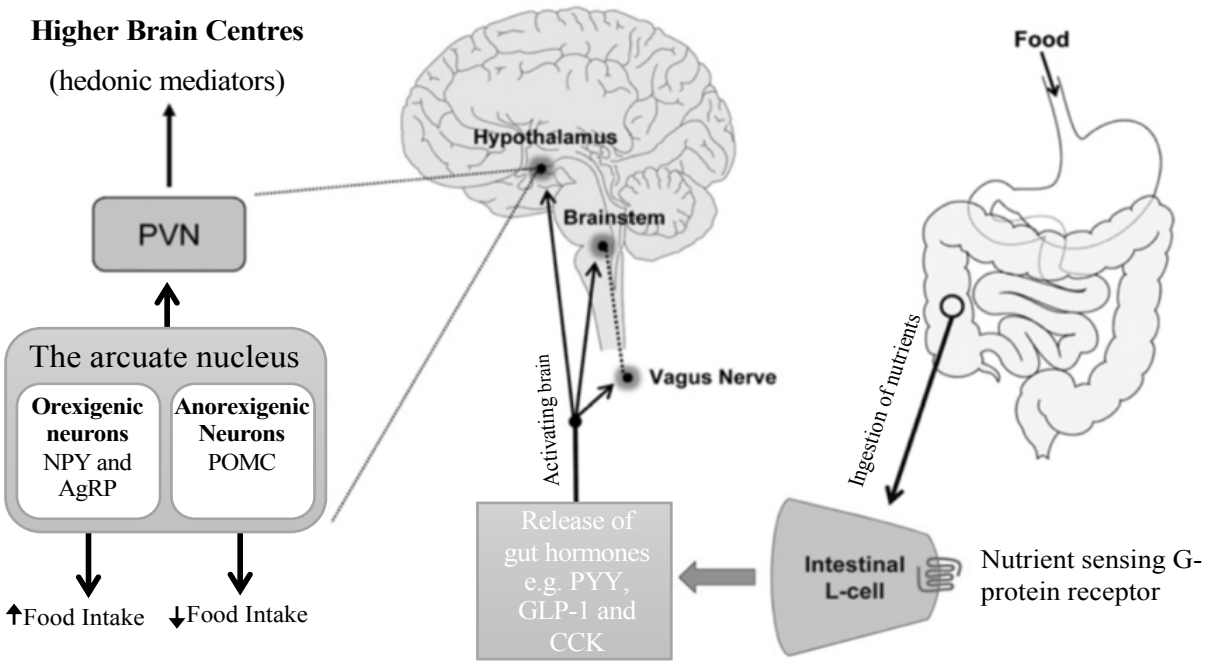

Figure 3. Gut-brain axis: regulation of food intake. GLP-1, Glucagon-Like Peptide-1; CCK, cholecystokinin; PYY, Peptide YY; PVN, paraventricular nucleus of hypothalamus (Modified by Sam et al., 2012).

Stimulation of gut hormones happens when nutrients after food digestion activates receptors in enteroendocrine cells. Gut hormones can influence food intake at either the vagus nerve, brainstem or hypothalamus (Sam et al., 2012). The hypothalamus and the brainstem are responsible for the regulation of energy homeostasis. They receive peripheral neural and hormonal signals giving information about short term nutritional state and fat reserves. Regulation of appetite and energy expenditure are controlled with signals to the brain center influenced by afferent neurons and hormones from the periphery (Sam et al., 2012). The ARC is having a key role in the regulation of food intake and energy expenditure and is containing two populations of neurons acting opposite of each other, supposing important key conduits for peripheral signals in altering food ingestion. These are orexigenic neurons expressing neuropeptide Y and Agouti-related protein, and anorexigenic POMC neurons illustrated in Figure 3 (Sam et al., 2012). A semi permeable blood brain barrier is located alongside the ARC, allowing hormones from the circulation and other peripheral signals to easily influence directly on the CNS (Hussain & bloom, 2013; Sam et al., 2012). Gut hormones signal short-term information after a meal to the ARC, and circulating peptides from adipose tissue, like insulin and leptin, inform about long term energy stores (Sam et al., 2012).

1.4 External factors impacting food intake

Food intake in humans is complex with a physiological basis, but is also influenced by emotional, social and learned behavior factors. Signals reflecting overall energy stores, recent energy intake and other specific nutrients within the brain are influenced by these external factors (Badman & Flier, 2005; Murray & Vickers, 2009). Body weight, age, gender, diet, such as liking and wanting food, and the social circumstances are confounding factors to pay attention to when measuring satiation (Benelam, 2009; Hall et al., 2012). Psychological and physical factors on an individual basis, like texture of the food, if it is salty or sweet, temperature, a participant's mood, and other feelings around the meal is likely affecting feelings of hunger and fullness (Murray & Vickers, 2009). In a setting with others, social influences of eating, like social modelling and norms, can affect how much and the way people eat (Higgs & Thomas, 2016).

Factors caused by the obesogenic environment we live in today influences quite heavily the choices we do about food consumption. The way hedonics impacts food intake does not necessarily have to do with the energy need or weight status of an individual (Beaulieu et al., 2017). The food environment affects the internal signals of hunger and satiety and make it

more difficult to regulate food intake. Examples of external factors influencing satiety are food commercials on TV, sight or smell of foods. Norms and traditions are also influencing both meal initiation and the social setting as food not only is about eating, but also about interaction among the people sharing the food. For instance, people can be in a social setting where they accept to eat, even if they just ate, thus overriding the feeling of satiety (Bilman, Kleef & Triip, 2015). According to Benelam (2009), studies has shown that people eat about 44% more when they are in a social setting with others, then they do when eating alone (Benelam, 2009). Highly palatable foods are easy accessible, affordable and highly promoted everywhere, and our appetite control system is challenged, possibly overpowered by habits, routines and cues in the external environment and act as determinants regulating food intake (Bilman et al., 2015). Palatability and satiety is shown to act opposite of each other, as satiety limits consumption and palatability increases appetite and food consumption. The most palatable foods are in other words the least satiating. Drewnowski (1998), found that high-energy dense foods are often selected in bigger portions because they are expected to be less satiating (Brunstrom & Rogers, 2009).

Palatability, which is defined as the "pleasurable experience when consuming food", being the individual experience when eating under a specific setting that can affects portion size and food perception (Benelam, 2009; Kral, 2006; Vartanian et al., 2017). Ways of increasing palatability could be adding more fat, and this can increase both appetite, portion size, duration and eating rate of a food or meal. Often, more palatable foods can be less satiating and more nutrient dense, and the composition of the food or meal is not controlled (Benelam, 2009). Furthermore, both the variety and portion size of a meal will influence how much people eat in a setting. Many studies have suggested that the more food choices, the more food is consumed. The same is seen with portion size, where the larger the portion size is, the more food is consumed. If food consumption only were affected by the internal mechanisms, people would not eat more when given larger portions (Benelam, 2009).

It is also suggested by some studies, according to Benelam (2009), that eating when watching television or having distractions, increases food consumption, because people get less sensitive to internal control systems regulating appetite and satiation. Even so, such distractions are complex, as it also means being sedentary for more or longer time while eating, and at the same time having the opportunity to eat more (Benelam, 2009).

In opposition to a sedentary lifestyle, physical activity is well known to have a positive impact on such as body composition and increase energy expenditure. It can also help with short term weight control and maintenance of a healthy weight (Benelam, 2009). However, according to Beaulieu et al., (2017), both the amount of physical activity, the amount of body fat people has, and weather the composition of the meal post exercise is high or low in fat, does play a significant role in the sensitivity to which it affects appetite control (Beaulieu et al., 2017).

1.5 Visual Analogue Scale to measure satiety

Satiety and appetite sensation can be measured both physiological and psychological, and VAS are one method commonly used to measure psychological sensation. A widely used scale is either 100 mm or 150 mm horizontal line, where one end is the extreme of subjective feeling of either "not hungry at all" or opposite "very hungry", in the case of hunger sensation assessment. According to how the subjects define the line, they are recording their satiety experience after a meal, and measurements using VAS is often taken at repeated time intervals. The measurement quantification is done by measuring the distance from left to right, and most often VAS is constructed on paper, however it can be done electronically (Stubbs et al., 2000). VAS can give important information when used appropriately in research related to food and satiety, even if it can be difficult to quantify, and several external factors must be accounted for (Livingstone et al., 2016). VAS validity and repeatability has been vastly studied in other research areas and are considered the "gold standard" in pain research. VAS is commonly used to measure satiety and are validated by several studies (Flint, Raben, Blundell & Astrup, 2000; Stubbs et al., 2000). Both repeatability and reproducibility may be assessed in the same way, because they are measures of reliability (Watson & Petrie, 2010). VAS is known to have good repeatability between groups, even if it has some limitations (Kaviani & Cooper, 2017; Lesdéma et al., 2015). In studies with single meals, appetite scores can be used as they are reproducible (Flint et al., 2000). Repeatability is the term used when there is identical conditions and measurements are made on the same subject, using the same instruments and method. On the other hand, the term reproducibility is the method used when conditions are changing (Barlett & Frost, 2008).

In a given situation perceived hunger or appetite that triggers feeding will greatly differ from individuals in different settings. The way we "feel" like eating or not, is determined by physiological signals, past experiences and other environmental influences, but these are not easy to measure directly. Blood glucose fluctuations, social occasions and previous food

poison experiences are examples of sources that are difficult to use as measurements for perceived hunger or appetite. Subjective hunger is best to track over time, in order to provide information about the actual feeding events such as diet composition, or physiological variables affecting the appetite control system (Stubbs et al., 2010). The actual sensation of hunger can greatly differ quantitatively and between subjects, and it is important to take into consideration what people mean using the terms hunger, appetite and satiety. People can use terms like this to describe many sensations they recognize and predict their normal behavior (Stubbs et al., 2010).

The subject´s emotional, mental and psychological status can affect VAS answers and cause bias when reporting the sensation of hunger and fullness (Kaviani & Cooper, 2017). It is important to have in mind, because when evaluating different meals and diets on appetite sensation, psychological factors can interfere with physiological factors affecting the meal. Indeed, hunger tolerance can vary from one day to another, and impact answers to be inaccurate on a test day (Kaviani & Cooper, 2017).

Some studies have found differences in hunger and satiety between meals with varying fatty acid composition, using VAS in addition to other hormones, whereas other studies have not found any relations between fatty acids quality and subjective satiety (Kaviani & Cooper, 2017).

1.6 Aim of the study

The aim of this study is to investigate how a test meal with different fat quality, SFA versus PUFA, may affect subjective satiety sensation in healthy individuals measured by VAS. In addition, to measure the effect of a meal with different fat quality on postprandial blood glucose levels.

Objectives of this master thesis were to:

- study the effect of SFA and PUFA on postprandial subjective satiety sensation in healthy subjects, using VAS.

- study the repeatability of the self-reporting satiety sensation (VAS), between two visits with intake of either SFA or PUFA.

- study if SFA and PUFA affect postprandial blood glucose levels differently in healthy subjects.

In this master thesis VAS is used as a method to further investigate the effect on fatty acid quality on subjective satiety sensation, as it is a non-invasive, easy and cost beneficial method. In addition, objective measures for blood glucose was measured to study any correlation between the subjective and objective measurements of satiety, or differences in blood glucose levels between a test meal rich in SFA compared to PUFA.

2.0 Materials and Method

The current study was carried out as a postprandial, double blinded, crossover study design. The study lasted for a total of 5 weeks, and one week prior to the first study period participants met for a screening day. Subjects were divided into two groups, and both groups came in 2 weeks each (V1 – V4), with one week washout in between. Each subject consumed two test-meals (Monday and Friday morning) each of the two interventions periods.

2.1 Recruitment and Subjects

The recruitment process was carried out from September 2017, until October 2017. The subjects were recruited from the student population at OsloMet, Kjeller. Information about recruitment was provided through social media, as well as presentations in classes at OsloMet. A total of 34 individuals showed interest in participating in the study, and of these 23 were included in the study (Figure 3).

All subjects were screened before included in the study. Screening was done to exclude subjects that could not participate due to the exclusion criteria set for this study. Exclusion criteria were self-reported current or previous chronic diseases, like diabetes mellitus (type 1 and 2), CVD and cancer for the last 6 months (Table 1). Any significant metabolic, endocrine or GI diseases were excluded. Food allergies or intolerances were also excluded, because the test meals could contain some of the allergens. The subjects could not be pregnant or lactating (Table 1). It was not permitted to plan a weight reduction or having changed weight during the last 3 months prior to the study. Subjects could not have given blood during the last two months before the study, or during the time of the study. Subjects had to be willing to stop using dietary supplements and stop hormonal treatment (except for contraceptives/birth control) from one week prior to study start, and throughout the study period. Lastly, they had to have a low alcohol consumption during the study period. None of the participants used medications known to affect appetite or weight regulation (Table 1).

Table 1. Exclusion criteria

Criteria	
Chronic metabolic diseases	Diabetes type 1 and 2, CVD, cancer last six months.
Intestine diseases	Chron´s disease, ulcerous colitis, celiac disease and IBS.
Food allergies and intolerances	Concerns foods as eggs, grains, milk.
Pregnant and nursing women	
BMI	<18.5 and > 27 kg/m^2
Reduction and/or weight change	5% weight change during the last three months.
Given blood	During the last two months and the study period.
Stop using dietary supplements	From one week before study start and throughout the study period.
High alcohol consumption	> 40g per day.
Hormonal treatment	Except use of oral contraceptives.

BMI, Body Mass Index; IBS, irritable bowel syndrome; CVD, cardiovascular diseases.

Due to exclusion criteria for BMI, three subjects were excluded, and after the screening and study start, two participants withdraw their consent due to personal reasons (Figure 3). Finally, a total of 21 healthy, normal weight men and women between 18 – 65 years of age, with a body mass index (BMI) between <18.5 and >27 kg/m^2 were attended the study. Of these, 18 subjects did both VAS (Appendix A) and blood glucose measurements, and 3 subjects did only VAS. BMI, body weight and height were measured and recorded while fasting, on two consecutive occasions, after the study start. Total registered interested participants were 34, but due to various reasons, seven did not participate. Of these, one participant did not show up on the planned screening day. Two participants were excluded due to high BMI, and one due to low BMI. Finally, two resigned after startup because of personal reasons and sickness. A total of 21 subjects participated in the study.

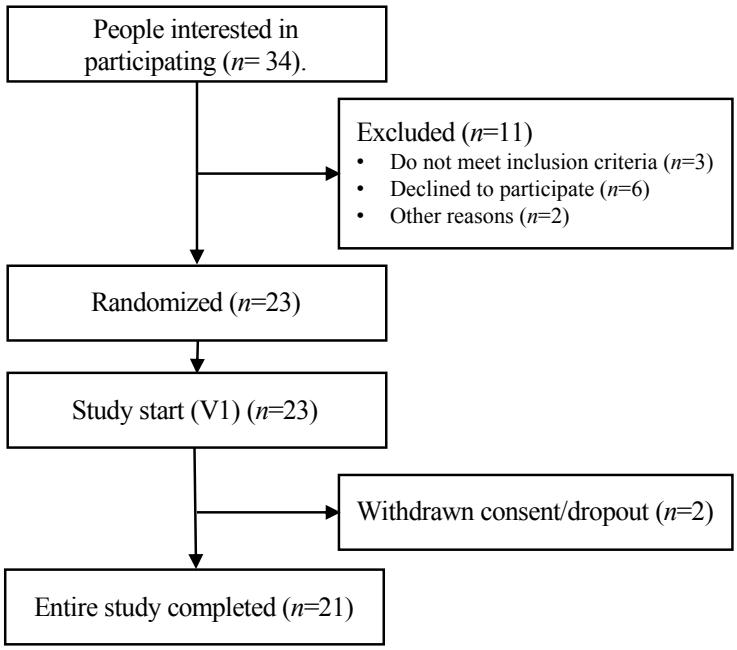

Figure 4. Flowchart with an overview of the study sample throughout the recruitment and screening process. V1, Visit 1.

2.2 Study design and implementation

This master thesis was conducted in cooperation with the research group in the study field Samfunnsernæring, at OsloMet. Subjective satiety effects of a high fat standardized breakfast muffins with either SFA or PUFA were assessed using a VAS questionnaire. The implementation of the study took place at OsloMet (Kjeller), where all subjects met fasting for the screening one week prior to the study start (V0), and further at four set occasions during the study period (V1 – V4) (Figure 4). This gives a total study duration of 5 weeks, and 5 visits (V0 – V4) for each participant. For both periods, they meet at Mondays and Fridays.

Anthropometric measures were performed fasting at screening and every visit. Height was preformed using, Holtain limited stadiometer, and body weight and body composition was done using, Tanita scale (BC-418 Segmental Body Composition Analyzer). Before measurement all subjects were asked to remove any metal, socks and shoes, and one kg was subtracted for clothes. Furthermore, the subjects completed a case report form (CRF, Appendix B) at each visit. The CRF included questions about diet, physical activity, use of medication or other supplements, and if they had followed the required restrictions. There was a one week washout between the two study periods, to avoid bias where participants could have remembered their VAS ratings.

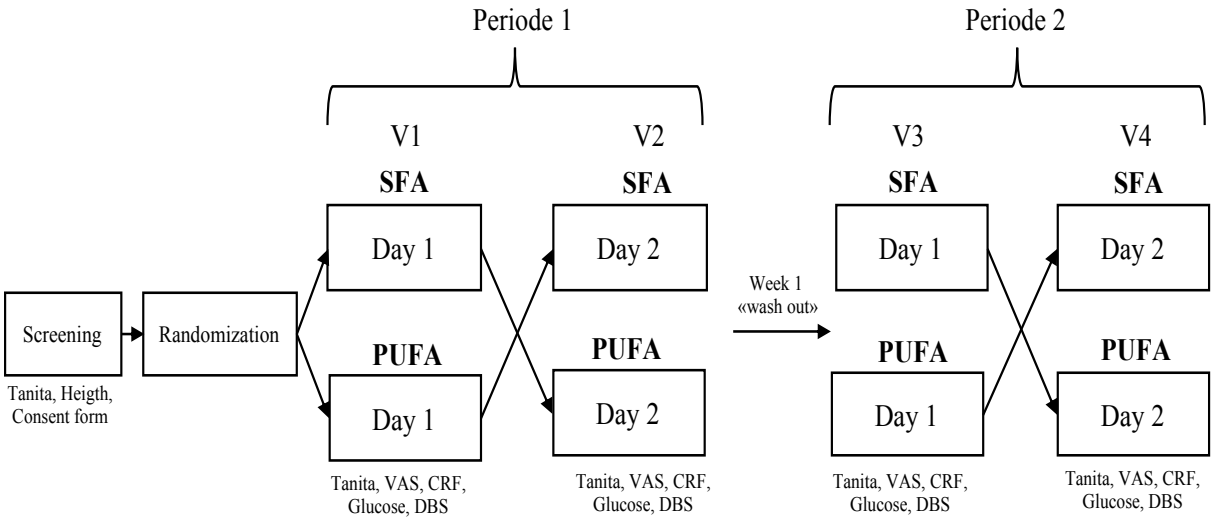

Figure 5. View of the study design.

V, Visit; VAS, Visual Analogue Scale; CRF, Case report form; DBS, dried blood spot; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids.

Restrictions

24 hours prior to each study-day, participants were asked to abstain from alcohol and heavy physical activity. The evening prior to each visit (V1 – V4) the participants last meal of the day had to be a restricted supper at home, no later than 20:00. This meal was supposed to be a low fat, and relatively low fiber meal, including bread (less than 50% wholegrain) with skimmed dairy products and/or lean meat products as they desired. Any buttery spreads, oil, nuts and fatty fish had to be avoided this meal (Appendix D). Information and restriction regarding the supper was described in an informative letter with illustrations of the food that were allowed and not allowed. This was given to all subjects after the screening, so they had time to read it and ask questions if they didn't understand it (Appendix D). Participants had to fast from 20:00 and until the next morning. The fasting included any liquids, except water. The participants were told not to change their diet during the study period, except for the evening meal as explained.

A member in the research group, not involved in the study implementation and analysis, were randomizing and distributing the meals for the subjects, and the subjects were not informed weather it was the PUFA or SFA meals they were eating. The standardized breakfast meals were given in random order and were served between 08:00 – 09:00, and the participants were asked to consume the entire muffin within 10 minutes. The exact duration of eating time was written down so all the subjects had their own time interval schedule to follow. VAS ratings

and blood glucose were measured throughout the next 180 minutes (eight times) and DBS was measured 4 times (Table 2). Participants remained in the study location during the three hours, and were allowed to read, write and similar activities. Participants were not allowed to consume any other food until the 180 minutes had passed. The first test meal was given at day one (Monday), and the second meal was given on day two (Friday).

Table 2. Time points for measurements in minutes after intake of HF testmeal

	Time (minutes) fasting and after intake of HF testmeal							
Measures	0	15	30	60	90	120	150	180
Tanita	x							
Glucose	x	x	x	x	x	x	x	x
Biochemical markers[1]	x		x	x		x		
VAS	x	x	x	x	x	x	x	x

[1] Dried blood spot; VAS, Visual Analogue Scale

2.3 Test meals

The two standardized breakfast test meals consisted of two chocolate muffins, with a weight of 100 grams. One was high in SFA, and the other high in PUFA. The PUFA muffin contained 443.8 kcal, whereas the SFA muffin contained 435.8 kcal, respectively.

Calculating the type of fat and amount of fat to be used were carried out using a diet planer, "Kostholdsplanleggeren.no". After having tested various types of fats in order to get SFA and PUFA as equal and standardized as possible, and MUFA as constant as possible, some good fat alternatives were selected for test baking. An easy standardized recipe with relatively few ingredients was used for the chocolate muffins (Appendix C). The muffins were prepared in two batches, and the ingredients were; sugar, egg, baking powder, wheat flour cocoa powder and fat source (Appendix C). Thus, the same amount, and the same ingredients, were used in the preparation of each of the two test meals, except for the fat source. *Bremykt* was used as the saturated fat source (47% SFA of total fat), and *Vita-Hjertego* was the polyunsaturated fat source (42% PUFA of total fat). Total Energy percent (E%) from fat was 61 in the PUFA-test meal, and 60 in the SFA test meal. After baking, the muffins were stored at -20°C, and thawed overnight at 4°C before consumption. The test meals were consumed with water.

Figure 6. The standardized breakfast muffins meal under production. The left picture shows test baking of two different alternatives, one sweet and one savory muffin. The middle image shows the finished product, and the third picture shows the labeling and packing of the meal.

Table 3. Distribution of the various fat types in the standardized breakfast muffins meal

	PUFA Testmeal	SFA Testmeal
Saturated fat % of total fat (SFA)	11	47
Monounsaturated fat % of total fat (MUFA)	40	35
Polyunsaturated fat % of total fat (PUFA)	42	11

SFA, Saturated fatty acid; MUFA, monounsaturated fatty acids, PUFA, Polyunsaturated fatty acids. Calculated using "Kostholdsplanleggeren".

Table 4. Nutrient content in the standardized breakfast muffins meal

Nutritional content (E%)	Testmeal PUFA	Testmeal SFA
Fat	61	60
Proteins	6	6
Carbohydrate	33	34
SFA	7	28
MUFA	25	21
PUFA	25	7

E%, Energy Percent; SFA, Saturated fatty acid; MUFA, monounsaturated fatty acids, PUFA, Polyunsaturated fatty acids. Calculated using "Kostholdsplanleggeren".

2.4 Sampling procedure

2.4.1 VAS measurements and adjustment

VAS, as described by Flint et al., (2000) were used to determine satiety before each test meal (fasted) and 15, 30, 60, 90, 120, 160 and 180 minutes after intake, as shown in Table 2. The VAS questionnaire was handed out to the participants at each time interval right after blood measurements were done (explained below). The subjects answered all the questions and put the sheet in an envelope, to prevent any looking back at what they had previously answered. All measurements were performed at day 1 and 2, in both study periods (Figure 4).

The VAS line is a standardized 100 mm (10 cm) horizontal line were the participants answered all the questions by putting a vertical line mark, depending on sensation of satiety/hunger. For example, "very hungry" or "not hungry at all". A vertical mark towards the left were indicating a low sensation of satiety or hunger, and stronger sensation the further right they answered. To measure the VAS scores, the same ruler was used throughout the study, and written down in millimeters. Measurements were done twice to avoid any human errors.

Table 5. Questions used to assess the subjective appetite sensation using VAS

Keywords	**Keywords**	**Response descriptors left scale**	**Response descriptors right scale**
Hunger (sult)	Hvorfor sulten er nå?	«Ikke sulten i det hele tatt»	«veldig sulten»
Satiety (metthet)	Hvor mett føler du deg nå?	«Ikke mett i det hele tatt»	«veldig mett»
Food cravings (Lyst på mat)	Hvor lyst har du på mat nå?	«Ikke lyst på mat i det hele tatt»	«veldig lyst på mat»
Eaten more (Spist mer)	Kunne du spist mer nå?	«Kunne ikke spist mer i det hele tatt»	«Kunne spist veldig mye mer»
Sugary food (Søt/sukkerholdig)	Hvor lyst har du på mat som er søt/sukkerholdig nå?	«Ikke lyst på søt mat i det hele tatt»	«Veldig lyst på søt mat»
Savory food (Salt)	Hvor lyst har du på salt mat nå?	«Ikke lyst på salt mat i det hele tatt»	«Veldig lyst på salt mat»
Fatty foods (Fettrik)	Hvor lyst har du fettrik mat nå?	«Ikke lyst på fettrik mat i det hele tatt»	«Veldig lyst på fettrik mat»

2.4.2 Blood measurements

Finger-prick capillary blood glucose was measured fasting, and at seven time points after intake of the test meals, as shown in Table 2. The finger-prick capillary blood samples were collected using a glucose meter Contour XT and Contour Next test stripes following a standardized procedure. This device was easy to handle and was used in room temperature. Plastic gloves were used during the procedure, and blood drops were applied to the test strip, and analyzed within 5 seconds. Blood glucose values are given in mmol/L. In addition, dried blood spots (DBS) were taken for different biochemical markers (insulin, blood lipids, and fatty acids), and stored in the freezer for future analysis.

2.5 Data processing and analysis

Data collected from this study were processed using Microsoft® Excel 2016 for Mac (version 16.12) and Windows 10 (version 15.32). The raw data was exported to IBM SPSS Statistics (version 23), where the statistical analyzes were conducted. Non-parametric statistics were used throughout the study due to the low number of participants (n=21), assuming the data was not normally distributed. When the normality is questionable, as it often is with a small sample sizes, one of the best tests to use is the Wilcoxon signed rank test, compared to the paired sample t-test (Imam, Mohammed & Abanyam, 2014). The Wilcoxon signed rank test (matched-pairs test) was used as participants were measured under two occasions, to detect any significant differences in satiety feeling after intake of the muffins with different fat quality.

Postprandial response in satiety and blood glucose was calculated as the incremental Area Under the Curve (iAUC) 0 – 180 minutes, by the trapezoid rule, using the average values for the two periods in the study.

$$A = \frac{(Y1 + Y2) \times (X2 - X1)}{2}$$

iAUC allows the fasting concentration (first observation) to be subtracted from each measurement. The statistical parameters median, minimum and maximum were used to present data.

Only at one occasion a subject did not answer all the questions (4 – 7) at 60 minutes at V1. The average of all the VAS values at 120 minutes at V4 was used as a substitute for the missing value, and in order to be able to include this subject's answers in our data. The significance level was set to 5% $P \leq 0.05$.
All calculations for the respected data set were done in Microsoft® Excel 2016.

To test repeatability between period 1 (V1/V2) and 2 (V3/V4), the method explained in Bland & Altmann, (1986) was used. For each subject the iAUC difference between period 1 and 2 was calculated for SFA and PUFA respectively. In addition, the average iAUC value for period 1 and 2 was calculated for SFA and PUFA. Bland-Altman plots were created by plotting the iAUC difference against the mean and adding two horizontals defined by mean $\pm$ Coefficient of Reparability (2xStandard Deviation). The difference between the two periods was thereafter tested towards a total agreement (difference equal to zero) with a one sample t-test. Lastly, a regression analysis was conducted to see if there was any relation between the difference in iAUC and mean, as a significant regression could indicate a proportional bias in the data. The significance level was set to 5% $P \leq 0.05$.

2.6 Ethical considerations

This project was approved by The Research Ethics in Norway, Regional Committee of Medical Ethics (REK 2017/1327REK Sør-Øst B). Before study start, all participants included in the study, signed a written informed consent (Appendix E). This consent included information about the background and purpose of the study, requirements from the participants, information about project leaders, what is measured, how data is registered, as well as processing and information about voluntary participation.

Participants were also informed about their rights to withdraw from the study after signing, if they had to for any reason. The consent stated that all personal data is unidentified. The data obtained in the study were non identifiable by providing the test subjects with an individual (ID) number. Personal information and IDs were kept strictly confidential and secured at the OsloMet, (Kjeller) both during and after the study. Only authorized persons in the project have access to personal information and IDs. No person-identifiable information will be recognized in published results.

2.7 Power calculation

The number of participants in the current study is based on a recent review by Kaviani and Cooper (2017), looking at appetite responses to high fat-meals with varying fat quality. The review included 24 studies, most of them were postprandial studies, investigating the effect directly after intake of a test meal. The number of participants in the studies were between 10-20. A recruitment target was set for 20 healthy subjects.

3.0 Results

3.1 Subjects characteristics

A total of 21 healthy individuals completed this postprandial crossover study; 18 women and 3 men. The participants were healthy, normal weight adults with a median age of 22 years and BMI of 22.9. Characteristics from screening and blood glucose from the first visit are shown in Table 6.

Table 6. Screening characteristics for gender, age, BMI, weight (*n*=21) and fasting blood glucose at the first visit (*n*=18)

Gender	
Male *(n)*	3
Female *(n)*	18
Age (years)	22 (21 – 27)
BMI (kg/m^2)	22.9 (21 – 23.3)
Weight	62.8 (59.7 – 68.2)
Blood glucose (mmol/L)	4.8 (4.6 – 5.1)

BMI, Body Mass Index. Values are presented as median and 25 – 75 percentile.

3.2 Subjective appetite sensation after intake of PUFA and SFA

Subjective satiety sensation (VAS) was tested after an overnight fast at day 1, in which participants were given a standardized breakfast meal containing a chocolate muffin, high in either PUFA (42% of total fat) or SFA (47% of total fat). The procedure was repeated at day 4 with the opposite muffins. The participants were asked to fill in VAS before consuming the muffin, and after 15, 30, 60, 90, 120, 150 and 180 minutes respectively. The experiment was repeated twice, with one week “wash out” in between.

No significant differences were detected for subjective sensation of hunger, satiety and desire to eat after intake of the test meals high in SFA or PUFA (Figure 5 a, b and c). To measure the subjective response for hunger, satiety and desire to eat throughout the time period incremental area under the curve (iAUC) was calculated for each participant in both periods and the average scores, are presented in Figure 5d, e, and f. No significant differences were detected between the two test meals.

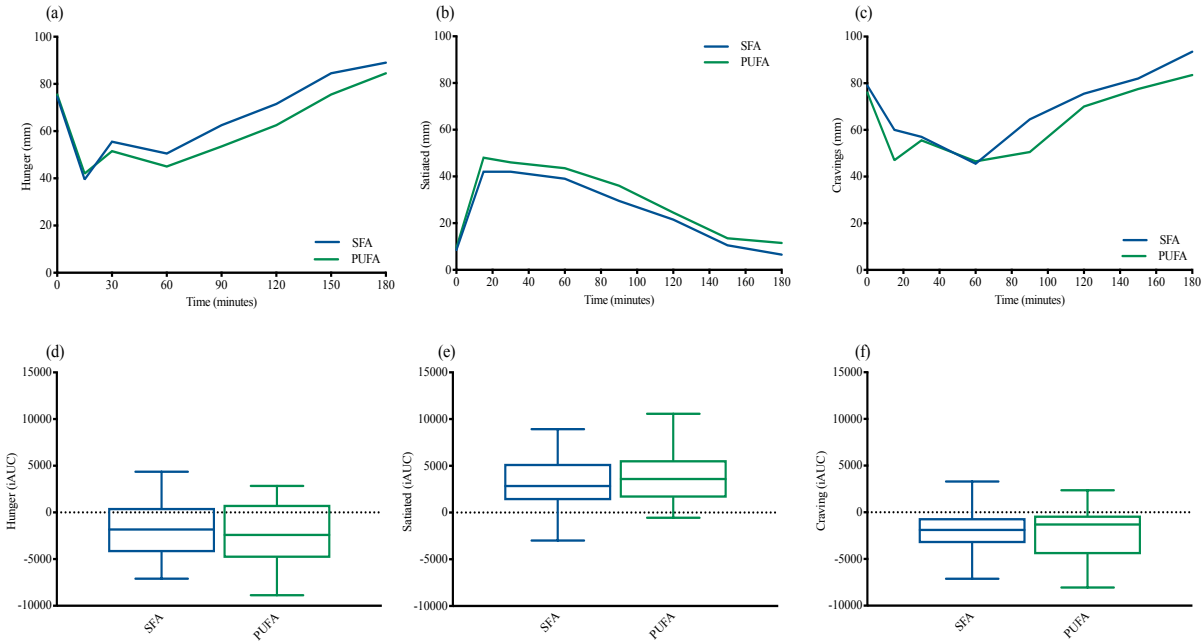

Figure 7. Postprandial subjective ratings of (a) hunger, (b) satiety and (c) food craving, measured using VAS (0 – 180 minutes) after consumption of the two breakfast meals. iAUC subjective rating scores for (d) hunger, (e) satiety and (f) food craving after consumption of the two different breakfast meals either high in SFA or PUFA are shown in the box-plots. Average value for both periods are presented by the median value of the subjects (*n*=21).
VAS, Visual Analogue Scale; iAUC, incremental area under the curve; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids.

No significant differences were observed between intake of test meals high in either SFA or PUFA on the participants' desire to eat more food, desire to eat sugary food, savory food or the desire to eat fatty food (Figure 6a – d). No significant differences were detected between the two test meals for iAUC response (Figure 6e – h)

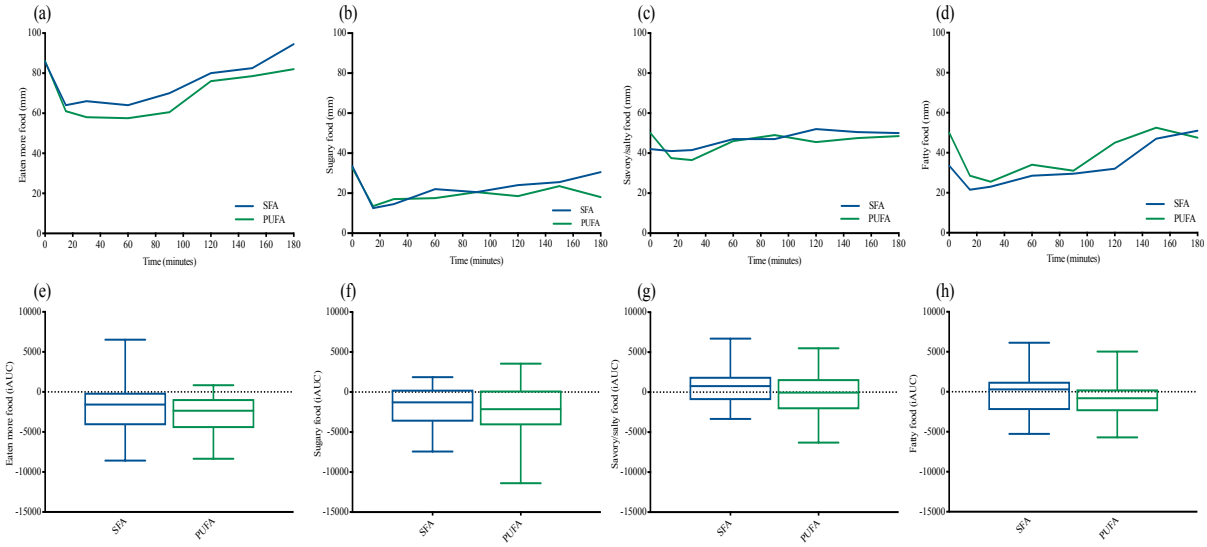

Figure 8. Postprandial subjective ratings of (a) eaten more, (b) sugary food, (c) savory food and (d) fatty food craving measured using VAS (0 – 180 minutes) after consumption of the two breakfast meals. iAUC subjective rating scores for iAUC for (a) eaten more, (b) sugary food craving, (c) savory food craving and (d) fatty food craving after consumption of the two different breakfast meals are shown in the box-plots. Average value for both periods are presented by the median value of the subjects (n=21). VAS, Visual Analogue Scale; iAUC, incremental area under the curve; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids.

3.3 Repeatability of VAS between subjective appetite sensation for two periods

Figure 9 illustrates the participants subjective sensation for period 1 and period 2, after intake of the test meals. There were no significant differences in the subjective appetite sensations between the two periods (Figure 9a – g). After intake of muffin high in PUFA a significant difference in the subjective sensation on food cravings (P = 0.023) and sugary foods (P = 0.004) between the two periods were observed (Figure 9c and e).

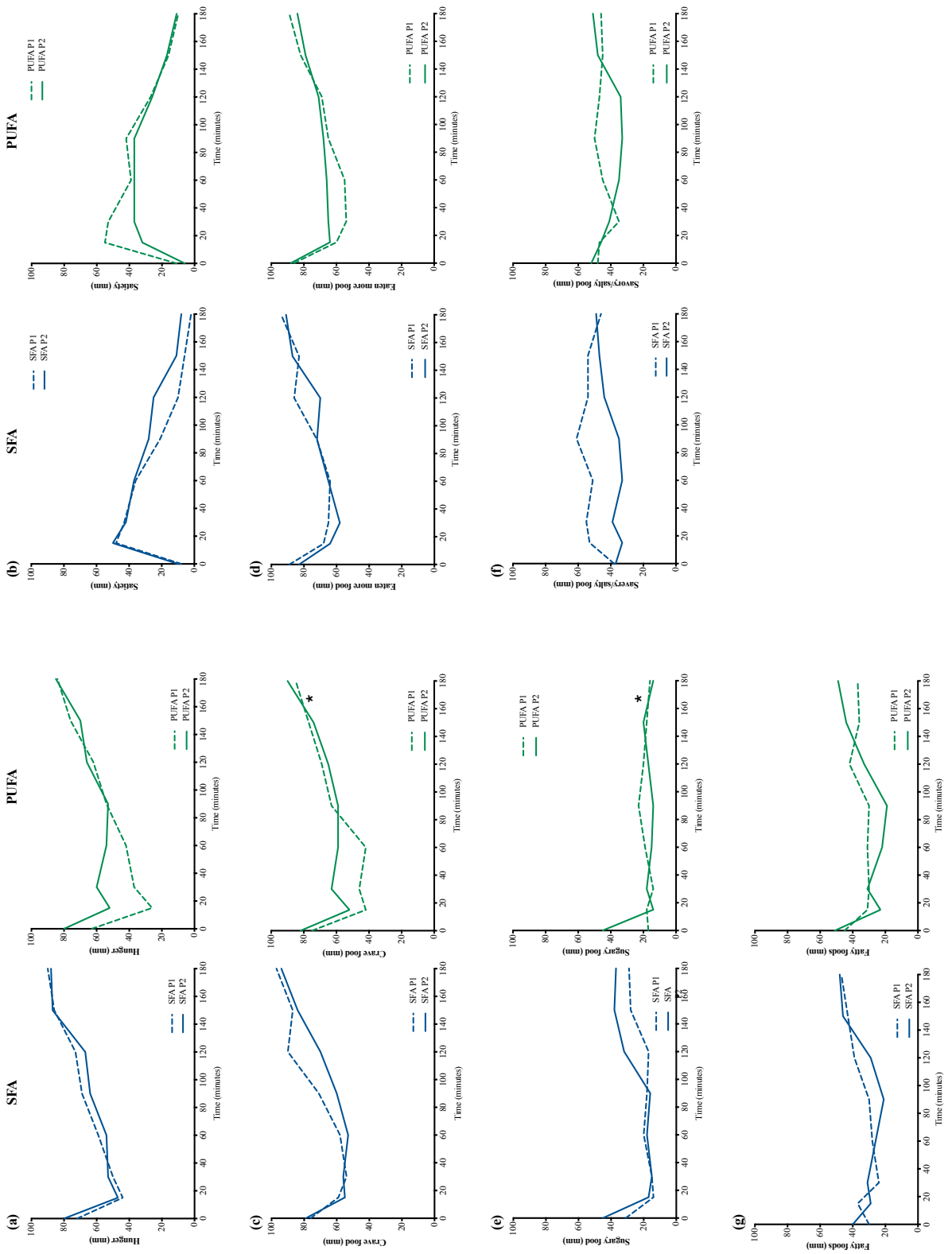

Figure 9. Postprandial subjective ratings of (a) hunger, (b) satiety (c) food cravings, (d) eaten more, (e) sugary food, (f) savory food and (g) fatty food craving, for both periods, measured using VAS (0 – 180 minutes) after consumption of the two breakfast meals. Average value for both periods are presented by the median value of the subjects (*n*=21). VAS, Visual Analogue Scale; iAUC, incremental area under the curve; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids.

3.4 Repeatability for VAS on subjective satiety sensation between two periods

Test of agreement was conducted based on the method by Bland & Altman (1986). The coefficient of repeatability, 2 times the SD, was calculated on the difference in iAUC between the two periods for each subject after intake of test meals high in SFA or PUFA and are shown in Table 7 and 8. Bland-Altman plots were also created by plotting the difference in iAUC between the two periods against the average value for the two periods. Each dot in the plots represents a subject. The average difference for all subjects is highlighted by a red line and the agreements levels, defined by the average value $\pm$ CR, are highlighted by green lines. These plots are shown in Appendix F.

It's was not a total agreement between the two periods (Table 8), because there was a significant difference in the subjective sensation for food cravings ($P = 0.02$) and sugary foods ($P = 0.002$) between the two periods after intake of PUFA. For SFA, there were no significant differences detected between the two periods for any of the questions (Table 7). Subjective sensation for hunger, satiety, eaten more, salty and fatty foods shows similar results for PUFA and SFA, indicating total agreement.

We thereafter used regressions analysis to test for proportional bias by testing if there was a relationship between the difference in VAS iAUC and the average value for the participants between the two periods for SFA and PUFA respectively. PUFA, "eaten more", displays a notable degree of proportional bias when comparing the mean difference to the average value. The regression was significant ($P = 0.01$). The other questions for PUFA shows no sign of proportional bias, with P-values ranging from 0.08 to 0.96. For SFA, regression analysis were not significant for any question, with P-values ranging from 0.14 to 0.96. This indicate that none of the questions shows sign of proportional bias.

For all VAS questions, the CR is higher for PUFA (Table 8) than the comparable VAS questions for SFA (Table 7). This may indicate a higher agreement between the two test periods after intake of SFA, compared to PUFA. The question with the best agreement for SFA, is "eaten more", with a CR of 4749. For PUFA, the question with the best agreement, and the lowest CR, is "satiety". However, the CR for "eaten more" PUFA, is very close with a value of 7692. Overall, "eaten more" seems to have the best agreement.

Table 7. Repeatability results presented for all seven VAS questions. Analyzed based on differences for SFA using iAUC values for both periods

	Difference iAUC SFA Period 1 and Period 2								
	Mean	SD	CR	-2SD	+ 2SD	t	p	r^2	p
Hunger	760	4236	8472	-7543	9062	0.82	0.42	0.09	0.17
Satiety	-1139	2926	5852	-6874	4596	-1.78	0.09	0.10	0.16
Food craving	1358	3457	6913	-5417	8133	1.80	0.09	0.03	0.49
Eaten more	545	2375	4749	-4109	5200	1.05	0.31	0.01	0.68
Sugary food	747	3150	6300	-5427	6920	1.09	0.29	0.00	0.96
Salty food	1281	4485	8971	-7510	10072	1.31	0.21	0.00	0.81
Fatty Food	1410	4019	8039	-6468	9288	1.61	0.12	0.11	0.14

SD, Standard Deviation; +2SD, mean + 2xSD; -2SD, mean – 2xSD; CR, Coefficient Repeatability; t, one sample t-test; p (t-test), p-value one sample t-test; r^2, regression analysis; p(reg.), p-value regression analysis; VAS, Visual Analogue Scale; iAUC, incremental area under the curve; SFA, Saturated fatty acids.

Table 8. Repeatability results presented for all seven VAS questions. Analyzed based on differences for PUFA using iAUC values for both periods

	Difference iAUC PUFA Period 1 and Period 2								
	Mean	SD	CR	-2SD	+ 2SD	t	p	r^2	p
Hunger	2215	5703	11406	-8963	13393	1.78	0.09	0.00	0.96
Satiety	-1029	3757	7513	-8392	6334	-1.26	0.22	0.15	0.08
Food craving	2545	4435	8871	-6148	11239	2.63	0.02	0.00	0.88
Eaten more	602	3846	7692	-6936	8141	0.68	0.48	0.28*	0.01
Sugary food	3397	4468	8936	-5360	12154	3.48**	0.002	0.00	0.95
Salty food	1135	5329	10658	-9310	11580	0.98	0.34	0.01*	0.71
Fatty Food	403	5075	10150	-9747	10553	0.36	0.72	0.00	0.92

SD, Standard Deviation; +2SD, mean + 2xSD; -2SD, mean – 2xSD; CR, Coefficient Repeatability; t, one sample t-test; p (t-test), p-value one sample t-test; r^2, regression analysis; p(reg.), p-value regression analysis; VAS, Visual Analogue Scale; iAUC, incremental area under the curve; PUFA, Polyunsaturated fatty acids.

*Difference between periods ($P < 0.05$), **difference between periods($P<0.001$)

3.5 Blood glucose response after intake of muffins with different fat quality

Blood glucose was measured fasting, and at 15, 30, 60, 90, 120, 150 and 180 minutes after intake of a muffin high in either SFA or PUFA, in both periods. Values for both periods are presented in figure 8a. No significant differences were detected for blood glucose in either periods between SFA or PUFA. Median fasting blood glucose were similar, with 4.8 mmol/L in both periods. The median for blood glucose for the two periods reached a peak at 5.6 mmol/L after intake of the meal high in PUFA, and 5.7 mmol/L after intake of the meal high in SFA. Median blood glucose values after 180 minutes were below fasting values (4.6 mmol/L), for both SFA and PUFA in the two periods.

Measurements for blood glucose (iAUC) were calculated using median values for each participant and take the average value for both periods for SFA and for PUFA. No significant differences were detected between intakes of SFA compared to PUFA muffins (Figure 8b).

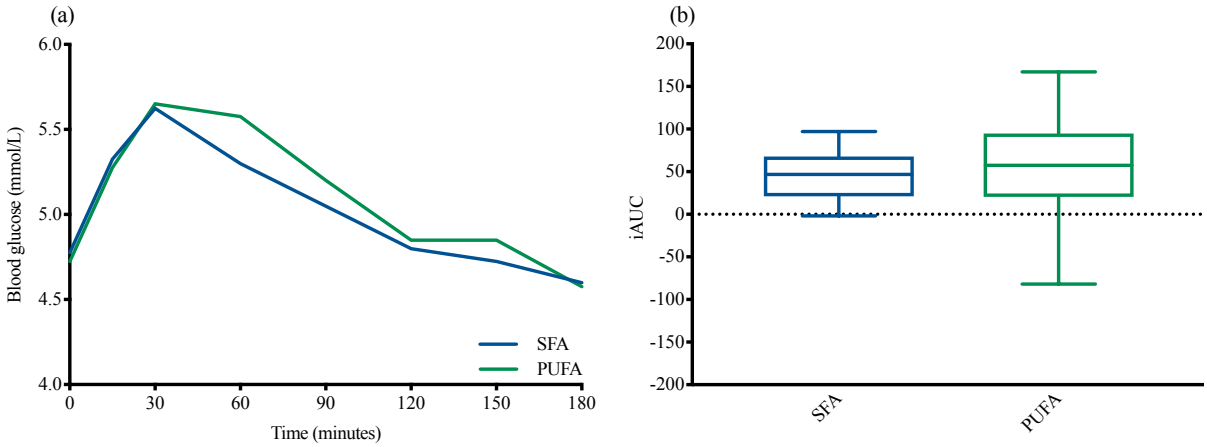

Figure 10. Postpranidal glycemic response (a) after intake of SFA and PUFA. iAUC average values (b) for both periods for SFA and PUFA are presented in the box plot, *n*=18. VAS, Visual Analogue Scale; iAUC, incremental area under the curve; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids.

4.0 Discussion

The aim of this crossover, double blinded intervention study was to investigate the effect of fat quality, on subjective satiety sensation in healthy individuals, using VAS. Our main finding is that intake of SFA and PUFA show no significant differences in how participants perceived satiety. When studying the repeatability of VAS, the two periods seemed to have good repeatability in subjective satiety sensations for most of the VAS question. We found good agreement in subjective satiety between the two periods after intake of SFA. However, after intake of PUFA, a significant difference in food cravings and desire to eat sugary foods, between the two periods were observed. Finally, no effect on postprandial blood glucose levels after intake of SFA and PUFA were detected.

The current study examined the subjective sensation of hunger, satiety, food cravings, eaten more food, desire to eat sugary-, savory- and fatty food using VAS. The main advantages of using VAS in assessing satiety sensation are first and foremost the ease of administration, and the fact that it is cost beneficial and non-invasive of nature. Nevertheless, there are some potential drawbacks of this tool. Firstly, interpretation of the scales may vary between subjects, and when reporting VAS, some may use the midsection of the scales and avoid the extremes, while others are using the very left or right end of the scales and avoiding the mid sections. Secondly, we cannot conclude that a mark of 20 mm indicated half the intensity of "desire to eat" as a mark of 40 mm did (Livingstone et al., 2016). The method of using VAS has some limitations when it comes to accuracy in reporting, due to it being a tool for subjective sensation and can be impacted by factors like emotional, mental and psychological status (Kaviani & Cooper, 2017).

In a review study by Kaviani and Cooper, (2017), they summarized studies that have investigated the effect of dietary fatty acid on hunger and satiety, including acute/short term (postprandial) meal challenges and long-term meal challenges. (Kaviani & Cooper, 2017). Of the postprandial studies, the study by Strik et al., (2010), found that a savory muffin with 50E% of fat (MUFA, SFA or PUFA) gave no significant difference in mean ratings when measuring appetite and satiety postprandially. The test meal in the current study was a 100-gram muffin, high in either SFA or PUFA, but similar in content of calories and macronutrients. It consisted of either 61 E% from PUFA or 60 E% from SFA, and the content of MUFA was similar in both muffins (25 E% for PUFA and 21 E% for SFA). In the SFA muffin, 47% of total fat was SFA, while the PUFA muffin contained of 42% PUFA of total

fat. This is in accordance with the study by Alfenas & Mattes (2003), who used a breakfast muffin with 54 E% from fat, where the SFA and PUFA amounts were similar to ours (66% and 59%) (Alfenas & Mattes, 2003). In the study by Flint et al., (2003) they used meals that consisted of rolls, cake and jam. This meal consisted of a total 60 E% from fat (70% PUFA and 83% MUFA), and the amount of energy in the test meal was calculated on an individual basis. Although they used a large amount of fat in the test meals, and calculated them on an individual basis, it did not seem to be affecting the results as they found no differences in postprandial satiety ratings. Hence, our findings correspond to previous findings, and indicate that VAS does not work to distinguish between fat qualities even with higher fat doses.

However, some studies have shown significant results on satiety sensation after intake of different fat quality. The main difference between these studies and the current study is the type of meal, whether it was whole foods or liquid meals.

Kozimor, Chang & Cooper, (2013) investigated high fat meals rich in SFA, PUFA or MUFA on the satiety hormone, PYY and subjective feelings using VAS. The study showed that postprandial PYY was higher for PUFA and SFA, than for MUFA, after consumption of a high fat liquid meal (70 E% from fat), and they found significant effect for subjective appetite sensation. This correspond to the results of Lawton, Delargy, Brockman, Smith & Blundell, (2000) and Stevenson, Clevenger & Cooper, (2015), which findings indicate that high fat meals rich in PUFAs elicit a greater satiety response compared to meals rich in SFA using VAS (Lawton et al., 2000; Stevenson et al., 2015). This may indicate that liquid meals and a higher fat content could affect the results. One factor that must be taken into account is that liquid meals will reach the small intestine more quickly, resulting in larger effective doses or concentrations (Hellström, Grybäck & Jacobsson, 2006). Studies that used liquid meals were more successful in keeping the percentages of different fatty acids close to each other, so that the comparison between meals was matched better regarding the percentage of fat coming from the fatty acids of interest, and importantly, these studies were the ones that also detected differences in gut hormones.

In the current study the majority of the participants were women and we cannot rule out that the results would have been different with a more equal number of gender. On the other hand, Kozimor et al., (2013) conducted a study with healthy women and demonstrated significant results between appetite response and different fat types (Kozimor et al., 2013). Likewise, in

the study by Stevenson et al., (2015), they used obese women, and found significant results regarding appetite response and fat quality. This indicates that a population with the majority of the participants being women still could find differences in acute postprandial appetite, although this was not the case in our study.

The repeatability of VAS after two test periods

Even if we could not detect any significant differences between subjective sensation comparing SFA and PUFA in a single test meal, when repeating the intervention, the two periods seemed to have good **repeatability** in subjective satiety sensations for most of the VAS question. Using the method as described in Bland & Altman, (1986) we found no significant differences in subjective satiety sensation between the two periods after intake of SFA. However, after intake of PUFA, we observed a significant difference in satiety sensations for food cravings and desire to eat sugary foods, between the two periods. When measuring repeatability of VAS in appetite research, external factors needs to be kept as constant as possible (Flint et al., 2000). There are not many studies that have tested the repeatability of VAS after two similar test periods, or on effects of different dietary fatty acids composition. According to Stratton et al., (1998), subjective ratings of satiety measured by VAS within subjects, seem to have overall good agreement, being reproducible and reliable. Raben, Tagliabue & Astrup, (1995), did their investigation similar to ours on reproducibility, where they had subjects eating the same meal, at two different days, under standardized conditions. They concluded that the CR (coefficient of repeatability) for subjective appetite ratings was quite large when tested twice in the same subjects on different days and found low degrees of agreement. Compared to our study they did only have nine subjects, and we had more than twice as many. This could possibly be the reason why we found good agreement using VAS in our intervention, and they did not find the same.

VAS as a method is prone to be sensitive due to contributing factors altering satiety (Kaviani & Cooper, 2017). Several studies have shown that a lot of factors can affect feelings of satiety and hunger. Factors like age, gender, physical activity level, mood swings, external stress, emotional state, sleep the previous night before test day and sickness during the study period can affect results when measuring satiety (Flint et al., 2000; Kral, 2006; Maher & Clegg, 2018; Murray & Vickers, 2009; Vartanian et al., 2017). Participants in the current study had restrictions to follow, and all participants were asked to eat a standardized supper at home (Appendix D), to fast from 20:00 the night before each test day, stop using dietary

supplements and hormonal treatment (except for contraceptives/birth control) from one week prior to study start and throughout the study period, and to abstain from alcohol and heavy physical activity the evening prior to the test day. For instance, physical activity may possible be a contributing factor to alterations in appetite and satiety. Exercise or deviation from the restrictions the day before may have affected the postprandially results, and according to Beaulieu et al., (2017), regular exercise could improve hormonal satiety signaling, and also body composition which can affect food intake and eating behavior. Postprandial secretion of GLP-1 and PYY may also increase after exercise (Beaulieu et al., 2017). On the opposite, Cooper et al, (2011) investigated exercise impact versus sedentary conditions and found no treatment effect differences, speculating that as long as individuals are in energy balance exercise may not affect perceived hunger or fullness using VAS. Taking our study into consideration, the participants were told not to alter their current level of physical activity and avoid hard exercise the night before the test day, and the most important thing was that the participants maintained their normal habits-apart from exercising the night before. The current study is a crossover and participants will probably do about the same before each test day. At the actual test day, there were some subjects biking or walking far to get to the location, which could impact satiety. Thus, what they ate and did the day and the night before test day, could actually have had greater impact than assumed. However, we still found that repeatability between two test days was good for most questions.

Blood glucose and satiety

In regards to blood glucose, we did not observe any significant differences between intake of SFA and PUFA. However, in a review by Imamura et al., (2016), substituting SFA or carbohydrates with PUFA was linked to lower fasting glucose and improved insulin secretion capacity. The reason why we did not see any effect in our study, is likely to be that we were just looking at a single test meal with either SFA or PUFA, and to see any effect, it probably should have been an intervention of sufficient duration, and a stricter diet standardizing for the participants. Clevenger, Kozimor, Paton & Cooper, (2014), also investigated the postprandial effect between three meals high in either SFA, MUFA and PUFA, on diet induced thermogenesis and oxidation rate. Fat content for PUFA was 42% of total fat, 40% for SFA and 42% for the MUFA meal. This fat content was similar to ours with 42% of total fat from PUFA and 47% from SFA. The AUC for plasma glucose did not show any significant differences between the three high fat meals, corresponding to our results.

Nevertheless, they found that the PUFA rich meal generated a greater diet induced thermogenesis than both MUFA and SFA (Clevenger et al., 2014).

Important to note is although participants in the current study were asked to consume a low fat, and relatively low fiber meal at home the night before test day (Appendix D), we cannot be sure if they actually followed this restriction, because the meal compliance for the day before visits was self-reported and could impact the outcomes tested in this study. We could anyway, assume that the subjects ate relatively similar the night before study visits, independent of if they followed low fat restrictions, and probably not impacting blood glucose measurement results.

In the study by Anderson & Woodend, (2003), they looked at blood glucose response after sugar intake. They speculated that the low glycemic index food signaling satiety could be an effect of that the actual test meal with lower glycemic index was higher in fat, causing satiety to be elevated longer postprandial, again related to fat, and not glucose by Anderson & Woodend, (2003). However, this makes us wonder that it could have been easier to see differences between SFA and PUFA in our study, if the muffins were even higher in sugar, causing blood sugars to spike even more.

It has been found that changes in blood glucose concentration, even those within the normal postprandial range, affect gastric emptying in rats. Hypoglycemia accelerates, and hyperglycemia slows gastric emptying. As a result, it has been proposed that blood glucose concentration also affects appetite and food intake. There is considerable evidence that a decrease in blood glucose concentration is associated with meal initiation. Thus, it is not clear whether blood glucose intrinsically is associated with short-term regulation of appetite (Salmenkallio-Marttila et al., 2009). Hormones related to satiety regulation is secreted postprandially in such small amounts, that speculating to find an effect, a longer intervention could possibly give other results.

Strength and limitations

Our investigation has several strengths, and most importantly, the double blinded crossover design is to note. Such a study design makes it possible to evaluate within the same subject, and hence the between-subjects variability is reduced. The crossover design also makes it possible to obtain equal statistical power with the limited number of subjects. The subjects

were not informed of witch muffins they got at each intervention, and this may reduce the risk of systematic bias. In addition, by keeping a washout period of one week between each intervention, we avoided that the subjects remembered their VAS answers between the periods. Subjects were asked to consume a standardized low-fat meal prior to each of their two study visits, in both periods. All subjects answered VAS without looking at each other's answers, and they were given one and one VAS sheet for each time interval. They had to put their VAS answers in an envelope between each time point, making it impossible to compare their own answers. Compared to a recent review by Kaviani & Cooper, (2017), our study had a fair number of participants.

Potential limitations should be considered, like the handling and processing of data, which is quite time consuming. There are furthermore potential for human error when measuring the answers on all the 100 mm horizontal lines by hand (Stratton et al., 1998). In order to limit potential error, all VAS responses were measured twice. It is possible that the results regarding VAS could have been different if the number of participants were even higher. Regarding test subjects, an uneven gender balance was present, and the majority of the participants were female students, mostly recruited from OsloMet. This reduces external validity and generalizability, as only three males compared with eighteen females were included in the study. Moreover, a possible limitation is the environment of witch the test meals was consumed, because all the participants was allowed to sit and talk together due to logistics and place limitations. We only regulated the meal the evening prior to the study visit, which could have had an effect on hunger and satiety the morning after. Although all subjects were able to eat each meal and stated verbally that they tasted good, we did not measure the palatability of each meal. If differences in palatability had existed, it could have affected the VAS responses. Palatability can be interfering with satiety and desire to eat, as seen in a study by Hill, Magson & Blundell, (1984). The physical activity status and meal compliance for the day before visits was self-reported which could impact the outcomes tested in this study. Furthermore, the energy content of the meals was not prescribed according to a percentage of subjects' individual estimated daily energy needs. Last, satiety studies are difficult to conduct because of the overlap between physiological and cognitive factors in the satiety response.

5.0 Conclusion

Although more studies are needed, our findings suggest that subjective ratings of satiety or blood glucose response do not differ after intake of a meal high in SFA compared to PUFA. We did however detect a high repeatability of VAS after intake of the same test meal at two different occasions. This indicates that VAS is a good tool to measure subjective satiety sensation and could be included in future studies.

5.1 Future

Further research is needed to understand the complexity of hunger and satiety mechanisms, and to investigate long-term consumption of meals with different fatty acid composition, their impact on altering energy intake in a variety of populations, and the mechanisms as to how this is occurring. It is an important note that measuring subjective sensation postprandially after only one meal with different fat quality might not be sufficient to distinguish between the different fatty acids. Longer diet interventions, instead of a single meal, investigating the effects of different fatty acids on hunger and satiety in free-living subjects using VAS as a method is needed. Preferably giving restrictions for the overall diet of the participants during the whole intervention period, and not only guidelines for the evening meal prior to the test day. In addition, satiety hormones, like the gut peptides, should also be measured. In addition, it would have been interesting to investigate if SFA or PUFA could show any effects on gut microbiota in acute or long-term trials, which has been suggested in some studies.

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Appendix A

Visual Analogue Scale (VAS)

Fettkvalitet og metthet (FM-studien)

Visuell Analog Skala (VAS)

I *Fettkvalitet og metthets*-studien ønsker vi å registrere sult og metthet ved bruk av en Visuell analog skala.

Vi ber deg derfor besvare et skjema med 7 spørsmål ved å sette en horisontal strek på linja som er beskrivende for hvordan du har det i forhold til hvert spørsmål. Du skal besvare skjemaet mandag og torsdag i forbindelse med frokostmåltidene du får utdelt. Du får utdelt 8 like skjemaer som skal besvares til bestemte tider. Tidspunktene for besvarelse er 0 min (rett før måltidet), 15 min, 30 min, 60 min, 90 min, 120 min, 150 min og 180 min etter at måltidet er ferdig spist.
Hvert av skjemaene har markert tidspunktet for besvarelse.

Det er viktig at du kommer fastende (minimum 12 timer siden forrige måltid) til HiOA de dagene (mandag og torsdag) du skal fylle ut skjemaet.

Dersom du skulle få problemer med å besvare ett av tidspunktene er det likevel viktig at du besvarer de resterende tidspunktene.

Fettkvalitet og metthet (FM-studien)

Tidspunkt: 0 min. (rett før måltidet).

Besvart klokken:

Visuell Analog Skala

1) Hvor sulten er du nå?

Ikke sulten i det hele tatt _____ veldig sulten

2) Hvor mett føler du deg nå?

Ikke mett i det hele tatt _____ veldig mett

3) Hvor lyst har du på mat nå?

Ikke lyst på mat i det hele tatt _____ veldig lyst på mat

4) Kunne du spist mer nå?

kunne ikke spist mer i det hele tatt _____ kunne spist veldig mye mer

5) Hvor lyst har du på mat som er søt/sukkerholdig nå?

Ikke lyst på søt mat i det hele tatt _____ veldig lyst på søt mat

6) Hvor lyst har du på salt mat nå?

Ikke lyst på salt mat i det hele tatt _____ veldig lyst på salt mat

7) Hvor lyst har du på fettrik mat nå?

Ikke lyst på fettrik mat i det hele tatt _____ veldig lyst på fettrik mat

Appendix B

Case report form V0 and V1 – V4

The effect on subjective appetite sensation after intake of dietary fat of different quality

Kort tittel: FM studien

Periode: Høst 2017

ID nummer: _____

Visitt: V0

Navn på personen som intervjuer: _____

ID nummer:	
Dato:	

Kartlegging:

	JA	**NEI**
Bruker du medisiner, inkludert hormonbehandling? Hvis ja, hvilke? _____		
Har du vært syk den siste uken/måneden?		
Driver du hardt fysisk aktivitet flere ganger i uken? Type og mengde fysisk aktivitet: _____ Hvor fysisk aktiv er du i gjennomsnitt per uke? _____		
Bruker du kosttilskudd?		

Forberedelse før blodprøvetaking: Forberedelse før blodprøvetaking:

	JA	NEI
Er du fastende i dag?		
Har du drukket alkohol 24 timer før blodprøvetaking?		
Trente du med høy intensitet i går?		

Laboratorieprøver

	JA	NEI
Har deltager målt fastende blodglukose?		

Antropometri:

	Ja	Nei
Vekt kg		
Er kroppssammensetning målt?		

KOSTHOLDSVANER

	JA	NEI
Har deltager fått påminnelse om kostrestriksjoner/alternativer?		

Er det avtalt tid for neste visitt V1/Er deltager påminnet tidspunkt for neste visitt og restriksjoner?

Dato og tid:

The effect on subjective appetite sensation after intake of dietary fat of different quality

Kort tittel: FM studien

Periode: Høst 2017

ID nummer: _____

Visitt: V1

Navn på personen som intervjuer: _____

ID nummer:	
Dato:	

Kartlegging:

	JA	**NEI**
Har du gjort endringer i bruk av medisiner, inkludert hormonbehandling, i løpet av perioden fra forrige visitt? Hvis ja, hvilke endringer er gjort? _____		
Har du vært syk i perioden fra forrige visitt? Hvis ja, har du brukt noen medisiner? Navn på medikament:_____ I hvilken periode benyttet du disse medisinene? Dato (f.o.m og t.o.m):_____		
Har du gjort endringer i kostholdet ditt annet enn å unngå inntak av fet fisk, spisefett, planteoljer, helfete meieri- og kjøttprodukter og nøtter kvelden før testdagen? _____ Hvis ja hva går de ut på? Beskriv kort:_____		
Har du gjort endringer i fysisk aktivitet? Type og mengde fysisk aktivitet: _____ Hvor fysisk aktiv er du i gjennomsnitt per uke? _____		
Bruker du fortsatt ikke kosttilskudd?		

Appendix C

Chocolate muffin recipe

Lettvinte sjokolademuffins

Ingredienser:

150 g fett
100 g sukker
100 g hvetemel
1 ts bakepulver
2 ss kakao
2 egg

Fremgangsmåte:

1. Smelt smør.
2. Pisk egg og sukker til eggedosis.
3. Bland godt sammen og sikt bakepulver, mel og kakao
4. Bland i smøret, og til sist hvetemelblandingen.
5. Fyll i muffinsformer av papir.
6. Stekes på 180°C i 12 – 18 minutter avhengig av størrelse.

Appendix D

Information letter with restrictions

Infoskriv om restriksjoner og gjennomføring av FM-studien

Husk å les denne informasjonen nøye, og gjør justeringene i god til før studiestart. Du skal ellers spise, drikke og trene som vanlig med unntak av begrensningene som nevnes under.

For å delta i studien kan du **ikke ha:** cøliaki (glutenintoleranse), eggallergi, diabetes, hveteallergi, kraftig laktoseintoleranse, melkeproteinallergi og/eller andre tarmsykdommer. Testproduktet som skal spises kan inneholde allergenene nevnt over (gluten, laktose, melkeprotein og egg).

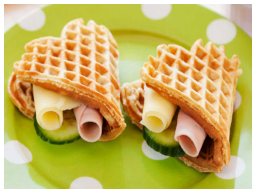
Du møter fastende på Høgskolen i Oslo og Akershus, Campus Kjeller, om morgenen til gitt tidspunkt. Fastende betyr at du ikke kan spise eller drikke noe annet enn vann minst 12 timer før besøket (ikke etter kl. 20 kvelden før). Det vil bli tatt blodprøver (fingerstikk) av deg.

For å standardisere matinntaket kvelden før må alle deltakere spise et brødmåltid som det siste måltidet kvelden før **hver** testdag. Det betyr at du må planlegge middag slik at den blir spist så tidlig på ettermiddagen at du rekker å spise et brødmåltid før kl. 20. Dersom middagen er så sen at det ikke vil være aktuelt å spise et kveldsmåltid i tillegg, må middagen være selve brødmåltidet.

Dette brødmåltidet skal bestå av brød med maks to kakestykker på brødskalaen (se bilde), **med magert** pålegg og uten helfete produkter**. Eksempler på magert pålegg: kyllingpålegg, mager leverpostei, kokt skinke, magerost, syltetøy, lett gulost, hamburgerrygg o.l.**

Kneip m/ kokt skinke

Lett gulost



Vaffel med pålegg

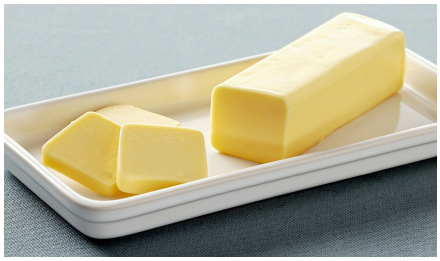
Philadelphia pålegg lett

Du må opprettholde ditt normale kosthold, men være villig til å unngå å spise **fet fisk, spisefett, planteoljer, helfete meieri- og kjøttprodukter og nøtter** til det siste måltidet kvelden før hver testdag. Du må også være villig til å slutte med kosttilskudd (inkludert fettsyrepreparater som tran og fiskeolje) **1 uke** før studiestart og så lenge studien varer.

Unngå inntak av disse matvarene kvelden før hver testdag

Fet fisk (som laks, ørret, makrell og sild)

Spisefett (smør, Bremykt ol.)



Planteoljer (oliven, solsikke, raps ol.)

Helfete meieri- og kjøttprodukter

Nøtter

De siste **24 timene** før du møter må du ikke drikke alkohol.
Du må unngå hard fysisk aktivitet dagen før testdagene. Utover det ovenfor nevnte skal du opprettholde din normale livsstil.

Appendix E

Written informed consent

FORESPØRSEL OM DELTAGELSE I FORSKNINGSPROSJEKT

FETTKVALITET OG METTHET

Dette er et spørsmål til deg om å delta i forskningsstudien «Fettkvalitet og metthet». Før du bestemmer deg for om du vil delta, er det viktig at du forstår hvorfor studien gjennomføres, hva den innebærer, og hvilke fordeler og eventuelle ulemper som kan være forbundet med å delta. Du bør derfor lese denne informasjonen nøye.

Overvekt og fedme øker i forekomst både i Norge og på verdensbasis, og gir økt risiko for livsstilssykdommer som hjerte- og karsykdommer og diabetes type 2. Økt kunnskap om hvordan maten, og næringsstoffene, påvirker sult- og metthetsreguleringen er viktig for å forstå utviklingen av overvekt og fedme. Fett er det mest energigivende næringsstoffet og vil derfor kunne påvirke vektutvikling. Vi har kunnskap om at fettkvalitet påvirker helsen vår, særlig i forhold til kolesterolverdier og risiko for å utvikle hjerte- og karsykdommer. Det er derimot ikke like mye kunnskap om hvordan fettkvalitet påvirker risiko for å utvikle overvekt og fedme via endringer i sult- og metthetsregulering. Noen nyere studier viser at økt inntak av mettet fett forstyrrer sult- og metthetsreguleringen i hjerne og dermed fører til økt matinntak og økt vekt. Det motsatte er vist for umettet fett. Sult- og metthetshormoner skilles ut i tarmen i forbindelse med måltider, og er viktige signalmolekyler til hjernen. Sult- og metthetshormoner er mulig å måle i blodet, men dette er svært kostbare analyser.

Visuell analog skala (VAS) er anerkjent som gullstandard innen selvrapportering av smerteopplevelse, og har blitt overført til registrering av subjektiv sult/metthetsfølelse. VAS er billig og enkelt i bruk, og det er også studier som viser at det er samsvar mellom mengde metthetshormoner i blodet og subjektiv metthetsfølelse målt med VAS. Det er derimot ikke nok kunnskap om hvordan enkeltnæringsstoffer, inkludert fettkvalitet, påvirker sult- og metthetsfølelse, om VAS klarer å fange opp eventuelle forskjeller og i hvor stor grad VAS er repeterbart.

Det er i tillegg flere studier som viser at fettkvalitet har betydning for blodglukosereguleringen, der mettet fett er vist å gi dårligere blodglukoseregulering mens umettet fett gir bedret blodglukoseregulering.

Dette prosjektet vil styrke kunnskapen om fettkvalitet og sult/metthetsfølelse målt med VAS, og blodsukkerregulering. VAS er enkelt og lite belastende for deltagerne. Blodprøvene er fingerstikk. Fingerstikkprøvene er mindre belastende enn venøse prøver.

Hensikten med dette prosjektet er å studere hvordan inntak av forskjellig fettkvalitet (mettet versus umettet fett) påvirker subjektiv sult/metthetsfølelse etter et måltid i en human intervensjonsstudie med friske individer. I tillegg ønsker vi å se på effekten av fettkvalitet på blodsukker og andre helsemarkører (som insulin og fettstoffer i blodet).

Studien gjennomføres ved Høgskolen i Oslo og Akershus. Faglige ansvarlige for gjennomføringen av studien er Førsteamanuensis Vibeke Telle-Hansen og Førsteamanuensis Mari Myhrstad ved Høgskolen i Oslo og Akershus.

HVEM SØKER VI?

Vi søker etter friske kvinner og menn i alderen 18-65 år, med stabil vekt og BMI mellom 18,5-27 kg/m^2. Du må være villig til å opprettholde ditt normale kosthold og fysiske aktivitetsnivå, og slutte med kosttilskudd (inkludert fettsyrepreparater som tran og fiskeolje) 1 uke før studiestart og så lenge studien varer. Du må også være villig til å spise et standardisert måltid kvelden før hver visitt hvor inntaket av fet fisk, spisefett, planteoljer, helfete meieri- og kjøttprodukter og nøtter unngås.

HVA INNEBÆRER PROSJEKTET?

Totalt innebærer studien at du møter på Høgskolen i Oslo og Akershus, avdeling Kjeller til tester i to perioder. Før du kan bli med i studien vil det også bli gjennomført en screening. Dersom du tilfredsstiller kravet til deltagelse, blir du bedt om å undertegne en erklæring på at du samtykker til å delta. Du kan likevel når som helst trekke deg fra studien. Den praktiske gjennomføringen av studien vil være i løpet av høsten 2017/våren 2018. Studien har en total varighet på 3 uker, som er delt i 2 ulike perioder der du får to forskjellige typer testmåltider. Hver periode har en varighet på 5 dager. I tillegg må du også møte til en screening kort tid før første periode. Totalt må du møte til 5 visitter.

Screening: Her vil vi finne ut om du kan være med i studien. Vi vil på denne visitten måle vekt, høyde, kroppssammensetning, blodsukker og noen kjente helsemarkører (som insulin og fettstoffer i blodet), i tillegg til at vi vil spørre deg noen spørsmål angående din helse. Dersom noen av opplysningene vi får tilsier at du ikke oppfyller kriteriene for deltagelse i intervensjonen, får du ikke delta videre i studien.

Periode 1-2: Dette er intervensjonsperiodene, hver av periodene varer i 5 dager. **Dag 1** kommer du fastende til høgskolen i Oslo og Akershus for å ta blodprøver og besvare VAS før og etter det første testmåltidet. Etter at du har inntatt testmåltidet (ila 15 min) skal du besvare VAS med gitte intervaller opp til 180 min etter inntak og gi blodprøver. Testmåltidet består av en muffins med 40-70 E% mettet fett eller umettet fett. **Dag 5** kommer du igjen fastende til visitt på Høgskolen i Oslo og Akershus hvor de samme målingene blir gjort (vekt, kroppssammensetning, VAS og blodprøver). VAS og blodprøver vil bli målt før og i et gitt tidsintervall etter inntatt frokost.

Du skal ellers spise, drikke og trene som vanlig bortsett fra at det siste måltidet du spiser dagen før hver visitt skal være et brødmåltid med maksimum 50% grovhet. Til dette måltidet skal du unngå inntak/pålegg av fet fisk, spisefett, planteoljer, helfete meieri- og kjøttprodukter og nøtter.

I løpet av de 3 ukene som studien varer vil det være totalt 5 visitter med måling av vekt, kroppssammensetning, blodprøvetaking og VAS. Hver av disse visittene innebærer følgende:

- Du møter fastende på Høgskolen i Oslo og Akershus om morgenen. Det betyr at du ikke skal spise eller drikke noe annet enn vann minst 12 timer før besøket (ikke etter kl. 20 kvelden før). Det vil bli tatt blodprøver av deg.
- Det siste måltidet dagen før visitt skal bestå av et brødmåltid (maksimum 50% grovt) uten fet fisk, spisefett, planteoljer, helfete meieri- og kjøttprodukter og nøtter.
- De siste 24 timene før du møter må du ikke drikke alkohol.
- Du må unngå hard fysisk aktivitet dagen før forsøksdagene.
- Du skal registrere sult og metthetsfølelse med et skjema (VAS) før/etter inntak av testprodukt.

På alle visittene vil vi stille deg spørsmål omkring kosthold, fysisk aktivitet og helse. Dersom vi avdekker forhold som krever medisinsk behandling eller videre undersøkelse av lege så vil vi informere deg om dette.

MULIGE FORDELER OG ULEMPER

Det er ingen direkte fordeler for deltagerne å bli med i studien, annet enn at din deltagelse vil være viktig for å bedre forstå betydningen av hvordan fettkvalitet påvirker sult- og metthetsføleselen vår og blodsukkerregulering, og dermed risiko for overvekt og type 2 diabetes.

HVA SKJER MED PRØVENE OG INFORMASJONEN OM DEG?

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte

gjenkjennende opplysninger. Det vil ikke være mulig å spore resultatene i studien tilbake til deg når disse publiseres.

En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg.

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.

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 Førsteamanuensis Vibeke Telle-Hansen e-mail: vibeke.telle-hansen@hioa.no eller Førsteamanuensis Mari Myhrstad e-mail: mari.myhrstad@hioa.no.

HVA SKJER MED PRØVER SOM BLIR TATT AV DEG?

Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Høgskolen i Oslo og Akershus. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Vibeke Telle-Hansen ved Høgskolen i Oslo og Akershus er ansvarshavende for forskningsbiobanken. Biobanken opphører etter prosjektslutt. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer.

Blodprøver er eneste biologiske prøve som vil bli lagret i biobanken (Fettkvalitet og metthet) ved Høgskolen i Oslo og Akershus. Førsteamanuensis Vibeke Telle-Hansen er ansvarshavende for denne biobanken.

PERSONVERN

Opplysninger som vi får i forbindelse med målingene og prøvene som blir tatt av deg (se over) blir registrert hos oss. Prøvene brukes til å undersøke om inntak av fettkvalitet påvirker sult- og metthetsfølelsen, i tillegg til å undersøke hvordan blodsukkeret påvirkes.

Det blir ikke kopling mot andre registre som har opplysninger om deg. Høgskolen i Oslo og Akershus ved prosjektleder Vibeke Telle-Hansen er ansvarlig for databehandlingen. Etter gjeldende regler er studien vurdert av Regionale komiteer for medisinsk og helsefaglig forskningsetikk.

UTLEVERING AV MATERIALE OG OPPLYSNINGER TIL ANDRE

INFORMASJON OM UTFALLET AV STUDIEN

Resultatene fra studien vil bli publisert, og deltagerne vil få informasjon om hvor publisering skjer.

FORSIKRING

Dersom et uhell eller en komplikasjon skulle inntreffe, er deltagerne forsikret gjennom pasientskadeforsikringen i prosjektperioden.

GODKJENNING

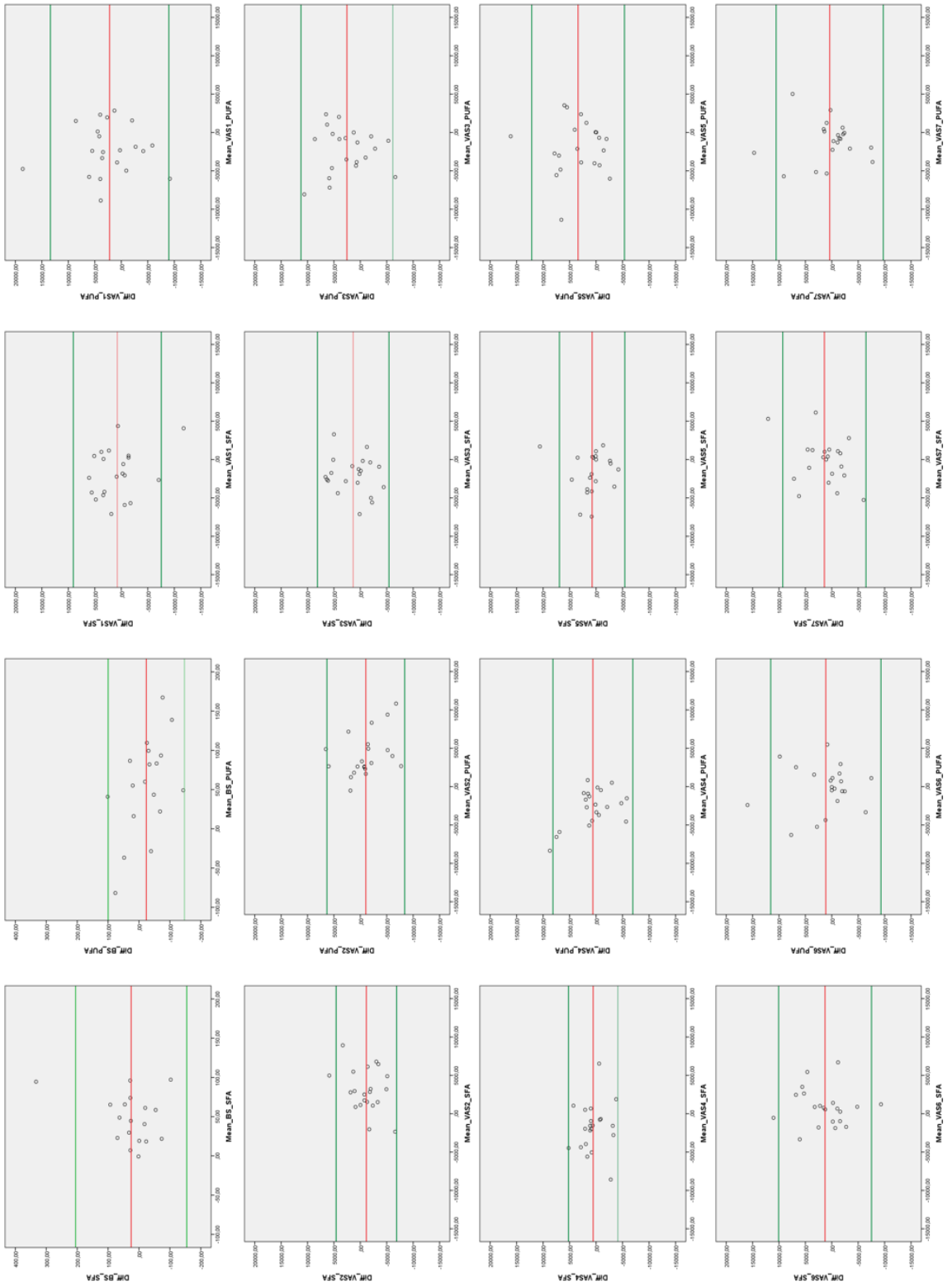
Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk (REK 2017/1327 REK sør-øst B).

JEG ER VILLIG TIL Å DELTA I PROSJEKTET

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver

Appendix F

Bland-Altman Plot comparing the difference in iAUC for period 1 and period 2 against the mean value for SFA and PUFA.



Red line, average; Green line, 2 x SD.