Role of dietary carbohydrate quality and quantity in visceral obesity reduction

Results from a randomized controlled trial (CARBFUNC)

Cathrine Horn

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Scientific environment

This PhD project was conducted from 2018 to 2022 at The Mohn Nutrition Research Laboratory, Centre for Nutrition, Department of Clinical Medicine, and the Department of Clinical Science, Faculty of Medicine at the University of Bergen. The main supervisor was Dr. Simon N. Dankel, and co-supervisors were Professor Jutta Dierkes and Professor Gunnar Mellgren.

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Abstract

Background: Increased visceral adipose tissue (VAT) volume is associated with common lifestyle diseases and poses an increasing health challenge worldwide. There is a great need for effective approaches that result in successful long-term VAT loss. Carbohydrate amount may be of importance as low-carbohydrate high-fat (LCHF) diets have been suggested to suppress increases in hunger feelings following diet-induced fat loss. Also, beyond established markers of carbohydrate quality, the food matrix and "cellularity" of carbohydrate-rich foods may influence both VAT volume and appetite.

Aims: The overall aim of this thesis was to investigate the effect of long-term dietary intervention approaches differing in carbohydrate quality (degree of cellularity) and amount on visceral adiposity and measures of appetite in adults with obesity.

Methods: All three papers included in this thesis are based on findings up to 12 months from a three-armed randomized clinical trial (RCT) of adults with obesity/central adiposity. In Paper I, we examined the habitual meal and dietary patterns, using exploratory hierarchical cluster and k-means cluster analyses, respectively, on cross-sectional preintervention dietary data using 6-day weighed food records. In Paper II, we compared changes in VAT volume, measured by abdominal computed tomography, after 1 year on three isocaloric eating patterns based on "acellular" carbohydrate sources (e.g., flour-based whole-grain products; comparator arm), "cellular" carbohydrate sources (minimally processed foods with intact cellular structures such as fruits, potatoes/tubers and rice), or LCHF principles. Finally, in Paper III we compared changes in fasting plasma concentrations of total ghrelin, the ketone body β-hydroxybutyrate (βHB) and subjective appetite (visual analog scale, VAS) after 3 and 12 months on the three diets. Outcomes were compared by an intention-to-treat (ITT) analysis using constrained linear mixed modeling (cLMM).

Results: In Paper I, we identified five clear temporal meal patterns among 192 (females: 53%, males: 47%) participants completing 99.1% of the planned preintervention dietary recording days. The reported energy intake (mean ± SD) was

highest and lowest among “midnight-eaters” (2551 ± 554 kcal/d) and “dinner-eaters” (2064 ± 546 kcal/d), respectively. Despite a reported difference of 490 kcal/d, there were no significant differences between these meal patterns in anthropometric measures or physical activity levels. In total, 57 participants (30%) completed 12 months with similar intakes of energy (females: 1820−2060 kcal, males: 2480−2550 kcal) and protein (16−17 energy percent, E%) throughout the intervention, and only modest reductions in energy from baseline. Reported mean dietary intakes were 11−15 (59−86 g/d) and 41−44 E% carbohydrate and 66−70 and 36−38 E% fat on the LCHF and HCLF diets, respectively. In Paper II, there were no significant between-group differences in VAT volume after 12 months (cellular vs. acellular [95% CI]: −122 cm^3 [−757, 514]; LCHF vs. acellular [95% CI]: −317 cm^3 [−943, 309]). VAT volume decreased significantly within all groups by 14−18% after 12 months. In Paper III, no significant between-group differences were seen in change scores of neither fasting ghrelin nor hunger feelings, despite a significant difference in βHB after 3 months. Fasting concentrations of ghrelin increased significantly from baseline to 3 months (mean [95% CI]) on the acellular (46 pg/ml [11, 81]) and cellular (54 pg/ml [21, 88]) diets, but not on the LCHF diet (11 pg/ml [−16, 38]).

Conclusions: Our data revealed five clearly distinct meal patterns among people with obesity, which may be important to consider for more effective individual follow-up. Results from the intervention suggest that the previously observed appetite suppression of carbohydrate restriction on very low-energy diets may be of less clinical relevance on more modest energy-restricted diets unless carbohydrate restriction is sufficient to induce greater ketosis. Overall, our findings indicate that similar and clinically relevant VAT reduction may be achieved on isocaloric dietary approaches differing in carbohydrate cellularity and amount in people with intra-abdominal obesity.

List of abbreviations

A-HCLF	Acellular high-carbohydrate low-fat diet
βHB	β-hydroxybutyrate
BMI	Body mass index
C-HCLF	Cellular high-carbohydrate low-fat diet
cLMM	Constrained linear mixed-effects model
CT	Computed tomography
E%	Energy percent
GI	Glycemic index
GL	Glycemic load
HEI	Healthy Eating Index
HU	Hounsfield units
ITT	Intention-to-treat
LCHF	Low-carbohydrate high-fat diet
MET	Metabolic equivalent
mGy-cm	Milligray cm
MRI	Magnetic resonance imaging
mSv	Millisievert
NHANES	National Health and Nutrition Examination Survey
PUFA	Polyunsaturated fatty acid
RCT	Randomized controlled trial
ROI	Region of interest
RUHS	Research Unit for Health Surveys
SAT	Subcutaneous adipose tissue

SFA	Saturated fatty acid
UPF	Ultra-processed food
VAS	Visual analog scale
VAT	Visceral adipose tissue
VLED	Very low-energy diet
WC	Waist circumference
WHO	World Health Organization

List of Publications

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1. Introduction

1.1 Abdominal obesity

1.1.1 Classification and measures

The World Health Organization (WHO) defines obesity as a medical condition in which excessive fat accumulation increases to the extent that it may impair individual health [1]. The definition does not state any specific amounts of excessive fat accumulation other than its abnormality and potential to negatively affect health.

Although the degree of excess fat is not part of the equation, the most frequently used measurement to define overweight and obesity is the body mass index (BMI), calculated by dividing an individual's body mass measured in kilograms by their height measured in meters squared (kg/m^2). In adults, obesity is defined as a BMI of ≥30.0 kg/m^2 (**Table 1**). In children and adolescents, age is an important factor when defining obesity, and growth charts (e.g., the WHO growth standards or the Norwegian curves for BMI) are most commonly used [1,2].

Table 1. Classification of body weight according to the World Health Organization [1].

Classification	Body mass index (kg/m^2)
Underweight	<18.5
Normal weight	18.5 – 24.9
Overweight	25.0 – 29.9
Obesity	≥30.0

A limitation of BMI as a defining criterion of obesity is the inability to distinguish between lean mass (e.g., muscle and bone) and fat mass. To address the element of excess fat accumulation in the WHO definition, simple clinically feasible measures

such as waist circumference (WC in cm), waist-to-hip ratio, and waist-to-height ratio provide additional information on the distribution of body fat, abdominal fat, or central adiposity [3]. The suggested cut-off points for WC to identify abdominal obesity in adult Caucasians are ≥88 cm in females and ≥102 cm in males as such levels have been associated with a substantial increase in health risk [4,5]. Available methods for more precise measures of whole-body fat mass include bioelectrical impedance analysis, air displacement plethysmography, dual-energy X-ray absorptiometry, magnetic resonance imaging (MRI), and computed tomography (CT) scan [6]. However, only MRI and CT scans (**Figure 1**) can distinguish between the subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT), by offering direct volumetric measures of these fat tissue compartments [7].

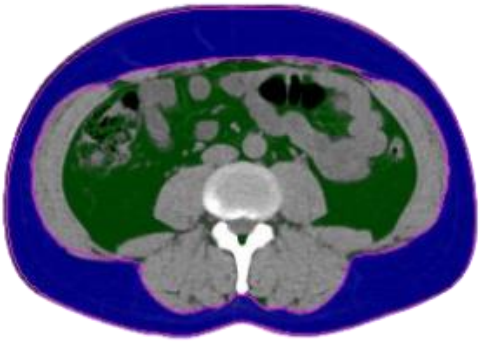

Figure 1. Cross-sectional axial image of the abdomen in which subcutaneous adipose tissue (blue) and visceral adipose tissue (green) may be distinguished. The image is obtained from a non-contrasted abdominal CT scan conducted for the CARBFUNC study at the Department of Heart Disease, Haukeland University Hospital.

1.1.2 Global and national prevalence trends

Since 1975, the prevalence of obesity has tripled worldwide and today over 650 million people have obesity, accounting for 13% of the world's adult population [1]. This astonishing rise in obesity prevalence has been described as an epidemic reflecting the high prevalence and the unexpectedly rapid increase [8]. However, although obesity is increasingly recognized as a chronic progressive disease [9], only 36% of adults with

obesity reported received an obesity diagnosis in a large international survey including 14 502 individuals with obesity from 11 countries [10]. In a Norwegian longitudinal population health study (the Nord-Trøndelag Health Study, HUNT), approximately 23% of both men and women were classified as having obesity corresponding to BMI ≥30 kg/m^2 in 2017−2019 [11], whilst in 2007−2008, 55% and 37% of men and women, respectively, were classified with abdominal obesity corresponding to WC >102 cm and >88 cm [12]. The findings of the HUNT study also show an increase in the prevalence of obesity at all ages, within both sexes, and especially among young adults [13]. Although measures of abdominal obesity, e.g., WC, in combination with BMI may better predict specific health outcomes, measurements of BMI alone are predominantly used when assessing the trends in obesity prevalence worldwide [14,15]. Interestingly, secular trends in obesity prevalence have been observed across populations, suggesting that increases in WC, a crude measure of abdominal obesity, may have increased beyond what is to be expected for a specific BMI category [16,17].

1.1.3 Impact on health

Obesity, as defined by BMI ≥30 kg/m^2, represents a significant risk to individual health and is one of the world's leading public health challenges [14]. The condition is associated with reduced life expectancy [18], quality of life [19], and disease-free years [20], with increased risk of several comorbidities including cardiovascular disease [21], type 2 diabetes [22], several types of cancer [23], hypertension [24], obstructive sleep apnea, and dyslipidemia [25]. In a Norwegian cross-sectional study, non-alcoholic fatty liver disease was the overall most prevalent obesity-related comorbidity in men with severe obesity (BMI ≥40.00 kg/m^2 or BMI ≥35.00 kg/m^2 with ≥1 obesity-related comorbidity), whilst dyslipidemia (defined as low-density lipoprotein ≥2.6 mmol/L) was the most prevalent comorbidity in women [26].

However, not all individuals with BMI ≥30 kg/m^2 exhibit the same metabolic risk factors, leading to the description of an obesity phenotype as "metabolically healthy obesity". Although a clear consensus is lacking, the term "metabolically healthy obesity" implies that these individuals do not have an increased risk of cardiometabolic complications, which may refer to the absence of the metabolic syndrome (including

elevated blood pressure, plasma glucose and triglycerides, and low high-density lipoprotein; HDL) and insulin resistance [27]. A recently published study using data from a large cohort (the third National Health and Nutrition Examination Survey, NHANES-III) found that people with the metabolically healthy phenotype were not at increased risk of cardiovascular disease or total mortality [28]. In this study, an empirically derived definition of "metabolically healthy obesity" was used based on the mortality follow-up of the NHANES-III cohort. Of note, there is inconsistency in the prevalence and clinical outcomes of this phenotype in the literature, resulting in an ongoing debate as to whether the state of metabolically healthy obesity is merely a transition stage leading to metabolically unhealthy obesity over time [29,30].

The higher-risk phenotype characterized by abdominal obesity, also referred to as central or visceral obesity, has been found to promote metabolic disturbances and cardiovascular disease, independent of overall obesity [7,31,32]. The association between "android" obesity (upper body predominance), as opposed to "gynoid" obesity (lower body predominance), and metabolic and cardiovascular disease was first described by Jean Vague in the 1950s [33]. Today the "android" obesity may be described as excess abdominal fat tissue. Abdominal fat tissue is distributed into two main depots, VAT surrounding the intra-abdominal organs in the abdominal cavity and SAT found beneath the skin. Increased WC mostly reflects increases in the visceral fat depot, whilst increased hip circumference largely reflects increased subcutaneous fat accumulation [34,35]. WC as a measure of abdominal obesity has been suggested as "a vital sign" to be identified and assessed in clinical practice to allow for improved targeted health care for people with this specific higher-risk obesity phenotype [35].

1.1.4 Development and aetiology

The capacity of the human body to store fat is a part of normal physiology and is an important survival trait to protect against starvation, as humans evolved in environments where food availability was limited and/or unpredictable. However, in our modern context, obesity has become a greater global threat to human health than starvation and is now linked with higher mortality rates worldwide than underweight [1]. Energy intake, energy expenditure, and energy storage constitute the basic components

of energy balance [36]. Obesity results from a chronic imbalance between energy intake and energy expenditure, creating an energy surplus resulting in energy storage and excessive fat accumulation [37]. However, it is increasingly recognized that this imbalance, in turn, results from a complex interplay between individual genetic predisposition, interacting through epigenetic mechanisms, and non-genetic environmental factors [38,39]. In addition to factors beyond individual control, modifiable behavioral factors may contribute to preventing the development of obesity. However, factors including dietary quality, genetics and hormonal state may influence to what degree excess fat is stored in SAT relative to VAT [32].

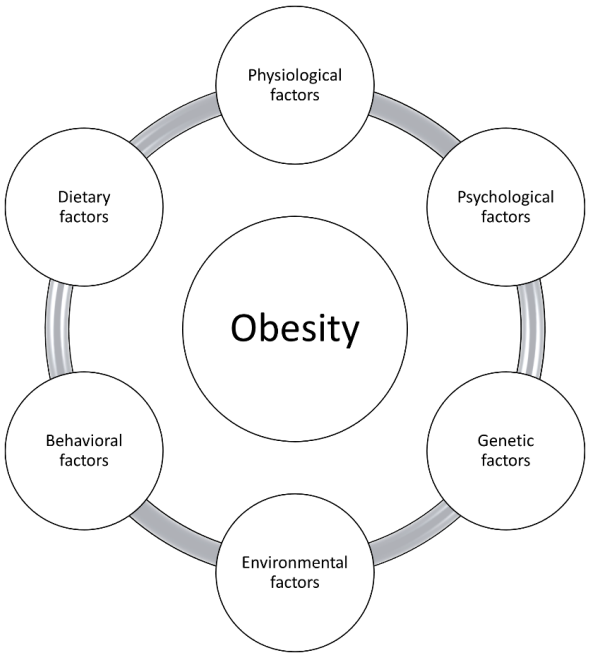

Figure 2. Multifactorial aetiology of obesity.

Physiological factors

The physiological signals associated with energy homeostasis are complex and interact in several parts of the human brain, particularly in the hypothalamus, to regulate eating behaviors [40]. The body's appetite control system is a critical part of these physiological mechanisms. Eating behaviors include processes to fulfill immediate energy

requirements, anticipate future energy demands, and provide nutrients for biological needs. Appetite control results from interactions between responses to internal biological cues for the maintenance of internal energy balance (homeostasis) and responses to external reward-based stimuli (hedonic responses) [40]. Important parts of the appetite control system include hunger (a conscious sensation that reflects a state of biological need), satiation (a process leading to the termination of eating), and satiety (the feeling of fullness after food intake) [41]. Although hunger is a strong physiological driver to eat, humans can choose to refrain from eating when hungry or choose to eat although experiencing feelings of fullness (satiated). Signals of hunger and satiety may follow the same biological pathways in humans, however individual responses to the same stimuli vary, and individuals may also override these signals (e.g., due to pleasurable aspects of food), increasing the risk of developing obesity through excess energy intake [41,42]. To assess subjective appetite in research, the most common method is the use of visual analogue scales (VAS) [43]. Using VAS, participants answer questions related to hunger and satiety by making a mark on a straight 10 cm line anchored by an extreme answer to each question.

The physiological regulation of appetite involves both anorexigenic (decreasing appetite) and orexigenic (increasing appetite) hormones. From this point forward, the main focus will be on the appetite hormone ghrelin as weight loss and ghrelin have consistently been inversely associated, whilst there are inconsistent patterns regarding the impact of weight loss on the postprandial release of satiety peptides [44–47]. Ghrelin is the only known orexigenic gut hormone stimulating appetite and is considered a part of both the episodic or short-term signaling, stimulating and initiating food intake, as well as a part of long-term energy balance by protecting against prolonged energy deficiency [48]. Total ghrelin is constituted by two circulating variants: acylated ghrelin and des-acyl ghrelin, with des-acyl ghrelin being the more abundant form [48]. Acylated ghrelin has a half-life of 240 minutes and measures of this variant in plasma samples require immediate treatment with protease inhibitors to prevent degradation [49]. Previous studies have shown that acylated ghrelin and total ghrelin have similar responses to macronutrient intake [50] as well as a similar relationship between total

ghrelin and hunger and acylated ghrelin and hunger [51]. Ghrelin is secreted in response to fasting with elevated concentrations observed before an eating occasion, whilst concentrations are reduced postprandially. In contrast, tonic or long-term signaling indicates the levels of energy storage in the body, contributing to the maintenance of more or less stable body weight [41]. Ghrelin also plays a part in long-term energy balance as fasting circulating ghrelin concentrations are inversely associated with levels of adipose tissue mass [52]. Importantly, ghrelin secretion is known to be upregulated during weight loss and has been associated with increased feelings of hunger during weight loss [53,54]. Although basal ghrelin concentrations are lower in people with obesity compared with lean individuals, postprandial ghrelin concentrations remain higher than in lean individuals, lengthening the time individuals with obesity feel hungry, potentially leading to further weight gain [55].

Psychological factors

Psychological factors associated with the development of obesity include named eating disorders, e.g., binge-eating disorder [56], emotional eating, and altered mood affecting food choices and energy intakes which may over time lead to obesity [57]. As a complete description of the psychological factors associated with the development of obesity is beyond the scope of this thesis, the following may be considered as a brief introduction. In the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders, DSM 5 [58], binge-eating disorder is defined based on several criteria related to recurrent binge eating episodes characterized by experiencing loss of control and consuming unusually large amounts of food over a short duration, without compensatory behavior. Prevalence of binge-eating disorder has previously been estimated to affect 1−2 % of the general population, whereof approximately 40% with BED have obesity [59]. In addition, chronic stress and depressed moods have been associated with increased intake of highly palatable foods as means of stress relief and reducing negative emotions, increasing vulnerability for developing obesity [57]. Of note, the relationship between psychological factors, e.g., depression and obesity, are often described as bidirectional, as shown by individuals with obesity having a 55% increased risk of

depression, whilst persons with depression have a 58% increased risk of developing obesity [60].

Genetic factors

Genetic factors play an important role in the individual predisposition for obesity, making some individuals more susceptible to changes in the environment [61,62]. Based on heritability studies, a substantial proportion of inter-individual differences in BMI, estimated at 40−70%, may be attributed to genetic factors [63,64]. However, although genome-wide association studies have identified several genetic variants associated with an increased risk of obesity, these variants only account for an estimated 2−5 % of the variation in BMI [65,66]. The inability of single variants to account for the majority of the heritability of a condition, e.g., obesity, is known as the missing heritability problem [67], which may be attributed to epigenetic changes or gene-environment interaction [68,69]. Interestingly, a specific genome-wide association study of the abdominal fat depots, measured with CT imaging, identified a locus for VAT in women [70], a finding highlighting that genetic factors may contribute to the variability in abdominal visceral fat level beyond general obesity levels. However, a comparison of individuals who were genetically predisposed for higher BMI with those genetically non-predisposed, showed that also individuals with a low-risk profile gained body weight during the recent decades, underscoring that environmental drivers of obesity affect the population as a whole, regardless of initial genetic predisposition [71]. Also, genetic alterations have not occurred at a population level at the same rapid pace as obesity prevalence has risen, supporting that genetic factors alone do not play the leading role in the recent increase in prevalence.

Environmental factors

An environment deemed to promote obesity and unhealthy lifestyle choices may be described as “obesogenic” [72]. Increased urbanization may result in more passive transportation such as more public transport and car travel, reducing walking and biking as means of transportation. Further distance to outdoor recreational space in urban living may reduce levels of physical activity. More urban occupations such as

desk jobs are sedentary compared to more physically active jobs in rural areas such as farming. These environmental factors may be categorized into the physical or built environment (e.g., neighbourhood characteristics), the economic environment (e.g., food prices, or taxes), the political environment (e.g., regulation of food advertising aimed at children or regulation entitling children to nutritious school meals), and the social-cultural environment (e.g., attitudes and believes), and these are all associated [72]. Although the physical environment has been associated with physical activity levels, characteristics of all four environmental categories have been suggested to play a part in the increases in obesity prevalence worldwide [73], however, comprehensive research on the causality of these obesity-promoting factors is lacking [38].

Behavioral factors

A vast number of dietary factors associated with the development of obesity have laid the foundation for a subsequently high number of dietary approaches designed to prevent and treat obesity. As the dietary factors associated with the development, prevention, and treatment of obesity are so closely connected, these factors will be presented in the following section on the treatment of abdominal obesity. Behavioral factors beyond diet affecting abdominal obesity include physical activity, sleep, stress, and sedentary behavior [38]. A sedentary lifestyle and low levels of physical activity increase the risk of positive energy balance, although it remains unestablished whether these lifestyle factors may specifically lead to increases in the VAT depot. In a 16-week exercise intervention, high-intensity exercise training reduced VAT to a greater extent than in the low-intensity exercise training, and in the control group, although the between-group differences were not statistically significant [74]. Also, in a review of exercise trials reporting non-significant changes in body weight, overall regular physical activity was associated with substantial reductions in WC, without affecting body weight [75]. These findings indicate that physical activity, in particular high-intensity exercise training, may specifically reduce intraabdominal obesity, and should therefore be controlled for when assessing the relationship between dietary factors and the VAT depot.

1.2 Treatment of abdominal obesity

Despite a complex aetiology including physiological, genetic, environmental and behavioral factors, abdominal obesity is to a large extent preventable and treatable through dietary management combined with behavior modification [38,76]. Although available options for obesity treatment also include pharmacotherapy and bariatric surgery, the focus of this thesis is on lifestyle interventions, and specifically on dietary approaches targeting abdominal obesity. Traditional weight-loss strategies include a variety of dietary approaches emphasizing either calorie restriction, implementing food-based eating patterns, or specific macronutrient profiles [77–80]. Although meta-analysis and systematic reviews of several different dietary approaches for obesity treatment find similar long-term (1-year) effects on weight loss [81,82], few studies have included more specific outcome measures that precisely quantify internal adiposity, including VAT [83,84]. In the following section, an overview of current dietary guidelines for the treatment of abdominal obesity will be presented, along with important dietary approaches associated with the reduction of abdominal obesity.

1.2.1 Dietary guidelines

Dietary management combined with behavior modification is a key element of obesity treatment [76,77,85]. National guidelines for the treatment of obesity highlight specifically the negative consequences of excess intraabdominal fat [86] but neither national nor international guidelines include specific treatment strategies or treatment goals for targeting abdominal obesity [86–88]. The guidelines from the Norwegian Directorate of Health suggest that a modest treatment target of 5−10 % should be used to indicate successful and clinically relevant weight loss as, despite initial body weight, intraabdominal fat loss is also expected to be relatively large (approximately 30%) at 5−10 % weight loss [86,89]. Overall, national and international guidelines emphasize reducing energy intake as the foundation of dietary treatment of obesity prescribing a 500−1000 kcal energy deficit [86,87], and supporting a variety of dietary approaches to achieve the sufficient energy deficit for inducing weight loss. These approaches include energy-restricted macronutrient-targeted diets including both low-carbohydrate and

low-fat diets, a low-glycemic-load diet, vegan or vegetarian diets, as well as very low-energy diets (VLEDs, ≤800 kcal), the latter only for a shorter period of time [87]. The guidelines also emphasize that the selected dietary approach must be based on the personal preferences of the individual with obesity and that successful diet-induced weight loss is dependent on long-term adherence to the dietary approach. Due to the diversity in the macronutrient composition in approaches for the treatment of obesity, these approaches may or may not comply with the National dietary guidelines for the general population recommending 25−40 E% fat, 45−60 E% carbohydrate, and 10−20 E% protein [90].

1.2.2 Dietary approaches

Although national guidelines do not include specific treatment strategies or treatment goals for targeting abdominal obesity, it remains highly debated in the literature whether certain dietary approaches are more effective than others for reducing visceral fat volume specifically. In the following section, an overview of important approaches addressing energy restriction, macronutrient composition, meal timing and diet quality will be presented, with particular emphasis on carbohydrate quality.

Energy restriction

A recent review investigating whether hypocaloric diets are effective strategies for the reduction of VAT specifically, concluded that hypocaloric diets are associated with important reductions in VAT, independent of ethnicity, age or biological sex [91]. Dietary approaches for the management of abdominal obesity include targeting overall energy reduction through conventional energy restriction of a specific amount of calories (e.g., 500−1000 kcal/d) or as a percentage of estimated energy requirements, low energy diets (e.g., 800−1200 kcal/d), or VLEDs (≤800 kcal/d). In a recent 1-year randomized controlled trial (RCT) [92], changes in abdominal fat mass, including VAT and SAT mass (measured with MRI), were compared between participants (postmenopausal women with obesity) following a severely (65−75% energy restriction for 4 months followed by 25−35% for the remaining 8 months) or moderately (25−35%) energy-restricted diet. VAT loss was greater in the severe group compared with the moderate group after

12 months, suggesting that initially large and rapid weight loss on VLEDs may be beneficial for the reduction of VAT mass. In a systematic review by Chaston and Dixon [93], early preferential loss of VAT mass using severe energy restriction is supported. However, the effect was found to be temporary, whilst preferential loss of VAT compared with SAT was greatest with modest weight loss in the long run.

In contrast to the more traditional continuous energy restriction approaches as described above, intermittent energy restriction has recently emerged as an alternative approach and involves, for example, cycling between days of fasting with extreme energy restriction (e.g., 500−800 kcal/d) and days with ad libitim intakes without energy restriction. Intermittent energy restriction gained increasing interest and attention in the literature after observations of similar or greater improvements in cardiometabolic risk factors and chronic disease prevention when comparing intermittent with continuous energy restriction in animal studies [94]. However, a 50-week RCT among adults with overweight or obesity (n = 150) found no significant differences in body weight or VAT (measured with MRI) at any of the follow-up time points [95]. These findings are in agreement with the similar effects of intermittent versus continuous energy restriction on weight loss and changes in abdominal obesity (WC) reported in a Norwegian 1-year RCT [96].

Macronutrient composition

A variety of dietary strategies for weight loss that target macronutrient composition have been extensively studied during the past few decades, with particular emphasis on the restriction of fat or carbohydrate intake [97–99], as these macronutrients are the main sources of energy in the human body and also have different hormonal (e.g., on leptin, insulin, and ghrelin) and other physiological effects [100–102]. Although the beneficial effects on weight loss appear similar between low-carbohydrate and low-fat diets with comparable energy intake levels in several RCTs [83,98,103,104], the degrees of energy restriction and definitions of these diets differ greatly [105].

Low-fat diets

Low-fat diets are often diets restricting fat intake to 10−30 E%, and also specifically limiting the intake of saturated fatty acids to a maximum of 10 E% [97,104]. Low-fat diets (e.g., the Ornish diet or the LEARN [Lifestyle, Exercise, Attitudes, Relationships, Nutrition] diet) may or may not have specific recommendations for energy intake, protein, or carbohydrate contribution. However, concern has been raised about insufficient nutrient intake on very low-fat diets, particularly the intake of essential fatty acids [106]. In general, low-fat diets, compared with high-fat diets, are more similar to the macronutrient composition recommended in the National and International dietary recommendations for the general population [90,107,108]. However, the recommended total fat intake has gradually increased, as exemplified by the recommended maximal fat intake in the US of <30E% in 1980, increasing to 20−35E% in 2005, whilst from 2015 the upper limit was removed [109].

Low-carbohydrate diets

Low-carbohydrate diets (e.g., the Atkins diet or Paleolithic diet) are often diets restricting carbohydrate intake to 10−30 E%, or ≤20 g/d for a restricted amount of time (e.g., 2 weeks or 2−3 months) or until desired weight loss is achieved, with a consequent increase of carbohydrate intake at a rate of 5 g/week up until ≤50 g/d [83,97–99,110]. Total energy, protein, and fat intake are commonly not restricted, but unintended energy restriction when following ad libitum low carbohydrate diets can occur [111]. Nutritional concerns associated with low-carbohydrate diets are insufficient intakes of fiber and micronutrients by limiting the intake of micronutrient-dense carbohydrate foods such as fruits, vegetables, legumes, and whole grains [112].

Overall, randomized controlled trials show beneficial effects of low-carbohydrate diets for reduction of abdominal obesity both in the short-term [83,103] and the long-term [98], although several studies report no significant differences in weight loss between low-carbohydrate or low-fat diets [83,98,99]. However, few randomized trials comparing low-carbohydrate (≤50 g/day) and low-fat (25−35 E%) diets have included precise quantification of VAT volume [83,84], and there is a lack of long-term studies as these

studies lasted only 2−3 months. Of note, low-carbohydrate diets have also shown rapid reductions in hepatic fat in humans, despite relatively high energy intake and minimal total weight loss [113].

Ketogenic diets

Both very-low-calorie diets and low-carbohydrate diets can lead to diet-induced ketosis as a result of severely restricted carbohydrate availability and depleted glycogen storage [114]. The production of ketone bodies may rise in response to inadequate glucose supply, becoming one of the main alternative sources of energy for the human brain [115]. Interestingly, ketogenic diets have been shown to induce significant weight loss while also suppressing the expected increase in appetite and hunger feelings following weight loss [116]. In a recent statement from the National Lipid Association Nutrition and Lifestyle Task Force [105], the authors pointed to several knowledge gaps, including the threshold for carbohydrate intake to achieve these suggested benefits, the exact mechanisms through which ketosis may regulate appetite, and at what level of ketosis these effects are evident [117]. Although one of the few RCTs investigating the effect of low-carbohydrate diets on VAT volume aimed at inducing ketosis through a very-low-calorie diet (800 kcal/d) in combination with restricted carbohydrate intake (<50 g/day), the authors did not report levels of ketosis or dietary intake to confirm that participants were in ketosis [84]. Studies that only categorically report whether participants are in ketosis or not, without reporting the specific levels of ketosis achieved or by failing to report the state of ketosis overall, is a common limitation in the current literature.

Meal timing

Recent hypotheses suggest that also meal timing and associated eating behavior may influence body weight regulation, and that timing of food intake is associated with the development of obesity [118–120]. Additionally, the distribution of energy intake throughout the day may have effects on metabolic health, such as when meal patterns conflict with internal circadian clocks (chronotype) [119,121]. An example of the association between meal patterns and abdominal obesity is the increased VAT

(measured with MRI) observed in Hispanic 18−19 year-olds when eating infrequently with <3 eating occasions (≥50 kcal and ≥15 min from the previous eating occasion) per day [122].

Despite the growing interest in chrononutrition [123] and the reported relationship between meal timing and the development of obesity [118–120], few meal timing interventions that assess changes in abdominal obesity have been conducted [124,125]. Two 12-week RCTs in women with overweight or obesity compared the effect of hypocaloric diets with 50 % of total daily energy intake at breakfast [124] or lunch [125] with consuming 50 % of total daily energy intake at dinner. These studies reported favorable changes in weight loss and reduction of WC in the participants consuming a higher proportion of their daily energy intake at breakfast or lunch compared with at dinner. In addition, although not addressing changes in abdominal obesity as an outcome measure, a recently published 11-week pilot randomized crossover trial in nineteen night-shift workers with abdominal obesity compared avoidance of energy intake during a fixed 5-hour window during the night shift (01:00−06:00) with the maintenance of usual dietary habits [126]. The pilot study showed that night shift workers were indeed able to maintain the short overnight fast by rearranging their meals over 24 hours promoting a small shift in body weight. These findings are in line with previous studies in normal-weight adults showing that consuming a high proportion of daily energy intake at night or late evening is associated with a higher total energy intake [127,128]. Although the evidence is limited, dietary approaches targeting meal patterns, including meal frequency and energy distribution, may prove beneficial for the reduction of visceral fat volume.

Dietary patterns and diet quality

Most studies examining the association between abdominal obesity and dietary intake have focused on a single or a few nutrients or foods. However, the single-nutrient approach in nutritional epidemiology may be insufficient in addressing the impact of overall diet on the risk of chronic diseases [129]. Beyond the traditional single-nutrient approach, the importance of understanding more complex dietary patterns and diet quality has gained interest in recent years [130]. This shift of focus may be seen in

connection with a change in the diet-related global disease burden, changing from undernutrition and deficiency of specific nutrients to non-communicable diseases [131,132]. Dietary pattern analysis allows for examination of the overall diet, including complex combinations of nutrients and food consumed together, and captures a greater picture of food choices and eating behaviors [129].

Diet quality is a broad concept involving assessment of the overall quality and variety of the diet as a whole, rather than nutrient quality alone, and is measured by the use of dietary quality indexes to evaluate adherence with dietary guidelines (e.g., the Healthy Eating Index, HEI) or other dietary patterns regarded as of high-quality (e.g., the Mediterranean Diet Score) [133]. In a systematic review of the association of diet quality indexes with obesity in adults, using a priori dietary pattern analysis [134], two of the included studies [135,136] investigated the effect of the adherence to the HEI on abdominal obesity (defined as WC ≥102 cm for males and ≥88 cm for females) as an outcome measure using NHANES data (NHANES III [1988−1994] and NHANES 2001−2008). The HEI is based on adherence to current and previous versions of the Dietary Guideline for Americans [137], and the two studies used either the original HEI from 1995 [135] or HEI-2005 [136]. Both studies concluded that the odds of having abdominal obesity were significantly higher when the HEI score was low and that higher overall dietary quality was associated with a lower risk of abdominal obesity.

Dietary quality indexes are not restricted to evaluating the intakes of food or beverages alone, but may also incorporate the intakes of specific nutrients, as exemplified in the Mediterranean Diet Score including the ratio of monounsaturated to saturated fat as an indicator of the intake levels of olive oil versus fats from animal sources [138]. Adherence to the Mediterranean dietary pattern, characterized by high intakes of fruit, vegetables, olive oil, nuts, and legumes, has been associated with lower abdominal obesity assessed by WC [139], truncal adiposity measured by dual-energy x-ray absorptiometry in women [140], and by VAT measured by ultrasonography [141] in cross-sectional studies. Dietary quality indexes may also be designed to score adherence with dietary patterns targeting specific risk factors such as the Dietary Approaches to Stop Hypertension (DASH) to help treat or prevent hypertension. Unfortunately, there are currently no dietary quality

indexes addressing adherence to the optimal diet for the reduction of VAT volume, as the major components of such a diet remain unknown, although adherence with the HEI and the Mediterranean dietary pattern has shown promising results in observational studies [135,136,139–141].

Carbohydrate quality

Established markers of carbohydrate quality include dietary fiber, added sugar, the glycemic index (GI) or glycemic load (GL), and whole grains [142]. The GI is defined as the blood glucose response, measured as the area under the curve (AUC), after consumption of a standard amount of carbohydrates from a test food relative to the response after consumption of a control food [143,144]. Whilst the GL of a food, meal or diet is determined by multiplying the GI by the amount of carbohydrates consumed [145,146]. Naturally occurring whole grains are plant seeds, also known as kernels, consisting of three components: the fat and protein-rich bran, the fiber-rich germ and the starchy endosperm [147]. In minimally processed or coarsely milled whole grains, all three components of the kernels are retained. Fiber content tends to be higher, and GI tends to be lower in minimally processed whole grain foods compared to more processed foods. More processed whole-grain foods, e.g., refined grains, may consist of only the starchy endosperm or include varying amounts of bran and germ which are reincorporated after being milled into fine particles [147]. In addition, whole-grain foods may contain a high amount of added sugar and have become more highly processed during recent decades [142].

Coinciding with the major increase in obesity prevalence over the last few decades, there has been extensive globalization of the food system and increased availability of processed foods high in added sugar and other refined carbohydrates [148,149]. In particular, there has been a marked increase in the consumption of sugar-sweetened beverages [150], and positive associations have been reported between intake of sugar-sweetened beverages and obesity and obesity-related chronic disease risk in systematic reviews and meta-analyses [151,152]. In a 10-week double-blinded parallel-arm study of 39 adults with overweight or obesity (BMI of 25–35 kg/m^2) consuming 25% of estimated daily energy requirements as either glucose- or fructose-sweetened

beverages, VAT increased significantly when consuming fructose-sweetened but not glucose-sweetened beverages, despite comparable weight gain [153]. Of note, the amount of sugar planned in this particular study (25 E%) was substantially higher than the mean intake of added sugars both in Norway (11 E% in 2019) and in the US (13 E% in 2015-2016) [154,155]. In an RCT published 2 years later, Maersk et al. [156] confirmed the observed negative effect of sugar-sweetened beverages on the VAT depot, in addition to liver fat accumulation, over 6 months when comparing daily intake of 1 L regular cola (50% glucose and 50% fructose) with 1 L diet cola, semi-skimmed milk, or still mineral water. In contrast, the results from a 2-year cohort study indicated a protective effect of higher fiber intakes, especially insoluble fiber from vegetables and fruit, on increases of visceral adiposity in Latino youth, independent of change in energy intake [157].

In addition to sugar and dietary fiber content, the glycemic index (GI) or glycemic load (GL) of foods or meals [158] may also be clinically useful markers of carbohydrate quality. For example, a 4-month trial found beneficial effects of low GL-diets, especially in females [159]. Specifically, this trial using GL as a marker of carbohydrate quality found an 11% reduction in VAT volume on an 8-week hypocaloric diet in the low-GL group compared to an increase of 1% in the high-GL group [159]. However, these diets differed not only in GL but also in macronutrient composition (low-GL: 43 E% carbohydrates; high-GL: 59% carbohydrates), preventing attribution of the VAT reduction to carbohydrate quality alone. In addition, in a recent review by Reynolds *et al*. [142], total dietary fiber and whole grains were reported as clinically relevant markers of carbohydrate quality associated with reduced incidence and mortality from NCDs, whilst GI and GL were considered less useful markers due to inconsistent findings and low-quality evidence (using the quality assessment protocols recommended by the GRADE Working Group [160]).

However, these traditional markers of carbohydrate quality do not adequately account for the possible metabolic impact of the food matrix [161], and there could be adverse metabolic effects of carbohydrate refining aside from added sugar (e.g., flour-based products, even whole-grain versions). An alternative marker of carbohydrate quality has therefore been proposed, “cellularity”, based on the degree of food matrix

breakdown and intactness of the cellular structures in plant-based foods [162]. The cellularity of the diet may affect oral processing, the composition, and function of the microbiota, and the degree of bioavailability and absorption of carbohydrates, also irrespective of GI [162]. The cellularity of carbohydrate foods, or more precisely the increased consumption of acellular forms of carbohydrate foods due to modern processing techniques, has been proposed to contribute to obesity [162]. However, this has not been tested in controlled trials. Generally, important questions remain regarding the impact of the carbohydrate restriction and the types of foods and carbohydrate sources consumed, especially on long-term effects.

2. Aims of the thesis

The overall aim of this PhD thesis was to determine the effect of dietary intervention approaches differing in carbohydrate quality and amount on visceral adiposity and measures of appetite in adults with obesity.

The specific aims of this thesis are as follows:

Paper I: To identify and describe food intake profiles, including dietary and meal patterns, for a population with obesity, and assess how the dietary and meal patterns are associated with caloric intake and/or anthropometric measures, using cross-sectional data.

Paper II: To determine long-term (up to 1 year) effects of isocaloric diets differing in dietary carbohydrate quality (cellularity) or amount on visceral fat volume and anthropometric measures in adults with obesity.

Paper III: To evaluate the relationship between subjective feelings of appetite, fasting plasma concentrations of ghrelin and ketones (β-hydroxybutyrate, βHB) on isocaloric diets within a moderate caloric range and varying in carbohydrates quality (cellularity) and quantity, both in the short-term (3-month) and long-term (1 year) in adults with obesity.

3. Methods

3.1 Study design and participants

The participants included in this study are participants of a 2-year RCT, the CARBFUNC study. The data included in this thesis were collected from enrolment to 1-year follow-up. The key features of Paper I, II and III are outlined in **Table 2**.

Table 2. Key features of the included papers.

Characteristic	Paper I	Paper II	Paper III
Study design	Cross-sectional	RCT	RCT
Sample size	192	192[1]	192[1]
Study visits	Baseline	Baseline, 3, 6, 9 and 12 months	Baseline, 3, 6, 9 and 12 months
Intervention	No intervention	1) Acellular diet 2) Cellular diet 3) Low-carbohydrate high-fat diet	1) Acellular diet 2) Cellular diet 3) Low-carbohydrate high-fat diet
Dietary data collection	2 x 3-day dietary recordings	3-day dietary recordings every second week	3-day dietary recordings every second week
Outcomes	Meal and dietary patterns Anthropometric measures	Abdominal fat volume Anthropometric measures	Total ghrelin Subjective feelings of appetite

[1] Baseline measures were collected from 192 participants and follow-up measurements from 118, 87, 64 and 57 participants at 3, 6, 9 and 12 months, respectively. Abbreviations: RCT, randomized controlled trial.

The CARBFUNC study was designed as a three-arm randomized controlled dietary intervention trial carried out in a real-life setting with visits to the Research Unit for Health Surveys at the University of Bergen for data collection and individual/group consultations/meetings. Participants were randomized to one of the three dietary interventions after the initial baseline study visit.

3.1.1 Ethical approval and trial registration

The CARBFUNC study was conducted in accordance with the guidelines in the Declaration of Helsinki [163] and was approved by the Regional Ethics Committee in Western Norway (2017/621/REC West). We registered the study protocol at ClinicalTrials.gov (NCT03401970) before enrolment. During screening visits, participants were provided with oral and written information about the study, and written informed consent was collected from all participants before any data collection commenced.

3.1.2 Recruitment and screening visits

Participant recruitment to the CARBFUNC study included a primary and a secondary recruitment period, taking place from September to December 2017 and February to May 2019. Participants were recruited through local newspaper advertisements, radio broadcasts and social media (including advertisements on Facebook and Instagram), in Bergen and the surrounding area. Candidate study participants registered their interest in participation by filling out an online form. All who fulfilled the self-reported non-sensitive eligibility criteria listed in the online form were contacted and invited to a screening visit at the research unit.

3.1.3 Inclusion and exclusion criteria

The participants recruited for the CARBFUNC study were relatively healthy adults with obesity. Participants were assessed for eligibility at the screening visit. Obesity was here defined as BMI ≥30 (kg/m2) or WC ≥102 cm for males and ≥88 cm for females or both BMI and WC above these cut-off values. In addition to obesity, inclusion criteria were age 20−55 years and <5% change in body weight within the last 2 months. Whilst exclusion criteria included smoking, known food allergies, habitual alcohol consumption of >2 alcohol units per day, recent surgical or antibiotics treatment during the past 2 months, use of statins and/or diabetes medication, severe diseases, including chronic inflammatory bowel disease, and for female subjects: pregnancy, breastfeeding and post-menopause. The rationale for excluding individuals over 55 years of age and postmenopausal females was to recruit a relatively

homogeneous study population, in particular, to limit the impact of menopause/loss of estrogen which could influence the outcome measures independent of the interventions [164].

Participants who fulfilled the initial inclusion criteria and gave written informed consent were invited to demonstrate the ability to complete online dietary recordings as a final prerequisite for enrolment.

3.1.4 Randomization and blinding

Research personnel not otherwise involved in the study randomly allocated participants to one of the three study diets after baseline data collection, using block randomization with block sizes of 6−9 and stratification by sex (R package *blockrand*, version 1.5, RStudio, Inc., Boston, MA) to ensure a balance in sample sizes and sex distribution across the diet groups. Participants taking part in the study together with a partner, family member or friend were allocated to the same intervention group to facilitate adherence and limit disclosure of the diets across groups.

Due to the nature of the dietary intervention, the study participants and staff were not blinded to the intervention group allocation after randomization, with exception of the study statistician who was blinded to the group identities until all outcome measures reported in this thesis had been analyzed.

3.1.5 Study diets

The three study diets of the CARBFUNC study were designed to compare the effect of dietary carbohydrates, both quality and quantity, on the study outcomes. The quality of dietary carbohydrate sources was defined based on the degree of cellularity as a guiding principle resulting in the following study arms:

1) an acellular high-carbohydrate low-fat diet (A-HCLF)
2) a cellular high-carbohydrate low-fat diet (C-HCLF)
3) a low-carbohydrate very-high-fat diet (LCHF)

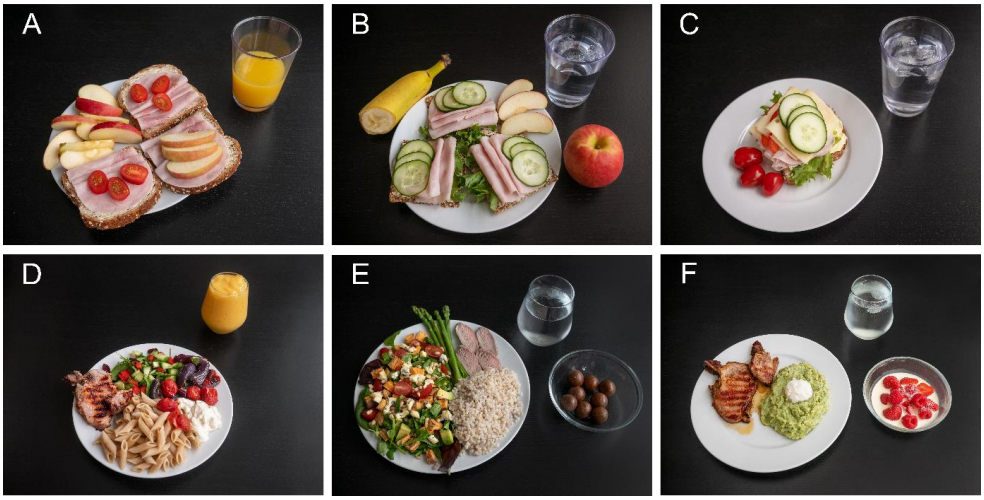

Figure 3. Images from the diet-specific recipes. Images A, B and C show breakfast/lunch options, whilst D, Ewere and F show dinner options. Images representing the specific diets are as follows: A and D, A-HCLF; B and E, C-HCLF; C and F, LCHF.

Energy and macronutrient composition

All three diets were planned as isocaloric diets with 2000 kcal and 2500 kcal per day for female and male participants, respectively. The planned macronutrient profiles of the A-HCLF and C-HCLF diets were 17 energy percent (E%) protein, 45 E% carbohydrate and 38 E% fat, differing only in the quality (cellularity) of dietary carbohydrates, and 17 E% protein, 8 E% carbohydrate and 75 E% fat for the LCHF diet. Although the diets were planned with a moderate energy intake, as opposed to low-energy or very low-energy diets, the planned carbohydrate restriction on the LCHF diet (8 E%, corresponding to 40 and 50 g/day for female and male participants) was below the threshold level suggested in the literature to lead to nutritionally induced ketosis. The planned contribution of saturated fatty acids was 10−12 E% for the A-HCLF and C-HCLF diets, and 30 E% for the LCHF diet. Finally, the planned intake of added sugar was <5 E% for the A-HCLF diet and <1 E% for the C-HCLF and LCHF diets.

Diet-specific recipes booklets

For each study diet, an extensive recipe booklet including diet-specific recipes was provided to all participants. The booklets were developed with the application FileMaker Pro 18 Advanced (Claris International Inc., Santa Clara, CA, USA), which also enabled the linking of recipes and ingredients to a comprehensive database of nutritional content as described in section 3.2.3 *Calculations of nutrient intake*. The participants were asked to choose two recipes from the breakfast/lunch recipes (50% of total daily energy intake) and one dinner recipe (50% of total daily energy intake) per day. Of note, the study diets were not eucaloric since tailoring the recipe booklets to individual energy requirements, rather than standardized according to sex, was not compatible with the recipe database. All recipes were given a unique identifier, included accurate amounts of each ingredient and food item, together with preparation instructions. To improve adherence with the dietary recordings during the study intervention, participants were encouraged to record these unique identifiers rather than each food item or ingredient from the recipes. The nutrient content of each recipe was pre-calculated so that all diet-specific recipes complied with the macronutrient profiles (both in grams and percentage of energy) and food profiles of the study diets. More than 175 recipes were provided for each diet, allowing the participants to choose from a variety of meals, both simple and more advanced.

Food profile

To distinguish between cellular and acellular carbohydrate sources when designing the diet-specific recipes, some assumptions were made based on previous knowledge of the food matrix and the effects of food processing on these structures. The degree of cellularity was considered as a continuum, where acellular carbohydrate sources included more processed/refined carbohydrate-rich products, such as bread, bakery products, fruit juice, pasta, and quick oats, whilst cellular carbohydrate sources included minimally processed or refined carbohydrate foods, such as whole (unground) grains, fruit and vegetables, unpolished rice, potatoes and rolled oats. In terms of cellularity, wheat flour with degraded cellular structures as opposed to naturally occurring wheat kernels with intact cells both represent extremes of the continuum.

On all three diets, the participants were encouraged to adhere to principles of a varied and nutritious diet by choosing recipes that included 2−3 dinners of fish per week, 2−3 portions of dairy products per day and ≥500 grams of fruits, berries and/or vegetables per day. Sugary drinks were not included in any of the study diets, and avoidance of artificial sweeteners was encouraged. By these measures, our goal was to avoid implementing a dietary intervention that favored one diet over the others with regards to overall dietary quality.

3.1.6 Study visits and participant follow-up

Data collection in the CARBFUNC study took place at the Research Unit for Health Surveys at six time points: baseline and 3, 6, 9, 12 and 24 months. As mentioned previously, findings from the 2-year follow-up were not included in this thesis. The participants arrived at each study visit in the morning after fasting for ≥12 hours, abstaining from alcohol consumption for 24 hours and avoiding any strenuous physical activity for the past 48 hours.

All participants received support to attain dietary adherence and underwent individual counseling/motivational interviewing with a member of staff between baseline and 3 months and were further given a choice of individual counseling or group sessions between the remaining study visits. The group sessions covered content appropriate for their respective diets, including food preparation, maintaining the assigned diet while traveling or dining at restaurants, and strategies for adherence throughout the intervention.

3.2 Dietary recordings

The participants' final prerequisite for enrolment in the CARBFUNC study was to demonstrate the ability to complete online dietary recordings. The results from these pre-intervention dietary recordings were used in Paper I to further investigate habitual meal and dietary patterns as described under the outcome measures section for Paper I

(*3.3.1* and *3.3.2*). In Papers II and III, the dietary records conducted during the study intervention were used as a measure of adherence to the assigned study diet.

3.2.1 Paper I

Before the participants started recording their dietary intake, they took part in training classes, led by a member of the research team, on the use of the online dietary recording system and received a digital kitchen scale. Each participant received a personal user ID in the online dietary recording system (www.diett.no; operated by Dietika AS, Slemmestad, Norway) to submit daily food consumption. Participants were asked to record the consumption of food and beverages, including all meals and snacks, the weight and amount of all consumed ingredients and products, the time of intake and any additional comments on their food intake.

Participants conducted three-day weighed food records for three consecutive days, including one weekend day. The procedure was repeated once more over the course of two weeks resulting in six days of dietary records (five consecutive days with a gap to include the last weekend day for most participants). The participants were explicitly asked not to change their dietary habits during the period of data collection, and the scientific value of honest and complete records was strongly emphasized.

3.2.2 Paper II and III

Whilst pre-intervention dietary recordings were used in Paper I, Papers II and III included dietary records conducted during the study intervention.

The participants were asked to record dietary intake for three days every second week, including two weekdays and one weekend day, throughout the intervention. As in Paper I, the participants used www.diett.no to record dietary intake, but during the intervention, the participants recorded the unique identifiers of the predefined recipes of choice, as opposed to recording single food items/ingredients. Alternatively, modifications of the recipes or own compositions of meals were recorded as in Paper I.

3.2.3 Calculations of nutrient intake

Following the export of the dietary recording data from www.diett.no, nutritional content was calculated largely based on the latest update of the official Norwegian Food Composition Table [165] or the nutrient declarations provided by the producer/retailer. Values from international databases were used (Danish or US food composition tables) when Norwegian data were not available. These comprehensive data were merged into a database tailored for the CARBFUNC study using the application FileMaker Pro 18 Advanced (Claris International Inc., Santa Clara, CA, USA).

Manual data integrity checks were performed for quality assurance of dietary recordings to help identify and correct possible errors caused by misunderstandings of responders, misinterpretations, or typing entry errors. Altogether, the database contained 2210 food and beverage items.

3.3 Outcome measures and procedures

The outcome measures included in Paper I were based on pre-intervention dietary recordings and baseline measures in the CARBFUNC study, whilst Paper II and III include outcome measures assessed from baseline to 1-year follow-up. The time points for data collection of measures of anthropometry, body composition, PAL, appetite and ketosis were at baseline and 3, 6, 9 and 12-months follow-up. Meal patterns and dietary patterns were only derived at baseline, and CT scans were performed at baseline and 6 and 12-months follow-up. In the following section, the outcome measures reported in this thesis will be described.

3.3.1 Meal patterns

To examine meal patterns in Paper I, we derived temporal patterns (related to timing and distribution of meals) using hierarchical cluster analysis based on total energy intake during different time intervals. This was possible because we collected data on the time point of consumption of all recorded meals and food items. We chose

hierarchical cluster analysis due to its flexibility when analyzing time-dependent data. We categorized and named all eating occasions into six four-hour periods based on the recorded time of intake: 04:00 ± 2 hours (Early morning meal), 08:00 ± 2 hours (Breakfast), 12:00 ± 2 hours (Lunch), 16:00 ± 2 hours (Dinner), 20:00 ± 2 hours (Supper) and 00:00 ± 2 hours (Midnight-meal). For each participant, we calculated the mean proportion of daily energy intake in each time interval across all six recording days and submitted these values to clustering.

3.3.2 Dietary patterns

Dietary pattern analysis includes a priori approaches characterized by dietary scores or indexes and a posteriori dietary pattern analysis characterized by data-driven approaches [138]. In Paper I, we used an a posteriori approach, k-means cluster analysis, based on the variation of the mean daily intake in grams of the food and beverage groups. We chose daily intake in grams rather than energy intake from the specific food and beverage groups due to the frequent use of low-calorie products reported in the dietary recording. Also, we observed that cluster membership was largely defined by liquid-based food items (i.e., coffee, alcohol, sugary drinks). Therefore, we separate food and beverage categories into two separate factor analyses. For a further description of the food and nutrient intake across the derived clusters, we calculated the nutrient intake and mean intake of foods from each food group.

3.3.3 Anthropometry

Height was measured in the upright position with the Frankfort plane horizontal, using a portable stadiometer (Seca 217, Seca GmbH & Co. KG). Bodyweight was measured with a Class III approved calibrated scale (Seca 877, Seca GmbH & Co. KG) to the nearest 100 g in light clothing without shoes. BMI was calculated as weight in kilograms divided by the square of the height in meters. In contrast to BMI, there is currently no single universally excepted method to measure WC, but two protocols are most often used [166]. We choose one of these two protocols and measured WC with a non-elastic tape halfway (the mid-point) between the point of the lowest rib and the

iliac crest. The measure was repeated three times and the average of the two last measurements was recorded.

3.3.4 Body composition

Body composition was measured by a segmental multifrequency bioelectrical impedance analyzer (Seca mBCA 514, Seca GmbH & Co. KG). The measurements were conducted following the device manufacturers' instructions. The body composition outcome included in this thesis was body fat mass at baseline in Paper I.

3.3.5 CT scans

In non-contrasted abdominal CT scan images, we quantified VAT volume (cm^3), SAT volume (cm^3), total abdominal fat volume (VAT+SAT, cm^3), liver density (in Hounsfield units, HU) and liver-to-spleen density ratio (calculated as liver/spleen attenuation index using Hounsfield units; mean hepatic HU/mean splenic HU) in the upper abdomen. The participants underwent CT scanning in a supine position using a 384-slice multidetector CT scanner (SOMATOM Force, Siemens; Siemens CARE Dose 4D automatic exposure control system; 120 peak kilovoltage; 20 milliamperes).

VAT, SAT and total abdominal fat volumes were quantified in iNtuition software (TeraRecon Inc., San Mateo, CA, USA) using a semi-automated method based on segmentation of pixels with values for HU corresponding to fat tissue (−195 to −45 HU) [167]. The segmentations were conducted on 5 mm thick cross-sectional CT scan images from the participant's upper right diaphragm to the vertebral corpus L5/S1 level. After the initial automatic segmentation of VAT and SAT, all segmented volumes were visually verified and manually adjusted if necessary (tracing the abdominal muscular wall separating the two compartments).

Liver and spleen HU densities were measured on single-slice CT images from the central liver and spleen, respectively. Trained personnel performed manual tracing of 15 mm^2 regions of interest (ROIs), three for each organ, and were instructed to avoid vessels and hepatic/splenic pathology when feasible. The average HU score of each

ROI was used to calculate liver and spleen density. Finally, the liver-to-spleen density ratio was calculated.

3.3.6 Physical activity levels

Physical activity recordings were collected pre-intervention for three days, parallel with the pre-intervention dietary recordings. During the intervention, physical activity was recorded for three consecutive days every three months to obtain a three-day recording between each study visit, to assess whether participants followed the encouragement to maintain a similar physical activity level throughout the intervention. Participants recorded the frequency, duration and intensity for all daily life activities and sports in the same online system as used for dietary recordings. The recordings were used to estimate a physical activity level for each participant based on the sum of estimated energy expenditure for each recorded activity and their associated metabolic equivalent (MET) values [168] divided by 24 hours.

3.3.7 Appetite measures

Subjective feelings of appetite (hunger, fullness, desire to eat and prospective food consumption) were measured by a validated visual analog scale (VAS) [43] to answer the following questions:

- How hungry are you? («Hvor sulten føler du deg?»)
- How full do you feel? («Hvor mett føler du deg?»)
- How strong is your desire to eat? («Hvor mye tror du at du kan spise?»)
- How much could you eat? («Hvor mye har du lyst til å spise?»)

Participants were instructed to answer these four questions by making a mark on a straight 10 cm line anchored by an extreme answer to each question.

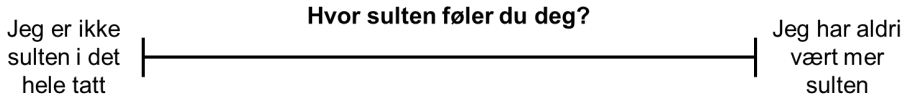

Figure 3. Visual analog scale (VAS) was used in the CARBFUNC study. "How hungry do you feel" was one of four questions regarding subjective feelings of appetite anchored with "not at all hungry" vs. "as hungry as I have ever felt".

The participants were asked to rate subjective feelings of appetite at three time points: in the fasting state and postprandially 120 minutes and 240 minutes after eating a mixed meal. VAS has previously been shown to exhibit a good degree of intrasubject reliability and is therefore suitable for repeated measurements [43].

For the objective measure of hunger, total concentrations (pg/mL) of the orexigenic hormone ghrelin were measured in fasting plasma by a Human Ghrelin (Total) ELISA kit (Millipore, EZGRT-89K). The analysis was conducted at the Medical Research Center at Oulu University Hospital (Oulu, Finland). Measurements of acylated ghrelin were not performed as plasma samples were not immediately treated with protease inhibitors as required to prevent degradation of acylated ghrelin.

3.3.8 Ketosis

To determine to what degree the low carbohydrate intake on the LCHF diet resulted in diet-induced ketosis, we measured concentrations of total βHB (the main circulating ketone body) and the ketone body acetoacetate (AcAc) in mmol/L were measured in fasting plasma samples by GC-MS/MS, a highly sensitive and accurate method [169].

3.4 Statistical analyses

The statistical analyses were performed in R version 4.0.3 for Paper I and version 3.6.1 for Papers II and III (https://www.R-project.org) by a statistician blinded to the groups.

Data transformation and exploration were performed with the *tidyverse* packages (https://tidyverse.tidyverse.org) and data were visualized in all three papers using the *ggplot2* package (https://ggplot2.tidyverse.org). The NbClust v3.0 R package was used to generate graphs to allow for visually deriving the number for "k" in the k-means cluster analysis [170]. In Papers I, II and III all inferential tests were two-tailed with a level of significance set at $P < 0.05$.

The effects of the three study diets on the primary and secondary outcome measures reported in Papers II and III were compared by an intention-to-treat (ITT) analysis including available data from all randomized participants (n = 192). The ITT analysis was conducted using baseline-adjusted constrained linear mixed-effects modeling (cLMMs). We did not use multiple imputation of missing values as linear mixed-effects modeling without prior imputation has been demonstrated, in studies with a high proportion of missing data, to be more powerful than other options for an ITT analysis [171–173].

In Papers II and III, all outcomes were reported as absolute or relative within- and between-group differences in change scores. In the between-group comparisons, the A-HCLF diet was a priori defined as the reference group (comparator arm). The primary outcome reported in Paper II was the between-group differences in absolute change scores in VAT volume, whilst the main outcomes reported in Paper III were the between-group differences in absolute changes scores in fasting total ghrelin and subjective appetite.

3.4.1 Sample size calculation

The a priori sample size calculation for the CARBFUNC study was based on the primary outcome measure (VAT volume, reported in Paper II) using previous research reporting the effects of different diets on visceral fat loss [83,174]. We chose not to conduct any post hoc sample size calculation for Papers II and III as such approaches have been considered flawed [175,176].

To detect a significant group difference in the study's primary outcome, expected to be around −1300 (500) cm^3 in one group and −1800 (700) cm^3 in the other, our power

calculation suggested that n = 18 in each group would be sufficient if the alpha level (type I error rate) was 0.05 and the beta level (type II error rate) was 0.2 (i.e., statistical power of 80%). More participants were recruited to account for dropouts, given the strict and long-term dietary program, and to increase power for exploratory analyses at the earlier follow-up time points.

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4. Results

4.1 Paper I

In Paper I, we set out to explore the habitual diets of people with obesity, analyzing dietary and meal patterns of 192 (females: 53%, males: 47%) adults using cluster analyses based on six days of dietary records. As recording habitual dietary intake was a prerequisite for participation in the CARBFUNC study, the compliance was extraordinarily high, with participants overall completing 99.1% of the planned dietary recording days.

Key baseline characteristics (mean (SD)) were age 41.6 (8.8) years, BMI 36.7 (4.8), body weight 111 (19) kg, and WC 113 (12) cm and 121 (11) cm for female and male participants, respectively. The recorded daily energy intake (mean ± SD kcal) was 2120 ± 520 kcal and 2550 ± 550 kcal for female and male participants, respectively. Self-reported occupational and leisure-time physical activity level was significantly higher ($P = 0.004$) in female (1.6 ± 0.2) compared to male (1.5 ± 0.2) participants.

The most important finding reported in Paper I was the differences in energy intake between the five clear temporal meal patterns that were identified and labelled "Dinner-eaters" (n = 16, females: 69%, males: 31%), "Lunch-eaters" (n = 13, females: 62%, males: 38%), "Supper-eaters" (n = 36 females: 58%, males: 42%), "Midnight-eaters" (n = 29, females: 58%, males: 42%), and "Regular-eaters" (n = 59, females: 49%, males: 51%). The daily reported energy intake was highest among "Midnight-eaters" (2551 ± 554 kcal), and significantly ($P <0.05$) higher than "Dinner-eaters" (2064 ± 546 kcal), "Lunch-eaters" (2082 ± 622 kcal) and "Supper-eaters" (2102 ± 461 kcal), but not "Regular-eaters" (2328 ± 653 kcal).

Despite a difference of up to 490 kcal in reported energy intake between the clusters with the highest and lowest energy intake, there were no significant differences between clusters in reported physical activity level or the anthropometric measures including body weight, BMI and WC.

In addition, four non-overlapping dietary patterns were identified based on the variation of the mean daily intake in grams of the food and beverage groups. These patterns were named according to the food groups characterizing each of them: 1: Meat, cheese, rice, pasta, pulses and oil; 2: Vegetables, fruit and seafood; 3: Sugary foods; and 4: Bread, cereal products and convenience foods.

4.2 Paper II

In Paper II, we investigated the effect of the three diets in the CARBFUNC study differing in carbohydrate quality (degree of carbohydrate cellularity) and amount on VAT volume over 12 months.

Of the 192 participants included in Paper I, 57 participants (30%) completed the 1-year follow-up with 14 (females: 50 %, males: 50%) in the A-HCLF group, 22 (females: 32%, males: 68%) in the C-HCLF group, and 21 (females: 29%, males: 71%) in the LCHF group.

Throughout the intervention, reported energy intake was only modestly reduced from baseline and similar energy intakes (females: 1,820−2,060 kcal, males: 2,480−2,550 kcal) were reported on the three study diets. Reported dietary intakes were 42−44, 41−42 and 11−15 E% carbohydrate and 36−38, 37−38 and 66−70 E% fat on the A-HCLF, C-HCLF, and LCHF diets, respectively. After a small reduction from baseline to 3 months, recorded protein intake remained stable at 16−17 E% on all three diets and did not differ between groups at any time point.

The most important finding reported in Paper II was that there were no statistically significant between-group differences in change scores for VAT volume from baseline to 6 or 12 months, nor for SAT, total abdominal fat volume, or any of the anthropometric measures. A significant and similar reduction of VAT volume after 12 months by 12%, 16% and 17% was achieved on the A-HCLF, C-HCLF and LCHF diets, respectively, despite a relatively high energy intake and a moderate weight loss (5−7%).

4.3 Paper III

In Paper III, the main goal was to examine the effects of the three diets in the CARBFUNC study on changes in fasting plasma ghrelin concentrations and subjective feelings of appetite, both in the short-term (3-month) and long-term (12-month).

As reported in Paper II, recorded carbohydrate intake differed substantially between diets, with an average intake in the LCHF group of 59 g (SD 13.6) at 3 months and 86 g (52.6) at 12 months, corresponding to 11−15 E%, compared with 245−252 g and 218−225 g, corresponding to 41−43 E%, on the A-HCLF and C-HCLF diets, respectively.

We found a significant between-group difference in βHB concentrations at 3 months when comparing the LCHF diet and the comparator diet arm (A-HCLF diet). On the LCHF diet, the concentration of βHB significantly increased after 3 months to 0.25 ± 0.25 mmol/L from baseline (0.06 ± 0.07 mmol/L), while the βHB concentration only slightly increased on the HCLF diets (~0.08 mmol/L). After 3 months, βHB gradually decreased on the LCHF diet to 0.08 ± 0.06 mmol/L at 12 months, no longer significantly different between groups or from baseline.

The most important finding reported in Paper III was that total ghrelin concentrations only increased significantly on the A-HCLF diet (46 pg/ml [11, 81]) and the C-HCLF diet (54 pg/ml [21, 88]) after 3 months, while the smaller increase observed after 3 months on the LCHF diet (11 pg/ml [−16, 38]) was not statistically significant.

Despite a significant increase in βHB concentrations and no increase in ghrelin concentrations on the LCHF diet after 3 months, we found no between-group differences in feelings of hunger, desire to eat or prospective food consumption, in the fasting state. Only feelings of fullness significantly differed between the LCHF diet and the A-HCLF diet at 6, 9, and 12 months, decreasing slightly on the LCHF diet and increasing on the A-HCLF diet.

5. Discussion

5.1 Methodological considerations

This thesis includes preintervention cross-sectional data (Paper I) as well as both primary (Paper II) and secondary (Papers II and III) outcome measures from an RCT in adults with obesity. In the following section, firstly (5.1.1), the methodological considerations of conducting and reporting on clinical trials will be discussed in general terms (using the principles of Good clinical practice) with particular emphasis on the specific considerations relevant for the CARBFUNC study. Secondly (5.1.2), important for the inference and generalizability of the findings from the RCT (Papers II and III), a discussion of sample size and dropout rates will follow. Thirdly (5.1.3), the methods used for dietary and physical activity data collection will be discussed (relevant for all three papers) followed by a specific discussion of the main methodological considerations in each paper separately.

5.1.1 Good clinical practice

Good clinical practice (GCP) is an international ethical and scientific quality standard for the design, conduct, recording and reporting of clinical trials with human participants [177]. Adherence to the principles of GCP ensures the safety, rights and well-being of study participants as well as the reliability of the reported study data. In the following section, the conduct and reporting of the CARBFUNC trial will be discussed considering the key principles of GCP.

Study initiation, enrolment, and data handling

Before the trial was initiated, a clear, detailed study protocol including written Participant Information, and an Informed Consent form, was approved by the Norwegian Regional Ethics Committees (REC West).

The written Participant Information included an outline of the study rationale, objectives, and interventions, a description of the potential risks and benefits, and

procedures for data storage to protect the confidentiality of subjects' identities, and data privacy. Of great importance, the Participant Information clearly stated that study participation was voluntary and that participants would be able to withdraw from the trial at any time. Informed consent to participate in the CARBFUNC trial was only collected after the participants had received oral and written information about the study during the initial screening visits.

The trial protocol was registered in the international publicly accessible web-based clinical trials registration site "ClinicalTrials.gov" before enrolment, an important first step to ensure transparency of the study protocol and the pre-defined primary and secondary outcome measures to be assessed. Initial registration of clinical trials is considered an important measure to prevent selective reporting and publication bias [178]. To have increased transparency further, we may have included a statistical analysis plan and data sharing plan which we did not [179]. However, emphasis on transparency has been made in Papers II and III by including visualizations of the distribution of data points of the main outcome measures and by the provision of a comprehensive description of the statistical analyses, allowing for the reproduction of the original analyses performed by the project's blinded statistician.

To allow for secure storage and processing of sensitive personal data collected in the CARBFUNC study, members of the research team were given access to a secure desktop provided by the University of Bergen, SAFE (secure access to research data and e-infrastructure).

Study design and population

RCTs are considered the most powerful and rigorous of study designs to determine whether a causal effect exists between an intervention and the outcomes of interest. However, several potential errors may challenge the conclusions from an RCT, which may be categorized as bias, confounding, and chance [180].

In research, bias refers to factors that may lead to systematic deviations between the observed outcome and the true outcome [180]. In RCTs, the process of randomization is to prevent selection bias that may occur when groups in a study systematically differ

in characteristics that may influence the outcome of interest [181]. However, the recruitment of study participants in the CARBFUNC study may be subject to volunteer bias, as the participants who volunteered may have characteristics that differ from the overall population meeting the inclusion criteria in the area of recruitment. Volunteer bias may limit the generalizability of the study results as the study population may overrepresent certain characteristics in comparison to the target population of interest [182].

A further measure to prevent selection bias in RCTs is blinding of intervention allocation [181]. Due to the nature of our intervention (following an assigned diet) and the close follow-up by the research team, blinding of participants and research staff was not possible or feasible. Therefore, any pre-knowledge of or experience with the allocated study diets may influence motivation and adherence among participants, and influence research staff in the advice given during group sessions and individual follow-up. However, the study statistician was blinded to the group identities until all outcome measures reported in this thesis had been analyzed. In addition, all laboratory analyses were performed without knowledge of the group identities.

The population of interest in the CARBFUNC study were relatively healthy adults (age 20−55 years) with obesity defined according to BMI ≥30 (kg/m^2) and/or WC ≥102 cm for males and ≥88 cm for females. In light of the literature supporting increased WC as a characteristic of a higher-risk obesity phenotype [4,35], the dietary interventions might have yielded slightly different results if we had specifically targeted these individuals by setting BMI *and* WC cut-offs as inclusion criteria rather than BMI *and/or* WC cut-offs. On the other hand, stricter inclusion criteria may reduce the number of participants volunteering for enrolment potentially limiting the sample size.

5.1.2 Sample size and dropout rates

Sample size calculations were conducted a priori for the primary outcome of the CARBFUNC study (reported in Paper II) to allow for the detection of a clinically relevant difference in VAT volume when comparing the intervention groups. A sufficient sample size may reduce the risk of random error due to chance alone [180].

Although a higher number of participants were recruited to account for dropouts, one of the groups ended up with four fewer participants than the calculated required sample size (n = 18 per group). Some level of participant dropout is inevitable in long-term intervention studies and missing data is a common challenge. A review of 121 RCTs for weight loss suggested that drop-out rates vary from 0 to 80%, and increase in long-term studies [183]. The overall dropout rate of 70% after 12 months in the CARBFUNC study was high, varying between 65-79% dropout in the three diet groups. Preconceptions of the assigned study diets may partially explain the higher initial dropout rates on the A-HCLF, as the macronutrient and food profile of this diet resembles the participant's baseline diet the most, whilst the lower dropout rate in the LCHF diet may be explained by the opposite. Unfortunately, we did not systematically collect data on the dropout reasons in our study. The authors of a previous RCT comparing four diets (the Atkins, Ornish, Weight Watchers, and Zone diet) varying in carbohydrate amount on weight loss suggested that dropout rates may be associated with participants finding the diets too extreme, as dropout rates were highest in the two diets with macronutrient profiles differing the most from baseline profiles (the Atkins and Ornish diet) [184].

Although the completion rate was sufficient in two of the diet groups (the C-HCLF and LCHF diets) based on the a priori power calculations, only 14 of the required 18 participants in the comparator arm (the A-HCLF diet) completed 12 months. Missing data may compromise the inferences of an RCT, especially in cases where missing data is not at random [185]. We cannot rule out that data was not missing at random, however, distinguishing between missing at random and not missing at random is generally impossible [185]. To increase the transparency of potential outcome variables associated with higher dropout rates, plots for completers vs. dropouts for the primary and secondary outcome measures were shown in the Supplementary Material of Paper II.

The study duration and the comprehensive nature of the interventions, including specific recipes for all meals and the frequency of dietary recordings, are likely to have affected the overall dropout rate. Also, an initially greater and more rapid weight loss may have resulted in lower dropout rates as weight loss may be highly motivating for

adherence to the study intervention and has been shown to result in greater long-term weight loss [186]. Insufficient weight loss and difficulties following the assigned diets have previously been reported as the most common reasons for dropout [184]. As we did not systematically collect data on the dropout reasons in our study, we can only speculate on the underlying missing data mechanism in our study. To mitigate the problem of missing data, statistical analyses were conducted using the ITT principles with a linear mixed-effects model approach. However, the high dropout rate is likely to have introduced selection bias, limiting the generalizability of the findings in Papers II and III.

5.1.3 Data collection and outcome measures

Dietary recordings and physical activity

Dietary intake data may be collected using several different instruments, and we used online dietary records (also named "food records") in this study, a prospective method allowing for relatively accurate real-time reporting of all foods and beverages consumed. Of particular importance for Paper I assessing the preintervention habitual diets of the participants, dietary recording in real-time is subject to reactivity where participants may change their eating behavior on reporting days. The participants may wish to present themselves to the research team in a positive manner, leading to underconsumption or underreporting of foods or beverages deemed unhealthy, introducing the concept of social desirability bias [187]. Participants may improve their dietary habits unintentionally by increasing awareness of food intakes during the days of dietary recording. Dietary recordings are therefore accurate tools to assess the actual food and beverage intake during recording days, however, due to reactiveness, dietary recordings may to a less extent capture the true habitual diet. As an attempt to mitigate this, participants were clearly instructed to eat normally during the dietary recordings. To validate the self-reported energy intake we may have used the doubly labeled water method, a gold standard approach, and a precise biomarker of energy expenditure [188]. This method requires participants to drink a dose of water enriched with stable isotopes (deuterium [^{2}H] and oxygen 18 [^{18}O]) followed by the collection of daily urine samples for 14 days to evaluate the rate of isotope disappearance which is further applied in

indirect calorimetric equations to finally estimate energy expenditure [188]. Although the participant burden of the doubly labeled water method is relatively low and reduces the risk of recall bias, social desirability bias and is not prone to reactiveness, the method is expensive and was not part of the CARBFUNC study protocol.

Of particular importance for Papers II and III was the length of the dietary recording period. Although participants were asked to only record three consecutive days, the frequency of dietary recordings (every second week) may have introduced respondent fatigue potentially leading to less complete recordings in the later stages of the intervention [189]. Also, participants may shift from prospective recording to retrospective recording by completing the dietary recording at the end of the day or on the following day, potentially introducing recall bias [187]. However, in Paper I, we may have increased the length of dietary recordings from 2 x 3 days to a 7-day dietary record as seven days would further reduce day-to-day variability.

As for the dietary data, we relied on self-reported physical activity data to detect changes in physical activity level during the intervention period. Objective data may have been collected by the use of accelerometers to complement the detailed information on, e.g., types of activities and intensity level reported in the online recording system, as combining both subjective and objective measures of physical activity are recommended in the OBEDIS (OBEsity Diverse Interventions Sharing – focusing on dietary and other interventions) expert guidelines [6].

Paper I

As discussed in Paper I, the lack of clear defining criteria and inconsistent approaches to describe meal timing in nutritional research challenges the interpretation of the relationship between meal patterns and obesity. The use of time-of-day to define meals, as used in Paper I, may not be appropriate for participants working alternate schedules or night shifts. However, we did not collect data on working hours, sleep patterns, or chronotypes in the CARBFUNC study to investigate this further.

In addition to the exploratory data-driven a posteriori analysis of dietary patterns (k-means cluster analysis) in Paper I, we may have complemented the analyses by

including an a priori approach to assessing the habitual diet quality. Strengths of a priori dietary pattern analysis include reproducibility and comparability of meaningful scores/indexes that are often more intuitive to interpret than the results of a posteriori approaches as derived in Paper I [138]. E.g., the healthy eating index (HEI) score where low scores have previously been associated with abdominal obesity in NHANES-data [135,136] would be a relevant approach in our study population.

Paper II

An important methodological consideration of Paper II is the exposure of the study participants to ionizing radiation from the CT scans to quantify abdominal fat volume. Each single radiation dose was <10 millisieverts (mSv), regarded as low-dose radiation without any direct epidemiological data supporting increased cancer risk [190]. Participants who underwent three CT scans from baseline to 1-year follow-up received on average a total radiation dose of 23.2 (SD 8.6) mSv, equivalent to 5 times the estimated natural yearly background exposure in Norway (approximately 4.5 mSv per year) [191]. Overall, the mean dose length product (DLP) was 489 (SD 204) milligray cm (mGy-cm), 383 (SD 154) mGy-cm, and 385 (SD 136) mGy-cm, at baseline, 6 months, and 12 months, respectively. The total radiation exposure in our study is considered to afflict a low risk of adverse effects compared to individual lifetime exposure. Importantly, the expected radiation dose for each CT scan (≤10 mSv) was explicitly addressed in the possible risk and benefits section in the study's written Participant Information which was, together with the study protocol, approved by the Norwegian Regional Ethics Committees (REC West). However, we cannot rule out that the radiation exposure may have been a reason for dropout for some participants. The use of MRI to directly quantify the abdominal and hepatic fat volume and distinguish between SAT and VAT would have the overall lack of ionizing radiation as a clear advantage over CT [7]. However, the use of MRI was not feasible due to the lesser accessibility of both MRI scanners and staff at the research site.

Paper III

In Paper III, we aimed at examining the relationship between subjective feelings of appetite, ketones, and plasma concentrations of ghrelin in the short and long term. However, due to the rebound of carbohydrate intake (11 to 15 E% from 3 months to 12 months) on the LCHF diet over time, there was not a sufficient difference in ketones on the diets to draw firm conclusions on the long-term relationship between ketones, ghrelin, and appetite. Adherence to the planned carbohydrate intake level of 8 E% on the LCHF diet may have been enhanced by the use of weekly measures of ketone bodies (acetoacetate) in the urine using Ketostix reagent strips for participants to self-monitor compliance. Ketostix reagent strips have previously been used as a marker of compliance in dietary intervention studies investigating the effect of VLED or LCHF diets and ketosis on changes in appetite [192–195].

On the LCHF diet, a measure to induce higher levels of ketosis by carbohydrate restriction would be to further restrict energy intake on the study diets without changing the energy percent contribution from carbohydrates or to decrease the planned energy contribution from carbohydrates. In a 12-week randomized clinical trial, Harvey et al. compared three low-carbohydrate diets matched from habitual energy intake differing in the magnitude of carbohydrate restriction (5, 15, or 25 E% carbohydrates) on anthropometric and cardiometabolic outcomes [196]. After 12 weeks, mean carbohydrate intakes were lower than allocated on the 15% and the 25% carbohydrate diets (14.1% and 22.5%, respectively), while mean carbohydrate intake was higher than allocated on the 5% (7.9%) carbohydrate diet. Accordingly, lowering the planned E% from carbohydrates on the LCHF diet in the CARBFUNC study to 5 E% may have resulted in overall lower carbohydrate intake and higher levels of ketosis, possibly also in the long term. Importantly, dropout rates were comparable between the three levels of carbohydrate restriction in the study by Harvey et al., suggesting that a stricter carbohydrate restriction in our study may not affect dropout rates.

5.2 Discussion of the results

In this study, we set out to explore the habitual diet of adults with obesity in a real-life setting and to further determine whether an acellular, cellular, or LCHF diet would differentially affect clinically relevant outcome measures. In the following section, the interpretations, implications and importance of our findings will be discussed in light of other relevant studies.

5.2.1 Dietary intake profiles

A common challenge in long-term dietary interventions is the progressive decline in adherence to assigned study diets with increasing deviations from planned energy restrictions or macronutrient profiles [97,99,184]. The preintervention macronutrient profile of the participants in our study was 42 E% carbohydrate, 40 E% fat, and 17 E% protein. This was close to the planned macronutrient profile of the A-HCLF and C-HCLF diets (45 E% carbohydrate, 38 E% fat, and 17 E% protein), but in large contrast to the macronutrient profile planned for the LCHF diet (8 E% carbohydrate, 75 E% fat, and 17 E% protein). Adherence to the macronutrient profiles was substantial with ≤5 E% recorded deviations from the planned macronutrient profiles after 12 months, except for higher carbohydrate (7 E% deviations) and lower fat (9 E% deviations) intakes on the LCHF diet.

In previous comparable long-term (1-year) RCTs, the reported deviation from planned macronutrient profiles of LCHF diets without specific goals for energy restriction has been substantially higher [97,99,184]. In two previous RCTs, participants were instructed to decrease carbohydrate intakes to 20 g/d with a gradual increase to 50 g/d, corresponding to 11 E% of the reported baseline energy intake [97,184]. After 12 months, reported carbohydrate intake was 35−40 E% or approximately 140−190 g/d, compared with a mean carbohydrate intake of 15 E% or 86 g/d on the LCHF diet in our study. In these previous long-term studies, results and conclusions were drawn based on the effects of assigning participants to LCHF diets (effectiveness), whilst in our study, the maintenance of long-term carbohydrate restriction on the LCHF diet may to a greater extent allow us to draw conclusions based on the effects of the diet itself (efficacy). In

contrast, smaller (n = 31−35) short-term (4−8 weeks) intervention trials examining weight-loss on ketogenic VLEDs have reported excellent compliance [192,195]. Of note, long-term (1-year) severe carbohydrate restriction (37 g/d or 9 E%) was achieved in the RCT by Brinkworth et al. [197] where the planned carbohydrate intake was substantially lower compared with our study (4 E% vs. 8 E%) and greater energy restrictions (1400−1700 kcal/d vs. 2000−2500 kcal/d) were included as part of the dietary intervention.

In our study, the recorded preintervention mean energy intake of 2120 ± 550 kcal and 2550 ± 550 kcal reported for females and males, respectively, was less than 150 kcal from the planned energy intakes of 2000 kcal and 2500 kcal during the study diet. For all three diets, the reduction in energy intake from baseline throughout the intervention was modest with comparable reported energy intakes (females: 1820−2060 kcal, males: 2480−2550 kcal). Despite the relatively high energy intake, after 3 months on the LCHF diet, the mean carbohydrate intake of 59 g/d or 11 E% was sufficiently low to induce ketosis as shown by significant group difference in βHB concentrations between the study diets. The presence of ketosis and the comparable protein levels of the study diets were important factors for the investigation of the suggested appetite-suppressing effect of LCHF diets, as high levels of both have previously been associated with appetite suppression [50,114].

An important feature of our study was the use of highly standardized diets and diet-specific recipes for all daily meals with predefined energy contents including 25% at breakfast, 25% at lunch, and 50% at dinner, corresponding to 500, 500, and 1000 kcal for female participants. The dinner recipes included primarily a main course with an additional dessert or evening snack, resulting in a meal pattern of 3−4 meals per day. The time of day for each meal was not specified. In Paper I, the energy intake from the largest meal in each of the five meal patterns varied from 916 to 1327 kcal (lunch, dinner, or evening meal), whilst none of the preintervention meal patterns, in particular, included a larger breakfast (ranging from 243 to 377 kcal). Eating frequency ranged from 3.6 to 4.7 meals/d in each meal pattern. None of the observed meal patterns in Paper I overlapped with the energy intake distribution planned for the intervention (25,

25, and 50%), suggesting that participants to a large extent had to change their habitual meal patterns to comply with the study intervention. In addition, participants may experience the reduction or increase of certain food groups on the assigned intervention diets as particularly challenging based on their habitual dietary patterns. For example, regardless of the assigned study diet, the participants in the dietary pattern "Sugary foods" consuming 110 g/day of sugary foods (e.g., chocolate, cakes, or ice cream) may have found the very low content of added sugar planned for all three study diets (<5 or <1 E%, i.e., 5−25 g/d for female participants) particularly challenging. Interestingly, in a study investigating predictors of dropping out of weight loss trials, the authors reported that the preintervention consumption of soft drinks was significantly higher among participants dropping out of the study [198]. The implications for dietary adherence of habitual preintervention dietary- and meal patterns warrant further investigation.

5.2.2 Meal patterns

The most important finding reported in Paper I was the differences in energy intake between the five clear temporal meal patterns, the "Dinner-eaters" (2064 kcal), "Lunch-eaters" (2082 kcal), "Supper-eaters" (2102 kcal), "Regular-eaters" (2328 kcal), and "Midnight-eaters" (2551 kcal). Previous observational studies support a shift in meal timing with the consumption of a greater proportion of total meals in the evening or night-time when comparing individuals with obesity with normal-weight individuals [199,200], a meal pattern observed in the "Midnight eaters" of our study. However, the overall lack of consensus regarding the definition of meals and meal timing has led to few and too diverse RCTs to conclude on the causality of meal patterns on health outcomes [201]. In light of the findings of Paper II, where different dietary approaches with predefined meals were successful in reducing VAT and body weight without substantial energy restrictions, interventions targeting meal patterns (as well as dietary quality) may be an alternative approach for promoting fat loss without focusing on energy restriction. Meal timing and frequency are the basic structures of an individual's diet and may provide a good starting point for clinicians to suggest a more intentional eating approach for the management of obesity that focuses on energy

distribution (meal timing and frequency) throughout the day rather than (or in addition to) energy restriction.

Although we observed distinct meal pattern clusters with significant differences in energy intake, there was a lack of significant differences in the anthropometric measures including body weight, BMI, and WC, between these clusters. Considering the basic components of energy balance, the differences in energy intake between the clusters, and the comparable reported levels of physical activity, one may expect that body weight should also differ. The lack of associations between energy intake and body weight may be attributed to the cross-sectional design used in Paper I, the complexity of the energy balance equation, and limitations of the methods we have applied to evaluate both sides of the equation (energy intake and energy expenditure). For example, due to the short duration of the three-day recordings of daily life activities and sports, we may have missed out on weekdays where the participants regularly engage in sports activities or exercise, contributing to habitual activity level over time. Exercise may be an important component contributing to energy balance [202]. However, the impact of exercise on total energy expenditure in a population with obesity may be limited. In a cross-sectional study of physical activity among >3000 Norwegian adults, accelerometers were used to objectively assess physical activity over 7 consecutive days [203]. The average amount of moderate (22 and 27 min/d for female and male participants, respectively) or vigorous (~1 min/d for both sexes) physical activity among individuals with obesity, was significantly lower compared to normal-weight participants of both sexes. In the general population, physical activity constitutes on average 25−35% of the total daily energy expenditure, whilst physical activity may account for up to 75% in extreme cases of sustained strenuous exercise [204]. As discussed under the "Methodological considerations" section, the dietary recordings were conducted as a "real-time snapshot" where participants may have changed their eating behavior on the specific reporting days, and therefore may not reflect a chronic balance or imbalance between energy intake, energy expenditure, and energy storage.

5.2.3 Visceral fat volume

The primary finding in Paper II was the significant and similar reduction of VAT volume (12−17%) on all three dietary interventions after 12 months, despite a relatively high energy intake compared to many other weight loss studies. However, previous comparisons of more energy-restricted diets have resulted in greater VAT loss. In the RCT by Seimon et. al [92] among postmenopausal women with obesity, substantial differences in VAT reduction after 12 months were observed when comparing severely (65−75% energy restriction for 4 months followed by 25−35% for the remaining 8 months) or moderately (25−35%) energy-restricted diet. The marginal mean VAT loss was −2379 cm^3 and −1077 cm^3 on the severely and moderately energy-restricted diet, respectively, with weight loss of 15.3 kg and 8.4 kg. Of note, the dropout rates were 3 times higher in the moderate vs. severely energy-restricted diet in the study by Seimon et al., possibly due to the rapid and large weight loss on the severely energy-restricted diet. In comparison, the mean reduction in VAT after 12 months in our study was −331 cm^3 on the A-HCLF diet and −502 cm^3 on the LCHF diet, suggesting that greater overall energy restriction may have resulted in greater VAT and weight loss in our study. These findings are in line with the results from a systematic review and meta-analysis on the effects of exercise versus hypocaloric diets for the reduction of VAT [205]. Data from Verheggen et al. indicated that body weight loss and VAT loss are linearly related, with 1% weight loss corresponding to approximately 2.5% VAT loss when weight loss is induced by hypocaloric diets alone without exercise training. Whilst exercise, in the absence of weight loss, induced a 6.1% decrease in VAT, diet without weight loss showed practically no change (1.1%). Based on the findings by Verheggen et al, the relative reduction in VAT (12−17%) in our study corresponds well to the expected VAT reduction following a 5−7% weight loss, as physical activity levels remained unchanged in our study.

Previous studies comparing the effect of low-carbohydrate and low-fat diets on VAT loss have produced similar results. In the shorter 6-month randomized comparison by Haufe et al. [206], both female and male participants with overweight or obesity were assigned to hypocaloric diets (−30% of baseline energy intake) differing in

carbohydrate and fat reduction. After 6 months, reductions of carbohydrate and fat were −25% and −9%, respectively, on the reduced carbohydrate group and −8% and −50%, respectively, on the reduced-fat group. Both diets resulted in similar VAT loss of 21% and 22% after 6 months, somewhat greater than at 12 months in the present study (12−17%). Also, in the 3-month RCT by Veum. et al. [83], VAT loss among men following a largely unprocessed HCLF diet (2200 kcal) and an LCHF diet (2100 kcal) resulted in comparable VAT loss of 21−27%. In accordance with previous studies, our findings support that comparable VAT loss can be achieved on both low- and high-carbohydrate diets with similar energy intake. Although greater VAT reduction may be obtained when following more severe energy restriction, our study indicates that also moderately energy-restricted (2000−2500 kcal/d) diets result in clinically meaningful VAT volume loss, implying that a focus on dietary composition and quality with only moderate energy restriction can produce long-term fat loss without a need for severe energy restriction. In support of the role of dietary quality, we found no clear relationship between changes in energy intake from baseline and changes in fat or weight loss, in line with the findings by Veum et al [83]. However, we cannot rule out that the dietary recordings were not accurate enough to correctly capture the preintervention or intervention intake, and that a relatively small energy deficit may produce significant fat loss over time.

5.2.4 Body weight

A second main finding in Paper II, also of importance for Paper III, was the clinically relevant and significant weight loss of 5−7% observed on all three study diets after 12 months. In agreement with the observed weight loss in our trial, two RCTs comparing low-carbohydrate and low-fat diets found that successful weight loss may be achieved on both dietary approaches after a 1-year follow-up, with no significant between-group differences [98,104]. Body weight was reduced by 5% and 6% on the low-fat and low-carbohydrate diets, respectively, in the study by Gardner *et al*. (DIETFITS) [98], and 11% in both groups in the study by Foster *et al*. [104], compared to 5−6% and 8% on the HCLF and LCHF diets in our study. However, the dietary interventions are not directly comparable with our study due to differences in recommended energy intake between

the studies. Also, the definitions of low-carbohydrate and low-fat diets that were applied differ as there are no standard definitions of these diets in terms of macronutrient composition in energy percent or grams per day. For example, the added sugar intake after 12 months on the A-HCLF diet (18 g/d) in our study was on average lower compared to the low-carbohydrate diet of the DIETFITS study (23 g/d). The study by Foster *et al*. restricted energy intake to 1200−1800 kcal/d on the low-fat diet and had no intentional energy restriction on the low-carbohydrate diet, while the DIETFITS study did not include any specific energy restriction as part of the dietary interventions. In contrast, our study included higher specific goals for energy intake within a more normal caloric range. Although energy restriction was not reported to be a part of the dietary intervention in the DIETFITS study, participants on both diets reduced energy intake similarly (by approximately 400−700 kcal/d), resulting in an overall greater reported reduction in energy intake compared with our study. Energy intake during the intervention period was not reported by Foster *et al*.

5.2.5 Appetite

Despite comparable energy intakes, similar VAT loss, and corresponding weight loss, total ghrelin concentrations were only significantly increased on the A-HCLF and C-HCLF diets and not on the LCHF diet after 3 months. Although we observed no significant group difference, these data are in agreement with several other studies reporting a suppression of ghrelin secretion during weight loss on ketogenic diets [54,192,193,207]. The significant group differences observed for βHB concentrations in our study support the presence of ketosis on the LCHF diet after 3 months. Although a clear consensus is lacking, the increased 3-month mean βHB concentration (0.25 ± 0.25 mmol/L) on the LCHF was below 0.30 mmol/L, an often-considered threshold for nutritionally induced ketosis [116]. Despite no increase in ghrelin concentrations on the LCHF diet at 3 months, suggesting a smaller increase in hunger on the LCHF diet, this was not observed for subjective feelings of appetite in our study.

Ketogenic diets have previously been shown to induce significant weight loss while also suppressing the expected increase in appetite and hunger feelings following weight loss [116]. Thus, ketogenic diets have been advocated as a strategy to counteract the

changes in appetite following weight loss thereby preventing weight regain [44]. Sumithran et al. found that even 1 year after weight loss, changes in appetite and concentrations of appetite-related hormones including ghrelin persisted, suggesting that strong physiological mechanisms promote weight regain after weight loss in persons with obesity [44]. Although we did not aim to measure changes in ghrelin concentrations and hunger feelings after an intentional weight maintenance phase in our study, the overall discussion of the relationship between weight regains and appetite after weight loss is highly relevant. In contrast to Sumithran et al., Martins et al. argue that these increases in appetite are, rather than a compensation, a normalization towards a lower body weight, fat mass and fat-free mass [208]. The increase in ghrelin concentrations should be expected as ghrelin concentrations are lower in individuals with obesity compared to age-matched lean control subjects [52]. In support of the normalization theory, the same group demonstrated that both hunger feelings and ghrelin secretion were not different when comparing individuals with obesity after weight loss with weight stable controls matched for body composition (fat mass and fat-free mass) [209]. Moreover, changes in appetite (ghrelin concentrations and subjective appetite feelings) following weight loss did not predict long-term weight regain in a study of 36 individuals with obesity who completed an initial 8-week weight loss phase on a VLED followed by a weight maintenance program up until 1 year [210]. However, well-controlled studies with larger sample sizes are needed to determine whether there is a causal relationship between changes in appetite after weight loss, food intake and weight regain.

5.2.6 Carbohydrate quality

In this thesis, cellularity has been used as a marker of carbohydrate quality based on the degree of food matrix breakdown and intactness of the cellular structures in plant-based foods [162] to determine the effects of carbohydrate quality on VAT volume, subjective feelings of appetite, and plasma ghrelin concentrations. Although distinct from previous markers of carbohydrate quality, the features differentiating the C-HCLF and A-HCLF study diets may also differ, or be more similar, according to other

established markers of carbohydrate quality, e.g., glycemic index, added sugar, fiber and whole-grain intake. In addition, the study diets may also, to some extent, differ or agree with the NOVA system of food classification, developed by Monteiro and colleagues, addressing diet quality based on the nature, degree and purpose of food processing [211]. Although the NOVA classification has been incorporated in food-based dietary guidelines by national governments [212], others have questioned the usefulness of the classification and caution against its use [213].

According to the definition by Spreadbury in the original article presenting the "cellularity hypothesis" [162], a cellular diet is a grain-free diet including ancestral foods (e.g., bananas, potatoes, or leafy vegetables) of lower carbohydrate density (g/100 g carbohydrate excluding fiber) whilst an acellular diet includes larger quantities of flour-based products, both white and whole-grain, sugars and processed foods with higher carbohydrate density. Although GI and GL were not assessed for the foods or meals included in our study diets, the degree of cellularity, according to the definition by Spreadbury, has not been shown to correlate with the glycemic index, as the GI of several ancestral and modern foods overlap [162]. However, the C-HCLF diet used in our study was not completely grain-free, with homemade bread, porridge and granola of primarily rolled oats among the most chosen breakfast and lunch menus. Sugar intakes (previously associated with increased VAT [153,156]) were low on both the acellular and cellular diet, with a planned exclusion of sugary drinks on both diets, whilst, fiber intakes (previously associated with decreased VAT [157]) were high with fruit and vegetable intakes above estimated average intakes in the Norwegian adult population [214].

Using the NOVA classification, foods are classified into one of four groups: 1) Unprocessed or minimally processed foods, 2) Processed culinary ingredients, 3) Processed foods, or 4) Ultra-processed foods [211]. The carbohydrate sources used on the C-HCLF diet of our study deemed as minimally processed (e.g., whole [unground] grains, fruit, and vegetables, unpolished rice, potatoes and rolled oats) are well in agreement with group 1 of the NOVA classification. However, there are a few important differences between the NOVA classification and the degree of cellularity.

For example, on the A-HCLF and C-HCLF diets, we differentiated between fresh fruit and fruit juice, brown and white rice, and rolled and quick oats, and flour and pasta were excluded from the C-HCLF diet. According to NOVA, all of these foods belong in group 1. For all three study diets, the recipe booklets included predominantly homecooked meals, using steaks, fillets, or other cuts of meat, fish, seafood, and poultry, belonging to group 1, vegetable oils and butter belonging to group 2, cheeses belonging to group 3, and overall avoidance of ultra-processed foods (e.g., pre-prepared pizza, pies, and pasta dishes). Additionally, all diets had a low intake of added sugar and encouraged avoidance of artificial sweetening.

Taken together, the concept of cellularity is unique compared to previous markers of carbohydrate quality and does not reflect the degree of processing as described based on the NOVA classification. However, both the A-HCLF and C-HCLF diets have several joint features (e.g., high intakes of fruit, vegetables, fiber and whole-grain food, and low intakes of sugar) associated with high carbohydrate quality, and may therefore both be appreciated as healthy dietary approaches for the reduction of VAT volume in adults with obesity.

6. Conclusions

The overall conclusions from this thesis are as follows:

- Five distinct preintervention meal patterns were identified and we found a strong relationship between the distribution pattern of daily energy intake and the total caloric intake.
- Four preintervention dietary patterns were identified but neither dietary nor meal patterns were associated with significant differences in anthropometric measures or physical activity levels.
- No significant differences were observed in the reduction of VAT volume up to one year on three isocaloric eating patterns differing in carbohydrate quality and quantity.
- All three study diets were successful in reducing VAT volume and resulted in clinically significant body weight- and fat loss, with similar improvements in all anthropometric outcomes measured.
- No significant differences were observed in fasting total ghrelin concentrations and subjective hunger feelings when comparing the study diets short-term (3 months) or long-term (1 year).
- Reported feelings of hunger increased on all three diets regardless of total ghrelin or βHB concentrations.

Taken together, our preintervention data highlight the significant variability in meal patterns in individuals with obesity which may be important to consider for personalized obesity treatment. The results of the intervention support that different nutritious dietary approaches, emphasizing dietary quality rather than strict energy restriction, may be recommendable for longer-term abdominal obesity reduction. The low-carbohydrate diet did not show greater appetite suppression during weight loss, suggesting that even stricter long-term carbohydrate restriction to achieve sufficient ketosis may be required for the reported benefits on appetite suppression.

7. Future perspectives

The results presented in this thesis do not support choosing only one of the studied dietary approaches for long-term VAT reduction. Overall, we observed a significant and clinically relevant reduction of VAT after one year on all three diets, despite marked differences in carbohydrate quality and quantity, but with similar energy intake. However, the translation of our research findings into actionable evidence, relevant for clinical practice in a more real-world setting, requires careful evaluation. In addition, based on the exploratory nature of Paper I, we may suggest new hypotheses and research questions for future research.

In the context of translational research moving from basic laboratory research to evidence-based practice [215], our randomized controlled trial should be considered an efficacy type trial, testing the biological effects of the study intervention. Efficacy research, as opposed to effectiveness research, emphasizes internal validity by minimizing bias that may lead to systematic deviations between the observed outcome and the true outcome [215]. Following a step-by-step approach, the next step in the context of translational research would be to conduct an effectiveness trial testing the three dietary approaches in a broader population and a more real-world setting without rigorous diet-specific recipe booklets of meals with pre-calculated nutrient contents and exact measures of each food item and ingredient. However, we experienced the challenging balance between scientific rigor and feasibility when conducting our study, as indicated by the high participant dropout rates, and therefore suggest considering a first step back to a preintervention setting before again moving forward.

Firstly, the concept of carbohydrate cellularity warrants further investigation as a marker of carbohydrate quality. In our study, the degree of cellularity was considered as a continuum based on the hypothesized degree of food matrix breakdown and intactness of the cellular structures in plant-based foods. However, further microscopic examination of pre-digestive processing – ranging from the use of simple household utilities to modern processing techniques – on food matrix breakdown and cellular intactness may contribute to a more nuanced and accurate categorization of

carbohydrate foods according to the principle of cellularity. Following more clear indexing of foods according to pre-digestive processing, a further step may be to investigate the effects of carbohydrate cellularity on bioavailability and absorption, e.g., by using in vitro models of the human GI tract [216].

Secondly, we acknowledge that the outcome measures included in this thesis are insufficient to adequately address the overall complexity of disease risk in individuals with obesity. Excessive VAT accumulation is associated with lipid profile abnormalities such as hypertriglyceridemia, low high-density lipoprotein (HDL) and small, dense low-density lipoprotein (LDL) particles [32,217]; however, assessment of these outcome measures was beyond the scope of this thesis. A combination of these three abnormalities is referred to as the "atherogenic lipid triad", recognized as a component of the metabolic syndrome and as an independent major risk factor of cardiovascular disease [218,219]. Therefore, we plan for further analyses to determine whether the three study diets differentially affect lipid profiles and additional metabolic syndrome components, including blood pressure and fasting and postprandial plasma glucose, insulin and triacylglycerol concentrations, to further expand on the clinical relevance of the diets of the CARBFUNC study.

Thirdly, several knowledge gaps remain regarding the relationship between ghrelin, subjective appetite, ketosis, carbohydrate intake and total energy intake, e.g., the threshold levels of both carbohydrate intake and βHB concentrations for nutritionally induced ketosis. A consensus statement addressing these threshold levels would contribute to a more unison definition of ketogenic diets to further investigate the promising appetite-suppressing effect following ketosis. However, the findings of our study suggest that the degree of energy restriction on LCHF diets also affects appetite despite strict carbohydrate restriction. As Harvey et al. compared low-carbohydrate diets matched from energy intake but differing in the magnitude of carbohydrate restriction [220], we may suggest comparing LCHF diets matched for carbohydrate restriction but differing in energy restriction to address this issue. Importantly, there is a need for long-term (≥1 year) studies with large sample sizes to determine whether

there is a causal relationship between changes in appetite after weight loss and weight regain.

Fourthly, the implications of a person's habitual meal and dietary patterns for adherence to different dietary approaches for fat loss warrants further investigation. For example, considering preintervention meal and dietary patterns may help to detect participants at particularly high risk of dropout and to tailor follow-up according to their needs. The observations in the meal pattern analysis encourage further research, in particular prospective studies, to explore whether changes in meal patterns and daily distribution of energy intake can lead to both beneficial changes in caloric intake and anthropometric measures in people with obesity.

Finally, although the overall focus of this thesis has been on the mean changes between and within diet groups, we observed substantial interindividual differences in the outcome measures. It would be useful to identify the characteristics, behavioral as well as biological, of the participants who were the least and the most successful on each diet. Based on knowledge of these characteristics, clinicians may guide individuals with obesity in choosing a more personalized dietary approach. Overall, the findings of this thesis may contribute to a shift in obesity management towards an increased focus on diet quality, intentional meal patterns, and flexibility in the proportion of carbohydrates and fat according to overall medical considerations and personal preference.

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Meal patterns associated with energy intake in people with obesity

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Abstract

It is widely assumed that people with obesity have several common eating patterns, including breakfast skipping, eating during the night and high fast-food consumption. However, differences in individual meal and dietary patterns may be crucial to optimising obesity treatment. Therefore, we investigated the inter-individual variation in eating patterns, hypothesising that individuals with obesity show different dietary and meal patterns, and that these associate with self-reported energy intake (rEI) and/or anthropometric measures. Cross-sectional data from 192 participants (aged 20–55 years) with obesity, including 6 d of weighed food records, were analysed. Meal patterns and dietary patterns were derived using exploratory hierarchical cluster analysis and k-means cluster analysis, respectively. Five clear meal patterns were found based on the time-of-day with the highest mean rEI. The daily rEI was highest among 'midnight-eaters' (10 669 (SD 2301) kJ), and significantly ($P < 0{\cdot}05$) higher than 'dinner-eaters' (8619 (SD 2301) kJ), 'lunch-eaters' (8703 (SD 2176) kJ) and 'supper-eaters' (8786 (SD 1925) kJ), but not 'regular-eaters' (9749 (SD 2720) kJ). Despite differences of up to 2050 kJ between meal patterns, there were no significant differences in anthropometric measures or physical activity level (PAL). Four dietary patterns were also found with significant differences in intake of specific food groups, but without significant differences in anthropometry, PAL or rEI. Our data highlight meal timing as a determinant of individual energy intake in people with obesity. The study supports the importance of considering a person's specific meal pattern, with possible implications for more person-focused guidelines and targeted advice.

Keywords: **Meal patterns: Dietary patterns: Energy intake: Obesity: Eating frequency**

Since 1975, the prevalence of obesity has tripled worldwide and today over 650 million people have obesity, accounting for 13 % of the world's adult population[4]. Obesity is a chronic disease and a metabolic risk factor of non-communicable diseases such as CVD, type 2 diabetes, obstructive sleep apnoea and several types of cancer[5]. Obesity is also associated with hypertension, elevated cholesterol levels and dyslipidaemia[6]. The burden of disease caused by obesity is still rising[4].

Obesity is caused by a chronic imbalance between energy intake and energy expenditure, resulting in excessive fat accumulation[7]. This imbalance results from a complex interplay between non-genetic environmental factors (e.g. energy intake and physical activity) and individual genetic predisposition, interacting through epigenetic mechanisms[8]. Diet is a modifiable factor, and strategies to avoid excess total energy intake are considered key for preventing and treating obesity[9].

Daily energy intake is determined by food choices and eating behaviour, influenced by a combination of physiological (e.g. appetite and hunger feeling)[10], psychological (e.g. perceived appropriateness or stress)[11,12] and environmental (e.g. portion size, availability, price, or convenience)[13–15] factors. Despite a complex aetiology, previous studies examining obesity and dietary intake have often focused on a single or a few nutrients or foods. The single nutrient approach in nutritional epidemiology may be insufficient in addressing the impact of overall diet on obesity, resulting in inconsistent findings[16,17].

Beyond the traditional single-nutrient approach, the importance of understanding more complex dietary patterns

Abbreviations: PAL, physical activity level; rEI, reported energy intake.

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has gained interest in recent years[18]. This shift of focus may be seen in connection with a change in the diet-related global disease burden, changing from undernutrition and deficiency of specific nutrients to non-communicable diseases[5,19]. Dietary pattern analysis allows for examination of the overall diet, including complex combinations of nutrients and food consumed together, and captures a greater picture of food choices and eating behaviours[20].

However, results from studies of dietary patterns in persons with obesity are inconsistent. When assessing longitudinal changes in anthropometric measures, dietary patterns rich in high-fibre foods and reduced-fat dairy products have been associated with lesser gains in BMI[21], while others conclude that no specific dietary pattern can predict changes in BMI[22].

Recent hypotheses suggest that also meal timing and associated eating behaviour may influence weight regulation, and that timing of food intake is associated with the development of obesity[23–25]. Additionally, the distribution of energy intake throughout the day may have effects on metabolic health, such as when meal patterns conflict with internal circadian clocks (chronotype)[24,26].

To our knowledge, few studies report combined data for meal patterns and dietary patterns, as previous studies of meal patterns in people with obesity focus primarily on the association between meal size[27], timing[28] and frequency[29], and less on the intake of nutrients and food groups. The present study, therefore, examined the food intake profiles of a population with obesity, including the timing of food intake. In particular, we conducted a parallel analysis of dietary and meal patterns, hypothesising that people with obesity have distinct dietary and meal patterns and that these differentially associate with energetic intake and/or anthropometric measures. Insight into specific dietary and meal patterns in a population with obesity may contribute to findings of clinical relevance for more targeted personalised intervention strategies.

Methods

Participants and study design

Data included in this exploratory analysis were collected before randomisation in a randomised controlled trial investigating the effects of dietary carbohydrates on internal body fat in men and women with obesity (Clinical Trials Identifier NCT03401970). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Regional Ethics Committee in Western Norway (Effects of carbohydrate quality and quantity in women and men with obesity, 2017/621/REK West). Written informed consent was obtained from all subjects. Participants were recruited through local newspaper advertisements, radio broadcasts and social media (including advertisement on Facebook), in and surrounding Bergen, Norway, between November 2017 and June 2019.

One hundred and ninety-two male and female subjects with abdominal obesity (BMI $\geq$ 30 kg/m^2) and/or waist circumference $\geq$ 102 cm for males and 88 cm for females were included in the randomised controlled trial, and the baseline data for these are analysed in the present study. Inclusion criteria were age 20–55 years and < 5 % change in body weight within the last 2 months. Exclusion criteria included smoking, known food allergies, alcohol consumption of > 2 alcohol units per day (1 unit being defined as 12 g of alcohol according to the definition of a unit used in the Nordic countries[30]), recent surgical or antibiotics treatment during the past 2 months, use of statins and/or diabetes medication, severe diseases, including chronic inflammatory bowel disease, pregnancy, breast-feeding and post-menopause.

Dietary recordings

Participants conducted 6 d of weighed food records, including four weekdays and two weekend days. Each participant received individually tailored recording days allowing for Fridays in some cases to be considered a weekend day. Therefore, 5/6 or 6/6 reporting days were consecutive days. Participants were asked to record consumption of food and beverages, including all meals and snacks, the weight and amount of all consumed ingredients and products, the time of intake and any additional comments on their food intake. Each participant received a personal user ID in an online dietary recording system (www.diett.no; operated by Dietika AS) to submit daily food consumption. Before data collection, the participants took part in training classes on the use of the dietary recording system and received a digital kitchen scale. The participants were explicitly asked to not change their dietary habits during the period of data collection, and the scientific value of honest and complete records was strongly emphasised.

Following the export of the dietary recording data from www.diett.no, nutritional content was calculated largely based on the latest update of the official Norwegian Food Composition Table[31], or the nutrient declarations provided by the producer/retailer. Values from international databases were used (Danish or US food composition tables, three and eight food items, respectively) when Norwegian data were not available. These comprehensive data were merged into a database using the application FileMaker Pro 18 Advanced (Claris International Inc.) where over 1000 individual products were added. In this database, all recorded consumed ingredients and products were categorised into fifteen food groups and five beverage groups to allow further investigation of the meal and dietary patterns (Table 1). Manual data integrity checks were performed for quality assurance of dietary recordings to help identify and correct possible errors caused by misunderstandings of responders, misinterpretations or keying entry errors. Altogether, the database contained 2210 food and beverage items.

Physical activity level and BMR

Concurrent with the first 3-d weighed food records, participants also recorded all daily life activities and sports for three consecutive 24-h periods to assess their daily level of physical activity. The frequency, duration and intensity for all daily life activities and sports were recorded in the same online system as used for dietary recordings. The recordings were used to estimate a physical activity level (PAL) for each participant based on the sum of estimated energy expenditure for each recorded activity

Table 1. Recorded food and beverage items categorised into twenty food groups and beverages groups

Food and beverage groups	Food and beverage items
Bread and cereal products	Multi-grain muesli, roasted granola, oatmeal (with fruits and nuts), cereal, cereal-based energy bars, bran flakes, sugary cereals (e.g. Cheerios), crispbread, crispbread (rye), crispbread (oats), crackers, bread, wholemeal bread, rolls, sandwich baguettes, baguettes, bread, rolls, oat bread, buns, rice porridge, oatmeal (prepared), porridge
Rice, pasta	Pasta, rice
Sugary foods	Chocolate cake, cake with cream, brownies, cheesecake, brownie cookies, brownies with chocolate icing, chocolate muffins, apple cake, ice cream (cream-based), ice cream (juice-based), rice pudding, chocolate mousse, chocolate sauce, vanilla sauce, milk chocolate, chocolate bar, licorice, milk chocolate with filling, cookies, biscuits, chocolate cookies, oat biscuits, jaffa cakes, cinnamon rolls, waffles, buns with icing, sweet roll (custard-filled), sweeteners, honey, sugar, brown sugar, nut-based chocolate spreads
Convenience foods	Pizza, ready meals, tacos, crisps, nachos
Cheese	White cheese, cream cheese, brown cheese (brunost)
Dairy products	Light cream, cream, sour cream dressing, creme fraiche, coffee cream, dairy butter, margarine, yogurt, yogurt (flavoured), yogurt (flavoured with toppings)
Meat	Chicken slices, beef mince, bacon pie, meat sausage, chicken fillet with spices, snack ham (diced), liver paste, ground beef patties, chicken breast fillet, chicken thighs, chicken (whole), ham, mutton sausage, liver paste, salami, lasagna, hamburger (home), hamburger (fast food), stew
Seafood	Cod roe, smoked salmon, mackerel in tomato sauce, sardines in tomato sauce, salmon fillet, fish pasta bake, sushi, fish soup, crabsticks, shrimp
Vegetables	Cucumber, red peppers, iceberg lettuce, carrot, red onion, white onion, tomato, broccoli, maize (canned), potato (boiled), potato puree, French fries, Greek salad (feta, olive), mixed bean stew, salad
Fruit	Banana, orange, blueberries, apple, grapes, strawberry, avocado, pear, lemon
Egg	Eggs (boiled), eggs (scrambled), eggs (fried)
Nuts	Walnuts, peanut butter, peanuts, almonds, chia seeds, hazelnuts, nut, and fruit mix
Cooking oils	Olive oil, rapeseed oil, coconut oil
Sauces and dressings	Mayonnaise, light mayonnaise, remoulade, salad dressing, taco seasoning mix, powdered mashed potatoes, bearnaise sauce, gravy (onion), gravy (meat), Mexican-style casserole with rice (powdered), tomato sauce (powder), pesto
Pulses	Sugar peas, chickpeas, baked beans (in tomato sauce), brown beans, green peas, peas, white beans
Alcohol	Red wine, beer, white wine, draft beer, sparkling rosé, liquor, 'light' beer
Coffee and tea	Coffee, carbonated water, black tea, fruit tea, caffe latte, green tea
Milk and milk-based drinks	Milk (full-fat), milk (skimmed), chocolate milk
Sweet drinks/juices (energetic)	Coca-Cola, soda, orange juice, apple juice
Sweet drinks/juices (non-energetic)	Coca-Cola zero, soda with artificial sweeteners

and their associated metabolic equivalent values[32] divided by 24 h. Estimated BMR was calculated with the Mifflin-St Jeor equation validated for individuals with obesity[33].

Body composition and clinical variables

Height was measured in the upright position with the Frankfort plane horizontal, using a portable stadiometer (Seca 217, Seca GmbH & Co. KG). Body weight was measured with a Class III approved calibrated scale (Seca 877, Seca GmbH & Co. KG) to the nearest 100 g in light clothing without shoes. Waist circumference was measured halfway between the point of the lowest rib and the iliac crest and was repeated three times. The average of the two last measurements was recorded. Body composition was measured by a segmental multifrequency bioelectrical impedance analyser (Seca mBCA 514, Seca GmbH & Co. KG) to assess body fat mass. The measurements were conducted following the device manufacturers' instructions.

Statistical analyses

Data are presented as raw unadjusted means and standard deviations unless otherwise stated. Statistical analyses were performed with R, version 4.0.3 (https://www.R-project.org). All inferential tests were two-tailed with a nominal alpha level of 0·05.

To examine meal patterns, we used Ward's hierarchical cluster method[34] to analyse participants based on total energy intake during different time intervals. We chose hierarchical clustering due to its flexibility when analysing time-dependent data. We categorised and named all eating occasions into six 4-h periods based on the registered time of intake: 04.00 ± 2 h (early morning meal), 08.00 ± 2 h (breakfast), 12.00 ± 2 h (lunch), 16.00 ± 2 h (dinner), 20.00 ± 2 h (supper) and 00.00 ± 2 h (midnight meal). For each participant, we calculated the mean daily energy percentage intake in each time interval across all six recording days and submitted these values to clustering.

When individual time measurements were missing, imputation was performed in R to assign missing time values to items that were eaten within the same meal period. For example, if a participant had recorded four entries for lunch on a specific day but had only recorded the time measurement for a single one of these items, the remaining three items were assigned to the same 4-h time period. For each participant-day combination, imputation was performed, and where imputation could not be performed to generate a complete time-record spanning 24 h, the day was excluded from the analysis. Participants who had no days with a full and valid time record were fully excluded from the hierarchical cluster analysis.

We generated hierarchical cluster solutions for both male and female participants separately, but similar results lead us to retain

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a cluster solution that included both male and female participants, allowing maximal statistical power.

Using k-means cluster analysis[35], we examined dietary patterns within the data based on the variation of the mean daily intake in g of the food and beverage groups. We chose k-means clustering using daily intake in g rather than energy intake from the specific food and beverage groups due to the widespread use of low-energy products in the dietary recordings. We observed that cluster membership was largely defined by liquid-based food items (i.e. coffee, alcohol, sugary drinks), even after performing scaling procedures, leading us to separate food and beverage categories into two separate factor analyses. Using graphs generated via the *NbClust* v3.0 R package, we visually derived the number for 'k'[36]. Energy contribution information from specific foods was not included in the clustering. For a further description of the food and nutrient intake across the derived clusters, we calculated the nutrient intake and mean intake of food from each food group.

Clusters derived from both dietary and meal pattern analyses in terms of their association with both categorical (e.g. sex) and continuous (e.g. BMI) variables using, respectively, Fisher's exact test or the Tukey test. Differences between meal patterns derived via clustering in terms of nutrient and food group intake were identified via an ANOVA model as implemented by the *aov* function in R, using the Tukey post-hoc test to correct for multiple comparisons between clusters[37].

Differences in energy, nutrient and food group intake, as well as participants' clinical information relating to body composition and PAL, were also identified between dietary and meal patterns utilising the same method. Finally, for participants derived from each meal pattern cluster, we tested for any significant overlap with their dietary pattern cluster via enrichment analysis using Fisher's exact test.

Results

The results presented are from 192 participants with obesity who we recruited for a randomised controlled trial in Norway (the CARBFUNC study). Although BMI and fat mass being similar between sexes, the male participants had a significantly higher body weight and waist circumference compared with the female participants (online Supplementary Table S1).

Dietary intake and physical activity level

As recording habitual dietary intake was a prerequisite for participation in the CARBFUNC study, the compliance was extraordinarily high. Dietary data collection could maximally give 1152 recording days, and the actual number of recording days was 1141, giving a completion percentage of 99·1% for all participants. 77 % of the participants reported 5 consecutive days with a 1-d gap to include a weekend day, while 19 % reported 6 consecutive days.

The recorded daily energy intake was not significantly different between females (8870 (SD 2176) kJ) and males (10 669 (SD 2301) kJ) (online Supplementary Table S2). The BMR was estimated to be non-significantly lower among females (7263 (SD 820) kJ) compared with male (8841 (SD 891) kJ) participants (online Supplementary Table S1). The ratio of energy intake to estimated BMR was 1·2 (SD 0·3) for both sexes, indicating a very low average PAL. The self-reported occupational and leisure-time physical activity showed, however, a higher average PAL of 1·5. PAL was significantly higher (95 % CI −0·17, −0·03, $P = 0·004$) in females (1·6 (SD 0·2)) than males (1·5 (SD 0·2)) (online Supplementary Table S1).

As expected, the male participants had a higher average intake of all macronutrients, contributing to the higher energy intake compared with the female participants (online Supplementary Table S2). The female participants had a non-significantly higher intake of added sugar, which is the only difference that becomes significant when adjusting for energy intake (95 % CI −20·7, −4·70, $P = 0·02$). Although mean alcohol consumption was 5 (SD 8) g/d, only half (48 %) of the participants reported alcohol consumption, resulting in an average intake of 10 (SD 9) g/d among the participants reporting alcohol consumption.

Meal patterns derived from hierarchical cluster analysis

We extracted five non-overlapping meal patterns based on total energy intake during six 4-h periods (Fig. 1(a)), including both male (M) and female (F) participants, and named them according to the time-of-day with the highest peak in mean energy intake when comparing meal patterns: (1) dinner-eaters; (2) lunch-eaters; (3) supper-eaters; (4) midnight-eaters; and (5) regular-eaters. In total, 153 participants (F: 84/M: 67) were included in the meal pattern analysis, with 16 (F: 11/M: 5), 13 (F: 8/M: 5), 36 (F: 21/M: 15), 29 (F: 16/M: 13) and 59 (F: 29F/M: 30M) participants belonging to meal patterns 1–5, respectively. Participants without full and valid time records were excluded (*n* 39).

Overall, the midnight-eaters had the highest reported mean energy intake (10 669 (SD 2301) kJ), and significantly higher than dinner-eaters (8619 (SD 2301) kJ, 95 % CI 137·7, 835·0, $P = 0·019$), lunch-eaters (8703 (SD 2176) kJ, 95 % CI 102·2, 834·4, $P = 0·007$) and supper-eaters (8786 (SD 1925) kJ, 95 % CI 190·8, 705·9, $P = 0·011$), but not regular-eaters (9749 (SD 2720) kJ, 95 % CI −44·42, 489·0, $P = 0·370$) (Table 2). As the meal pattern clusters were based on all recordings for every 4 h combined, we also analysed frequency directly by counting each unique eating occasion throughout the day. The dinner-eaters showed the lowest eating frequency (3·56 (SD 1·04) times per day on average), which was significantly lower than regular-eaters (4·7 (SD 1·01), 95 % CI −2·64, −0·39, $P = 0·013$), lunch-eaters (4·0 (SD 1·53), 95 % CI −3·08, −0·39, $P = 0·367$) and supper-eaters (4·68 (SD 1·27), 95 % CI −2·55, −0·18, $P = 0·026$), but not the or the midnight-eaters (4·58 (SD 1·49), 95 % CI −1·37, 1·74, $P = 0·068$) (Fig. 1(b)). Eating frequency also correlated positively with total daily energy intake across the meal patterns (Fig. 1(c)).

Comparing each 4-h period throughout the day, the energy intake during the first two periods of the day did not significantly differ between the meal patterns. During the early morning (04.00 ± 2 h), the energy intake reported was clinically insignificant for all meal patterns, while energy intake at breakfast (08.00 ± 2 h) ranged from 1017 to 1577 kJ (Table 2). For lunchtime (12.00 ± 2 h), the mean energy intake varied from 3833 kJ

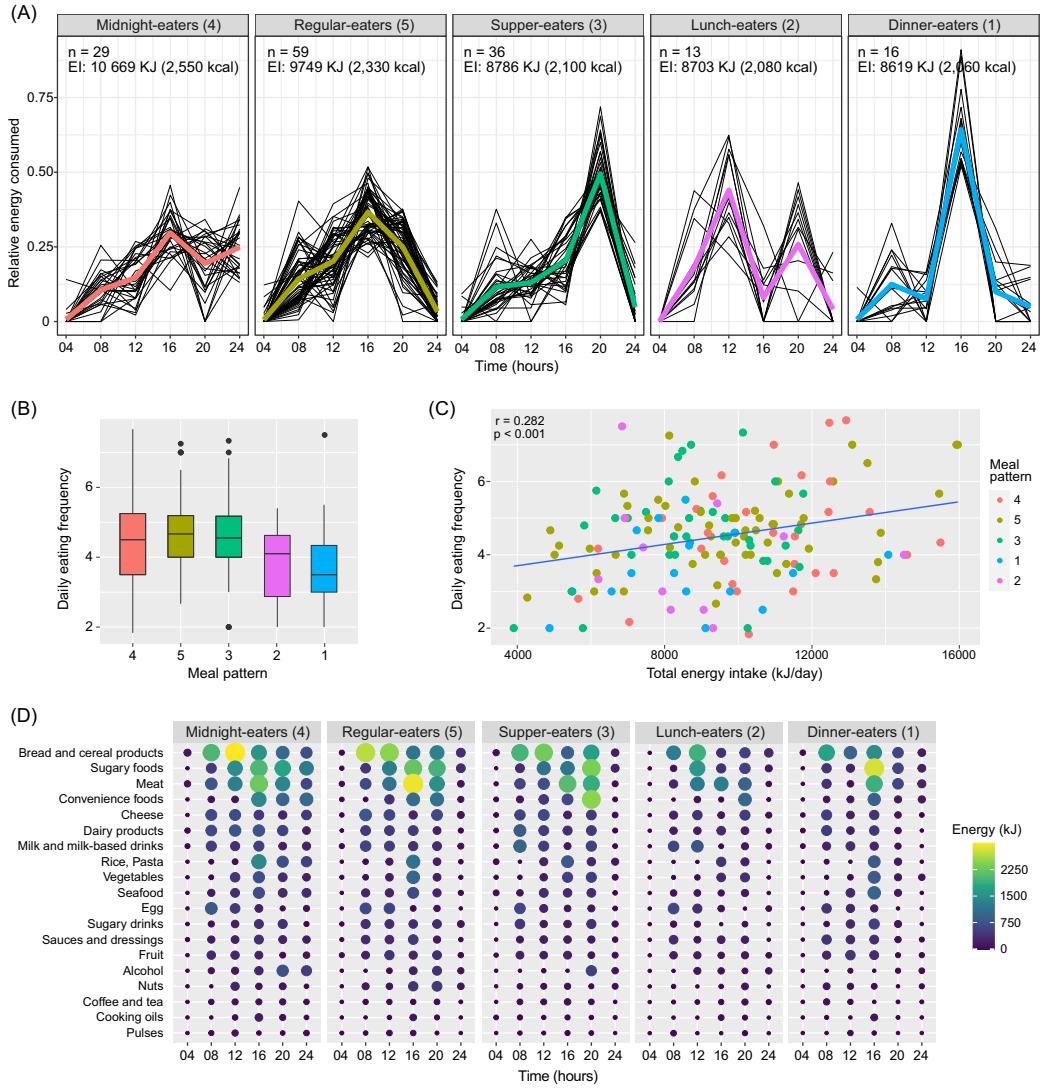

Fig. 1. Meal patterns and associated eating frequencies and food groups. **A**: Relative energy intake for all six four-hour periods in the five meal patterns ordered from highest total energy intake (Midnight-eaters) to lowest (Dinner-eaters) from right to left. **B**: Box-whisker plots showing mean daily eating frequency of the five meal patterns, obtained by counting the number of time points of recorded dietary data for each participant throughout each of the six recording days. **C**: The relationship between mean daily eating frequency and reported total daily energy intake in the total study population, each point representing a participant. **D**: Energy intake from the fifteen food and beverage groups for every four-hour period in the five meal patterns. The brighter color and larger dots indicate higher energy contribution from a specific food or beverage group.

for the lunch-eaters compared with 669–1966 kJ in the other meal patterns. The largest variation between the meal patterns was at dinnertime between the lunch-eaters and dinner-eaters, consuming on average 711 and 5565 kJ, respectively. The supper-eaters consumed 4351 kJ on average in the evening compared with 879–2427 kJ in the other meal patterns. During the last period of the day, the energy intake of the midnight-eaters (2657 (SD 1046) kJ) was significantly higher compared with the four remaining meal patterns (regular-eaters: 95 % CI 288·3, 684·1, $P < 0·001$; supper-eaters: 95 % CI 318·7, 718·6, $P < 0·001$; lunch-eaters: 95 % CI 454·4, 849·8, $P < 0·001$; dinner-eaters: 95 % CI 334·0, 758·3, $P < 0·001$).

Further, we compared energy intake from each of the fifteen food and beverage groups for every 4-h period between the

Table 2. Mean energy intake (kJ) in total and during every 4-h period in the five meal patterns ordered from highest total energy intake (midnight-eaters) to lowest (dinner-eaters)
(Mean values and standard deviation)

	04.00		08.00		12.00		16.00		20.00		00.00		Total energy	
Meal pattern	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Midnight-eaters (*n* 29)	75	310	1130	812	1565	929	3171	941	2075	1397	2657	1046	10 673	2318
Regular-eaters (*n* 59)	59	109	1389	925	1966	1151	3569	1084	2435	1197	326	485	9740	2732
Supper-eaters (*n* 36)	63	255	1017	933	1146	854	1799	879	4351	1381	427	732	8795	1929
Lunch-eaters (*n* 13)	0	0	1577	1536	3833	1891	699	941	2247	1837	356	623	8711	2602
Dinner-eaters (*n* 16)	63	615	1079	983	653	925	5552	1527	858	845	435	623	8636	2284

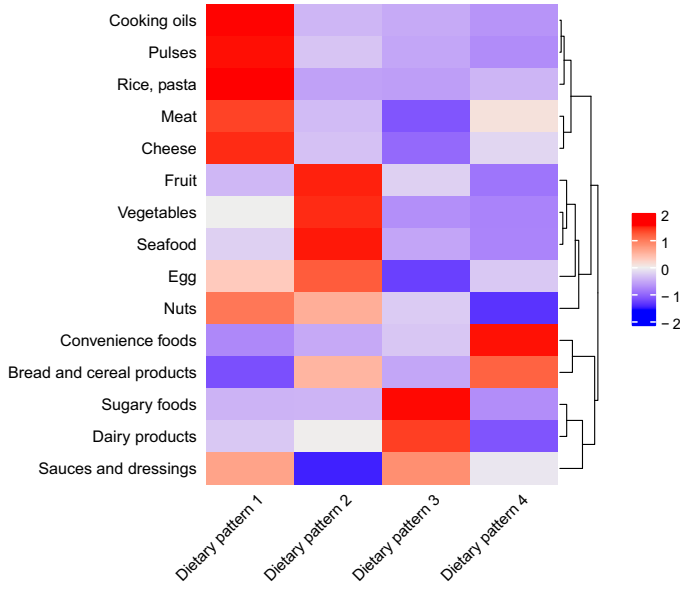

Fig. 2. Dietary patterns and their defining food groups. Heat map and k-means clustering showing the four dietary patterns and the fifteen food groups. The colors correspond to the mean intake of each food group in the four dietary patterns compared to the overall mean intake in the study population (red: above mean intake; white: at mean intake; blue: below mean intake). The four dietary patterns are named according to their defining food groups: 1: Meat, rice, pasta, pulses and oil, 2: Vegetables, fruit, and seafood, 3: Sugary foods, 4: Bread, cereal products, and convenience foods.

meal patterns (Fig. 1(d)). The midnight-eaters with the overall highest energy intake (10 669 (SD 2301) kJ) had the highest mean intake of alcohol, oil, rice, pasta and sugary foods compared with the other meal patterns (online Supplementary Fig. S1). The highest mean intake of fish, fruit and vegetables was found in the dinner-eaters with the overall lowest energy intake (8619 (SD 2301) kJ). The lunch-eaters consumed the highest amounts of bread and cereal products, cheese, pulses, milk and milk-based drinks (online Supplementary Fig. S1). However, these differences in specific food group intakes were not statistically significant between the meal patterns.

Despite a difference of 2050 kJ in reported energy intake (rEI) between the midnight-eaters and the dinner-eaters (lowest energy intake), we did not find any significant differences in the anthropometric measures including body weight, BMI and waist circumference.

Dietary patterns derived from k-means cluster analysis

Including both female and male participants, we extracted four non-overlapping dietary patterns based on the variation of the mean daily intake in g of the food and beverage groups (Fig. 2). These patterns were named according to the food groups characterising each of them: (1) meat, rice, pasta, pulses and oil; (2) vegetables, fruit and seafood; (3) sugary foods; and (4) bread, cereal products and convenience foods. The number of participants was 39 (F: 19/M: 20), 44 (F: 26/M: 18), 55 (F: 36/M: 19) and 54 (F: 20/M: 34) for dietary patterns 1–4, respectively.

In dietary pattern 1 (meat, rice and pasta, pulses and oil), the intake of each of the four food groups was significantly higher compared with dietary patterns 2–4 (online Supplementary Table S3). Meat intake in dietary pattern 1 was on average 180 g/d (1250 g/week) and was 30 % higher compared with

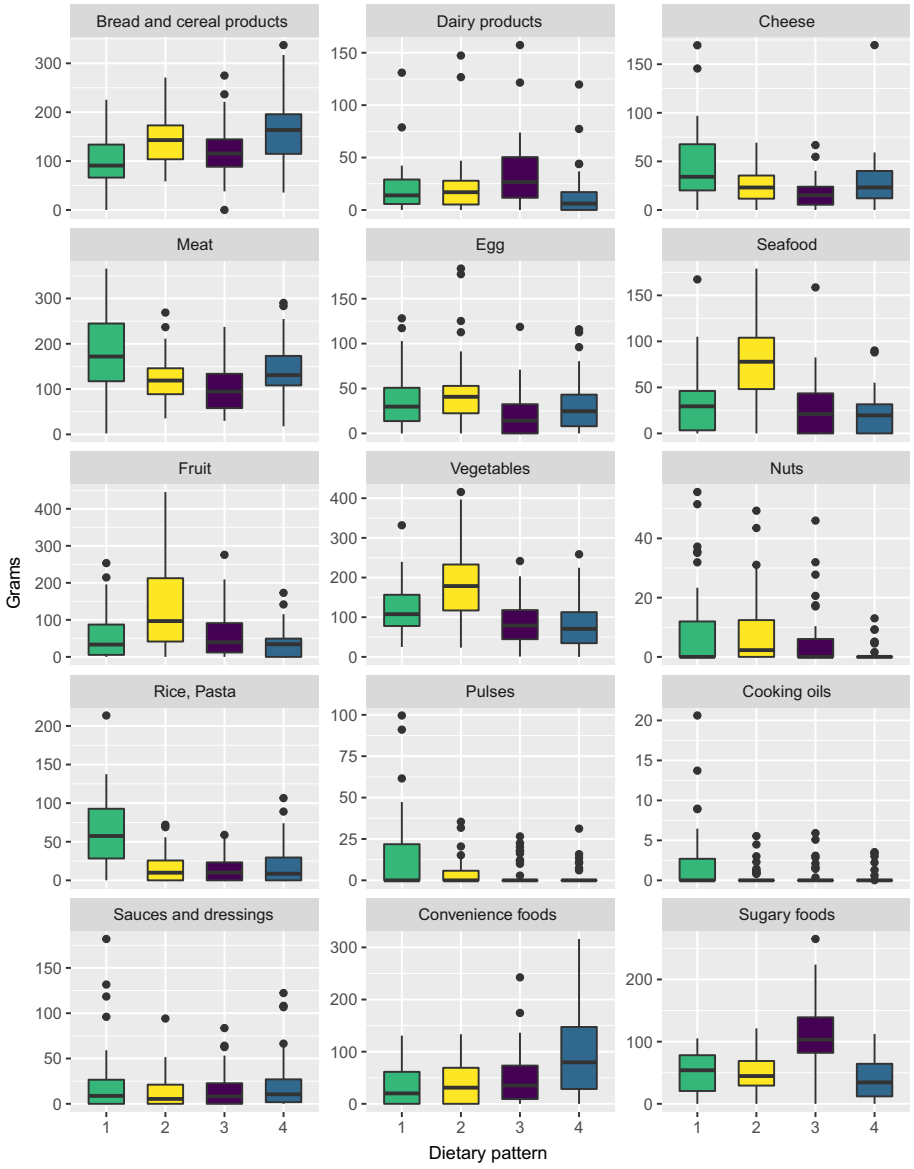

Fig. 3. Box-whisker plots showing the mean intake (in grams) of the fifteen food groups in the four dietary patterns. The different colors correspond to the four different dietary patterns: pattern 1: green; pattern 2: yellow; pattern 3: purple; pattern 4: blue. The dietary patterns are named according to their defining food groups: pattern 1: Meat, rice, pasta, pulses, and oil; pattern 2: Vegetables, fruit, and seafood; pattern 3: Sugary foods; pattern 4: Bread, cereal products, and convenience foods.

dietary pattern 4 with the second highest meat intake of 140 g/d (980 g/week) (Fig. 3).

In dietary pattern 2 (vegetables, fruit and seafood), the intake of each of the three food groups was significantly higher compared with dietary patterns 1 and 3–4 (online Supplementary Table S4). Although non-significant, fibre intake was highest in dietary patterns 2 and 4 (24 g/d) compared with dietary patterns 1 and 3 (20 g/d).

In dietary pattern 3 (sugary foods), the intake of sugary foods was significantly higher compared with dietary patterns 1–2 and 4 (online Supplementary Table S5). The intake of 110 g/d of sugary foods was over twice as high as in dietary pattern 2 with the

second highest intake of 50 g/d. The mean intake of added sugar was 56 g. This was significantly higher compared with each of the remaining dietary patterns, and 50 % higher compared with dietary pattern 4 with the second highest intake of added sugar (Fig. 3). Mean carbohydrate intake was significantly higher for dietary patterns 3 (253 g) and 4 (247 g) compared with dietary patterns 1 (200 g) and 2 (209 g).

In dietary pattern 4 (bread, cereal products and convenience foods), the intake of convenience foods was significantly higher compared with dietary patterns 1–3, while the intake of bread and cereal products was only significantly higher compared with dietary patterns 1 and 3, not 2 (online Supplementary Table S6).

Mean energy intake was highest in dietary pattern 3 (10 167 (SD 2473) kJ), followed by dietary pattern 4 (10 104 (SD 2569) kJ), dietary pattern 1 (9288 (SD 2276) kJ) and dietary pattern 2 (9079 (SD 2033) kcal). Although there were significant differences in the intake of food groups, the differences in energy intake were not significant.

Discussion

In the present exploratory analysis, we analysed specific dietary and meal patterns in community-dwelling adults with obesity, to gain deeper insight into the diversity of typical dietary habits. By including several approaches to examine the habitual diet, we identified five meal patterns by time-of-intake of daily energy consumption, and four dietary patterns derived from the intake of specific food groups. Specific meal patterns, and to a lesser extent dietary patterns, corresponded to differences in total daily energy intake.

Meal timing and total energy intake

A primary finding in our meal pattern analysis is that participants with the highest energetic intake around midnight had the highest overall daily energy intake (10 669 kcal/d), corresponding to more frequent eating. These findings are partly consistent with previous studies suggesting that, in adults with BMI within the normal range, consuming a high proportion of daily energy intake at night or late evening is associated with higher total energy intake[38,39]. On the other hand, these studies found that a higher intake in the morning or afternoon was associated with a lower total energy intake, which is only partially in agreement with the current study. In the present study, although the dinner-eaters with the lowest overall energy intake reported consuming the highest proportion of their energy intake in the afternoon, we found no association between energy intake in the morning, for example, eating breakfast (08.00 ± 2 h), and total energy intake.

Intake of food and beverage groups among meal patterns

Our data reveal intake of specific food and beverage groups for different meal patterns. Intake of foods with relatively low energy density and glycaemic index, such as fruits and vegetables, was highest in the dinner-eaters with the overall lowest reported daily energy intake. Conversely, the consumption of energy-dense foods and beverages such as alcoholic beverages and foods with higher glycaemic index, for example, sugary foods and pasta, was highest among the midnight-eaters, likely contributing to higher glycaemic load as well as overall energy intake in this group. Differences in energy density might at least partly explain the different total energy intakes[40]. Of note, although alcohol consumption contributed to the overall higher energy intake in the midnight-eaters, the differences in alcohol consumption between the meal patterns were small. This may partially be explained by the exclusion of participants with an alcohol consumption of > 2 alcohol units per day from our study.

Dietary patterns and associated food groups and energy intake

Unlike the results for meal patterns, we did not find significant differences in energy intake between dietary patterns. Nonetheless, the difference in mean energy intake between the two dietary patterns 'sugary foods' and 'vegetables, fruit and seafood' was 11 %, which may be clinically relevant. When comparing intake of specific food groups between the dietary pattern and the meal pattern with the overall lowest energy intakes (the vegetables, fruit and seafood pattern and the dinner-eaters), we found that both patterns had the highest mean intake of vegetables, fruit and seafood compared with the other dietary and meal patterns. As for the midnight-eaters in the meal pattern analysis, the daily intake of sugary foods was highest in the dietary pattern with the overall highest energy intake (sugary foods pattern). In agreement with our findings, dietary patterns dominated by sugary foods have previously been associated with the highest overall energy intake[41].

Differences in reported energy intake and anthropometric measures

Although differences in reported mean energy intake accounted for up to 2050 kJ/d between the clusters in the meal pattern analysis, we found no difference in anthropometric measures including waist circumference and body weight. We also found no differences in estimated BMR using the Mifflin St Jeor predictive equation nor in reported PAL (self-reported and leisure-time physical activity) between clusters. In addition, possible confounders including age and distribution of male and female participants did not differ between clusters. In a Swedish study comparing eating patterns in people with normal weight or overweight (BMI < 30) and with obesity (BMI ≥ 30), no significant differences were found in overall energy intake, even after adjusting for PAL[42]. However, the authors found an association between obesity and both night-time meals and portion sizes, even though there were no differences in rEI. A recent study investigating meal timing and BMI dependent on chronotype found on average only a 728 kJ difference in mean daily energy intake when comparing normal-weight subjects (8322 kcal) and subjects with obesity (9050 kcal)[43].

Underestimation of actual intake and under-reporting among adults with obesity is a well-described phenomenon[44,45], and this might partly explain the non-significant differences in self-rEI between normal-weight subjects and subjects with obesity in previous studies. To further explore whether the differences in self-rEI could be explained by differences in the level of under-reporting in our study, we used both recommended

cut-off levels (lower cut-off < 2092 and < 3347 kJ (< 500 and < 800 kcal), upper cut-off > 14 644 and > 16 736 kJ (> 3500 and > 4000 kcal) for women and men, respectively) and the Goldberg cut-off, revealing no significant differences in the estimated level of under- or over-reporting between clusters. These data suggest that the differences in rEI between meal patterns cannot be attributed to under-reporting being overrepresented in any of the clusters. We cannot rule out that the lack of association between total energy intake and anthropometric measures was due to differences in biological regulatory mechanisms that were not measured in the present study, including circadian, thermogenic, hormonal and other effects of the different meal and dietary patterns.

Overall, our data highlight the potential importance of identifying a person's habitual meal pattern, beyond general dietary pattern, to inform more precise interventions aligned with a person's specific sources of excess energy intake. Additionally, awareness that groups of people with obesity can have meal patterns with relatively lower energy intakes, as observed in our study, may help to gain a more nuanced understanding of the different biological as well as lifestyle factors that promote or maintain obesity in a given individual.

Strengths and limitations

A strength of our study is the detailed real-time dietary data obtained using multiple consecutive days of weighed dietary records to account for day-to-day variability in energy intake and eating behaviours[46]. Furthermore, our database was manually curated to include all food and beverage items consumed during the study period. This resulted in a comprehensive database adapted for the current study. Also, all recordings were manually quality controlled by trained personnel and the recording completion rate was high (99 %). To our knowledge, no previous studies have explored the habitual diet, with the inclusion of data on nutrient intake, dietary patterns and meal patterns, in a healthy, weight-stable, non-smoking, population with obesity.

Generalisability of our findings is, however, limited as our study participants were recruited for a weight-loss trial and are not necessarily representative of the general population with obesity. Overall, comparing our findings of meal patterns with previous relevant studies is challenging for several reasons. The use of different defining criteria for meals is widespread, including meals defined by time-of-day[47], timing relative to individual sleep/wake timing[43] and self-identified eating occasions as a snack or a meal[48]. Also, differences in categorisation of time-of-day occur, including dividing 24 h into morning, midday and evening[49], morning, afternoon and night[38], or into periods of varying duration and number, as in 24 h divided into five 4-h periods excluding 02·00–05·59[39], or six 4-h periods as in the current study. We cannot conclude that our approach is superior to other defining criteria.

Among the limitations of this study is the lack of an a priori power calculation to assess adequate sample size to detect possible differences in the reported outcome measures. Power calculation based on the primary outcome from the dietary trial was conducted a priori but not for the secondary outcomes included in this cross-sectional analysis. We chose not to conduct post-hoc analyses as this approach is considered flawed[50]. However, we did observe a statistically significant difference in energy intake between meal pattern clusters, supporting sufficient power.

Conclusions

In conclusion, our data reveal five distinct meal patterns in a population with obesity, and a clear relationship between daily meal patterns, eating frequency and total energy intake, although no correlations with anthropometric measures were found. Our data support that the identification of individual meal patterns may facilitate the clinical application of more personalised prevention and treatment strategies. For example, identification of a 'midnight-eater' or a 'supper-eater' can allow targeted, time-specific adjustments of the person's food-, beverage- and energy intake, beyond general advice to reduce energy intake throughout the whole day. Our observations encourage further research, in particular prospective studies, to explore whether changes in meal patterns and daily distribution of energy intake can lead to both beneficial changes in energetic intake and anthropometric measures in people with obesity.

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The authors' responsibilities were as follows – C. H.: conceptualisation, writing – original draft preparation, investigation, data curation, methodology, validation, project administration. J. L.-B.: software, data curation, writing – review and editing. A. I. O. A.: investigation, data curation, resources, validation, writing – review and editing. L. D.: formal analysis, visualisation, writing – review and editing. I. R.: investigation, resources, writing – review and editing. T. L.: investigation, resources. N. T. N.: investigation, resources. I. S.: investigation, resources. K. K. J.: investigation, resources. G. M.: writing – review and editing, resources, funding acquisition. J. D.: writing – review and editing, resources, funding acquisition. S. N. D.: conceptualisation, investigation, supervision, writing – review and editing, project administration, methodology, resources, funding acquisition, validation. All authors: contributed to the manuscript review and read and approved the final manuscript.

The authors have no financial or personal conflicts of interest to declare.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521002580

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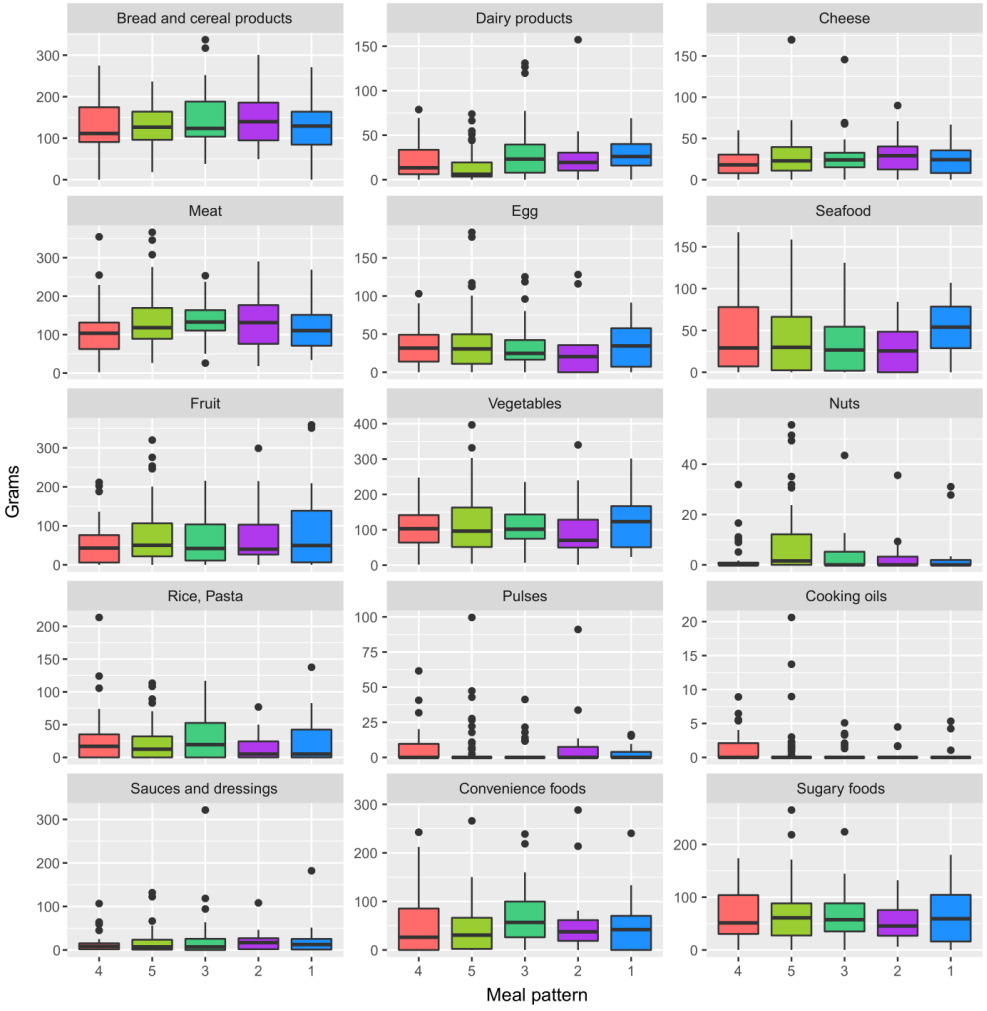

Supplementary Figure 1 Box-whisker plots comparing the mean intake (in grams) of the fifteen food groups in the five meal patterns. The meal patterns are ordered from the highest total energy intake (4: Midnight-eaters) to the lowest (1: Dinner-eaters) from right to left. 1: Dinner-eaters; 2: Lunch-eaters; 3: Supper-eaters; 4: Midnight-eaters; and 5: Regular-eaters.

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