UiT The Arctic University of Norway

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The long-term effect of carbohydrate intake differing in quantity and quality on appetite in people with obesity

- results from a randomized controlled trial (CARBFUNC)

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Abstract

Background: Overweight and obesity are major risk factors for å series of health consequences. A better understanding of appetite regulation can be an important part of providing treatment strategies. When weight is lost, several physiological adaptations, including changes in appetite, take place. Dietary composition seems to affect appetite, and both carbohydrate quantity and quality may play a role. It has been suggested that ketogenic diets may suppress the expected rise in appetite during weight loss. Several studies have investigated the effects of ketogenic diets on appetite, but not in the long term.

Aims: The primary aim is to determine if a VLCHF diet results in a lesser short-term and long-term increase in ghrelin, as well as subjective hunger, compared to HCLF diets despite expected weight loss in all groups. The secondary aims are to determine if there are differences between the cellular and acellular HCLF diets in regard to appetite and if there are sex differences in regard to appetite between all three groups.

Methods: 192 obese participants (20-55 years) were randomized to one of three normocaloric diet interventions: 1) a high-carbohydrate low-fat (HCLF) diet with mostly cellular carbohydrate sources, 2) a HCLF with mostly acellular carbohydrate sources, and 3) a very-low-carbohydrate high-fat (VLCHF) diet. Subjective appetite was assessed with VAS. Fasting levels of ghrelin and ketones were analyzed in blood samples.

Results: Change in ghrelin was significantly lower in the VLCHF group compared to the HCLF groups at three months. Change in subjective fasting hunger was significantly lower in the HCLF- a group at three months compared to the other groups. Sex differences were shown for both changes in ghrelin and change in subjective hunger.

Conclusion: The current thesis suggests that both carbohydrate quantity and quality may affect appetite and that there may be sex differences in response to the diets in regard to appetite.

Abbreviations: HCLF, high-carbohydrate low-fat diet; VLCHF, very-low-carbohydrate high-fat diet; VAS, visual analog scale.

Key words: Ketogenic diet, Low-carbohydrate, Ghrelin, Appetite, Subjective hunger, VAS, Ketosis, Carbohydrate quality, Carbohydrate cellularity

Sammendrag

Bakgrunn: Flere helserelaterte risikofaktorer er knyttet til overvekt og fedme. For å utvikle gode behandlingsstrategier kan en bedre forståelse av appetittregulering være nyttig. Ved vektnedgang skjer det en rekke fysiologiske adaptasjoner, inkludert endringer i appetitt. Diettsammensetning kan trolig påvirke appetitt, og bade karbohydrat kvalitet og kvalitet kan være viktige. Det har blitt foreslått at ketogene dietter kan begrense den forventede økningen i appetitt under vektnedgang. Flere studier har undersøkt effekten av ketogene dietter på appetitt, men ikke over lang tid.

Formål: Formålet med oppgaven er å finne ut om en VLCHF diett resulterer I en mindre økning i ghrelin og subjektiv sultfølelse sammenliknet med HCLF dietter, på kort og lang sikt. I tillegg er de sekundære formålene å finne ut om det er appetittforskjeller mellom den cellulære og det acellulære HCLF dietten, og om det er kjønnsforskjeller i appetitt mellom alle diettgruppene.

Metode: 192 deltakere med fedme (20-55 år) ble randomisert til en av tre normokaloriske diettintervensjoner: 1) en høy karbohydrat, lav fett diett (HCLF) med hovedsakelig acellulære karbohydratkilder, 2) en HCLF med hovedsakelig cellulære karbohydratkilder, og 3) en veldig lavkarbohydrat, høy fett diett (VLCHF). Subjektiv appetitt ble vurdert ved hjelp av VAS, og fastende ghrelin og ketoner ble målt i blodprøver.

Resultater: Endringen i ghrelin var signifikant lavere i VLCHF gruppen sammenliknet med de to HCLF gruppene ved tre måneder. Endringen i subjektiv sultfølelse var signifikant lavere i den acellulære HCLF gruppen ved tre måneders visitten sammenliknet med de andre gruppene. Det var forskjell i endring i ghrelin og subjektiv sultfølelse mellom menn og kvinner.

Konklusjon: Det foreslås at både kvantiteten og kvaliteten av karbohydrater i kosten kan påvirke appetitt, og at det kan være ulik appetittrespons av diettene mellom kjønnene.

Forkortelser: HCLF, høy karbohydrat, lav fett diet; VLCHF, veldig lavkarbohydrat, høy fett diett; VAS, visuell skale («visual analog scale»).

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Abbreviations

AcAc	Acetoacetate
AG	Acylated ghrelin
AgRP	Agouti-related protein
AUC	Area under the curve
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CI	Confidence interval
E%	Energy percent
FA	Fatty acid
GC-MS/MS	Gas chromatography-tandem mass spectrometer
GLP-1	Glucagon-like peptide-1
HCLF	High-carbohydrate low-fat
HCLF-a	High-carbohydrate low-fat acellular diet
HCLF-c	High-carbohydrate low-fat cellular diet
HDL	High-density lipoprotein
IQR	Interquartile range
KLCD	Ketogenic low-carbohydrate diet
LDL	Low-density lipoprotein
ln(b-HB)	Log-transformed level of β-hydroxybutyrate
MAR	Missing at random
MCAR	Missing completely at random
METs	Metabolic equivalents
MNAR	Missing not at random
NPY	Neuropeptide Y
PAL	Physical activity level
PUFA	Poly unsaturated fatty acid
PYY	Peptide YY
RCT	Randomized controlled trial
REK	Norwegian regional ethics committees
ROS	Reactive oxygen species
RUHS	Research unit for health surveys
SD	Standard deviation
TAG	Triacylglycerol
UoB	University of Bergen
VAS	Visual analog scale
VLCHF	Very-low-carbohydrate high-fat
VLED	Very low energy diet
WC	Waist circumference
WHO	World health organization
β–HB	β-hydroxybutyrate

1 Introduction

1.1 Rationale

Overweight and obesity are major risk factors for å series of health consequences (1). Weight loss and maintenance are often challenging (2). One of the many aspects of weight loss is appetite. A better understanding of appetite regulation can be an important part of providing treatment strategies. When weight is lost, several physiological adaptations take place. These adaptations have been thought of as compensatory responses, but this has been challenged (3). Part of these adaptive responses are changes in appetite. When adipose tissue mass is reduced, levels of the hormone ghrelin rise, and the levels of several other hormones, including leptin, are reduced (4). This might contribute to increased hunger as a side effect of weight loss. The increased appetite associated with weight loss may be a factor in making weight loss even more challenging. It has been suggested that ketogenic diets may suppress this rise in appetite. This effect is thought to be due to ketosis (5). A review and meta-analysis on ketogenic low carbohydrate diets (KLCD) and very low energy diets (VLED) from 2014 (6) found decreased hunger when adhering to said diets. A clear lack of the expected increase in hunger when losing weight was seen. The duration of the ketogenic interventions ranged from four to twelve weeks.

It is possible that not only the amount but also the quality of carbohydrates may affect appetite. Hall et al. (7) found that ghrelin was decreased when following an unprocessed diet compared to a processed diet, but energy intake-adjusted subjective appetite did not differ significantly. It has been hypothesized that a diet with high-density carbohydrates may lead to increased leptin resistance, and hence increased appetite, through the promotion of a more inflammatory microbiota (8).

Both high- and low-carbohydrate diets are popular among the general public. Some popular diets restrict one group of macronutrients; others exclude certain food groups. Globally, diets such as “ketogenic diet”, “low-carb diet”, “low-fat diet”, and “paleolithic diet” were all among the top ten most *“googled”* diets between 2004 and 2019 (9).

More research on the effects of diets with differing carbohydrate quantity and quality on appetite in the long term is needed for clinicians to be able to tell their patients what to expect when choosing to follow such diets. Tightening knowledge gaps in this field of research may also be an important part of understanding the complexity of weight loss and how to manage the obesity epidemic.

1.2 The obesity challenge

Overweight and obesity are commonly classified based on body mass index (BMI), an index that puts weight in proportion to height squared. A BMI ≥ 25 kg/m^2 is classified as overweight, and a BMI ≥ 30 kg/m^2 is classified as obesity. More than two decades ago, the World Health Organization (WHO) called obesity a global epidemic (1). Today the epidemic continues to rise among both adults and children (10, 11). This is also true in Norway, where 1 in 4 men and 1 in 5 women have a BMI > 30 kg/m^2 (12). The health consequences of overweight and obesity are many. They include an increased risk of premature death and a series of non-communicable diseases like diabetes mellitus type 2, cardiovascular disease, and several types of cancer (10). It is estimated that greater BMI scores contributed to approximately 4 million deaths globally and almost 2400 deaths (5.7 % of all deaths) in Norway in 2015 (12, 13).

Even a modest weight loss of up to 10 % is associated with health benefits, such as improved glycemic control, reduced blood pressure, and more favorable blood lipid levels (10). Change in body weight, both loss, and gain, is associated with an energy imbalance (14). If a negative energy balance is sustained over time, weight loss will be achieved (15). In other words, all that is needed to lose weight is to have a lower energy intake than the energy expenditure. This might seem simple on paper, but in reality, it seems to be more complicated to lose weight and maintain weight loss. Weight loss is commonly regained after both surgical and non-surgical interventions (16). The lack of success can be explained by failure to adhere to the intervention and metabolic and behavioral responses to weight change (15, 16). These responses include changes in energy expenditure and appetite and have been thought of as compensatory responses (3, 14, 15). Martins et al. (3) has challenged this view and argue that the changes reflect a normalization towards a lower body weight. Either way, these changes may contribute to making weight loss and maintenance challenging. Polidori et al. (17) found that the adaptation in appetite counters weight loss more strongly than the adaptation in energy expenditure.

The etiology of overweight and obesity is also complex (10). Individual susceptibility may be affected by genetic factors, sex, age, and other factors that cannot be controlled. Other important aspects may include social and cultural elements, structural environment, and psychological factors (10). It has also been hypothesized that the obesity epidemic is, at least in part, driven by changes in the global food system that have contributed to an obesogenic environment (18). When the prevalence of obesity started rising in the 1970s, there was

simultaneously a rise in available calories and refined carbohydrates and fats (18). Spreadbury et al. (8) proposed that “Westernized foods”, which contain more acellular carbohydrate, produce an inflammatory microbiota that may lead to obesity through leptin resistance and an increased appetite.

1.3 Appetite

When discussing terms like “appetite”, “hunger”, “fullness”, and other related terms, it is important to define and distinguish the terms. Appetite can be thought of as a sum of factors leading to food intake, including preference and motivation. It can also refer to the qualitative aspects of eating, including sensory and environmental aspects, that can be contrasted with physiological stimuli and energy deficit (19). Hunger can be thought of as a construct of appetite. It is a conscious sensation that reflects a state of biological need (20). Even so, hunger is not necessary for eating, nor is it enough on its own to initiate eating, but it encourages eating behavior (21). Satiety or fullness can be thought of as both the opposite of hunger and as the absence of hunger. Satiety can be defined as a decline in hunger, incline in fullness, and inhibition of further eating (19).

1.3.1 Appetite regulation

Both physiological and psychological factors influence appetite, and the experience of appetite can differ between groups, for instance, age groups (20). Several studies have shown that aging leads to a decline in food intake and that older subjects rate hunger and prospective consumption lower, and satiety and fullness higher than younger subjects (20). Gregersen et al. (20) also found that appetite varied based on sex, menstrual cycle, smoking habits, and physical activity. They did not find any differences in appetite based on BMI classes, but it has been proposed that dysregulation of appetite can contribute to overeating in overweight subjects (20, 22).

Several organs and systems are involved in appetite regulation. This includes the brain and nervous system, the hormonal system, the gut, and adipose tissue (23). The hypothalamus is important in the hormonal signaling of appetite. Many peripheral appetite-related hormones mediate their effect via the vagus nerve (24). The vagus nerve is also involved in appetite regulation in several other ways, making it another important structure in appetite regulation (25). The gut and adipose tissue secrete appetite-related hormones. The microbiota may also affect appetite through the gut-brain-axis, also involving the vagus nerve (25).

1.3.1.1 Appetite hormones

The physiological regulation of appetite involves several appetite-related hormones. Some hormones are thought to be anorexigenic (decreases appetite), and others are thought to be orexigenic (increases appetite) (23, 24, 26). Amongst the hormones that are thought to be orexigenic are agouti-related protein (AgRP), neuropeptide Y (NPY), orexin, endocannabinoids, and ghrelin. Leptin, insulin, peptide YY (PYY), GLP-1, and several others are considered anorexigenic (23, 24).

1.3.1.2 Ghrelin

Ghrelin is an orexigenic peptide hormone (27) and, in fact, the only known peripheral orexigenic hormone (28). The hormone is mainly produced by endocrine cells in the gastric fundus and requires acyl-modification to become the active form, acylated ghrelin (AG). The AG is then enzymatically des-acylated, and the AG form has a half-life of 240 minutes (29). Most circulating ghrelin is in the inactive form, which has no known receptor (27). It has been suggested that all circulating ghrelin is AG and that des-acyl ghrelin in samples may be due to sample handling (30).

Ghrelin plays a role in normal feeding behavior, meal initiation, and hunger. It is involved in the regulation of food intake and body weight. Several areas of the central nervous system, including the hypothalamus, seem to be involved in mediating ghrelin´s orexigenic effects. These effects include hyperphagia and an increased drive to eat. It is also found to have a series of other actions, both central and peripheral, including growth hormone stimulation and effects on metabolism (27). By stimulating glucagon secretion and inhibiting insulin secretion, it has an elevating effect on blood glucose (31). It may also promote energy conservation by affecting adipose tissue cell metabolism. Promotion of fatty acid storage and decreased fat oxidation has been seen in rodent studies (32).

Ghrelin has an indirect relationship with adipose tissue mass. When adipose tissue mass is reduced, levels of the orexigenic hormone rises (4). Several studies have found that ghrelin rises when more than 10 % of the weight is lost (33, 34).

The Mayo clinic's laboratories state that the reference values for total ghrelin are 340-450 pg/mL for obese subjects and 520-700 pg/mL for normal-weight subjects (35). Beasley et al. (36) investigated ghrelin 163 subjects with a mean BMI of 30.3 ± 6.1 and found that mean fasting total ghrelin was 817.0 ± 355.5 pg/mL. When split into BMI categories, they found that overweight subjects´ ghrelin concentration was 161.7 ± 69 pg/mL lower compared to

normal-weight subjects. Obese subjects´ ghrelin concentration was 288.7 ± 66.5 pg/mL lower compared to normal-weight subjects.

The hormone has both episodic and tonic properties (21). On an episodic level, ghrelin is secreted in response to fasting, and levels are normally elevated pre-prandially and reduced post-prandially, proportionally to the caloric load ingested (27, 37). It has been proposed that these episodic changes related to food intake indicate that ghrelin is a trigger for hunger (38). The tonic properties are more stable over days and are related to body composition (21).
The post-prandial fall in ghrelin is induced when nutrients are administered to the upper parts of the small intestine (27). The post-prandial fall is also affected by the type of nutrient ingested. Protein is the most effective macronutrient group in reducing ghrelin levels, while lipids reduce the levels the least (39). Effects of habitual macronutrient intake have been less investigated (40). Ellis et al. (40) investigated the effect of 8-week habituation to either a lower-carbohydrate diet (43 % carbohydrate, 18 % protein, 39 % fat) or a higher-carbohydrate diet (55 % carbohydrate, 18 % protein, 27 % fat) on fasting ghrelin. They found that habituation to the differently composed diets did not affect fasting ghrelin nor postprandial ghrelin response to a mixed meal. There were also no between-group differences in subjective hunger.

1.3.2 Quantification of subjective appetite

Since appetite, hunger, and satiety have both physiological and learned components and the sensations are subjective and cannot be directly measured. Several different systems to quantify subjective sensations have been devised, and the use of a visual analog scale (VAS) has become popular in appetite research (41).
VAS has previously been shown to exhibit a good degree of intrasubject reliability and is suitable for repeated measurements (41). The method is also sensitive to experimental manipulations and shows some ability to predict aspects of feeding behavior (41).

1.3.3 Appetite and weight loss

When weight is lost, there is an expected increase in Ghrelin due to its indirect relationship with adipose tissue mass (4, 33, 34). Appetite has also been found to increase after weight loss. Martins et al. (42) found that fasting subjective hunger significantly increased from 4.1 ± 1.6 cm to 6.5 ± 2.5 cm after 12 weeks, and a mean exercise-induced weight loss of 3.5 kg. Outside of ketosis, both ghrelin and appetite have been found to be increased after a weight

loss and remain increased compared to baseline even after long periods of weight maintenance. Sumithran et al. (34) found that circulating mediators of appetite, including ghrelin, did not revert to baseline within 12 months after a diet-induced weight loss and a 12-month maintenance phase. Subjective appetite was also higher after the 12-month maintenance phase compared to baseline, and mean body weight was also lower compared to baseline (34). Nymo et al. (4) found that both ghrelin and subjective hunger increased when refeeding occurred after ketogenic diet-induced weight loss.

1.4 Ketosis

When glycogen storages are low, the liver oxidizes fatty acids to form ketone bodies to serve as fuel. The most abundant ketone bodies are β-hydroxybutyrate (β-HB) and acetoacetate (AcAc). After an overnight fast, the blood concentration of β-HB reaches approximately 0.1 mmol/L (6). Nutritionally induced physiological ketosis is generally considered to be safe and must not be confused with the pathological ketosis that can occur in diabetic patients (43, 44). Nutritionally induced ketosis can be achieved by limiting the intake of carbohydrates. Both KLCDs and VLEDs lead to a low intake of carbohydrates and a subsequent increase in the production of ketone bodies. There is no clear consensus on a carbohydrate limit to ensure ketosis. The amount of carbohydrate allowed in ketogenic diets vary, but the limit is often set to 50 g per day or lower. For diets that are not calorie-restricted, a carbohydrate limit of 50 g per day or a maximum of 8-10 E% from carbohydrates is likely to be sufficient to reach ketosis (6, 45).

1.4.1 Ketosis and appetite

It has been suggested that ketogenic diets may suppress the expected increase in appetite during weight loss. This has been shown several times in both VLEDs and KLCDs (6). The effect is thought to be due to ketosis (5), but the exact mechanisms are not fully understood (46). Ketone bodies could potentially have both direct and indirect effects on appetite regulation. Questions regarding its potential direct effects, for instance, if it could act as a satiety signal in the brain, remain unanswered. The potential indirect effects could be via appetite-related hormones or in synergy with butyrate production by the gut microbiota. It has also been hypothesized that ketosis may have some orexigenic effects, partly through decreasing the production of reactive oxygen species (ROS) (46). Stubbs et al. (47) investigated the effects of exogenous ketone ester on appetite and found that both appetite-

related biomarkers, including ghrelin and subjective appetite, were decreased postprandially after consumption of ketone esters.

Several studies have investigated ghrelin levels under ketogenic conditions and found no change in ghrelin despite weight loss (4, 5, 48-50). During refeeding and out of ketosis, several studies have found an increase in ghrelin (4, 5, 48).

Just like there is no clear consensus on how low in carbohydrates a diet needs to be to be ketogenic, there is no consensus on a limit of β-HB blood concentration to achieve the appetite suppressing effects of a ketogenic diet. Gibson et al. (6) hypothesized that a minimum level of 0.3 – 0.5 mmol/L of β-HB would be sufficient to achieve the effects. On the other hand, Lodi et al. (51) found that a much higher minimum level of 1.48 mmol/L was required.

Some studies suggest that it takes some time on the ketogenic diet before the appetite suppressing effect commences (4). Nymo et al. (4) concluded that weight loss, even with a ketogenic diet, increased fasting hunger up to 5 % weight loss despite no change in ghrelin, but when weight loss was 10-17 %, it was no longer associated with increased appetite. Lodi et al. (51) found an immediate appetite suppressing effect of the ketogenic diet. They speculate that this may be due to a relatively high level of ketosis in their participants. The longevity of the appetite-suppressing effects is not established as it has not been thoroughly investigated for more than 12 weeks.

1.5 Existing literature on non-energy restricted or normocaloric KLCD effects on appetite

A review and meta-analysis by Gibson et al. from 2014 (6) were conducted to determine the effect of ketogenic diets on appetite. They found that overall satiety increased after VLED interventions and that both hunger and desire to eat decreased after KLCD interventions. They concluded that the true benefit of VLEDs is that it seems to prevent an increase in appetite during weight loss. Ketosis was pointed out as a plausible explanatory factor for this effect. Gibson et al. (6) identified 26 publications on the effects of adhering to a KLCD, or VLED that was likely to be ketogenic, on subjective appetite. Only three of the included studies were KLCD. These were all carbohydrate-restricted but not energy-restricted (50, 52, 53).

To identify new literature on this field, a similar literature search to that of Gibson et al. was conducted in November 2020. The search strategy can be found in **Appendix I**. One additional publication was identified (51). Including the once from Gibson et al., a total of

four publications investigating the effects of ketogenic diets without energy restriction on subjective appetite using VAS-scores were identified (50-53). The number of participants varied from 17 to 134. BMI was at least 25 or above at baseline, and participants lost weight in all four publications. The duration of the interventions varied from 10 days to 2 years. Only one study had a duration longer than 12 weeks, and its duration is debatable in this setting. This study´s (53) low carbohydrate diet had a duration of 2 years, but the diet changed during that time. The planned intervention started with 20 g of carbohydrate per day for the first 12 weeks and then allowed a weekly increase of 5 g of carbohydrate. The authors did not report measured levels of ketones. It is therefore unclear how long the diet could be considered to be a KLCD. Data were collected at 3 and 6 months, but already at six months, the daily carbohydrate allowance would be 80 g per day, which is often not considered to be ketogenic (45). For the sake of this study, only the first 12 weeks of this intervention can be considered ketogenic. Martin et al. (53) found that participants, especially male participants, allocated to the low-carbohydrate diet experienced reduced appetite and less hunger than participants in the low-fat allocation.

Johnstone et al. (52) found less hunger and greater weight loss when following a low-carbohydrate diet compared to a medium-carbohydrate diet. Ratliff et al. (50) found that participants were less hungry and more satisfied after following a low-carbohydrate diet, both with and without the addition of cholesterol from eggs. Lodi et al. (51) similarly found that a ketogenic diet reduced appetite more compared to a Mediterranean diet.

Only one of these publications also reported levels of ghrelin. Ratliff et al. (50) found that ghrelin levels did not change for either of the two low-carbohydrate groups, despite also finding that both groups lost weight. They also found a change in subjective appetite measured with VAS scores, as a reduction in “desire to eat” and an increase in “satiety” and “fullness”. The authors speculated that the observed reduction in appetite could be due to the high level of protein in the diet since ghrelin was not affected.

Other studies have investigated the effect of KLCDs on appetite without assessing VAS scores. One study (54) reported data on fasting appetite-related hormones, including ghrelin, and self-reported change in appetite over 12 months. The 148 participants were allocated to either a low-carbohydrate diet or a low-fat control diet. The planned low-carbohydrate diet is likely to be ketogenic, as it allowed only 40 g of carbohydrate per day. Concentrations of ketone bodies were not measured. Hu et al. (54) collected data at baseline, 3, 6, 9, and 12 months. They found that neither the mean differences in change in ghrelin nor self-reported change in appetite (yes/no question, not VAS-score) differed significantly between the diet

groups. Peptide YY (PYY), a satiety hormone, was reduced in both groups at twelve months but to a lesser extent in the low-carbohydrate group.

1.6 The role of KLCD in the management of obesity

Several studies have investigated the role of KLCD in the management of obesity, both in the short and long term (55). Bueno et al. (55) conducted a meta-analysis investigating the long-term (at least 12 months) effects of KLCD compared to energy-restricted low-fat diets. The KLCD investigated allowed a maximum of 50 g per day or 10 E% from carbohydrates, and the low-fat diet allowed a maximum of 30 E% from fat. They found that following a KLCD led to a greater long-term weight reduction, a greater reduction in diastolic blood pressure and triacylglycerol (TAG), but also a greater increase in both LDL and HDL cholesterol.

Several mechanisms have been suggested for the effect of ketogenic diets on weight loss. They include reduced appetite, increased lipolysis, increased metabolic costs of gluconeogenesis, and the thermic effect of proteins (56).

Even though ketogenic diets have been shown to be effective in reducing weight and even seem beneficial to improve several biomarkers (55), several concerns have been raised about the diet (56). One concern is that the macronutrient composition of the diet may lead to a more unfavorable lipid profile with increased LDL and TAG. The meta-analysis by Bueno et al. (55) found a reduction in TAG and an increase in both LDL and HDL. It has also been reported that ketogenic diets may lead to an increase in the size of LDL particles, which is considered to reduce the risk of cardiovascular disease (56, 57).

Another concern is that ketogenic diets for weight loss may lead to a "yo-yo effect". This is a reasonable concern, as it has been demonstrated that the appetite suppressing effect of ketogenic diets disappears when subjects come out of ketosis, and an increase in hunger and ghrelin is seen during refeeding and weight stabilization (4). On the other hand, Paoli et al. (58) found that their participants succeeded in long-term weight loss after two periods of a ketogenic diet and longer periods of weight maintenance on a Mediterranean diet.

Yet another concern, and the main proposed risk, is possible renal damage due to increased nitrogen excretion during protein metabolism (58). There are conflicting results in the literature regarding this (58). The concern is mainly applicable for ketogenic diets with high levels of protein, which ketogenic diets do not have to be (56).

1.7 Carbohydrate quality

Carbohydrate quality can refer to the glycemic index, amount of fiber in the carbohydrate source, or the degree of processing of the carbohydrate source. Either way, it is possible that the carbohydrate quality may affect appetite, and several types of fiber have been shown to reduce it (59, 60). Carbohydrate quality is also associated with a series of health benefits. It has been found that dietary fiber and whole grains have a dose-response relationship with several non-communicable diseases (61). A high fiber intake is also associated with improved insulin sensitivity and glucose homeostasis (61, 62). Levels of ghrelin are modulated by both glucose and insulin (62, 63). It is reasonable to think that this could be a link between the intake of dietary fiber and appetite regulation. St-Pierre et al. (62) found that fiber intake was significantly associated with ghrelin levels, even after adjusting for insulin sensitivity.

It is important to separate the effects on within-meal appetite and between-meals appetite. Within-meal appetite referring to the staying effect of the meal, which can lead to a lower ad libitum intake, and between-meals appetite referring to appetite leading up to the next meal or in the fasting state. Fiber can promote both within and between meals satiety (64). This is thought to be partly due to its bulking effect, but it can also be influenced by glycemia, hormones, and transit time (64). Nilsson et al. (65) found that fiber intake in an evening meal affected subjective appetite after a standardized breakfast meal the morning after. This was associated with colonic fermentation and increased transit time.

Hall et al. (7) found that fasting ghrelin was decreased and PYY was increased when following an unprocessed diet compared to a processed diet, but energy intake-adjusted subjective appetite did not differ significantly. The potential negative effects of ultra-processed foods have been thought to be related to low fiber and high sugar content. In this study, the intervention diets were matched for sugar, fiber, and macronutrients. This indicates that there might be another explanation for the differences in appetite when following a processed diet versus an unprocessed diet.

It has been hypothesized that a diet with high-density carbohydrates may lead to increased leptin resistance and hence increased appetite through the promotion of a more inflammatory microbiota (8). In this setting, carbohydrate density is linked to cellularity. Sources such as flours, sugar, and processed starchy plant products consist of carbohydrates in an acellular form and will be denser. Carbohydrates locked in an intact cell structure will be less dense and will maintain their low density until the cell walls are destroyed during digestion (8). It has been found that western populations have elevated leptin levels compared to people living in non-westernized communities and eating a cellular diet (8).

1.8 Knowledge gaps

Several questions on these topics remain unanswered. Ketosis is thought to be involved in the observed lack of increase in appetite when adhering to ketogenic diets, but it is not established whether ketosis has direct or indirect effects (6). There is no consensus on how strictly carbohydrates must be restricted to induce ketosis. Also, there is no clear consensus on what concentration of β-HB is needed to affect appetite. Perhaps most importantly, long-term appetite-suppressing effects are not established as it has not been thoroughly investigated for more than twelve weeks.

Most studies investigating the effect of a ketogenic diet on appetite were conducted in young female participants (6). Few studies have evaluated the potential sex differences in appetite response to different diets (66). Lyngstad et al. (48) investigated if changes in appetite differed between sexes even when subjects were in ketosis. They found that changes in most appetite-related hormones and subjective appetite were similar for both sexes regardless of ketosis, even though subjects had a lower appetite when in ketosis. Glucagon-like peptide-1 (GLP-1) was more favorable in females while in ketosis and was the only appetite-related hormone that differed between the sexes.

Carbohydrate quality could potentially affect appetite in several ways (59, 60). The differences in leptin levels between western populations and non-western populations eating a cellular diet could indicate that the cellularity of carbohydrates correlates with increased appetite, but this has not been found under experimental conditions (8).

1.9 Aims & objectives

The primary aim of this study is to determine if a VLCHF diet results in a lesser short-term (three months) and long-term (one year) increase in ghrelin, as well as subjective hunger, compared to high-carbohydrate, low-fat diets despite expected weight loss in all three groups. The secondary aims are to determine if there are differences between the cellular and acellular HCLF diets in regard to appetite and if there are sex differences in regard to appetite between all three groups.

The main hypothesis of this project is that there is an appetite suppressing effect of the VLCHF diet mediated through ketosis and that it will persist in the long run.

To fulfill the primary aim the following objectives have been set:

- Compare changes in fasting plasma concentration of total ghrelin and subjective appetite (VAS) between the intervention groups over the duration of twelve months.
- Evaluate the level of ketosis by analyzing β-hydroxybutyrate (β-HB) in plasma samples drawn in the fasting state.
- Evaluate the correlation between measurements of appetite, ketosis, and weight loss.

To fulfill the secondary aims the following objective has been set:

- Compare changes in ghrelin and VAS score between the two HCLF groups over the duration of 12 months.
- Compare changes in ghrelin and VAS score between male and female participants in all three groups.

2 Methods

2.1 Study Design

The CARBFUNC study (ClinicalTrials.gov identifier: NCT03401970) is an ongoing two-year randomized controlled trial (RCT) investigating the effect of dietary carbohydrates on changes in visceral fat mass (67). The study was conducted in Bergen, Norway, started in 2018, and will finish in May 2021.

Participants were recruited through social media, local newspaper advertisements, radio broadcasts, and flyers.

2.1.1 Research environment

The CARBFUNC-study is conducted in collaboration between the Mohn research laboratory and the Centre for Nutrition at the University of Bergen (UoB) and Haukeland University Hospital. Data collection is carried out at the Research Unit for Health Surveys (RUHS) at the University of Bergen (https://www.uib.no/fhu).

2.2 Study Population

2.2.1 Inclusion and Exclusion Criteria

To participate in the study one had to fulfill the inclusion and exclusion criteria (**Table 1)**.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Sex: all	Statin medication
Age: 20-55 years	Diabetes medication
Obesity*	Surgical treatment last two months
Able to follow diet	Antibiotics treatment last two months
Able to record food intake	Smoking
	Pregnancy or lactation
	Alcohol intake > 2 units** per day

*Obesity = BMI: > 30kg/m2 or WC > 88 cm for females, > 102 for males; **one alcohol unit = 15 mL of pure alcohol.

2.2.2 Screening

People interested in participating in the study filled out an online screening form and were invited to a screening session where they were given information about the project. The screening session was completed at the RUHS. Before any sensitive information was collected, written consent was received. Those who met the criteria described above were invited to participate in the study.

2.2.3 Randomization

Participants were allocated to one of the three intervention diets using block randomization. The allocation was stratified by sex. Participants who were living together or had other social relations were allocated to the same intervention diet.

2.3 Study visits

Participants were invited to study visits at baseline, 3, 6, 9, 12, and 24 months. Only data from the first five (12 months) visits will be assessed in this thesis. At each visit, participants provided biological samples and anthropometrical data. Some data was collected in the fasting state, others after the intake of a standardized meal.

On the days of the visits, participants came in after an overnight fast. During the fasting period of 12 hours, they should also abstain from using chewing tobacco. 48 hours before the visits, they should avoid intense physical activity. 24 hours before the visits, they should abstain from alcohol consumption. On the day of the visit, they should also avoid using chewing gum or toothpaste.

2.4 Study intervention

The intervention diets were 1) a high-carbohydrate, low-fat diet with mostly acellular carbohydrate sources (HCLF-a), 2) a high-carbohydrate low-fat diet with mostly cellular carbohydrate sources (HCLF-c), and 3) a very low-carbohydrate high-fat diet (VLCHF) (**Table 2**). The two HCLF diets had a planned carbohydrate content of 45 E% and 30 E% from fat. The VLCHF diet was planned to have a carbohydrate content of only 8 E% and 75 E% from fat. More details on the macronutrient composition of the intervention diets can be found in table 2. Participants were asked to consume 2000-2500 kcals from their allocated diet and not change their level of physical activity during the intervention. All diets should contain at least 500 grams of fruit and/or vegetables per day and cover all basic nutritional requirements. Participants allocated to the HCLF-a diet were encouraged to choose carbohydrate sources with the “keyhole” symbol, which indicates that the labeled product is

relatively healthy compared to similar products (68). Participants in the VLCHF group were encouraged to consume more cheese and less butter and meat compared to a previously conducted trial with a similar diet (69). There were no planned limits or goals set for the daily intake of dietary fiber for either intervention group.

Regarding the diets carbohydrate cellularity, both cellularity index and degree of processing are considered. The list of foods with estimated dietary carbohydrate cellularity can be found in **Appendix VII**. The acellular carbohydrate sources are refined products such as flour, bread, and pasta. The cellular carbohydrate sources are unrefined and minimally processed products such as minimally processed cereals, vegetables, fruit, and berries.
As the VLCHF diet have a very low carbohydrate allowance and encourages the intake of fruit and vegetables, the carbohydrate sources in this diet are also mainly cellular.

Table 2. Planned intervention diets

	HCLF-a	**HCLF-c**	**VLCHF**
Energy, kcal/d, female(male)	2000 (2500)	2000 (2500)	2000 (2500)
Carbohydrate	**45 E%**	**45 E%**	**8 E%**
Added sugar	<= 5 E%	<= 1 E%	<= 1 E%
Fat	**30 E%**	**30 E%**	**75 E%**
Saturated FA	10-12 E%	10-12 E%	25-30 E%
PUFA	7-10 E%	7-10 E%	7-10 E%
Protein	**17 E%**	**17 E%**	**17 E%**

Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate; VLCHF, Very-low-carbohydrate high-fat diet; E%, energy percent; FA, fatty acid; PUFA, polyunsaturated fatty acid

All participants were given access to recipes of hundreds of meal options suited for their sex and allocated diet. The HCLF-a participants were given 203 recipes, the HCLF-c participants were given 177 recipes, and the VLCHF participants were given 428 recipes. The recipes ranged from simple to more advanced recipes, and the participants were encouraged to vary their choice of meal recipe. All recipes were designed to fit the allocated diets planned macronutrient composition, both in grams and in energy percent.

2.4.1 Adherence to intervention

Participants were asked to record dietary intake for three days (one day from the weekend) two times before the baseline visit and for three days every 14 days between study visits. This was done using an electronic dietary record tool, "Diett.no" (www.diett.no; operated by Dietika AS, Slemmestad, Norway). All participants were given a food weighing scale and were encouraged to use it when recording dietary intake.

They also registered their physical activity, using the same electronic tool, for three days every three months. Physical activity level (PAL) was calculated by multiplying the time spent on different activities throughout the day with the metabolic equivalent of the task (MET). METs for the different activities were found in the Compendium of Physical Activities (70).

For assessment of adherence, the participants were asked to rate their dietary adherence from no adherence (0 %) to complete adherence (100 %). Participants also recorded deviations from the diet in their dietary records.

2.5 Data collection

Data were collected at baseline and at visits at each visit. Some measurements and samples were taken in the fasting state, others after a standardized meal. This, and handling and storage of blood samples, is illustrated in **Figure 1**. Blood was also drawn postprandially, and several circulating biochemical variables, fats, and other metabolites were analyzed at the

routine laboratory at Haukeland University Hospital (Medical biochemistry and pharmacology, MBF). This is described in further detail in CARBFUNCs protocol (67).

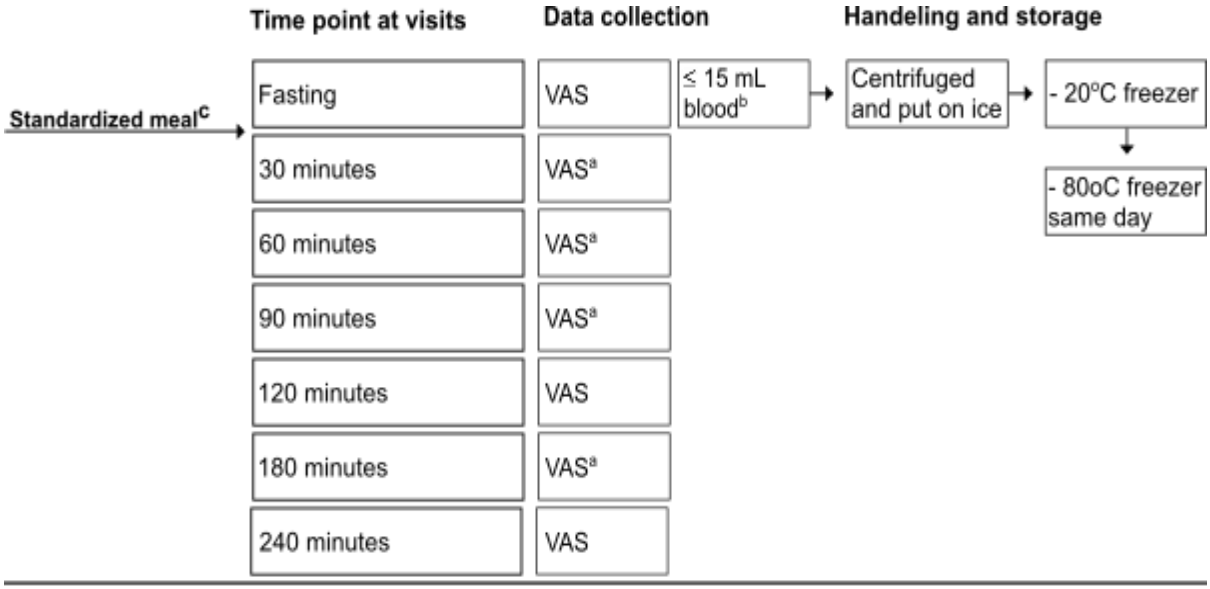

Figure 1. Data collection at the study visits.
[a] Added to VAS protocol after the study started.
[b] Blood was drawn by a project nurse through a peripheral venous catheter.
[c] The standardized meal consisted of cooked oatmeal with added butter and rapeseed oil. This gives 50 grams of carbohydrate and 40 grams of fat.
Abbreviations: VAS, visual analog scale.

2.5.1 Standardized meal

To assess postprandial variables, participants were asked to consume a standardized meal was at every study visit. The meal was a mixed meal, oatmeal providing 700 kcal. It consisted of 50 g of "Bremykt", a butter-and-oil spread with 70 % butter and 30 % rapeseed oil, 80 g of "Bjørn Lettkokte Havregryn" (AXA, rolled oats), 5 grams of sugar, and 200 mL of water. Approximately 32 E% came from carbohydrates (56 g), 61 E% from fat (47 g), and 5 E% from protein (9 g). Participants were instructed to consume the meal in 10 minutes.

2.5.2 Anthropometry

Weight in kg and waist circumference (WC) in cm were measured at each study visit. Height was measured with a stadiometer at the baseline visit. Height and WC were measured three times, and the mean value of the two last measurements was registered. This was done to minimize measurement errors.

2.5.3 Subjective appetite

During the visits, the participants were asked to fill out VAS- questionnaires to assess subjective appetite. VAS has previously been shown to exhibit a good degree of intrasubject reliability and is suitable for repeated measurements (41).
In the first year of the study, the VAS questionnaire was filled out a total of three times. After one year, VAS-questionnaire routines were changed. Participants should now fill out a total of seven VAS questionnaires at each visit. This is illustrated in **Figure 1**. Because the participants entered the study at different times, some participants only filled out three VAS questionnaires, and others filled out seven VAS questionnaires at each visit. The data from participants who filled out seven questionnaires will be analyzed as the area under the curve (AUC). All data can be used to analyze changes from the fasting state to two hours postprandially, from the fasting state to four hours postprandially, and from two to four hours postprandially.

When filling out the VAS questionnaire, the participants were instructed to mark a ten-centimeter line to indicate how they felt at that exact time. One end of the line represented "not at all", while the other end represented the most intense version of the feeling. The questions they were asked to answer were:

- How hungry do you feel right now?
- How strongly do you desire to eat right now?
- How full do you feel right now?
- How much do you think you can eat right now?

2.6 Plasma analyses

2.6.1 Ketone bodies

Concentrations of total β-HB in µmol/mL and AcAc in µmol/mL were measured in the frozen plasma samples drawn in the fasting state. The analyses were performed by trained personnel. Because mmol/L is more commonly seen in the literature, concentrations of the ketone bodies were converted to mmol/L (µmol/mL/1000 = mmol/L) and given as mmol/L in the current thesis.

The analyses were conducted at the BEVITAL laboratory (Bergen, Norway) (https://folk.uib.no/mfapu/Pages/BV/BVSite/index.html) using gas chromatography-tandem mass spectrometers (GC-MS/MS).

2.6.2 Total ghrelin

Concentrations of total ghrelin in pg/mL were measured in the frozen plasma samples drawn in the fasting state. The analyses were performed by trained personnel.

The analysis of total ghrelin was conducted at the Medical Research Center at Oulu University Hospital (Oulu, Finland). Total ghrelin was measured with a Human Ghrelin (Total) ELISA kit (Millipore, EZGRT-89K). All samples were measured in duplicate with internal controls (1 male and one female). The samples were measured in 14 plates in two weeks period. 40µL of plasma per sample is needed for this analysis.

2.7 Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics version 26 (IBM Corp., Armonk, N.Y., USA) (71).

Visualizations of data are made with RStudio version 1.3.1093 (https://www.R-project.org, RStudio, Inc., Boston, MA) (72-74), Microsoft® Excel for Macintosh (version 16.48), and Inkscape version 1.0.1 (75). All data are presented as means ± standard deviations (SD) or 95 % confidence interval (CI) if not otherwise specified.

2.7.1 Distribution of data

To check for normality, Shapiro-Wilk´s test was performed, and Q-Q plots and histograms were assessed. As normality is best checked based on residuals (76), Q-Q plots of residuals were used when checking the assumptions for a linear model. Diagnostic plots are more useful than normality tests (76); therefore, the emphasis was put on the diagnostic plots rather than the Shapiro-Wilk´s test if the results differed.
For data that were not normally distributed, natural log transformation was used, however, means, SDs, and CIs are reported on the original scale.
To test for homogeneity of variances, Levene´s test was used.

When relevant, homogeneity of regression slopes and linearity were checked by assessing scatterplots and fitted regression lines. If necessary, linearity was further checked by assessing the regression coefficients.

Box-plots were used to detect outliers in the dataset. Outliers were defined as 1.5 times the interquartile range (IQR) outside the first and third quartile. Extreme outliers were defined as 2 x 1.5IQR outside the first and third quartile.

2.7.2 Analyses

For between groups analysis, the study outcomes were analyzed with the parametric tests "one-way analysis of variance" (ANOVA) or "one-way analysis of covariance" (ANCOVA) if assumptions were met. The assumptions for these tests are that observations should be independent, the dependent variable should be normally distributed, and there should be homogeneity of variance (77). For the ANCOVA test, there are two additional assumptions: that the relationship between the dependent variable and the covariate should be linear and that the regression slopes are equal among the groups (78).

In the ANCOVA analysis, both diet and a categorical variable for level of β-HB will be used as a fixed factor in the analysis. Baseline values and other relevant variables were added as covariates and hence adjusted for. P values < 0.05 will be considered to be statistically significant. If results from the ANOVA or the ANCOVA were statistically significant, post hoc tests were assessed. For the ANOVA, the post hoc test is Tukey HSD. For the ANCOVA, the post hoc test is a pair-wise comparison. The post-hoc tests assess which of the groups were statistically significantly different from each other.

The categorical variable for the level of β-HB will be created with visual binning of the continuous variable. Cut points will be the level of β-HB seen after an overnight fast in previous studies (~0.1 mmol/L) and the level of β-HB that has previously been thought to be sufficient to have effects on appetite (0.3 mmol/l) (6).

Paired t-tests were used to examine within-group changes. When making multiple comparisons, the p-value level for significance was adjusted with the Bonferroni adjustment.

For correlation analysis, Pearson´s correlation was used when appropriate. For this test to be appropriate, both variables should be continuous and have a linear association, the dependent

variable should be normally distributed, and there should be no outliers as the test is sensitive to outliers (79). If Pearson´s was not appropriate, for instance, if data contained outliers, Spearman's correlation was used instead.

The area under the curve (AUC) for subjective hunger measured with the VAS questionnaire was calculated with the trapezoidal method using both three and seven timepoints per visit.

2.7.3 Missing data

Missing data occurs when participants drop out or fail to contribute to data collection points. The Little´s missing completely at random (MCAR) test (80) was conducted to test if the dropouts were MCAR.
Missing not due to drop out, but to failure to contribute to data collection points will not be included in the analysis as the chosen statistical methods exclude incomplete cases.
As missing data are unknown, observed data cannot be used to determine if missing is missing at random (MAR) or missing not at random (MNAR). Comparisons of observed data before the missing occurred will be conducted to assess whether there were any differences in between the participants who participated and the once who dropped out before they dropped out.

2.7.4 Post hoc power calculation

Post hoc power calculations and sample size estimations for a power of 80 % and a significance level of 5 % were conducted using G*Power version 3.1 (81).

2.8 Ethical considerations

The CARBFUNC study is conducted according to the guidelines laid down in the Declaration of Helsinki (82), and the protocol and patient information and consent form were approved by the Norwegian Regional Ethics Committees (REK) (REK Vest nr:2017/621). The study is registered at Clinical Trials (ref.: NCT03401970). All participants gave full written consent after receiving adequate information about the study.

2.8.1 Confidentiality

Data from the CARBFUNC study will not contain any participant identifiers.

The data will be available via UoBs secure system, "SAFE" (https://www.uib.no/safe).

3 Results

3.1 Participants

193 participants entered the study and were randomized to one of three intervention groups (**Figure 2**). One participant withdrew consent after the three-month visit, resulting in analyzed data from 192 participants (**Table 3**). Mean age and BMI at baseline were 42 ± 8.8 years and 36.6 ± 4.8 years, respectively. As randomization was successful, mean age and anthropometric measurements did not significantly differ between the intervention groups. For the same reason, any other potential differences at baseline are considered to be attributed to chance alone.

Table 3. Baseline characteristics

Characteristics	HCLF-a	HCLF-c	VLCHF	Total
Sex, n (female %)	67 (51 %)	62 (55 %)	63 (52 %)	192 (53 %)
Age, mean (SD), years	41 (8.8)	43 (8.7)	41 (8.8)	42 (8.8)
Weight, mean (SD), kg	111 (19.6)	114 (17.6)	108 (17.9)	111 (18.5)
BMI, mean (SD), kg/m2	36.4 (4.3)	37.7 (5.1)	35.9 (4.8)	36.6 (4.8)
WC, mean (SD), cm	116 (11.6)	119 (13.3)	115 (12.1)	117 (12.4)

Abbreviations: HCLF-a, high-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, high-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; SD, standard deviation; BMI, body mass index; WC, waist circumference.

3.1.1 Dropout and loss-to-follow up

A portion of the participants dropped out of the study between each study visit, and some were lost to follow-up. As a result, the number of participants dropped for each study visit (**Figure 2**). At the 12-month study visit, data were collected for a total of 57 (30 %) participants. Of these participants, 14 were in the HCLF-a group, 22 were in the HCLF-c group, and 21 were in the VLCHF group.

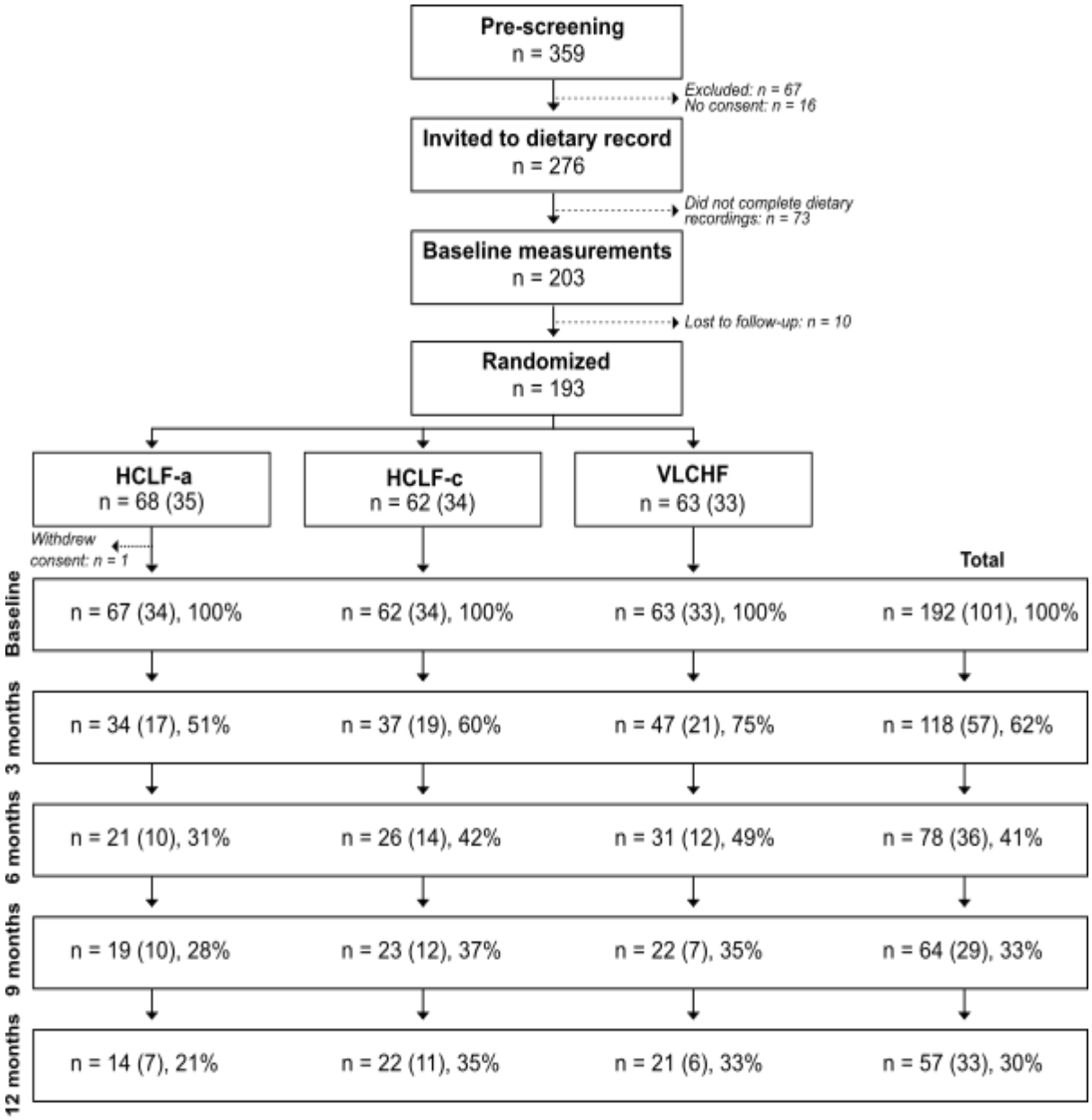

Figure 2. Flow chart of participants included at each period. Reported as "number of participants (female), % remaining participants from baseline". Abbreviations HCLF-a, high-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, high-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet.

The participants who dropped out were not systematically asked to report reasons for their drop out, however, a large proportion of the participants who dropped out of the study did state a reason. Several different reasons for the drop out were stated. The different reasons can be categorized into five general reasons for drop out: 1) Unknown (participants who did not give a reason), 2) Personal or health-related, 3) Intervention-related, 4) Lost-to-follow up, 5) Multiple reasons. A table of the distribution of the different reasons for drop out can be found in **Appendix II.**

The Little´s MCAR test indicated that missing was not completely at random, $p < 0.001$. However, this test does not indicate if the missing was MAR or MNAR. Comparisons of observed data before the missing occurred were conducted to assess whether there were any differences in between the participants who participated and the participants who dropped out. As missing data are unknown, this cannot determine whether the missing is MAR or MNAR (83), but it can provide information about differences between the participants and the participants who dropped out before they dropped out of the study. This might provide an indication of any patterns in the drop out. A table of the comparisons can be found in **Appendix III**. Body weight, BMI, weight change, and fasting subjective hunger measured at three months was significantly different between the participants who participated at the six-month study visit and those who had dropped out between the three- and six-month visit. Body weight (kg) and BMI were significantly higher, while weight change (%) and fasting subjective hunger were lower in the participants who had dropped out. Drop out at three months also differed between the diet allocations ($p = 0.019$). Of the 74 participants who did not attend the three-month visit, 45 % were allocated to the HCLF-a diet, 34 % were allocated to the HCLF-c diet, and 22 % were allocated to the VLCHF diet. At the nine-month visit mean age was significantly lower in the participants who had dropped out compared to those still participating in the study. No other tested variables differed between the participants and the participants who later dropped out.

3.2 Adherence to the dietary intervention

The participants were asked to rate their dietary adherence from no adherence (0 %) to complete adherence (100 %). Mean subjective adherence was between 62.9 % and 80 % throughout the twelve months of the study for all participants. At the three-month visit, the mean self-reported adherence in the VLCHF group was 80 %, which was significantly higher than the HCLF-a group with a mean self-reported adherence of 70.6 %. The HCLF-c group had a mean self-reported adherence of 78.4 % at the three-month study visit and did not differ significantly from any of the other groups. Mean self-reported subjective adherence to the diet did not differ between groups at any other study visit (**Table 4**). Also, self-reported subjective adherence to the intervention did not differ between sexes at any study visit.

Table 4. Self-reported adherence to the diet.

Study visit	HCLF-a	HCLF-c	VLCHF	P - value		
				HCLF-a vs. HCLF-c	HCLF-a vs. VLCHF	VLCHF vs. HCLF-c
3 month	70.6 % (63.7 - 77.5)	78.4 % (71.7 - 85.0)	80.0 % (74.1 - 85.9)	0.110	**0.042**	0.718
6 month	74.3 % (65.7 - 82.9)	72.3 % (64.6 - 80.0)	74.2 % (57.1 - 81.2)	0.733	0.987	0.720
9 month	69.5 % (60.4 - 78.5)	70.4 % (62.2 - 78.7)	72.7 % (64.3 - 81.2)	0.876	0.601	0.699
12 month	70.0 % (58.3 - 81.7)	66.7 % (57.1 - 76.2)	62.9 % (53.3 - 72.4)	0.659	0.346	0.573

Mean % subjective adherence (95 % confidence interval). No adherence = 0 %, complete adherence = 100 %.
Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; CI, confidence interval.

3.3 Physical activity

The mean PAL (**Table 5**) during the intervention did not differ between the intervention groups or sexes.

Table 5. PAL during the intervention

				P - values		
	HCLF-a (n = 28)	**HCLF-c (n = 30)**	**VLCHF (n = 36)**	**HCLF-a vs. HCLF-c**	**HCLF-a vs. VLCHF**	**VLCHF vs. HCLF-c**
PAL, Mean	1.5	1.6	1.6	0.127	0.100	0.955
(95 % CI)	(1.4 - 1.6)	(1.5 - 1.7)	(1.5 - 1.7)			

PAL is calculated based on self-reported physical activity data and is calculated using METs.
Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; CI, confidence interval; PAL, physical activity level; MET, metabolic equivalent

3.4 Dietary intake

There were no significant differences in dietary intake at baseline, in line with successful randomization. During the intervention, the mean energy intake per day was 1998 ± 410 kcal for the female and 2533 ± 435 kcal for the male participants, in line with the recommended

intake of 2000 and 2500 kcal for female and male participants, respectively. The reported daily energy intake did not differ significantly between the intervention groups at any time, with the exception of daily energy intake for men between baseline and the three-month visit (**Figure 3**). The mean daily calorie intake among male participants differed between the two HCLF groups (p = 0.016) with a mean difference of 269 (95 % CI: 79.4 – 457.9) kcal.

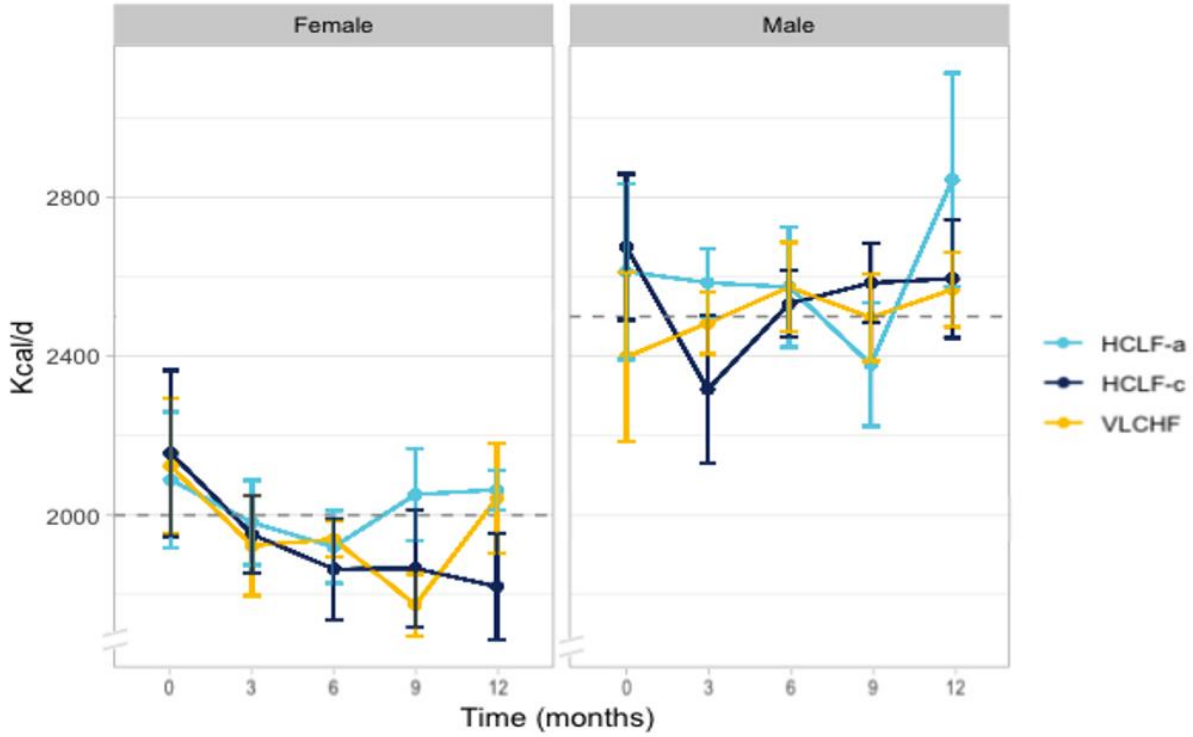

Figure 3. Mean daily energy intake in kcal for male and female participants. Error bars represent 95% CI. Data is based on dietary records. Dotted lines represent the planned intake of 2000 kcal/d for females and 2500 kcal/d for males.
Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; E%, Energy percent; g, grams; CI, Confidence interval.

Between baseline and the three-month study visit, the reported mean carbohydrate intake was 43.4 ± 3.5 E%, 41.5 ± 3.7 E%, 11.4 ± 5.0 E% for HCLF-a, HCLF-c, and VLCHF, respectively. Only one participant in the latter group had an intake of carbohydrates below the planned 8 E%, while 61 % of the participants had an intake ≤ 10 E%.

The whole-grain profiles of the higher carbohydrate diets corresponded to a 1.5-2 times increase in fiber intake on the interventions compared to baseline (from around 20 to 33-42), and up to twice as high fiber intakes compared to the VLCHF group, which showed no clear change in fiber intake from baseline (**Figure 4**).

At baseline mean dietary intake of protein was 17.7 E% before randomization. During the intervention, it varied between 15.7 and 17.0 E% (**Figure 4**). It did not differ between the

diets at any study visit, with the exception of a statistically significant difference between the two HCLF groups at the six-month visit (p = 0.035).

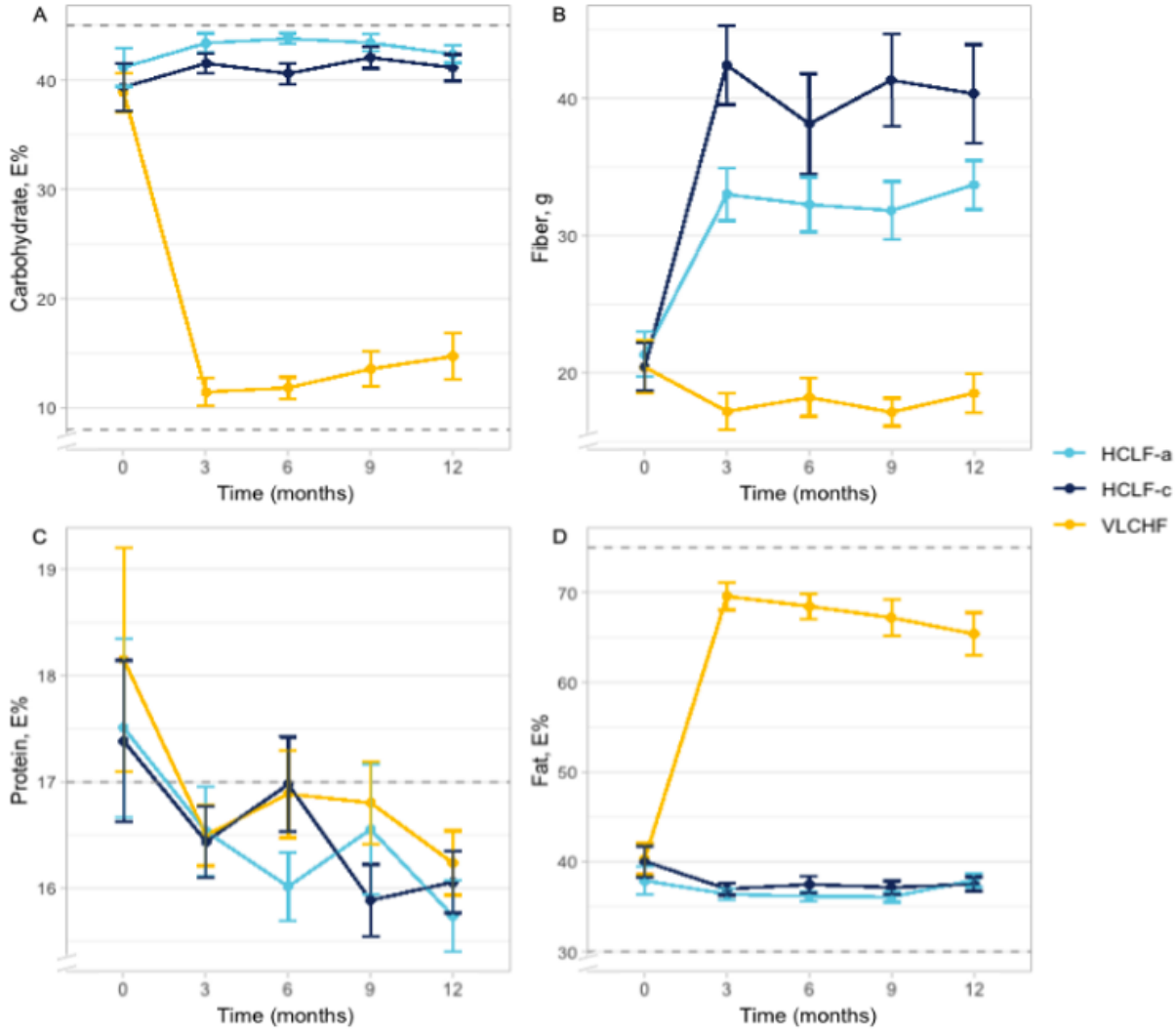

Figure 4. Dietary intake of carbohydrate, fiber, protein, and fat. Error bars represent 95 % CI. Data is based on dietary records. Dotted lines represent the planned intakes for the intervention diets. A: Mean carbohydrate intake per day, E%. 45 E%, represented by the upper dotted line, was the planned intake for the two HCLF groups, whilst 8 E%, represented by the lower dotted line, was the planned intake for the VLCHF group. B: Mean intake of fiber per day, g. C: Mean intake of protein per day, E%. The dotted line represents 17 E%, which was the planned intake for all diet groups. D: Mean intake of fat per day, E%. Dotted lines represent 30 and 75 E%, the planned intake of fat in the HCLF and VLCHF diets, respectively. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; E%, Energy percent; g, grams; CI, Confidence interval.

3.4.1 Sex differences in dietary intake

There were no significant differences in reported energy intake from carbohydrates between the sexes in the two HCLF groups. At the three- and six-month visit, there were no significant differences between the sexes in the VLCHF group., However, at the nine- and twelve-month visits, the female participants had a higher reported energy intake from carbohydrates

compared to the male participants. Mean E% from carbohydrate in the female participants increased from 17.9 (13.6 – 22.4) E% between the six and nine-month study visit to 23.5 (17.3 – 29.7) E% between the nine-month and twelve-month study visit. The mean difference in reported intake of carbohydrates was ~6.5 E% around the nine-month study visit and ~12 E% around the twelve-month study visit.

Reported dietary intake of fiber (g) was significantly higher in male participants compared to female participants in the HCLF-a group at both the three- and six-month study visit, but not at the nine- or twelve-month study visit. At the two study visits where the intake of fiber differed between the sexes, the male participants had a mean intake of ~10 g/d more than the female participants. In the HCLF-c group, there were also some sex differences in intake of dietary fiber. The male participants had a mean intake that was ~15g/d higher than the female participants at the six-, nine- and twelve-month study visit. The intake of dietary fiber did not differ between the sexes in this group at the three-month study visit. In the VLCHF group, there were no sex differences in intake of dietary fiber at any study visit.

Reported intake of protein in E% differed significantly between the sexes around the three-month study visit for the VLCHF group, but not in any other groups or at any other time points. Around the three-month study visit, the reported intake of dietary fiber was ~1 E% higher in the male participants compared to the female participants in the HCLF-c group.

There were no significant differences in reported intake of dietary fat around any study visit between the sexes in the HCLF groups. In the VLCHF group, the reported intake of dietary fat was significantly lower in the female compared to the male participants around the nine- and twelve-month study visit. The mean difference between the sexes was ~8 E% around the nine-month study visit and ~21 E% around the twelve-month study visit.

3.5 Anthropometry

At 12 months, body weight, WC, and BMI were all significantly reduced compared to baseline for all three groups (**Figure 5**). Weight change (%) from baseline values did not significantly differ between the three diet groups at any time point. Mean body weight was significantly reduced from baseline to three months and from three to six months in all three groups. From nine to twelve months, only the VLCHF group had a significant weight gain ($p = 0.016$), while the two other groups remained weight stable.

When adjusted for baseline values, mean WC did not differ between the groups at any time, with one exception being a difference of on average 7 cm (p = 0.018) between the HCLF-a group and VLCHF group at the nine-month visit for male participants only.
BMI did not differ significantly between the diet groups at any time point when adjusted for baseline values.

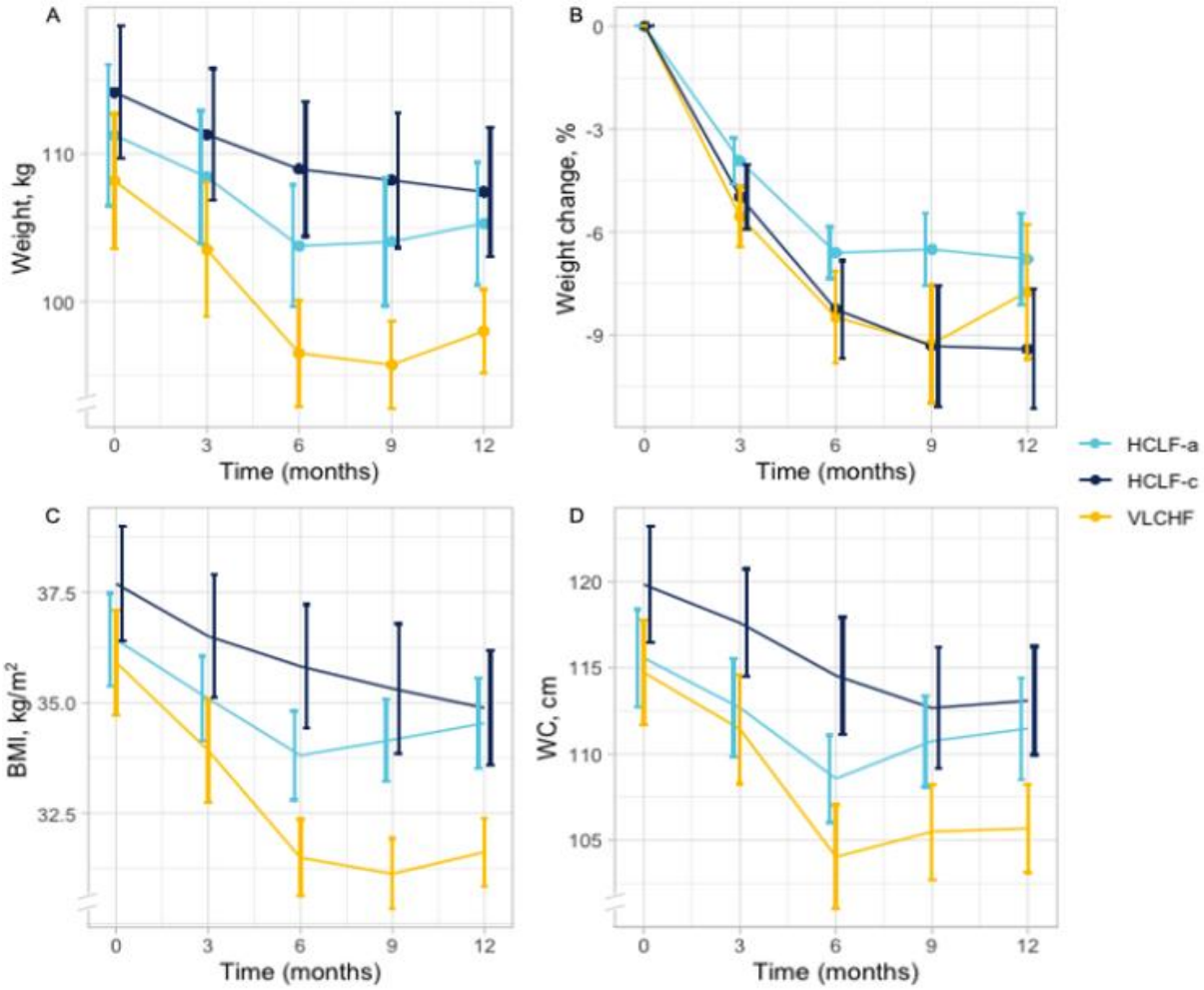

Figure 5. Anthropometry. A: Mean weight (kg). B: mean weight loss relative to baseline (%) C: Mean BMI (kg/m^2). D: Mean WC (cm). Error bars represent 95% CI. Abbreviations: WC, waist circumference; BMI, body mass index; HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; CI, Confidence interval.

3.5.1 Sex differences in anthropometry

Naturally, there were some sex differences in absolute anthropometric measurements such as body weight in kg and waist circumference in cm. Relative measurements such as change in body weight and BMI did not differ between the sexes in any of the intervention groups at any study visit.

3.6 Ketosis

Data on fasting β-HB was not normally distributed, therefore log-transformed data were used in the analyses. The log-transformed data (ln(β-HB)) was normally distributed and had few outliers and no extreme outliers.

Baseline β-HB ranged between 0.01 and 0.63 mmol/L with a median of 0.04 mmol/L and an unadjusted mean of 0.6 mmol/L. Mean ln(β-HB) was 3.78 ± 0.75 (SD), which corresponds to 0.04 ± 0.002 mmol/L in the original scale.

In the VLCHF group at the three-month visit, 28 participants (60 %) had a fasting β-HB ≥ 0.1 mmol/L, and 16 participants (34 %) had a level ≥ 0.3 mmol/L. The unadjusted group mean was 0.25 ± 0.25 mmol/L, and the median was 0.15 mmol/L. At the twelve-month visit, the number of participants with a β-HB level ≥ 0.1 mmol/L had decreased to 6 (28.6 %), and no participants had a β-HB level ≥ 0.3 mmol/L. Levels of fasting β-HB are visualized in **Figure 6**.

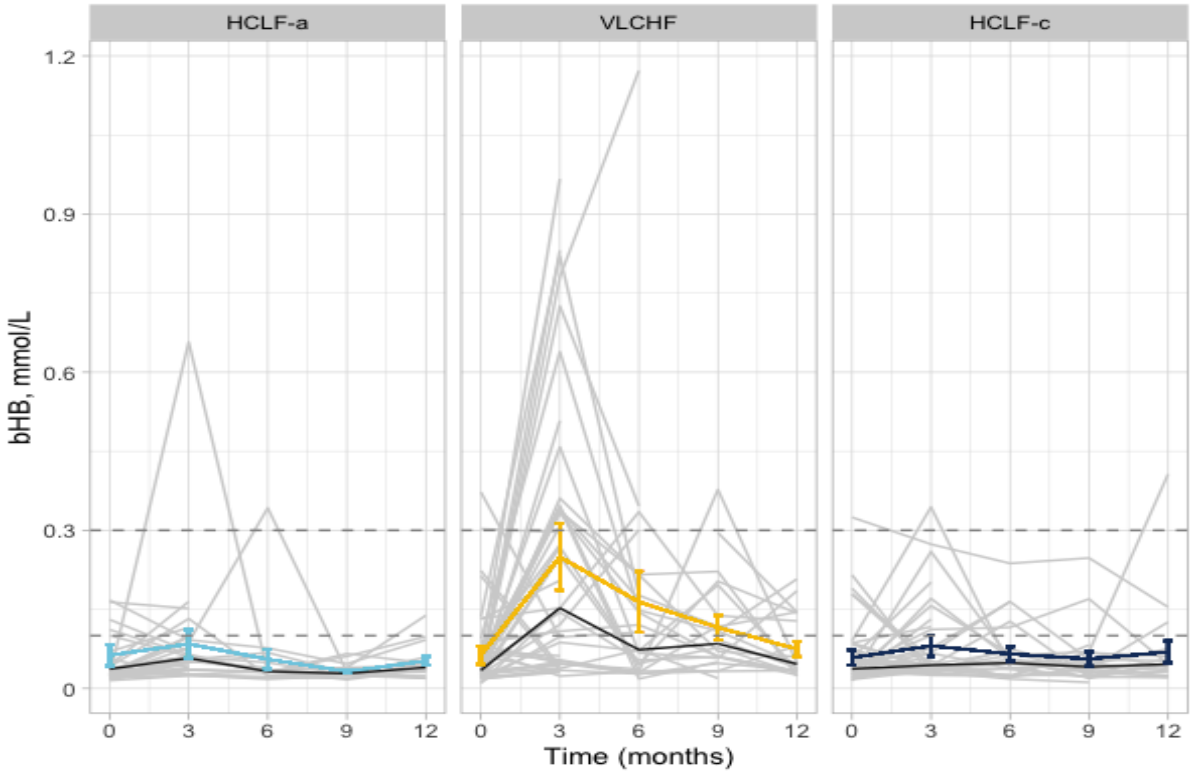

Figure 6. Mean and individual levels of fasting β-HB (mmol/L). Colored lines represent the mean. Error bars represent 95 % CI. The black line represents the median. Grey lines represent individual observations. Dotted lines represent 0.1 and 0.3 mmol/L. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; β-HB, ß-hydroxybutyrate; mmol/L, millimoles per liter.

3.6.1 Sex differences in β-HB-levels

At the three-month study visit, 10 (38 %) of the male participants and 6 (27 %) of the female participants had a fasting β-HB of 0.3 mmol/L or above. At the six-month study visit, only one male participant (5.6 %) and 4 (44 %) of the female participants have a β-HB of 0.3 mmol/L or above. Despite this, there was no statistically significant difference ($p = 0.056$) in mean level of fasting β-HB between the male and female participants at any study visit. A figure illustrating the fasting plasma level of β-HB for females and males can be found in **Appendix IV**.

3.6.2 Between and within-group differences in β-HB-levels

The VLCHF group had a significantly higher β-HB level at all study visits, except the twelve-month visit when none of the groups significantly differed. The two HCLF groups did not significantly differ at any study visit.

Mean β-HB in the HCLF-c group did not significantly differ from baseline at any other study visits. In the HCLF-a group, it differed significantly ($p < 0.001$) at the three-month visit when mean β-HB had increased to 0.06 ± 0.002. In the VLCHF group, mean β-HB differed significantly from the baseline mean at the three-, six-, and nine-month visits, but only the difference between baseline and the three-month visit remained significant after adjusting for multiple comparisons. At the three-month visit mean β-HB increased to 0.15 ± 0.003 mmol/L (calculated with ln(β-HB)) ($p < 0.001$).

3.6.3 Ketosis and dietary intake

There were no correlations between registered energy intake and level of β-HB at any study visit for either sex. Nor were there any significant correlations between the level of β-HB and registered dietary intake of protein at any study visit.

When all groups were analyzed together, there was a weak to moderate negative correlations ($r = -0.324$ – $r = -0.585$, $p < 0.05$) between the level of β-HB and registered dietary intake of carbohydrate (E%) at all study visits.
In the VLCHF group, there was a significant difference in carbohydrate intake in the participants who had a β-HB level ≥ 0.1 (0 = 0.031) and ≥ 0.3 mmol/L ($p = 0.045$) compared to those who had a lower level at the three-month visit. The participants who had a β-HB

level ≥ 0.1 mmol/L had a mean carbohydrate intake of 10.3 E% (CI: 9.3-11.3), while those who had a β-HB level of < 0.1 mmol/L had a mean carbohydrate intake of 12.2 E% (CI: 10.9-13.5). The participants who had a β-HB level ≥ 0.3 mmol/L had a mean carbohydrate intake of 9.9 E% (CI: 8.5-11.2), while those who had a β-HB level of < 0.3 mmol/L had a mean carbohydrate intake of 11.6 E% (CI: 10.6-12.7). When analyzing intake of carbohydrates in g/d, the same differences are not seen in the three-month study visit between the participants who had a β-HB level of < 0.1 mmol/L compared to those who did not ($p = 0.655$), nor between the participants who had a β-HB level of < 0.3 mmol/L compared to those who did not ($p = 0.650$). This was also true when the sex-separate analysis.

3.6.4 Ketosis and weight loss

In the VLCHF group, change in body weight (%) and β-HB was moderately inversely correlated at the three-month ($r = -0.494$, $p < 0.001$) and nine-month visit ($r = -0.518$, $p = 0.014$), but not at other visits. The two variables were not correlated in the HCLF groups (**Figure 7**). When the sexes were analyzed separately, the correlation only remained significant at the three-month visit for the male participants. No other correlations were significant for male nor female participants.

At the three-month study visit, the participants in the VLCHF group with β-HB ≥ 0.1 mmol/L had a mean relative weight change of -6.7 % (CI: -7.9 – 5.5), which is significantly greater ($p = 0.005$) compared to the participants with β-HB < 0.1 mmol/L who had a mean weight change of -3.9 (CI: -5.3 - -2.4). The participants in the VLCHF group with β-HB ≥ 0.3 at the three-month visit also significantly differed from those who had a β-HB level < 0.3. The participants with a β-HB level ≥ 0.3 had a mean relative weight change of -7.3 % (CI:-9.0 - -5.7). Relative weight change did not differ between participants with ≥ 0.1 mmol/L β-HB compared to participants with < 0.1 β-HB, nor between the participants with ≥ 0.3 mmol/L β-HB compared to participants with < 0.3 β-HB at any other study visit.

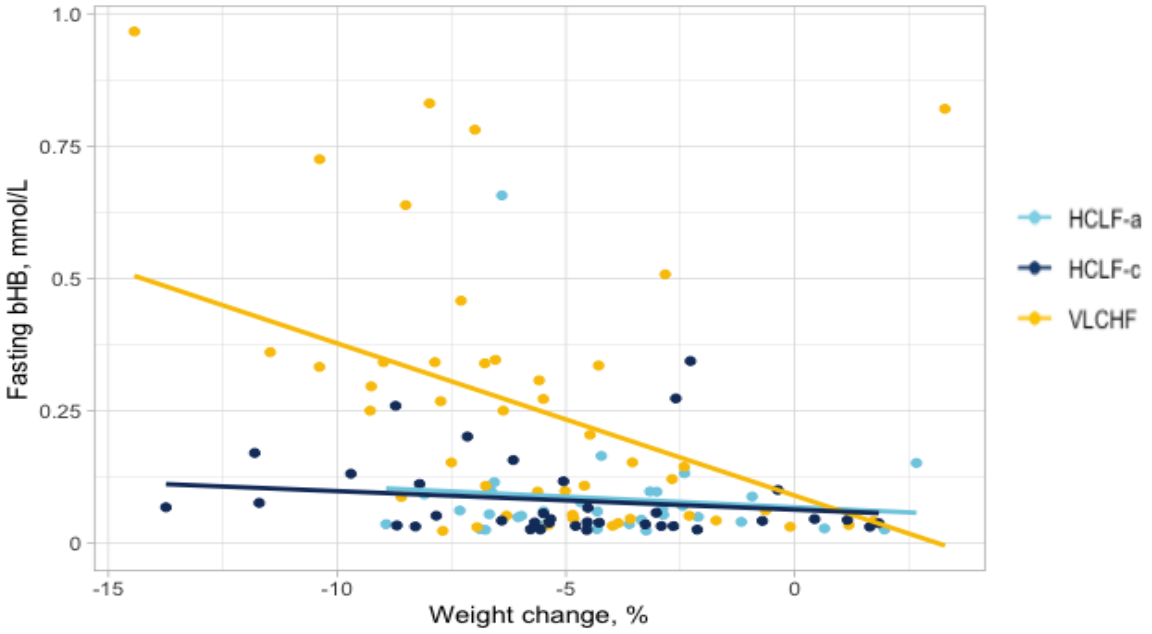

Figure 7. Scatterplot illustrating the correlations between relative weight change and level of fasting β-HB at the three-month study visit. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; bHB, ß-hydroxybutyrate; mmol/L, millimoles per liter.

3.7 Subjective appetite

3.7.1 Correlation of the VAS-questions

To assess subjective appetite, including feelings of hunger, desire to, and prospective food consumption, participants answered four questions using a VAS scale. Violin and box plots showing the distribution of the raw data from the VAS-scores for subjective appetite can be found in **Appendix V**. The associations between the VAS questions were assessed with Pearson´s correlation test. This was done to determine if there was a logical correlation between the questions and to help decide whether to include one of the questions or on a combination of the questions going forward.

The four VAS questions showed weak to strong correlations. The strongest correlation was between the questions “How hungry do you feel right now?” and “How strongly do you desire to eat right now?” ($r = 0.739$, $p = < 0.001$). The weakest correlations were between the question “How full do you feel right now?” and the other three questions. It was negatively correlated to the other questions with a Pearson´s correlation coefficient of -0.311 with “How hungry do you feel right now?”, -0.372 with “How strongly do you desire to eat right now?”, and -0.237 with “How much do you think you can eat right now?”. These correlations were all significant at a $p \leq 0.001$ level.

As the VAS questions did correlate, the main focus will be on the question “How hungry do you feel right now?” going forward.

3.7.2 Area under the curve

All participants were asked to answer the VAS questionnaire at each study visit in the fasting state and at 120 and 240 minutes after a standardized mixed meal. A proportion of the participants (21 %) were asked to answer the VAS questionnaire an additional four times, at 30, 60, 90, and 180 minutes postprandially. With data from the participants who were asked to answer the questionnaire a total of seven times, the area under the curve (AUC) was calculated using the trapezoidal method with seven (AUC7) and three time points (AUC3). AUC7 and AUC3 were found to be strongly correlated ($r = 0.931$, $p = <0.001$), using the Pearson´s correlation test. This led to the conclusion that AUC using only three time points may be a useful measure of subjective appetite in these data.

3.7.3 Subjective hunger

At baseline mean fasting subjective hunger using the 10 cm VAS scale was 3.95 ± 2.1 cm. At the same time, the mean AUC calculated with fasting, 120 minutes, and 240 minutes postprandial subjective hunger VAS-score was 918.7 ± 393.0 cm^2. Mean fasting subjective hunger at baseline did not differ between the sexes, but the baseline AUC did differ ($p = 0.027$) between the sexes. Mean baseline AUC for female participants was 858 ± 40 cm^2 and 988 ± 42 cm^2 for the male participants. The mean and individual levels of subjective hunger are visualized in **Figure 8**.

3.7.3.1 Between-group differences in subjective hunger

Fasting subjective hunger did not differ significantly between the groups at any study visit when adjusted for baseline values, neither with both sexes analyzed together nor separately. For male participants, the AUC was significantly higher in the HCLF-c group compared to the VLCHF group at the twelve-month (mean dif. = 419 cm^2, $p = 0.008$, $n = 21$), but not at any other study visits or compared to the HCLF-a group.

Change in fasting subjective hunger (study visit – baseline) differed significantly between the groups at the nine-month study visit, with a significantly greater change in the VLCHF group compared with the HCLF-a group ($p = 0.007$). The change in fasting subjective hunger did not differ significantly between the groups at any other study visit (**Figure 9**). When change

in body weight was added as a covariate in the model, the change in fasting subjective appetite was significantly lower in the HCLF-a group compared to the HCLF-c ($p = 0.030$) group at the six-month study visit and significantly lower compared to both the VLCHF ($p = 0.003$) and the HCLF-c ($p = 0.023$) group at the nine-month study visit.

In the sex-separate analysis, no significant differences in change in fasting subjective hunger were found in the female participants at any study visit. In the male participants change in the HCLF-c group were significantly different ($p < 0.05$) from the two other groups at both the three- and six-month study visit. At the nine- and twelve-month visit, the HCLF-a group differed significantly from the VLCHF (respectively $p = 0.047$, $p = 0.021$) group but not the HCLF-c group.

Neither change in 120 minutes nor 240 minutes postprandial subjective hunger, adjusted for change in body weight, did not differ significantly between the diet groups at any study visit.

When the sexes were analyzed separately, it was found that change in subjective hunger at 240 minutes postprandially at the twelve-month study visit was significantly lower ($p = 0.020$) in the HCLF-a group compared to the HCLF-c group for the female, but not the male, participants. The change in subjective hunger (study visit - baseline) at 240 minutes postprandially at the twelve-month study visit was -1.6 (-2.9 - -0.2) cm for female participants in the HCLF-a group and 0.6 (-0.5 – 1.7) cm for the female participants in the HCLF-c group. No other changes in postprandial subjective hunger, neither at 120 minutes nor 240 minutes, were found to be significantly different between the diet groups for either sex. A visualization of the changes in postprandial subjective hunger can be found in **Appendix VI**.

3.7.3.2 Within-group differences in subjective hunger

When looking at within-group effects, twelve-month AUC was significantly higher compared to baseline (mean increase from baseline = 275 cm^2, $p = 0.01$) in the HCLF-c group, but not for any other visits or intervention groups. When analyzing the sexes separately, this difference was only seen in the female participants (mean increase from baseline = 287 cm^2, $p = 0.013$). When adjusting for multiple comparisons with the Bonferroni adjustment, the difference was no longer statistically significant.

Mean fasting subjective hunger significantly increased from baseline to all other study visits for the HCLF-c and VLCHF groups. In the HCLF-a group, there was no significant change in

fasting subjective hunger from baseline. These results are from a paired samples t-test. When adjusting for multiple comparisons with the Bonferroni adjustment (adjusted $\alpha = 0.0125$), the increase in fasting subjective hunger from baseline only remained significant at the nine-month visit in the VLCHF group (3.75 ± 1.9 to 5.75 ± 1.9, $p < 0.001$). Also, when the sexes were analyzed separately, there were no significant differences between baseline and the other study visits after adjustment.

Compared to baseline, the measure of mean 120 minutes postprandial hunger did not differ within the three diet groups at any of the study visits. The same is seen with mean 240 minutes postprandial hunger at baseline compared to the same variable at any other study visit. This was true, not only for the total groups but also when the sexes were analyzed separately.

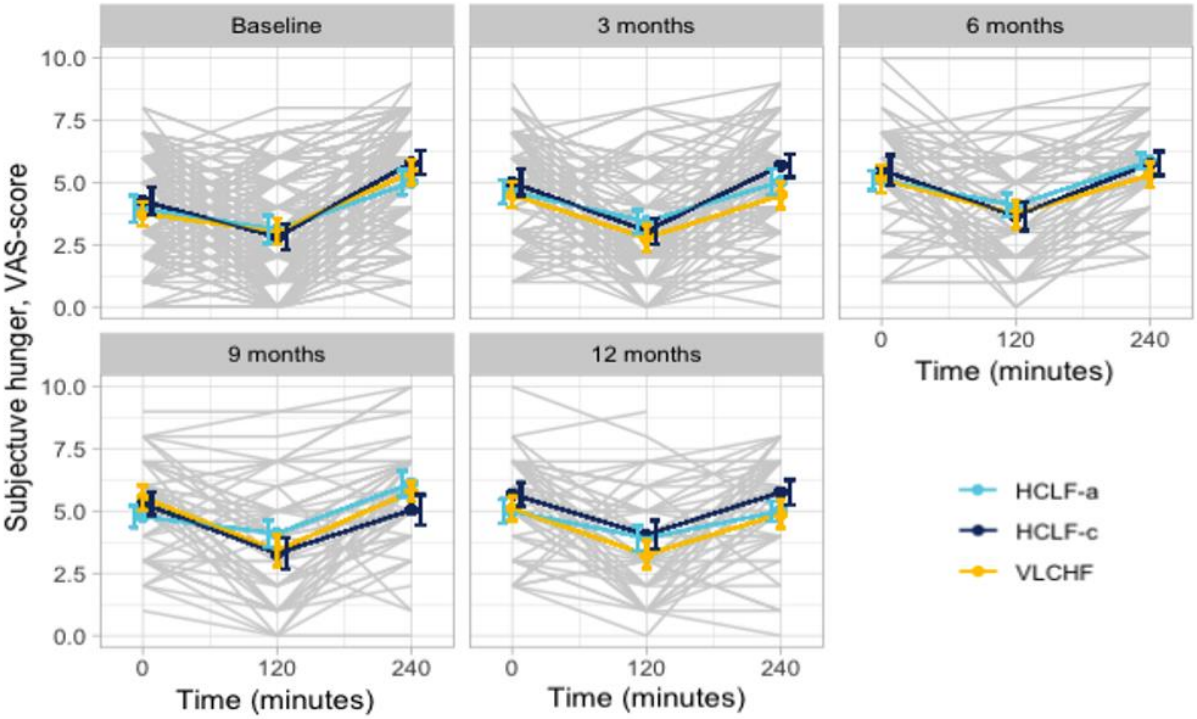

Figure 8. Subjective hunger. Mean VAS-score for subjective hunger. Grey lines represent individual observations. On the y-axis, 0 represents "not at all" and 10 represents "as hungry at imaginable" to the question "how hungry do you feel right now?". On the x-axis, 0 minutes is in the fasting state, while the two other time points are postprandial. Error bars represent 95 % CI.
Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; CI, confidence interval.

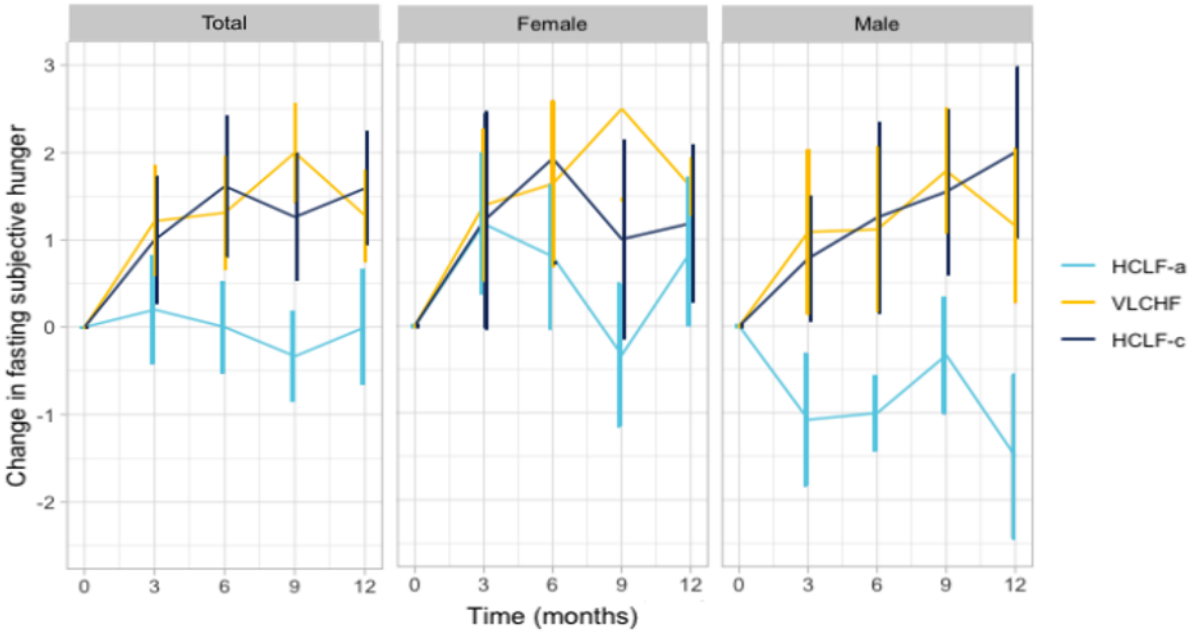

Figure 9. Change in fasting subjective hunger (VAS) from baseline. Error bars represent 95 % CI. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; CI, confidence interval; VAS, visual analog scale.

3.7.4 Subjective hunger, ketosis, and change in subjective hunger

In the VLCHF group, there were no significant correlations between fasting plasma levels of β-HB and subjective hunger, neither as fasting subjective hunger nor as AUC calculated with one fasting and two postprandial (120, 240 minutes) VAS-scores.

There were no significant differences in fasting subjective hunger between participants who had a β-HB level ≥ 0.1 or 0.3 mmol/L to those who did not, even when adjusted for age and baseline fasting subjective hunger, at any study visit.

Change in fasting subjective hunger from baseline was not significantly correlated with the level of β-HB for either group at any study visit. When the sexes were analyzed separately, there was a significant moderate correlation between the change in fasting subjective hunger and level of β-HB ($r = 0.556$, $p = 0.049$, $n = 13$) at the twelve-month study visit in the VLCHF group. No other correlations were statistically significant.

3.8 Ghrelin

3.8.1 Between-group differences in fasting total ghrelin

Fasting plasma concentrations of ghrelin (**Figure 10**) did not differ significantly between the diet groups at any of the study visits, even with baseline ghrelin as a covariate in the ANCOVA model. Since adipose tissue mass has an indirect relationship with ghrelin, weight

change was used as a covariate as well. When weight change (%) was added as a covariate, the VLCHF group differed significantly from the HCLF-a group (p = 0.046) and the HCLF-c group (p = 0.028) at three months.

The absolute change in fasting total ghrelin (ghrelin at study visit – ghrelin at baseline) (**Figure 11**) did not differ significantly between the groups at any other study visits when no covariates were added to the analysis. When weight change (%) was added as a covariate, the absolute change in fasting total ghrelin differed significantly between the groups at three months but not at any other study visits. At the three-month visit, the absolute change in ghrelin was significantly smaller in the VLCHF group compared to the HCLF-a (mean dif. = -49.5 (-92 - -7) pg/ml, p = 0.022) and the HCLF-c group (mean dif. = -48.4 (-88 - -8) pg/ml, p = 0.018).

When the sexes were analyzed separately, the significantly smaller change in ghrelin at the three-month study visit only remained significant for the female participants. In addition, the difference in ghrelin was also significantly smaller between the VLCHF and HCLF-c group at six months for female participants. There were no differences in change in ghrelin in the male participants when analyzed separately. Also, no differences in change in ghrelin were found between the sexes.

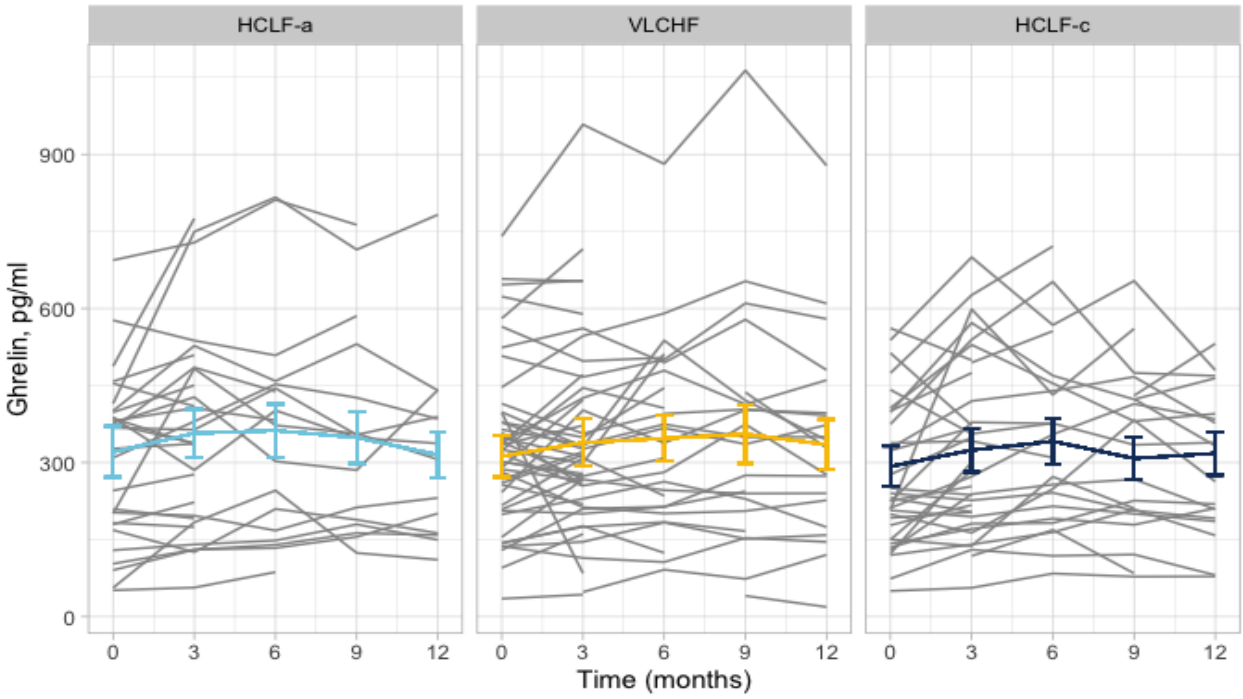

Figure 10. Fasting total ghrelin, including group means and individual values for the three dietary intervention groups. Error bars represent 95 % CI. Colored lines represent total ghrelin, and the grey lines represent individual observations. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly

cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; pg/ml, picograms per milliliter; CI, confidence interval.

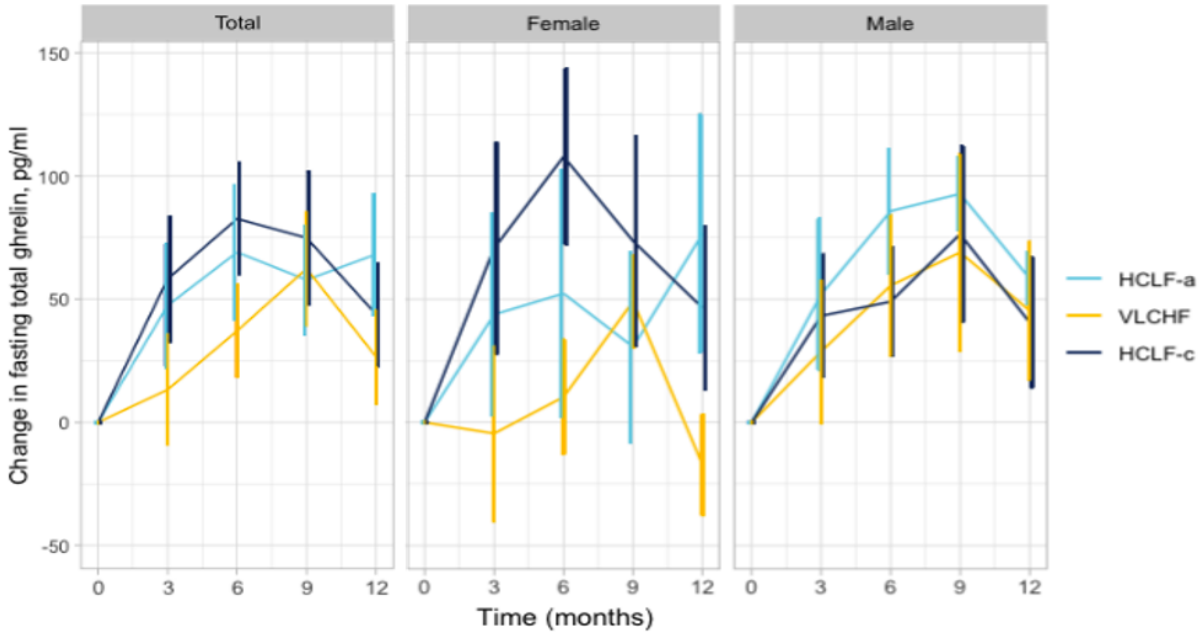

Figure 11. Change in plasma level of fasting total ghrelin. Change calculated as plasma level at study visit – plasma level at baseline. Error bars represent 95 % CI. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; pg/ml, picograms per milliliter; CI, confidence interval.

There were several outliers (1.5 x IQR outside the first and third quartile) in the ghrelin data, but no extreme outliers (2 x 1.5 x IQR outside the first and third quartile). If all outliers are removed, the main results of the ANCOVA remain the same. The only group that differs from the others at the same visit is still the VLCHF group at the three-month visit.

3.8.2 Within groups, differences in fasting total ghrelin

Within-group changes in fasting ghrelin (pg/mL) from baseline were assessed with a paired samples t-test. At the three- and twelve-month visits, ghrelin was not significantly different from baseline in the VLCHF group. With this exception, fasting total ghrelin was significantly increased for all diets at all study visits compared to baseline at the 0.05 level. However, only the HCLF-c group at six months remains significant after adjusting for multiple comparisons. Results from the test are shown in **Table 6**.

Table 6. Changes in fasting total ghrelin from baseline

Diet	**Baseline,** mean (SD), pg/ml*	**Study visit (n)**	**Δ,** pg/ml (visit -baseline)	**SD**	**p - value**
HCLF-a	309.6 (158)	3 mo (29)	47.5	102.8	0.019
	293.2 (181)	6 mo (18)	69.1	110.6	0.017
	308.3 (183)	9 mo (16)	57.8	90.2	0.022
	246.8 (133)	12 mo (13)	68.2	100.0	0.030
HCLF-c	267.6 (138)	3 mo (33)	58.2	99.2	0.002
	256.6 (140)	6 mo (21)	82.7	88.6	0.000**
	257.1 (146)	9 mo (20)	74.9	106.3	0.005
	274.1 (151)	12 mo (20)	43.8	81.3	0.026
VLCHF	332.0 (161)	3 mo (45)	13.3	88.7	0.322
	319.2 (152)	6 mo (27)	37.1	73.7	0.015
	306.5 (168)	9 mo (21)	62.4	90.5	0.005
	319.0 (164)	12 mo (20)	26.6	74.2	0.125

*Mean fasting ghrelin at baseline. As the values are from a paired samples t-test, which is a complete case analysis, the baseline mean is calculated based on complete data from baseline and the study visit it is being compared to. Hence, the baseline mean value varies in this table. **Still significant after adjusting for multiple comparisons. Adjusted alpha = 0.00125. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; vs., versus; SD, Standard deviation; dif., difference.

3.8.3 Ghrelin and subjective hunger

Spearman´s correlation was used to assess the association between subjective hunger and fasting total ghrelin. At baseline, no significant correlation between the level of total ghrelin and subjective appetite was found. The correlation coefficient and dot plot illustrating the association can be found in **Figure 12**.

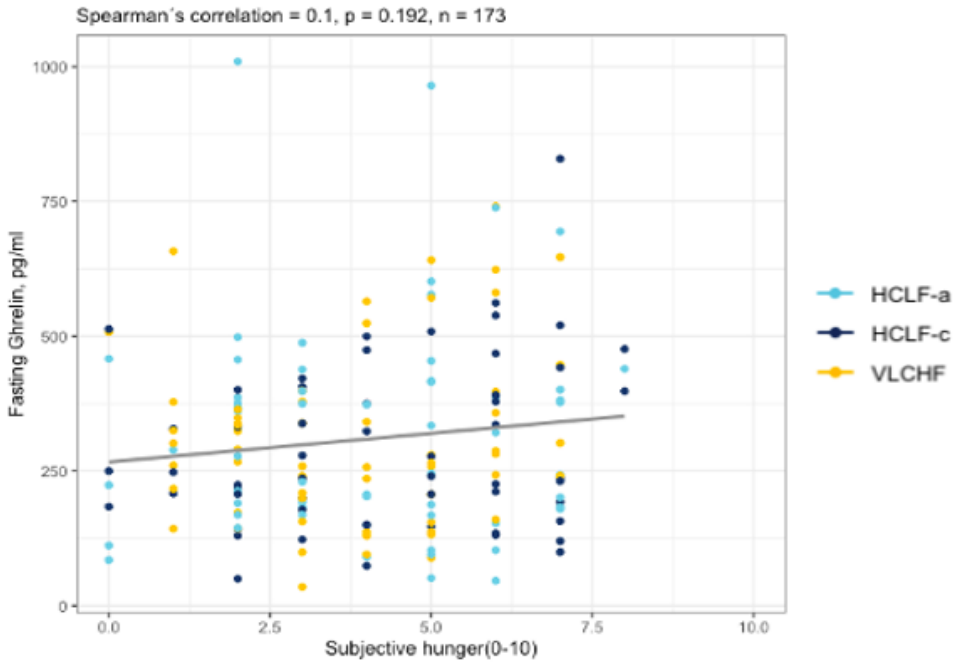

Figure 12. Fasting ghrelin and subjective hunger at baseline for the three intervention groups. Dot plot with fitted line illustrating the correlation between fasting total ghrelin and subjective hunger at baseline. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; pg/ml, picogram per milliliter.

Absolute change in ghrelin was not significantly correlated with absolute change in fasting subjective hunger for any of the intervention groups at any study visit, neither in the main analysis nor in the sex-specific analyses.

3.8.4 Ghrelin and body weight

Spearman´s correlation test was used to assess associations between total ghrelin and body weight (**Figure 13**). At baseline, the fasting plasma level of total ghrelin and weight in kg was significantly correlated with a correlation coefficient of -0.364 ($p < 0.001$, $n = 183$). When the sexes were analyzed separately the correlation remained significant for the female participants ($r = -0.272$, $p = 0.006$, $n = 99$), but not for the male participants ($r = -0.184$, $p = 0.096$, $n = 83$).

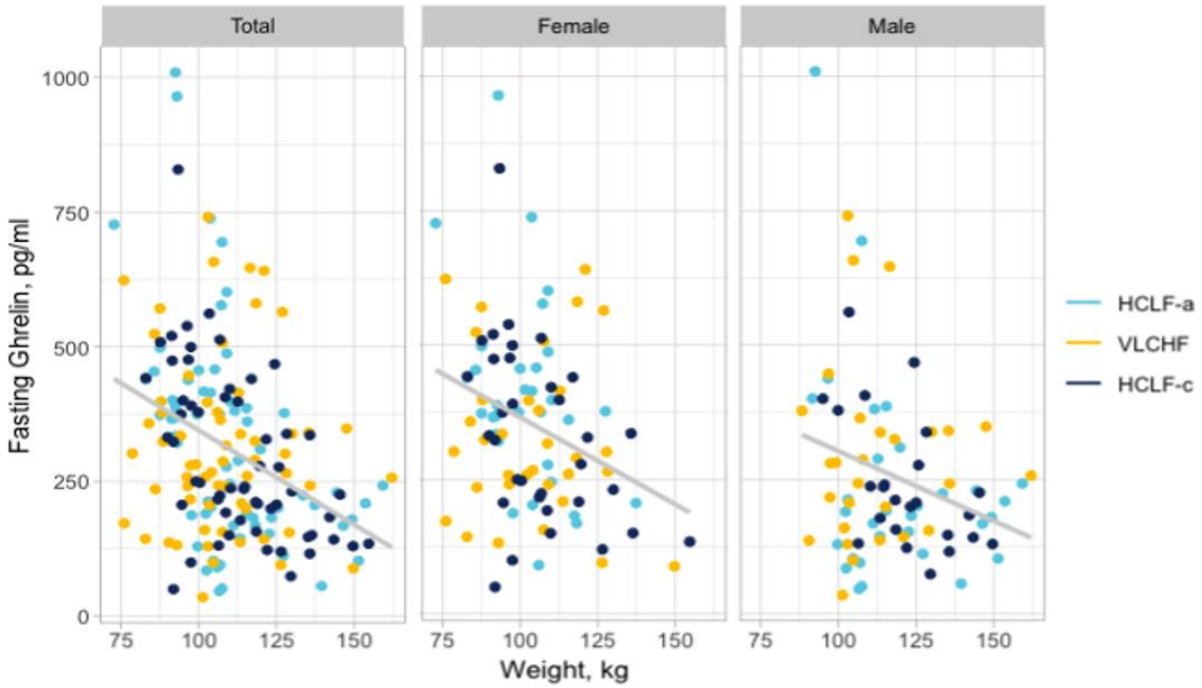

Figure 13. Scatter plot with fitted lines for fasting total ghrelin and body weight (kg) at baseline. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; pg/ml, picogram per milliliter.

Change in body weight (kg at study visit – kg at baseline) and change in fasting total ghrelin were not significantly correlated in the HCLF-a group, except for one strong negative correlation at the six-month study visit in only male participants. In the HCLF-c group, the two variables were correlated at all study visits when the sexes were analyzed together and for male participants, but not at any study visit for female participants. In the VLCHF group, the two variables were significantly correlated at the three-, nine- and twelve-month study visits, but not at the six-month study visit. In the separate sex analysis, only the correlation at the three-month visit remained significant for male participants, and no correlations were significant for the female participants.

3.8.5 Ghrelin, change in ghrelin and ketosis

There were no correlations between the level of β-HB and fasting level of total ghrelin (pg/ml) in the VLCHF group. With the exception of a moderate significant correlation at the six-month visit ($r = -0.437$, $p = 0.033$, $n = 24$), there were also no significant correlations between level of β-HB and absolute change in ghrelin (level at study visit – level at baseline) in the VLCHF group. This correlation was not significant for either sex in the separate analysis.

Levels of ghrelin were compared between participants who had a β-HB level ≥ 0.1 and ≥ 0.3 mmol/L versus in those who had a β-HB level of < 0.1 and < 0.3 or less in the VLCHF group. Weight loss and ghrelin at baseline were added as covariates in the analyses. At the three-month visit, participants who had a β-HB level of < 0.1 mmol/L had a significantly higher mean level of ghrelin compared to the participants who had a β-HB level of ≥ 0.1 mmol/L ($p = 0.029$). The same is true at six months ($p = 0.021$), but not at nine or twelve months. At the three-month visit, mean level of ghrelin was 358.5 pg/ml (CI: 335.8-380.5) in the < 0.1 group, and 315.7 pg/ml (CI: 286.3-345.1) in the ≥ 0.1 group. At the six-month visit, mean level of ghrelin was 365.8 pg/ml (CI: 341.9-389.6) in the < 0.1 group, and 312.0 pg/ml (CI: 273.7-350.3) in the ≥ 0.1 group. There was no difference in ghrelin between participants who had a β-HB level ≥ 0.3 mmol/L compared to those who had a level of 0.3 or less at any study visit, even with weight loss and baseline ghrelin as covariates.

3.9 Post hoc power calculations

As the main outcomes of this thesis were change in fasting subjective hunger and change in fasting total ghrelin, these variables were included in the post hoc power calculations.
Post hoc power was calculated for change in fasting total ghrelin at the three-month visit. With an alpha level of 0.05, a total sample size of 107 participants, and the effect size of 0.279, the power was calculated to 0.72 at the three-month study visit. Post hoc power was also calculated for change in ghrelin at the twelve-month visit. At the twelve-month visit, the power was calculated to 0.41, with an effect size of 0.283, an alpha of 0.05, and a total sample size of 53. For the power to be 0.8 with the same effect size, the required minimum total sample size was estimated to be 124 participants at the twelve-month study visit.

For change in fasting subjective hunger, the effect size was 0.16, and the total sample size was 109 at the three-month visit. With an alpha level of 0.05, the power was calculated to be 0.31.
At the twelve-month visit, the effect size for change in fasting subjective hunger was 0.266, and the total sample size was 51. With an alpha level of 0.05, the power at twelve months was calculated to be 0.36. For the power to be 0.8 with the same effect size, the required minimum total sample size was estimated to be 140 participants at the twelve-month study visit.

4 Discussion

Previous dietary intervention studies for fat loss have suggested that increases in appetite due to energy restriction may contribute to loss of diet adherence in the long term (17). Dietary strategies that attenuate this effect on appetite may facilitate long-term fat loss or at least reduce fat regain, which is often seen in longer-term dietary interventions (16). Studies have shown that levels of the orexigenic hormone ghrelin increase during fat loss, while ketosis may have an appetite-suppressing effect. The primary aim of this study was to determine if a VLCHF diet might result in a lesser short-term (three months) and long-term (one year) increase in fasting levels of ghrelin, as well as subjective hunger, compared to high-carbohydrate, low-fat diets, despite expected weight loss in all three groups. The secondary aims were to determine if there are differences between the cellular and acellular HCLF diets in regard to appetite and if there are sex differences in regard to appetite between all three groups. To address the aims, the fasting levels of ketone bodies β-HB (mmol/L), fasting levels of total ghrelin (pg/ml), and both fasting and postprandial subjective hunger (VAS-score) were evaluated and compared in the three intervention groups of the CARBFUNC study.

In the following sections, methodological strengths, limitations, and factors that could have affected the results will be discussed. Subsequently, the results and their relation to existing literature will be discussed.

4.1 Methodological discussion

4.1.1 Intervention

To support that the participants in the CARBFUNC trial adhered to their allocated diet in their everyday life, several measures were taken to facilitate good adherence. Participants who lived together or had close relations were randomized together, as it would be more practical for them to follow the same diet. Numerous easy-to-prepare recipes were available and tailored to each intervention diet. This might have aided adherence in several ways, both because of the large number of options and because the tailored recipes were available electronically.

To further aid adherence throughout the trial, 45-minute motivational interviews were conducted between baseline and the three-month study visit. Furthermore, participants were given a choice of individual counseling or group sessions between the remaining study visits. Not only ensuring that the participants follow their allocated diet but also ensuring that they honestly record their intake was important. The participants were instructed to perform two 3-day dietary records before the baseline visit and 3-day dietary records every two weeks between study visits. To ease the recording, a digital platform for recording dietary intake electronically (www.diett.no, Dietika AS) was used. To assess dietary intake, 7-day dietary records are sometimes considered a "gold standard" (84). Recording dietary intake may be considered a burden to the participants, but when recording less frequently, all foods or nutrients may not be captured (84). When using 3-day dietary records, foods that are only eaten episodically may not be reported, but it is also likely less of a burden on the participants compared to 7-day dietary records. The repetition of the 3-day dietary record every two weeks may help capture seasonal variations in the diet.

Even though several measures were taken to ensure adherence throughout the trial, following the intervention diets may have been demanding as it required the participants to strictly follow a diet that was likely to be different from their habitual diet. When eating out, during travels, holidays, or celebrations, and in social settings, following the allocated diet may have been particularly challenging. This has been seen in other studies (85). Adherence to the intervention diet is a well-known challenge in human nutrition research, and adherence has previously been shown to be a predictor of long-term weight loss success (86).

4.1.1.1 Intervention diets

In the CARBFUNC study, the two HCLF diets had similar proportions of macronutrients but were distinguished by the carbohydrate quality. The HCLF-a diet had a higher amount of powdered carbohydrate sources such as flour. Store-bought bread was the primary source of carbohydrates in the HCLF-a group, and fruit was the primary source of carbohydrates in the HCLF-c groups (Data not shown). Flours can differ in grade of milling. Participants randomized to this diet were encouraged to choose products with the "keyhole" label. For products like bread to use the "keyhole" label, it must contain at least 30 % whole grain flour (68). The HCLF-a diet was planned to be more processed than the HCLF-c diet.

Both the HCLF-a and the HCLF-c diets were relatively high in dietary fiber content, however, the type of fiber may vary between the diets. Naturally, different fruits and different breads contain different types of fiber, so it is difficult to determine what types of fiber were

frequently eaten on the different diets without investigating the dietary records in more detail. In general, fruits are often a good source of pectin, which is a water-soluble type of fiber, and fruit can also be a good source of insoluble fiber such as cellulose (87). The fiber content in bread may also vary greatly in the type of fiber, based on the types of cereal grain that are used in the bread. Many cereal grains are high in hemicellulose, which is a water-insoluble type of fiber (87). Cereal grains such as oats and barley are good sources of ß-glucan, which is a water-soluble type of fiber (88).

As the VLCHF diet had a planned carbohydrate content of 8 E% from carbohydrates, it was expected to be a ketogenic diet if followed as planned, although the total amount of energy must also be taken into account. Ketogenic diets have previously been shown to lead to several beneficial effects such as weight loss, reduction in hyperinsulinemia, and improvement in insulin sensitivity (89), but there are also some negative effects associated with ketogenic diets (45, 89, 90). Vitamin and mineral deficiencies might be a concern (89), especially if the diet is very one-sided and excludes certain food groups, but this is not limited to ketogenic diets. It has previously been reported thiamine deficiency in an individual consuming only foods of animal origin and no vegetables (45). However, this was not a major concern for the VLCHF intervention diet in the CARBFUNC study, as it is designed to cover all nutritional needs, and participants were encouraged to eat at least five portions of fruits and vegetables per day. Other potential adverse effects are negative effects on blood lipids and kidneys. As ketogenic diets are typically high-fat diets, concerns regarding blood lipids are common, but on the contrary, there is evidence that ketogenic diets might improve blood lipids (56, 89). A meta-analysis by Mansoor et al. (91) found that low-carbohydrate diets may lead to some favorable changes in some blood lipids, but it may also lead to less favorable changes specifically in LDL cholesterol and that the changes must be weighed against each other. Several studies and meta-analyses have found that intake of saturated fats and coronary heart disease is not associated (92). On the other hand, the general recommendation is still to have an intake of 25-40 E% for fat, whereof less than 10 E% from saturated fatty acids (93). Potential negative effects on the kidneys have been suggested to be linked to high levels of nitrogen excretion and a high intake of dietary protein (56). However, ketogenic diets are not necessarily high-protein diets. The ketogenic diet in the CARBFUNC study has a planned proportion of protein of 17 E%, which is in line with the Nordic Nutrition recommendations (2012) of 10-20 E% from protein (93).

4.1.2 Outcome measures

4.1.2.1 Subjective appetite

Subjective appetite was measured in the fasting state as well as postprandially. The postprandial measures were taken after the ingestion of a standardized mixed meal. The meal was the same for all participants, regardless of the intervention group. For this reason, the postprandial measurements do not reflect acute effects of the intervention diets but could reveal more long-term or tonic effects of the intervention diets.

Subjective appetite was measured using VAS questionnaires. When VAS data are collected in the fasting state and several times postprandially, AUC can be calculated, and it may be used to assess the overall postprandial response in appetite. How many times participants are asked to fill out the VAS questionnaire varies in the literature but is typically done up to 2-4 hours postprandially (5, 6). In the CARBFUNC trial, participants initially filled out three VAS questionnaires in conjunction with the meal test (fasting, 120 and 240 minutes postprandially), but to gain more detailed data, this was expanded by also including 30, 60, 90, and 180 minutes postprandially for a subset of participants (n = 40, 21 %). With only three VAS-questionnaires filled out at each study visit by most participants, AUC was not necessarily an appropriate measure for appetite during the study visits. However, the more detailed data for the subset could be used to assess the relevance of the 120 and 240 min time points. In the current study, the AUC calculated with the three time points were correlated with AUC calculated with seven time points in the subset, which indicates that AUC calculated with only the three timepoints may be appropriate to assess appetite. The paper VAS lines were measured and plotted by the researchers. Unfortunately, the printed VAS lines were not consistently 10.0 cm (some were 9.6 cm), so this had to be taken into account when measuring. This might have led to some errors in the measurement of reported subjective appetite, although it is unlikely as the individual VAS scores were plotted as whole centimeters without decimals.

4.1.2.2 Ghrelin

Ghrelin was measured as total ghrelin in fasting plasma samples, and measurements of the bioactive form acylated ghrelin (AG) were not performed. To measure AG, the plasma samples must be treated to prevent degradation, a procedure not performed in the CARBFUNC-study (67). However, most of circulating ghrelin is acylated (27). Several studies indicate that des-acyl ghrelin, the inactive form, has some effects of its own, but its

effect on food intake remains unclear. It has also been suggested that des-acyl ghrelin might be a product of sample handling (27). Previous studies have shown that AG and total ghrelin have similar responses to macronutrient intake (39) and a similar relationship between total ghrelin and hunger and acylated ghrelin and hunger (94). In our study, only fasting total ghrelin was analyzed. There has previously been seen a postprandial fall in ghrelin when nutrients are administered to the upper parts of the small intestine (27). This postprandial fall is affected by the type of nutrient ingested. As postprandial ghrelin was not analyzed, it was unfortunately not assessed in this study.

4.1.2.3 Ketone bodies

The two most abundant ketone bodies, β-HB and AcAc were both measured in fasting plasma samples using GC-MS/MS, an accurate and precise method (95).
In the literature, the primary focus is on β-HB (6). Other studies have measured level of ketosis using urine ketone strips or blood test strips (4, 51). Urine or blood strips were not used in the CARBFUNC study. These types of tests are quick and may be practical for controlling that participants were ketotic at the study visits (4). Doing this might give an indication of adherence during the intervention and might help motivate participants to adhere closely to the intervention. On the other hand, it may also increase the burden of the intervention, and it may also affect how the participants experience the intervention.

4.1.3 Strengths

A strength of this study is the RCT study design. Part of the design is randomization, which minimizes the risk of bias, particularly allocation bias (96). Another part of the study design is that the study was controlled. The control groups were active controls receiving another intervention and can be considered active comparator groups (97). This allows the interventions to be compared to determine which is more favorable. Another benefit of this control design is that it may be considered more ethical as all participants receive an intervention (96).
Another strength of this study is the comparability of the three intervention diets. They are all normocaloric and have the same planned proportion of energy from protein. Most studies investigating the effects of ketogenic diets on appetite assess the effects of either a VLED or an ad libitum KLCD (6). The CARBFUNC trial stands out by having only a modest calorie restriction and normocaloric interventions of 2000 (females) or 2500 (males) kcal per day. The modest calorie restriction is sufficient to result in weight loss, but it might not be as rapid

or great as what could be expected in a more calorie-restricted diet. Because of the normocaloric nature of the diets, possible confounding effects of ad libitum intake, low energy intake, and rapid weight loss will not influence the results. Also, the planned proportion of energy from protein was 17 E% in all three intervention diets, increasing the likelihood of affecting all groups equally and not influencing the results. This is especially relevant as protein is known to affect appetite and is the most effective macronutrient group in reducing ghrelin levels (39).

The relatively long duration of the study is a further strength. The recently completed CARBFUNC trial had a duration of two years, but not all two-year data were available to be included in this thesis. However, the one-year data included in the current thesis provides a unique opportunity to investigate the effects of a normocaloric ketogenic diet on appetite in the long run, as it has not been investigated for more than 12 weeks in previous literature. The long duration may also be a strength as it may, at least to a certain degree, prevent the results from been affected by seasonal or cyclic changes. The menstrual cycle, for instance, is known to affect appetite (98), but the relatively long duration of the study and the multiple study visits may contribute to the attenuation of such effects.

4.1.4 Limitations and possible sources of bias

Despite efforts to minimize drop out, a main limitation is the relatively large attrition rate of 70 % at the twelve-month study visit. A proportion of missing of 40 % or more is often considered very large (83). The Little´s MCAR test indicated that the missing was not MCAR. However, this does not indicate whether the missing is MAR or MNAR. By definition, the missing data are unknown (83). Hence, one cannot determine if the missing is MAR or MNAR based on the observed data.

Missing data were to a certain degree handled by the chosen statistical methods (ANOVA, ANCOVA, and paired t-test), which are all complete case analyses. Complete case analysis may handle missing data, however, using this approach also has some disadvantages, including a reduced number of observations and loss of power (97). For the results of a complete case analysis to be unbiased, the missing excluded should be MCAR. Since the missing is unlikely to be MCAR, the results of these analyses could be biased. This is important to keep in mind when assessing the results.

Power calculations regarding the primary outcome of the CARBFUNC study (change from baseline in visceral fat volume measured by CT) were conducted *a priori* based on previous research (99). The power calculations indicated that a minimum of 18 participants should be

included in each intervention group to determine group differences on this primary outcome. However, the main outcomes of the current thesis are change in subjective appetite quantified by VAS and change in fasting total ghrelin. No *a priori* power calculations regarding these outcomes were conducted. Hence, the study might not have the appropriate power to determine group differences on these outcomes. *Post hoc* power calculations were conducted, and the results may indicate that the statistical power was below 0.8 for both outcome measures used in the calculations, with an alpha-level of 0.05. This may be interpreted as an indication that the study was not appropriately sized for these outcome measures and that the probability of rejecting a false null hypothesis was suboptimal (i.e., higher type II error rate than wanted). However, calculating post hoc power is not always considered good practice, and the confidence intervals indicate the range of likely effect sizes, given the observed data (100).

Another limitation of the study is that it is an open-label study. With the exception of a blinded study statistician, both the researchers and the participant knew which intervention arm the participant was randomized to. This could potentially affect how the participant reported data and how the researcher interpreted them. Awareness of this potential bias and emphasizing the importance of honest reporting of data to the participants may have helped minimize the risk of this. Ideally, the study should have been double-blinded, but this was not possible due to the food-based design of the dietary interventions. This limitation is not unique for the CARBFUNC study, as blinding is a common challenge in dietary research (96).

As ghrelin is not the only appetite-related hormone (23, 24, 26), the choice of only measuring ghrelin can also be considered a limitation. Other studies investigating the effect of ketogenic diets on appetite have analyzed other hormones in addition to ghrelin, such as PYY, GLP-1, and CCK (4, 54).

In line with the inclusion and exclusion criteria, all participants were obese and otherwise relatively healthy. Individuals with diabetes, resent treatment with surgery or antibiotics, and individuals treated with statins were all excluded. This could both affect the generalizability of the findings and potentially lead to “healthy worker”-bias, which is a type of selection bias. However, blood samples and anthropometric measurements were taken by trained study personnel following a predefined study protocol. This may have reduced the risk of both systematic and random errors, but the risk of information bias, such as measurement errors, cannot be ruled out entirely.

4.2 Discussion of results

The results of the current thesis suggest that there may be a ghrelin suppressing effect of the VLCHF diet, however, the expected effect of the VLCHF diet on subjective appetite was not seen. The results also suggest that the two HCLF diets affect subjective appetite differently. In this study, all intervention groups lost weight, and the relative weight loss did not differ significantly between the groups. Hence, all groups were expected to experience an increase in ghrelin and subjective appetite, but the increase was expected to be smaller in the VLCHF group compared to the HCLF groups.

4.2.1 Adherence

In any dietary intervention, especially when long-term, the participant's ability to follow the prescribed diets is key to enabling meaningful interpretation. What constitutes sufficient adherence is a subject for discussion. In the present study, we had two HCLF groups that were relatively similar and one VLCHF group that was markedly different from both of these. Self-reported subjective adherence to the allocated diet was higher earlier in the intervention compared to at the end of the twelve-month period for all intervention diets. At the three-month visit, mean self-reported adherence to the intervention was 71 %, 78 %, and 80 % for the HCLF-a, HCLF-c, and the VLCHF groups, respectively. At this time point, adherence statistically differed between the VLCHF group and the HCLF-a group, with a mean difference of less than 10 %. The clinical significance of this difference may therefore be debatable. At the twelve-month visit, the mean self-reported adherence had fallen to 70 %, 67 %, and 63 % for the HCLF-a, HCLF-c, and the VLCHF group, respectively. The degree of adherence to the diets might be considered relatively high, considering that the diets were followed for a longer period of time. On the other hand, this level of adherence might not be high enough to determine the effects of the planned diets. This concern is especially raised regarding the intake of carbohydrates in the VLCHF diet, which needs to be followed relatively strictly to reach a state of nutritionally induced ketosis (6, 45).
However, the dietary records support that considerable differences in both dietary quality and quantity were achieved. Furthermore, the self-reported adherence in CARBFUNC is similar to the self-reported adherence found in another long-term dietary intervention study with different interventions (85) and higher compared to the estimated adherence in some studies (101).

The mean reported energy intake throughout the intervention was in line with the planned intake for all three diets, with few exceptions. This indicates that in regards to caloric load, the participants complied well to the study intervention. As this is based on self-reported data, the data could be affected by self-report bias.

4.2.2 Dietary intake

Carbohydrate intake was in line with the planned energy percent on both high-carbohydrate diets. However, only one participant in the VLCHF group reported an intake of ≤ 8 E% in line with the planned diet. Mean intake of carbohydrates was 11.4 E% in the VLCHF group at the three-month visit, and it increased to close to 15 E% at the twelve-month visit. This increase in intake of carbohydrates may be seen in relation to the decrease in self-reported adherence to the diet. Both the increase in carbohydrate intake and the decrease in self-reported adherence may further be related to the fall in mean β-HB level in this group after the three-month visit. Interestingly, for the VLCHF group, there were significant sex differences in the reported intake of carbohydrates around the nine- and twelve-month study visit. This indicates that the male participants in the VLCHF group adhered more strictly to the intervention diet than the female participants in the second half of the trial, even though there were no significant sex differences in self-reported adherence to the diet. This might demonstrate a discrepancy between the self-reported adherence and the recorded dietary intake.

Differences in intake of dietary fiber may affect appetite (102). The VLCHF group had no clear change from baseline (fiber intake ~20 g/d), while the intake of dietary fiber increased to ~40 g/d in the HCLF-a group and to ~33 g/d in the HCLF-c group. As expected, male participants with a higher planned energy intake (2500 vs. 2000 kcal/d) also had a significantly higher intake of dietary fiber compared to female participants in both HCLF groups. The intake of dietary fiber did not differ significantly between the sexes in the VLCHF group. Fiber can affect appetite in several ways, including by contributing to bulk and viscosity, increasing transit time, and through colonic fermentation (103). Hence, the differences in fiber in the diets could potentially have an effect on appetite.

Although there were statistically significant differences in protein intake, the difference was less than 1 E%, and the clinical significance is questionable. Studies that have investigated the effects of protein on appetite have typically investigated a much higher protein content, for

instance, dietary interventions with 30 E% from proteins or test meals with 80 E% from proteins (39, 52). The intake was close to the planned intake of 17 E% for all groups at all study visits. Male participants in the HCLF-c group also had a significantly higher intake of protein in E% compared to female participants around the three-month study visit. Although statistically significantly different, the mean difference was only 0.9 E%, and it is not likely to have a clinical significance.

The mean daily intake of dietary fat was planned to be 75 E% in the VLCHF diet and 30 E% in the two HCLF diets. In the VLCHF group, the reported mean intake of fat was approximately 70 E% at the three-month visit and gradually decreased to approximately 65 E% at the twelve-month visit. This can be seen in relation to the simultaneous increase in dietary intake of carbohydrates in this group. In the HCLF groups, mean reported intake of dietary fat was higher than the planned content of 30 E%, and above 35 E% and below 40 E% throughout the intervention. As the reported intake of protein was relatively close to the planned limit, the reason why the intake of fat was higher than the planned limit can in part be because the intake of carbohydrates was a little lower than the planned limit and because the planned limits of 17 E% from protein, 45 E% from carbohydrates and 30 E% from fat does not add up to 100 E%. There were also some sex differences in reported intake of fat. Female participants in the VLCHF group reported a lower intake of dietary fat around the nine- and twelve-month study visits compared to male participants. This is related to female participants having a greater intake of carbohydrates compared to male participants at the same time. Again, this might indicate that male participants have adhered more strictly to the VLCHF diet in the last six months of the twelve-month intervention.

4.2.3 Ketosis

Although the energy intake was relatively high in the CARBFUNC study, the planned low carbohydrate intake was expected to induce at least some degree of ketosis, and based on previous studies (6, 45), we hypothesized that this might induce suppression of appetite on the VLCHF diet. Mean fasting levels of β-HB at baseline was in the expected range, below 0.1 mmol/L, for all three groups (5). As expected, the mean fasting β-HB was below 0.1 mmol/L throughout the trial, even though there were some outliers in both HCLF groups. The higher level of β-HB in the few HCLF outliers might be due to prolonged fasting, as fasting can induce ketosis (56), but the time of the last consumed meal was not assessed in the current

thesis. In the VLCHF group, 34 % of the participants had a fasting level of β-HB of 0.3 mmol/L or above, and 60 % of the participants had a level of β-HB of 0.1 or above at the three-month visit. 0.3 mmol/L is sometimes considered a threshold for nutritionally induced ketosis, even though there is not a clear consensus for this (6). It is also the lowest level of β-HB that Gibson et al. (6) suggested to be sufficient to achieve effects on appetite. After the three-month study visit, the mean and median level of β-HB gradually decreased. At the twelve-month study visit, no participants in the VLCHF group had a β-HB level of 0.3 mmol/L or above, and only 27 % (6 participants) had a level of β-HB of 0.1 or above. The VLCHF group did not at any time have a mean or median level of β-HB above the 0.3 mmol/L threshold, but it was well above 0.1 mmol/L the three-month visit. The group did not differ significantly from baseline at any other visit. Even though there were some sex differences in reported dietary intake of carbohydrates, there was no statistically significant sex differences in level of fasting β-HB for any of the intervention groups at any study visit. There may be several explanations for the relatively low level of β-HB in the VLCHF. One explanation, and perhaps the most important one, is that many participants had an intake of carbohydrates that were likely to be too high to induce ketosis (6, 45). Another explanation is that the diet is only moderately energy-restricted and gives larger amounts of protein than a VLED would. The studied VLEDs with 500-600 kcal typically contains 50-60 g protein per day (6), while the planned VLCHF diet had a protein content of 17 E% and 2000 - 2500 kcal (~85 - 105 g/d). It is known that several amino acids, especially alanine, can serve as gluconeogenic precursors (23). For this reason, absolute amounts of protein in the diet may contribute to counteracting nutritionally induced ketosis. Of the four identified studies investigating the effects on appetite of non-energy restricted or normocaloric KLCD (50-53), only one measured the achieved level of ketosis. Lodi et al. (51) reported that mean β-HB increased with 2.1 ± 1.6 mmol/L from baseline in participants adhering to an *ad libitum* KLCD and with 1.5 ± 0.8 mmol/L from baseline in participants adhering to an energy-restricted (1000 - 1100 kcal/d) ketogenic diet. Previous studies investigating the effect of VLEDs have reported relatively higher β-HB levels. Nymo et al. (4) reported a level of β-HB of 0.6 ± 0.13 mmol/L after just three days on a VLED. Both Lodi et al. (51) and Nymo et al. (4) found that the ketogenic diets were associated with suppression of an increase in appetite. In the review and meta-analysis by Gibson et al. (6), the studied levels of β-HB was also > 0.3 mmol/L. If the relatively low level of β-HB reported in the current study does affect appetite,

it could indicate that only very mild ketosis is needed, and it would be interesting for future research.

4.2.4 Subjective appetite

As all groups in the CARBFUNC trial lost weight, one might expect that the HCLF groups, and to a lesser extent, the VLCHF group, would have reported an increase in hunger. However, this was not the case. When change in body weight was added as a covariate in the model, the change in fasting subjective hunger differed significantly between the groups at the six- and nine-month study visit. The change in the HCLF-a group was smaller compared to the HCLF-c group at six months and smaller than both the HCLF-c and the VLCHF group at nine months. When the sexes were analyzed separately, there were no significant differences in change in fasting subjective hunger in the female participants. In the male participants, the change was significantly smaller in the HCLF-a group compared to the HCLF-c group at three and six months and smaller than both the HCLF-c and the VLCHF group at nine and twelve months. This is surprising as the subjective hunger was expected to increase in both HCLF diets as participants lose weight outside of ketosis (6, 42).

In the sex-separate analysis of change in postprandial subjective hunger, female participants in the HCLF-a group had a lower change in subjective hunger at 240 minutes postprandially at the twelve-month study visit, compared to the HCLF-c group. This was unique for this study visit, and it is possible that it has been affected by the relatively low sample size at the end of the trial.

Neither of the within-group changes was in line with what could be expected based on previous studies. Martins et al. (42) found that fasting subjective hunger significantly increased from 4.1 ± 1.6 cm to 6.5 ± 2.5 cm after 12 weeks and after an exercise-induced weight loss of 3.5 kg. In the HCLF-a group, mean fasting subjective hunger varied between 4.4 cm and 5.3 cm but did not significantly differ from baseline to any other study visit. In the HCLF-c and the VLCHF group, mean fasting subjective hunger was lower at baseline compared to all other study visits, but the only difference that was still significant after adjusting for multiple comparisons was the increase in fasting subjective hunger from baseline to three months in the VLCHF group. Here, the mean fasting subjective hunger increased from 3.75 ± 1.9 cm to 5.75 ± 1.9 cm.

Even though there were some differences in change in subjective hunger between the groups, absolute subjective hunger did not differ between the intervention groups at any study visit, neither as fasting subjective hunger nor as AUC calculated with fasting and two postprandial

(120, 240 min) VAS-scores. In the meta-analysis by Gibson et al. (6), subjective hunger measured with VAS was found to decrease significantly by 0.55 cm when adhering to a KLCD, despite a mean weight loss of > 6 kg.

In the current study, mean body weight was significantly reduced from baseline by 4.5 kg, 5.9 kg, and 6.1 kg in the HCLF-a, HCLF-c, and VLCHF group, respectively, at the three-month visit. At the twelve-month visit, mean body weight was significantly reduced from baseline by 8.2 kg, 10.7 kg, and 9.1 kg for the same groups. The weight loss did not differ significantly between the groups at any study visit. Weight loss in the VLCHF group may have been affected by loss of stored glycogen (104). A mean glycogen loss of ~400 g has previously been seen in VLED, and because glycogen is associated with an additional 3-4 parts water, a total weight loss due to loss of glycogen stores is likely to be ~1.5-2 kg (104). The mean weight loss in the VLCHF group was, although not significantly different from the HCLF groups, ~1.0-1.5 kg lower than the HCLF groups at the three-month visit. Also, a large proportion of the participants in the VLCHF group were not in ketosis. The loss of glycogen stores was not estimated in this study, but it is likely that the weight loss did not differ between the groups even if some of the weight loss in the VLCHF group is due to loss of water and glycogen.

This raises a series of questions: Why was the change in subjective hunger smaller in the HCLF-a group compared to the other groups at several study visits? Why did the between-group differences in change in subjective hunger show sex differences? And finally, why did the fasting subjective hunger increase significantly (after adjustment) within the VLCHF but not within the HCLF groups?

To start with the latter question, one reason might be that the VLCHF diet did not have the appetite-suppressing effects previously seen in VLEDs or KLCDs (6). This could be due to other traits of the diet than the carbohydrate restriction, for instance, the protein or fat content. Gibson et al. (6) could not determine whether the appetite suppression was due to ketosis or other factors, and the proportion of protein in the VLEDs was relatively high (50-60 g protein, 500-600 kcal total energy). Alternatively, the adherence to the diet and the levels of ketone bodies might have been too low in the VLCHF group to see an effect. The latter will be discussed in further detail in later sections. Another possible explanation is that the weight loss of less than 5 % at the three-month study visit was too low to expect suppression of the increase in subjective hunger on the VLCHF diet, even if the participants were ketotic. Nymo et al. have seen that fasting hunger increases with weight loss of up to 5 %, even if participants are in ketosis (4). At three months, mean weight loss in the VLCHF group was 4

± 2.8 %. So, an increase in subjective hunger may still have been expected in the VLCHF group at this time. What is still surprising is that the increase in subjective hunger does not seem to be smaller than in the HCLF groups. In fact, the change in subjective appetite was the smallest in the HCLF-a group.

This leads to one of the other questions; why was the change in subjective hunger smaller in the HCLF-a group, compared to the other groups, at several study visits? This must be explained by something other than ketosis. One explanation might be the differences in carbohydrate quality, but not necessarily cellularity. Both HCLF diets gave relatively large amounts of dietary fiber, but the types of fiber are likely to differ as the carbohydrate sources differed. Different types of dietary fiber may affect both appetite-related hormones and subjective appetite differently (105). In the sex-separate analysis, the differences in change in subjective hunger were only significant for the male participants. The male participants in the HCLF-a group had a higher intake of dietary fiber in grams per day compared to the female participants, so one might speculate that the type and amount of fiber may have had an effect. Different types of fiber have been shown to have an effect on appetite-related hormones, decrease appetite in the short term (four hours), and decrease ad libitum energy intake in the following meal after fiber intake (105, 106). However, it is important to note that the results on fasting subjective appetite found in CARBFUNC are measured after an overnight fast, and the effect of fiber on appetite is often studied in a shorter-term (~4 hours). Nilsson et al. (65) studied the effect of fiber intake in the evening and found that it did affect subjective satiety the morning after. However, the sample size in the study was relatively small (n = 6), and all participants were female. The mechanism was hypothesized to involve colonic fermentation (65). In the current thesis, information about the last meal consumed before the study visit was not assessed in this study. Both composition and timing of the previous meal may affect the appetite in the next meal (65).

A possible answer to all the questions above might also be that the results might have been biased by the relatively large attrition rate. Analysis of differences at the three-month visit between the participants who dropped out by six months and those who participated at this visit indicated that there were differences between the participants and those who dropped out. Three-month fasting subjective hunger was significantly higher in the 78 participants that still participated at the six-month visit, compared to the 41 who had dopped out between the three- and six-month visit ($p = 0.020$). This difference is likely to be, at least in part, mediated through differences in weight loss. Those who had dropped out between three and six months had a significantly smaller weight loss at the three-month study visit, compared to those who

still participated ($p < 0.001$). As the missing data are unknown, one cannot know for certain that the results would differ between the participants who dropped out and the participants who still participated, but based on the differences between them before they drop out, it seems plausible, and it may have biased the results.

4.2.5 Ghrelin

Fasting plasma concentrations of ghrelin did not differ significantly between the diet groups at any of the study visits, even with baseline ghrelin as a covariate in the ANCOVA model. Despite this, there were significant differences between the change in ghrelin between the groups when weight was added as a covariate. The absolute change in fasting total ghrelin differed significantly between the groups at three months but not at any other study visits. When the sexes were analyzed separately, the significantly smaller change in ghrelin at the three-month study visit only remained significant for the female participants. In addition, the difference change in ghrelin was also significantly smaller in the VLCHF compared to the HCLF-c group at six months for female participants.

The differences in change in ghrelin are in line with what would be expected if ketosis does suppress the increase in ghrelin seen with weight loss. When both sexes are analyzed together, the significant difference is only seen at the three-month visit. For the same group, the three-month visit is the only study visit when median β-HB is above 0.1 mmol/L and mean β-HB is close to 0.3 mmol/L. At the three- and six-month visits, participants who had a β-HB level of <0.1 mmol/L had a significantly higher mean level of ghrelin compared to the participants who had a β-HB level of ≥0.1 mmol/L. At these study visits, female participants have a median β-HB above 0.1 mmol/L and a mean β-HB close to 0.3 mmol/L. Hence, it seems plausible that the level of β-HB may be the explanation for the apparent sex differences in change in ghrelin. It also seems plausible that an increase in β-HB is associated with a smaller change in ghrelin and that a fasting plasma β-HB level above 0.1 is necessary and sufficient to see an effect. Even if there is an effect, it is relatively small. The fasting levels of ghrelin did not differ significantly, even though the change in ghrelin did differ significantly. Also, the level of ghrelin and differences in change in ghrelin did not seem to affect the subjective hunger.

Unfortunately, the level of β-HB was relatively low in the VLCHF group and did not differ from baseline β-HB level for the twelve-month study visit. Hence, the current thesis cannot conclude that there is an appetite suppressing effect of the VLCHF diet in the long run.

4.3 Future perspectives

A better understanding of appetite regulation in response to weight change can be an important part of providing treatment strategies for overweight and obesity. Unfortunately, the current thesis could not determine if ketosis suppressed subjective appetite, neither in the short nor long run, as the participants in the VLCHF group did not have relatively high levels of β-HB and the diet did not seem to have an effect on subjective appetite. The results did, however, suggest that a level of β-HB as low as above 0.1 mmol/L may be sufficient to affect the change in fasting ghrelin during weight loss. We encourage future research to investigate the effect of nutritionally induced ketosis in non-energy-restricted diet intervention in the long run and to investigate what levels of β-HB may affect ghrelin and appetite. In future research, we advise that measures be taken to reduce the attrition rate. Other studies have measured ketones using urine or blood sticks, and used this information to advise participants on continued or improved adherence to ketogenic interventions (48). Perhaps doing so, in addition to other strategies used in the CARBFUNC study, could contribute to less drop out in future studies. In the current thesis, it is speculated that fiber type and amount might affect subjective appetite. Hence, another advice for future studies would be to control or assess the types and amounts of fiber in more detail compared to the current thesis.

In the current thesis, weight change was associated with an increase in fasting ghrelin. Because ghrelin has an inverse relationship with adipose tissue mass (4), we suggest that future studies investigate correlations between changes in ghrelin and change in adipose tissue mass, in addition to change in body weight. In addition to loss of adipose tissue mass, weight loss may lead to loss of glycogen storage and water (104). Hence, it might be beneficial to estimate the proportion of weight loss from glycogen and water losses in future studies.

In the current thesis, only ghrelin and no other appetite-related hormones were measured. As appetite regulation is complex, we advise that additional appetite-related hormones, such as GLP-1, PYY, and CCK, be measured in future studies.

The current thesis suggests that carbohydrate quality can affect subjective hunger in the long run (effect seen up to six months). For this reason, we also encourage future studies to further investigate the effects of carbohydrate quality on appetite.

5 Conclusion

The current thesis suggests that both carbohydrate quantity and quality may affect appetite. There were two main findings: The VLCHF diet may affect the change in fasting ghrelin levels, despite the level of β-HB being relatively low. The absolute fasting ghrelin level was not affected, and the effect did not transfer to an effect in fasting subjective hunger. The indicated effect was only seen in the three- and six-month study visits. If the effect on change in ghrelin is due to ketosis, this may explain why the effect was not seen in later study visits. The level of β-HB reached in the VLCHF group was relatively low, especially after the three-month study visit. Hence, the findings indicate that even a low level of β-HB, reached under normocaloric conditions, may affect appetite. Unfortunately, the question of the effect persists, in the long run, could not be answered in the current thesis, as levels of β-HB was not above baseline values after the three-month study visit for male participants and after the six-month study visit for female participants.

The other main finding was that the fasting subjective appetite did not increase despite losing weight when adhering to the HCLF-a diet. The results indicate that there are differences in subjective appetite between the two HCLF-groups. Appetite did not change significantly from baseline throughout the twelve months of the trial for the HCLF-a group, despite the relative weight loss being similar between all intervention arms. This effect may be due to the carbohydrate quality of the diet, perhaps the type and amount of fiber.

The results also indicate that there may be sex differences in regard to appetite response on the different diets, but the differences could also be influenced by differences in adherence or dietary intake.

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Appendix

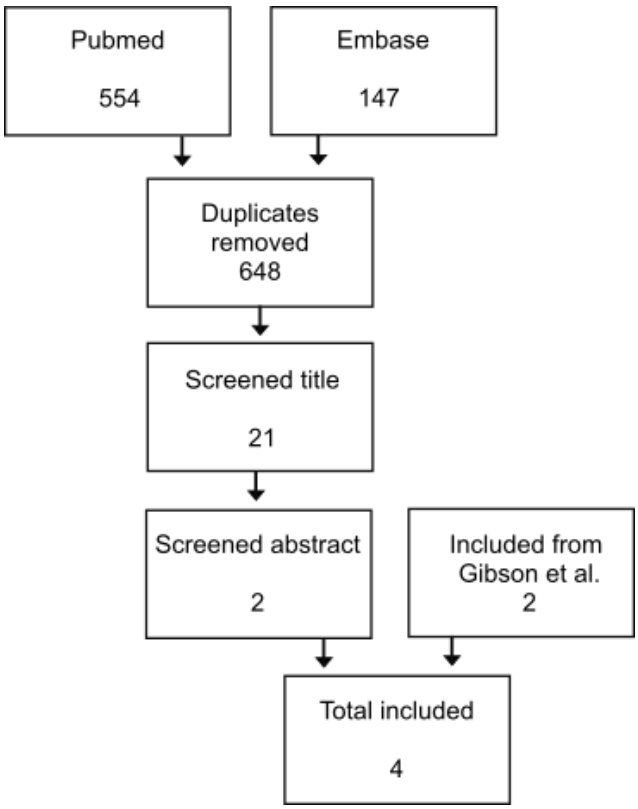
Appendix I: Systematic search

A systematic literature search was conducted on November 1st 2020. The aim of this was to identify all publications on the effect of normocaloric ketogenic diets on appetite. Gibson et al. (6) conducted a similar search in 2013, but not exclusively for non-energy restricted interventions. This search was an extension Gibson´s search. Publications were considered relevant if whey fit the inclusion- and exclusion criteria listed below:

Inclusion criteria:	Exclusion criteria:
• Investigates appetite using VAS	• Non-human subjects
• Investigates normo-caloric, ketogenic diet	• Conference paper /post paper
• Obese, non-diabetic subjects	• Other than English or Scandinavian language

Search strategy:

Combine with OR		Combine with OR
Low carb* Carbohydrate-restric* Diet, ketogenic(/) Ketogenic Ketosis Keto acids (/) Ketone bodies Ketones	AND	Appetite (/) Hunger (/) Satiation (/) Satiety Desire to eat Visual analogue scale Visual analog scale VAS



Boxes were combined with AND, words inside boxes were combined with OR. Words were used as MeSH-terms, free-text searches or both. The search was run in PubMed and Embase. Publication time limited to: 13 December 2013 - 1 November 2020.

Four studies were identified (50-53).

Appendix II: Reasons for drop out.

	0-3 months			3-6 months			6-9 months			9-12 months		
	HCLF-a	**HCLF-c**	**VLCHF**	**HCLF-a**	**HCLF-c**	**VLCHF**	**HCLF-a**	**HCLF-c**	**VLCHF**	**HCLF-a**	**HCLF-c**	**VLCHF**
Unknown	8 (4)	8 (3)	1 (1)	1 (1)	3 (2)	4 (2)	2 (1)	1	2 (2)			
Personal or health												
Personal reasons	11 (5)	8 (4)	5 (3)	6 (2)	2 (2)	7 (4)		1	1	2 (1)		
Health related	3 (2)		1 (1)						2 (1)			1 (1)
Pregnant			1 (1)									
Intervention related												
To demanding	6 (5)	3 (3)	2 (2)	3 (2)	2 (1)	2 (1)		1 (1)	2 (2)		1 (1)	1
Other	2	2 (1)	2 (1)	1 (1)	1	1						
Loss to follow-up												
Does not attend	3 (1)	2 (1)	2 (1)	1	2	1 (1)			2 (1)	1		
Multiple reasons		2 (2)	1 (1)			2 (1)	1			1 (1)	1 (1)	
Total	33 (17)	25 (14)	15 (11)	12 (6)	10 (5)	17 (9)	3 (1)	3 (1)	9 (6)	4 (2)	2 (2)	2 (1)

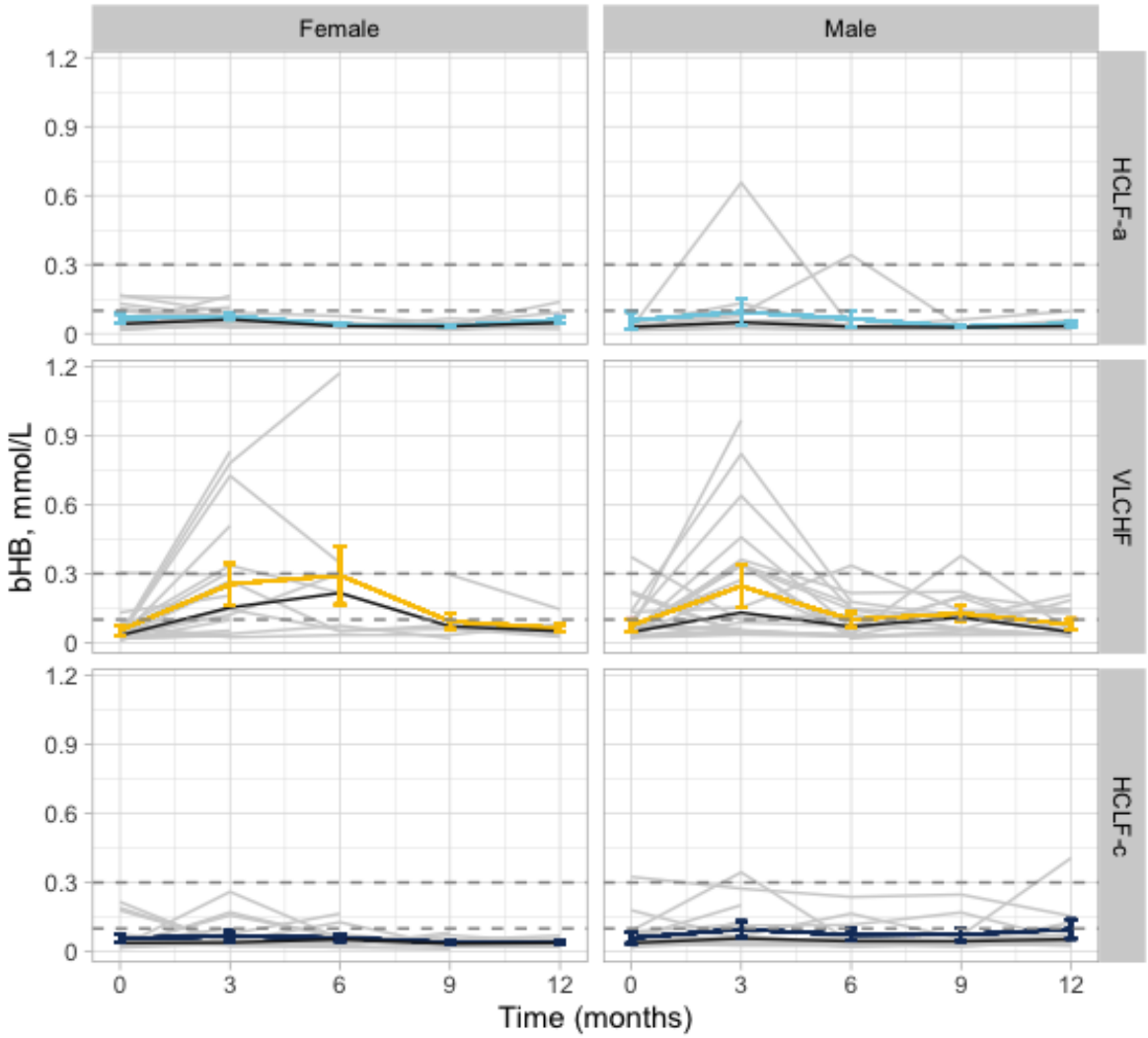
Participants given as number of participant (number of female participants). Abbreviations: HCLF-a, high-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, high-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet.

Appendix III: Comparison of drop outs vs. participants before drop out.

	3 months		
Measures at baseline, mean (95 % CI)	**Participated (n = 118)**	**Missing (n = 74)**	**p - value**
Age, years	42.4 (40.8-44.0)	40.4 (38.4 - 42.4)	0.117
Weight, kg	112.9 (109.6 - 116.3)	108.4 (104.2 - 112.6)	0.099
BMI, km/m2	36.9 (35.9 - 37.7)	36.4 (35.3 - 37.5)	0.483
Fasting hunger, VAS	3.9 (3.5 - 4.3)	4.1 (3.7 - 4.6)	0.418
Ghrelin, pg/ml	303.2 (270.1 - 336.3)	317.0 (276.5 - 357.4)	0.604
Diet[a]			**0.019**[b]
	6 months		
Measures at 3 mo, mean (95 % CI)	**Participated (n = 78)**	**Missing (n = 41)**	**p - value**
Age, years	42.3 (40.9 - 44.8)	40.8 (39.2 - 42.4)	0.114
Weight, kg	104.9 (100.9 - 108.9)	112.0 (106.4 - 117.5)	**0.043**
BMI, km/m2	34.4 (33.3 - 35.5)	36.3 (34.9 - 37.8)	**0.036**
Weight change, %	-5.7 (-6.5 - -5.0)	-3.4 (-4.4 - -2.4)	< **0.001**
Fasting hunger, VAS*	5.0 (4.6 - 5.5)	4.2 (3.6 - 4.8)	**0.020**
Ghrelin, pg/ml*	348.3 (325.2 - 371.5)	330.8 (298.9 - 362.7)	0.381
Diet[a]			0.728
	9 months		
Measures at 6 mo, mean (95 % CI)	**Participated (n = 64)**	**Missing (n = 17)**	**p - value**
Age, years	43.5 (41.4 - 45.7)	40.7 (39.2 - 42.2)	**0.033**
Weight, kg*	102.2 (100.8 - 103.6)	104.1 (101.4 - 106.7)	0.227
BMI, km/m2*	33.4 (32.9 - 33.9)	34.1 (33.3 - 35.0)	0.147
Weight change, %**	-8.0 (-8.6--7.4)	-7.5 (-8.6 - -6.3)	0.387
Fasting hunger, VAS	5.5 (5.0 - 6.0)	4.4 (3.4 - 5.4)	0.048
Ghrelin, pg/ml*	358.2 (333.1 - 383.2)	329.5 (278.9 - 380.1)	0.314
Diet[a]			0.280
	12 months		
Measures at 9 mo, mean (95% CI)	**Participated (n = 57)**	**Missing (n = 9)**	**p - value**
Age, years	43.4 (41.2 - 45.7)	40.8 (39.4 - 42.3)	0.060
Weight, kg*	102.2 (100.4 - 104. 0)	105.6 (101.1 - 110.1)	0.162
BMI, km/m2*	33.4 (32.8 - 34.0)	34.6 (33.1 - 36.1)	0.120
Weight change, %***	-8.5 (-9.2 - -7.9)	-6.8 (-8.6 - -5.1)	0.128
Fasting hunger, VAS	5.2 (4.7 - 5.7)	5.4 (4.2 - 6.7)	0.722
Ghrelin, pg/ml*	351.6 (324.3 - 378.8)	381.1 (308.1 - 454.2)	0.451
Diet[a]			0.394

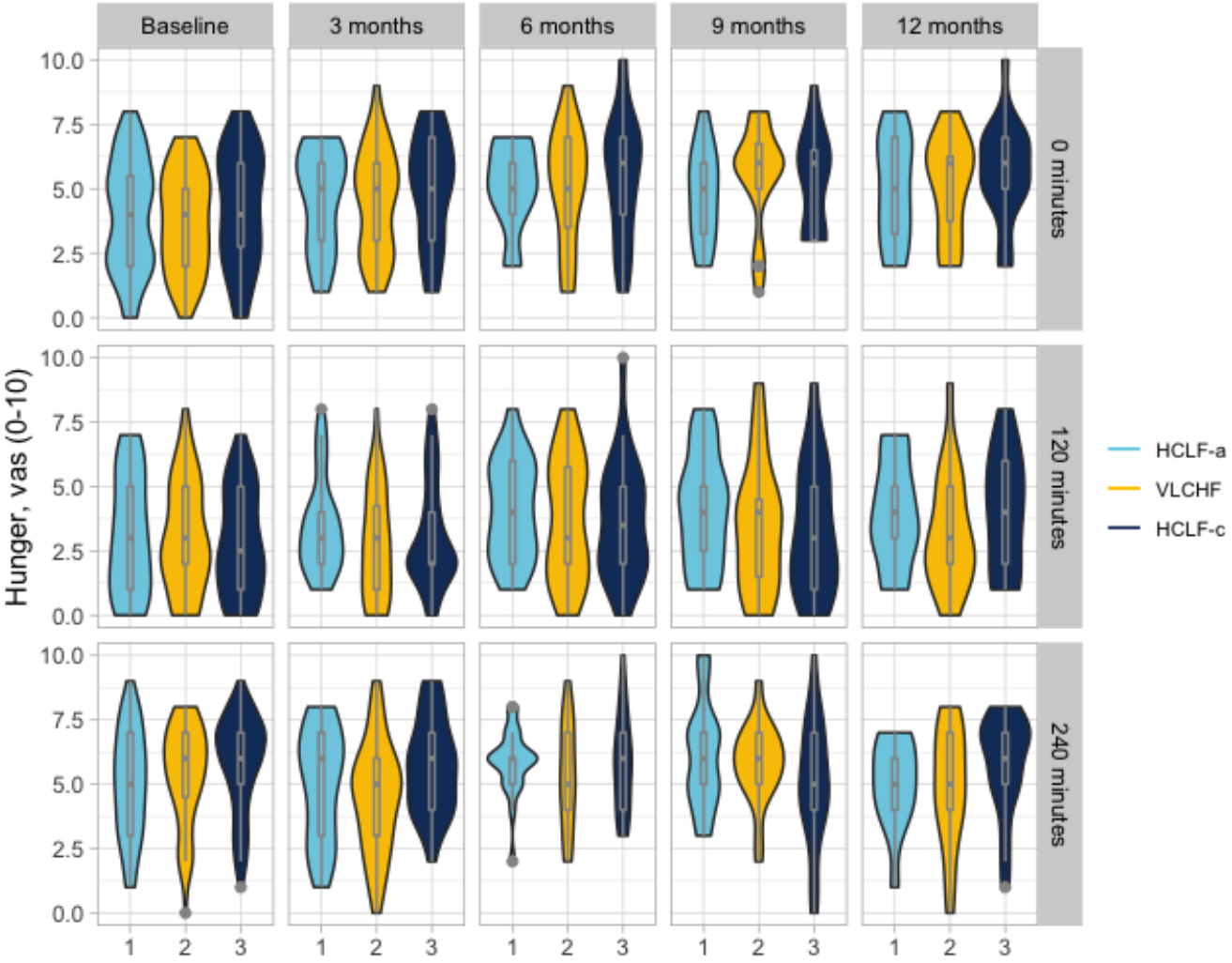
*Baseline value added as covariate in the model. **3 month value added as covariate in the model. ***6 month value added as covariate in the model. [a]Analysed with Chi-square (2x3). [b]49 % dropped out from the HCLF-a group, 40 % from the HCLF-c group, and 25 % from the VLCHF group. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate; VLCHF, Very-low-carbohydrate high-fat diet; VAS, visual analog scale; mo, months.

Appendix IV: Fasting level of β-HB (mmol/L) for all three groups, split by sex.



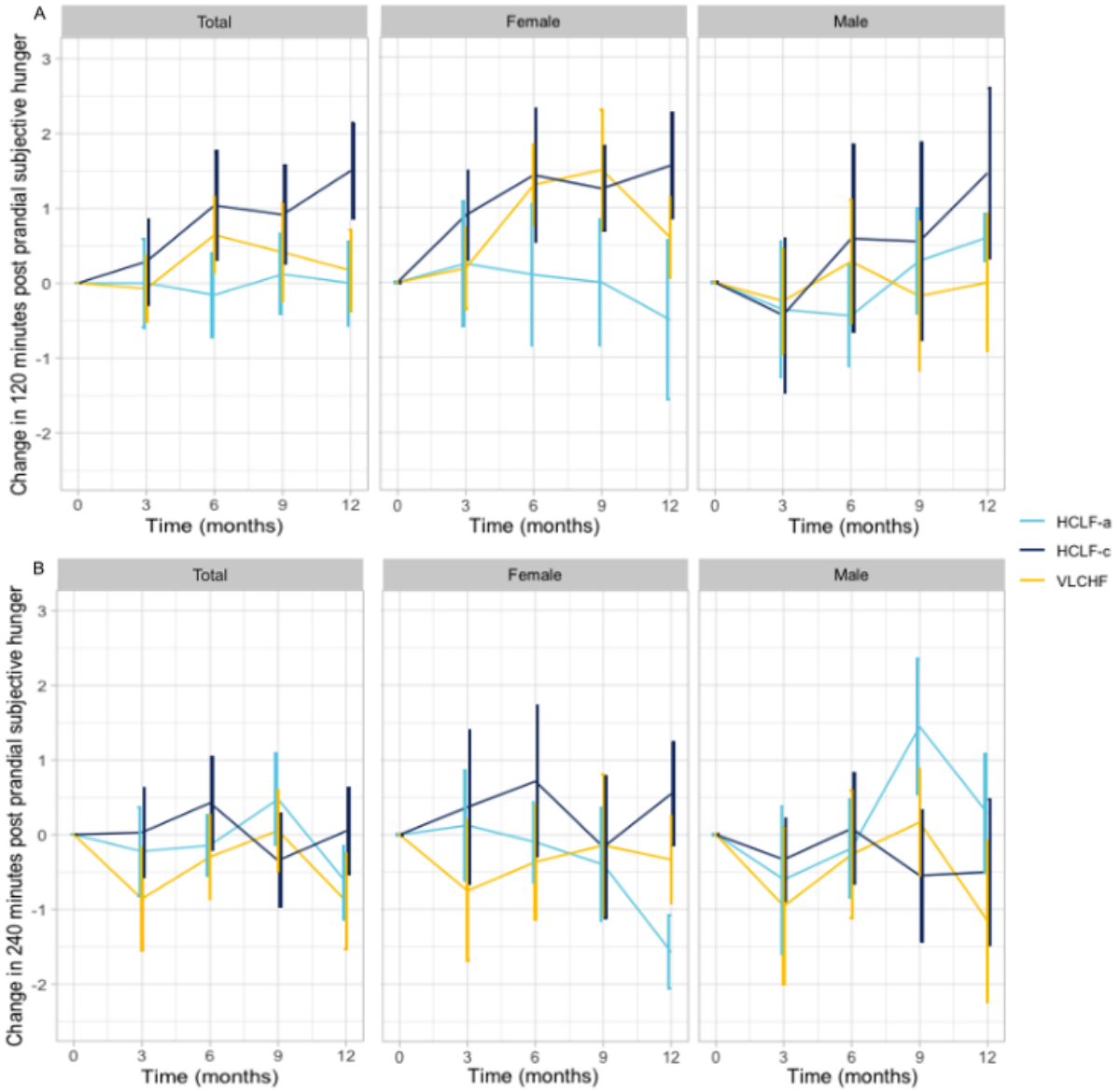
Mean ± 95 % CI. Black line represents median. Grey lines represent individual observations. Dotted lines represent the set thresholds at 0.1 and 0.3 mmol/L. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate; VLCHF, Very-low-carbohydrate high-fat diet; bHB, b-hydroxybutyrate; CI, Confidence interval.

Appendix V: Distribution of VAS-scores for subjective hunger.



Violin plots with box plots visualizing the distribution of the VAS scores (0-10) for subjective hunger at all study visits in all three groups. The box of the grey box plots represents the IQR, the line in the middle of the plot represents the median, and the whiskers represent 1.5 x IQR. The colored violin plots represents density. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate; VLCHF, Very-low-carbohydrate high-fat diet; VAS, visual analog scale; IQR, Inter quartile range.

Appendix VI: Change in postprandial subjective hunger (VAS).



Error bars represent 95 % CI. Panels A: Change in subjective hunger measures with VAS (0-10) 120 minutes postprandial. Panels B: Change in subjective hunger measures with VAS (0-10) 240 minutes postprandial. Abbreviations: HCLF-a, High-carbohydrate low-fat diet with mostly acellular carbohydrate sources; HCLF-c, High-carbohydrate low-fat diet with mostly cellular carbohydrate sources; VLCHF, very-low-carbohydrate high-fat diet; CI, confidence interval; VAS, visual analog scale.

Appendix VII: The list of foods with estimated dietary carbohydrate cellularity

Cellular index	Grains/flour	Fruit/berries	Nuts/seeds	Vegetables	Dairy	Sugars
1	Whole grains/rice	Whole fruit/berries	Nuts/seeds	Whole vegetables		
2	Steel cut grains, puffed grains, polished grains (including rice)	Dried fruit/berries, fruit/berry jam (no sugar)				
3	Rolled grains	Smoothies		Mashed vegetables, homemade		
4				Mashed vegetables, commercial products		
5-8						
9	Ground grains	Fruit/berry juice		Powdered vegetables		
10			Coconut milk		All lactose containing dairy products	Fructose, sucrose, maltose, lactose, honey, agave, etc.

Food items were scored based on assumed or actual cellularity. The planned HCLF-a diet was based on food items with a cellularity index of five and above, with only few exceptions of food items with an index of 1-4. The HCLF-c diet was based on food items with a score of 1-3.