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Doctoral thesis

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Most of those who are obese

Will not go into obesity treatment

Most of those who go into treatment

Will not lose weight and most

Of those who lose weight will regain it

Stunkard AJ, 1958

Photo «La familia Colombiana», 1973 by Fernando Botero. Musa de Antioquia, Medellin, Antioquia, Colimbua.

Tidslinje for endring i adaptive fysiologiske mekanismer ved vektreduksjon

Fysiologiske mekanismer som appetittregulering og energiomsetning ble undersøkt i forbindelse med rask vektreduksjon ved hjelp av en 8-ukers pulverkur hos personer med fedme. Etter kuren var 16 % vektreduksjon oppnådd og metabolske endringer ble målt seks ganger underveis og etter et år. Pulverkuren, som hadde svært lavt energiinnhold, var utfordrende å følge de første ukene på grunn av sult. Appetitten ble målt både subjektivt (endringer i rapportert sult- og metthetsfølelse) og objektivt (endringer i nivået av sult- og metthetshormoner). Samtidig med vektreduksjonen ble energibehovet redusert både i hvile og under trening. En slik longitudinal studie med gjentatte målinger gir et innblikk i kroppens måte å regulere eller motsette seg endringer av kroppsvekt. Ett år etter var sultfølelse og sulthormonet fortsatt økt og vekten stabil. Det var overraskende at også metthetsfølelsen var økt etter et år. Det kan kanskje motvirke (den uønskede) effekten av økt sult.

Forskningsprosjektet har gitt nyttig kunnskap for pasienter og klinikere om utfordrende faser ved vektreduksjon og hvordan disse kan mestres. Tidslinjen viser at en sunnere kroppsvekt kan gi tydeligere sult- og metthetsfølelse. Det kan bety at det er mulig å holde vekta i og med at det er lettere å regulere matinntaket i tillegg til at en lettere kropp kan være mer aktiv.

Bakgrunn for undersøkelsen er at flere enn hver 5. voksne nordmann har fedme, som er en utløsende faktor for mange livsstilssykdommer. Vektreduksjon er mulig for de fleste, men å holde vekta etter slanking er den største utfordringen i fedmebehandling. Vi ønsket å undersøke når og hvordan ulike fysiologiske mekanismer (appetitt og energiomsetning) påvirket vektreduksjonen med standariserte metoder.

Kandidat Siren Nymo

Institutt for klinisk og molekylærmedisin ved det Medisinske fakultet ved NTNU

Hovedveileder er Cátia Patricia Alho Letra Martins, assosiert professor

Biveileder er Bård Kulseng, professor MD.

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à votre santé!

Siren

Abstract

Relapse after weight loss remains the biggest challenge in obesity management, likely due to a combination of reduced compliance with diets and exercise regimes and adaptive physiological responses to weight loss. Despite reduced total energy expenditure, the drive to eat is known to increase after weight loss. However, when weight loss is induced with ketogenic diets the expected increase in appetite seems to be absent.

The aim of this PhD thesis was to identify the timeline of changes in both appetite and energy expenditure variables with progressive weight loss in individuals with obesity on a ketogenic very-low energy diet (VLED).

One hundred adult (43±10 years of age) participants (45 males) with obesity (BMI: 37±4 kg/m^2) underwent 8 weeks of a ketogenic VLED, followed by 4 weeks refeeding and 1-year weight maintenance program. Body weight and composition, resting metabolic rate (RMR), exercise induced energy expenditure (EIEE) and appetite (subjective appetite feelings and plasma concentration of appetite hormones: active ghrelin (AG), glucagon-like peptide 1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK)) were assessed at baseline and week 13, and 1-year (Study III). In a sub-study (31 participants) the same variables were additionally collected at day-3, 5 and 10 % weight loss and week 9 (Studies I and II).

In study I: Hunger and desire to eat feelings in fasting were increased at day-3 and 5 % weight loss despite ketosis and no changes in basal AG plasma concentration (compared with baseline). After refeeding and with a 17 % weight loss, both fasting hunger and AG plasma concentration were increased compared with baseline.

In study II: RMR was reduced for the first time at 5 % weight loss, and EIEE at all levels of power (10, 25 and 50 Watt (W)) reduced at 10 % weight loss, followed by stabilisation. Adaptive thermogenesis was only seen transiently at 10 % weight loss. Refeeding had no significant impact on the above variables.

In study III: A 16 % WL outside of ketosis was associated with an increase in fasting and postprandial hunger ratings and postprandial fullness. These were accompanied by a significant rise in basal and postprandial AG concentrations and a reduction in postprandial CCK. At 1-year follow-up, with sustained WL, fasting hunger and postprandial fullness

ratings remained increased. Basal and postprandial AG remained elevated, while postprandial CCK was increased and PYY decreased.

In conclusion, an increased drive to eat should be expected up to 3 weeks (5 % weight loss) on a ketogenic VLED, but thereafter, appetite feelings return to baseline levels for as long as participants are ketotic. A fall in RMR should be anticipated at ≥5 % weight loss and a reduction in EIEE at ≥10 % weight loss. With refeeding, the drive to eat increases and this is sustained up to 1-year follow-up with weight loss maintenance, but it may be balanced out by increased fullness after a meal.

Patients with obesity, and health professionals working with this patient group, should be aware of these findings. This should be discussed against patient's expectations and strategies need to be put in place to support those undergoing weight loss during the critical periods.

Table of contents

Table of figures

List of papers included in this thesis

I. Nymo S, Coutinho SR, Jorgensen J, Rehfeld JF, Truby H, Kulseng B, et al. Timeline of changes in appetite during weight loss with a ketogenic diet. Int J Obes (Lond). 2017;41(8):1224-31. https://www.ncbi.nlm.nih.gov/pubmed/28439092

II. Siren Nymo, Silvia Ribeiro Coutinho, Linn-Christin H Torgersen, Ola Jakob Bomo, Ingrid Haugvaldstad, Helen Truby, Bård Kulseng and Catia Martins. Timeline of changes in adaptive physiological responses, at the level of energy expenditure, with progressive weight loss.

Publiched in British Journal of Nutrition.
https://www.ncbi.nlm.nih.gov/pubmed/29733003

III. Nymo S, Coutinho SR, Eknes PH, Vestbostad I, Rehfeld JF, Truby H, Kulseng B and Martins C. Investigation of the long-term sustainability of changes in appetite after weight loss.
Accepted for publication in International Journal Obesity

Abbreviations

ADP: air-displacement plethysmography

AG: active ghrelin

AgRP: agouti-related peptide

AUC: area under the curve

AT: adaptive thermogenesis

β-HB: β-hydroxybutyric acid

BMI: body mass index

CCK: cholecystokinin

CV: coefficient of variation

DIT: diet-induced thermogenesis

DTE: desire to eat

EB: energy balance

EE: energy expenditure

EIEE: exercise induced energy expenditure

ER: energy restriction

ExEff: exercise efficiency

FFM: fat free mass

FM: fat mass

GLP-1: glucagon-like peptide 1

KLCD: ketogenic low-carbohydrate diet

LED: low energy diet

LMM: linear mixed model

VLED: very-low energy diet

METs: metabolic equivalents

NEAT: non-exercise activity thermogenesis

NPY: neuropeptide Y

NREE: non-resting energy expenditure

PA: physical activity

PFC: prospective food consumption

PYY: peptide YY

RMR: resting metabolic rate

SD: standard deviation

SEM: standard error of the mean

TEE: total energy expenditure

VAS: visual analogue scale

VCO_2: volume of carbon dioxide

VO_2: volume of oxygen

W: Watt

WL: weight loss

1 Introduction

1.1 Obesity

Obesity is now a larger health problem than starvation worldwide (1). The world is challenged by a double burden of disease with 462 million being underweight and 1.9 billion overweight.

Obesity is associated with increased morbidity, such as type-2 diabetes, heart disease, high blood pressure, stroke, some kinds of cancer, sleep apnoea, hernia and arthritis, and also increased overall mortality (1). Type-2 diabetes is increasing, and is likely to reach 366 million cases by 2030 (2). Obesity is the largest risk factor for type-2 diabetes, and both weight loss (WL), dietary changes and increased physical activity (PA) should be the first choices of treatment for metabolic control. Obesity is a major cause of economic loss, death and suffering that shows no indication of abatement. Every year 2.9 million people die due to overweight or obesity (1).

Even though the reasons for the epidemic proportions of obesity are complex and not completely understood, it is generally accepted that it results from a state of chronic positive energy balance (EB), with energy intake (EI) being higher than energy expenditure (EE) (3).

EB regulation is asymmetrical, with WL being more difficult to achieve than weight gain (3). Disturbances in body weight lead to the activation of compensatory mechanisms that try to restore the system back to its set-point, and the strength of these mechanisms are likely to be larger with underfeeding (*dieting*) versus overfeeding (4). Obesity is considered to have an weakening effect on the homeostatic regulation of body weight (5).

1.1.1 Definition and causes

Obesity comes from the Latin word “Obesitas”, and is defined as an abnormal or excessive fat accumulation that may impair health (1). Body Mass Index (BMI) is the most commonly used proxy to define and categorize obesity. At the population level, BMI is well correlated with the degree of adiposity (fat mass–(FM)), and the different categories (normal weight, overweight and obesity) indicate increased risk of non-communicable diseases (NCD) (6). Obesity is defined as a BMI >30 kg/m^2 and is divided into different classes (obese class I; 30-34.99, obese class II; 35-39.99 and obese class III; ≥ 40 kg/m^2) (6). However, BMI has also

a number of limitations, particularly when used on an individual basis, and should ideally be used together with other indexes, namely waist circumference (7, 8).

Obesity is in most cases (> 95 %) a result of the interaction between genetic susceptibility (9) and an obesogenic environment characterised by overconsumption of energy dense food and reduced PA levels (10). However, certain drugs, endocrine diseases and inherited syndromes can also lead to weight gain and obesity (11).

1.1.2 Prevalence

The prevalence of overweight and obesity is increasing worldwide (12) and Norway (13) is no exception. In 2014, 1.9 billion (39 % of the population) adults over 18 years were overweight and 600 million (13 %) were obese (12). Around 11 % of males and 15 % of females worldwide were obese in 2015 (1).

Obesity was initially especially prevalent in high-income countries, but has also spread to low and middle-income countries (14). In Norway, obesity among adults from the Nord-Trøndelag Health Study (The HUNT 3 study) was reported to be 22 and 23 % in 2006/08, for males and females respectively (13).

1.2 Energy balance and body weight homeostasis

1.2.1 Energy balance (EB)

EB is achieved when EI equals EE. If EI is lower than EE, then a person would be in negative EB, while if EI is higher than EE, a person would be in positive EB, which would translate in the long-term into WL and weight gain, respectively. Even though the time scale of EB is not clear, it is well known that human beings do not balance EI and EE on a day-to-day basis, and an equilibrium seems to be reached after a couple of weeks (15).

When discussing EB it is important to emphasize that EI is 100 % behaviour, it is discontinuous (we eat at meals, not all the time) and highly variable (it can range from 0 to 5000 kcal/day). On the opposite, EE is a continuous process (we are spending energy all the time, even when we sleep) and only a fraction is under our control (11).

1.2.2 Energy expenditure

Total energy expenditure (TEE) varies greatly among individuals, and is dependent on many factors such as body weight and composition, gender, age and PA levels, but also affected by growth (childhood, pregnancy and catabolic states) and genetics (11). TEE is the sum of resting metabolic rate (RMR), diet induced thermogenesis (DIT) and activity thermogenesis (made of non-exercise activity thermogenesis (NEAT) and PA) (See Figure 1.).

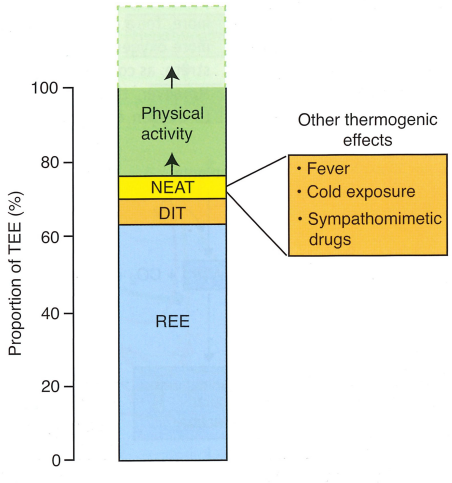


Figure 1. Components of total energy expenditure. TEE: Total energy expenditure. REE: resting energy expenditure. DIT: diet induced thermogenesis. NEAT: non-exercise activity thermogenesis. Taken from Williams & Frubeck (11).

It is well known that TEE rises with increased BMI, and that individuals with obesity present with a higher TEE compared with their normal-weight counterparts (16). The increased TEE seen in individuals with obesity is due to their higher body weight, not to increased PA levels, since PAL seems to be quite similar at different BMI (16). RMR is the main component of TEE (60-75 %) in a sedentary person, and covers the energy needs of the vital organs in the body. Vital organs such as the brain, liver, heart and kidney contribute to 60 % of RMR. Even though fat free mass (FFM) is the main determinant of RMR, there is a large variation in RMR that cannot be explained by body composition (~250 kcal/day) (17). DIT is the amount of energy needed to digest, absorb and utilize food (up to 8-10 % of energy intake).

NEAT is defined as low level involuntary activity, which is spontaneous. NEAT is a large contributor to the inter-individual variation (100-800 kcal) in TEE (18), and has been shown to be lower in people with obesity compared with those who are normal-weight . NEAT may therefore, in the long-term contribute to weight gain (19).

PA is the most variable component of TEE, from 30 % in sedentary individuals, up to 70 % in those doing strenuous work (11, 20). Several factors influence the energy spent on PA, namely type, intensity and duration (11). Individuals with obesity usually spend more energy to perform a given volume PA (with the same duration and intensity) compared with normal-weight individuals, particularly for weight bearing exercises.

1.2.3 Appetite

Food intake is regulated by metabolic, endocrine and neural signals arising from the hypothalamus, brainstem and reward circuits. The hypothalamus is one of the main regions of the brain involved in the control of appetite. Within the hypothalamus, the arcuate nucleus (ARC) contains two neuronal populations: one expressing orexigenic neuropeptides (neuropeptide Y (NPY) and agouti-related peptide (AgRP)) and another anorexigenic neuropeptides (melanocortin and, α-melancocyte stimulating hormone (α-MSH)). The blood-brain barrier inside the ARC transports the hormones insulin, leptin and ghrelin, among others, to cognate receptors to regulate the activity of NPY/AgRP and α-MSH neurons to other hypothalamic areas involved in the regulation of eating. The ARC also has connections with ventromedial hypothalamic area, the dorsomedial hypothalamus and the lateral hypothalamic area, where neurones expressing orexigenic peptides are present (11, 21).

The gastrointestinal tract (GI) communicates with the brain through both hormonal and neural signals via vagus nerve. The stomach is also innervated with mechanoreceptors that respond to gastric filling and gastric distension signals that signal the brain via both vagal and spinal afferents. Gastric distention can contribute to the control of eating (satiation) (22, 23).

Post-ingestive signals can be divided into short- (episodic) and long-term (tonic). Meal-related signals are episodic, arise mainly from the gut and their plasma concentration oscillates according to our meal patterns. They include the hunger signals, ghrelin and satiety

peptides such as glucagon-like peptide 1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK). Insulin, produced by the pancreas, is also an episodic signal (24). Tonic signals arise mainly from tissue stores and exert a constant pressure on the appetite system signalling chronic nutritional status. They include leptin, produced by adipose tissue, and insulin and reflect overall metabolic status (25). Together, episodic and tonic signals, reflect metabolic status (both acute and chronic) and lead to the inhibition or stimulation of neuropeptides within the ARC and other brain areas, modulating EI and EE and therefore EB (see Figure 2.).

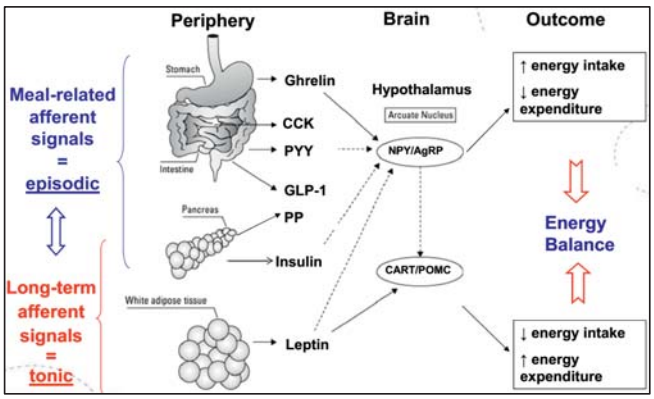


Figure 2. Circulating hormones influencing energy homeostasis. CCK: cholecystokinin. PYY: peptide YY. GLP-1: glucagon-like peptide-1. PP: pancreatic polypeptide. NPY: neuropeptide Y. AgRP: agouti-related peptide. (The figure is modified from Murphy and Bloom by Martins C (24)).

Eating is organised into meals, whose initiation and size is determining by both inhibitory and excitatory signs. To eat is a decision made from coordinated body feelings reflected by changes in metabolic biomarkers, often described as hunger, but hunger is also strongly conditioned by other factors like timing and social settings. Hunger describes the process that initiates a meal, and the amount of food eaten depends upon the hedonics linked to pleasure (26).

The "Satiety Cascade" is a framework to explain the impact of foods on satiation and satiety. Satiation signals generated by food ingestion activate several inhibitory signals that induce meal termination. Satiety signals inhibit eating until the next meal and will determine inter-meal interval (25).

The post-ingestive phase involves the stomach and gastric stretch receptors (23), and involves both ghrelin and the satiety peptides GLP-1, PYY and CCK (26-28), released from the proximal small intestine when food is released to the gut and both signals are carried to the NTS via the vagus nerve. The post-absorptive phase is influenced by nutrient status (absorption of glucose and amino acids) and involves endocrine organs like the liver for fat oxidation and pancreas for release of insulin (25).

Gut hormones and episodic signals

CCK is both a peptide hormone and a neurotransmitter communicating with the central nerve system (CNS). CCK is produced in the duodenum and ileum during and after a meal, stimulated by protein and long-chain fatty acids in the lumen. CCK stimulates gallbladder contraction, pancreatic and gastric acid secretion and slows gastric emptying. It is the most important satiating signal and is involved in meal termination and possible early satiety, by reducing meal size and hunger feelings between meals (29). The effect of CCK is mediated by CCK receptors on vagal nerve, and acts together with other signals like gastric distention to inhibit eating (27, 29).

GLP-1 is produced by intestinal L-cells, and is both an incretin hormone and a specific neuron in the nucleus tracts solitaire (NTS) and brainstem. It is released from the intestine during and after a meal in response to nutrients (specifically carbohydrates), and leads to satiation and post-meal satiety, when GLP-1 reaches receptors in the paraventricular nucleus (PVN) and other areas in the brain to inhibit eating (11).

PYY is produced by the intestine L-cells in response to nutrients including fat, and leads to a decrease in gastric emptying (ileal brake), and is mainly involved in post-meal satiety. PYY works in two separate ways, via the vagus nerve and through the traditional endocrine way. PYY has the ability to cross the blood-brain barrier, and inhibits eating by an indirect action in the ARC of hypothalamus (30).

Ghrelin is an endogenous ligand of the growth hormone secretagoge receptor, and is mainly produced in the stomach by neuroendocrine cells, but also in the hypothalamus. Its secretion increases during fasting and falls during and after a meal (11). Ghrelin is thought to increase

hunger and food intake in humans (31), however it remains to be fully elucidated if ghrelin initiates a meal or increases in anticipation of eating, given that the timing of ghrelin peaks has been shown to be related to habitual meal patterns (32). The orexigenic (appetite-stimulating) effect of ghrelin is achieved through the stimulation of neurons that express GHS-R in ARC at the base of hypothalamus (33).

Tonic signals

Leptin is the most important adiponectin and is produced by the adipocytes in direct proportion to the amount of fat stored. The role of leptin is to regulate eating behaviour by modulating appetite (11, 34). Leptin reduces food intake by reducing meal size, as well as enhancing the satiation effect of CCK (35, 36). Leptin supresses feeding through the interaction with both orexigenic and anorexigenic signals in hypothalamus and other brain areas (11).

The basal concentration of insulin is positively correlated with FM content (37). Insulin enters the medio-basal hypothalamus and cerebrospinal fluid by a receptor mediated transportation, insulin receptors are present in hypothalamus and related areas and their deletion has been shown to lead to hyperphagia. Central administrated insulin inhibits eating in a dose dependent manner, and acts through the same pathway as leptin (38).

1.3 Weight loss management

WL management aims to reduce obesity related morbidity and mortality. Obesity management should lead the patient to success in losing weight and sustaining it, in the long-term, in order to achieve the above-mentioned aims. Clinically relevant health benefits can be achieved with a WL between 5-10 % of initial body weight (39, 40). This magnitude of WL has been shown to improve blood pressure, blood lipids and glycaemic control and decrease cardiovascular risk by 40-50 % (39). The available strategies for obesity management include lifestyle treatment (also known as conservative treatment), pharmacological treatment and bariatric surgery.

1.3.1 Lifestyle treatment

The cornerstone of lifestyle treatment of obesity should be like a triad of increased PA, dietary restriction and behavioural therapy, followed by a long-term maintenance program to prevent relapse.

Which lifestyle intervention is the bests remains a matter of continued scientific and popular discussion. Some programs emphasize energy-restricted diets, others increased PA levels and even others the combination of both. Individual preferences, skills and motivation to follow an intervention should be taken into account, but compliance has been shown to be the key factor determining magnitude of WL (41). Even though the impact of PA on WL is limited, there is a dose-response relationship between PA duration and WL (42). Regardless of the impact of PA on WL, exercise should always be present in any lifestyle intervention due to its health benefits (42). Diet-induced energy restriction (ER) can be achieved in many ways, including continuous ER (43), intermittent ER (44), low-energy diets (LEDs) (45), low-fat, low-carbohydrate and very-low energy diets (VLEDs) (46), among others. However, the most common WL strategy used in obesity management is continuous ER (-600 kcal/day) (43).

1.3.2 Very-low energy diets

There has been scepticism on losing weight fast, namely with VLEDs (< 800 kcal/day), due to myths that faster WL would lead to a larger loss of lean tissue and therefore also a larger reduction in RMR, and also more weight regain in the long-term compared to LEDs (47, 48). However, this has been refuted (49, 50), and there is even evidence that it may be easier to lose weight faster, with a VLED, compared with more gradually, with a LED, as shown by a larger number of participants reaching their WL goals (50). Losing weight fast may work as a motivational factor and help with further WL. Moreover, the fact that WL with VLEDs is not associated with the expected increase in hunger otherwise seen with weight reduction, likely due to the fact that these diets are ketogenic (51), may potentially also contribute to improved compliance.

Ketogenic diets can either be VLEDs or ketogenic low-carbohydrate diets (KLCD), which are severely restricted in dietary carbohydrates, but allow ad libitum consumption of protein and fat (52). With ketogenic diets, ketone bodies (KB) (acetoacetate (AcAc), ß-

hydroxybutyric acid (ß-HB) and acetone) are produced in the liver from free fatty acids (FFA) and become an alternative source of energy, especially to the brain, given their capacity to cross the blood brain barrier (53). Diets with severe ER and/or limited carbohydrate content lead to ketosis (51). When carbohydrate intake is limited with a VLED or KLED, ketosis is induced and, unexpectedly, appetite seems to be supressed (51, 54). However, the timeline of changes in appetite with progressive WL on a VLED has never been investigated and it is not known how long does it take for appetite suppression to be seen with VLEDs.

VLEDs are very effective in inducing a large WL over a short period of time (51). These kind of diets provide between 40-60 g protein/day (0.8-1.5 g/kg ideal body weight), and induce a rapid WL of 1.5-2.5 kg/week or between 10-15 % of baseline weight, depending on duration (46). With a substantial WL, it effectively improves many obesity-related comorbidities and risk factors (55-57). Clinical supervision is necessary when treatment extends over more than 4 weeks, and the usual standard duration of treatment is restricted to 12-16 weeks (58, 59).

The most common side effects of VLEDs are dizziness, constipation, fatigue/ weakness and nausea, abdominal discomfort, diarrhea, headache, transient increased uric acid and liver enzymes. These are usually mild symptoms that can easily be managed and that disappear when the diet is resumed (60). The most serious side effect is the elevated risk of gallstone formation, which may require cholecystectomy, and is caused by the large WL (60). The risk of gallstone formation is three-fold higher with VLEDs compared with LEDs (61).

VLEDs are also proven to be efficient in terms of comorbidity resolution in a variety of patient groups, like those presenting with type-2 diabetes, sleep apnea, knee osteoarthritis etc. (56, 57, 62). A 12-week VLED, including a long-term follow-up program over 1-year, including individual and group sessions, has been shown to be cost efficient compared to pharmacological treatment (63). A greater short-term WL has been consistently shown with VLEDs compared with LEDs (46), while a similar long-term WL maintenance has been reported (46, 50). Moreover, several meta-analysis and reviews have found that a larger initial WL, with a VLED is, in fact, associated with a better WL maintenance (40, 64-66). So, the available evidence shows that VLEDs are one among many tools in obesity management, and that they are at least as good as other approaches for WL.

1.4 Weight regain/maintenance

Obesity is a chronic relapsing disease and maintaining WL in the long-term is the biggest challenge in obesity management (67). Relapse is common, and the majority of obese patients will experience weight regain in the long term (68, 69). Only 20 % of those who lose 10 % WL with diet are able to maintain it for 1-year (40, 70). Moreover, a meta-analysis has shown that after a 5 year follow-up of different lifestyle interventions, including a variety of dietary interventions, alone or in combination with exercise, the observed WL was of only 3 kg (3 % WL) (40).

The high rate of recidivism observed after WL (70) is potentially a combination of reduced motivation and compliance to the diet and exercise regimes (71, 72), together with the activation of potent and redundant metabolic, behavioral, neuroendocrine and autonomic responses that oppose the maintenance of a reduced body weight (73, 74). It is important to understand the mechanisms behind weight regain in order to be able to design strategies aiming at minimizing relapse.

1.5 Adaptive physiological responses to WL

WL leads to adaptive physiological responses on both sides of the EB equation, with a reduction in TEE (75), changes in substrate metabolism towards reduced fat oxidation (76) and an increased drive to eat, motivated, at least partially, by changes in the appetite control system towards increased hunger (73, 77, 78). These mechanisms are likely to slow WL rate, facilitate fat storage and may increase the risk of weight regain (54, 75). Clearly, the weight-reduced state is counter-regulated by adaptive mechanisms that try to restore body weight back to its original level.

1.5.1 Adaptations at the level of appetite

The weight reduced state is characterised by increased drive to eat, likely due to changes in the plasma concentration of appetite-regulating hormones, with an increase in the hunger hormone ghrelin, and potentially also a reduction in satiety peptides (GLP-1, PYY and CCK) (73, 79-81), even though the latest remains controversial (49, 82-84). Changes in non-

homeostatic signals such as motivation, reward attention and behaviour may also be important (65).

Changes in appetite with LED interventions

Several studies have shown that acute ER has a significant impact on appetite, even before significant WL is achieved (85-87). Moderate ER has been shown to result in a significant increase in the feelings of hunger, desire to eat (DTE) and prospective food consumption (PFC) after 1-4 days (WL 0-2 kg) (85, 86), despite no changes in basal or postprandial concentration of total ghrelin during the first 2-5 days (85, 88, 89). These studies were, unfortunately, done mainly in lean and overweight younger males.

Studies where a 5-10 % WL was achieved show controversial results regarding changes in appetite. Basal ghrelin plasma concentration has been reported to increase (90-92) or not to change (49, 93). Similarly, some studies have shown no significant changes in the postprandial secretion of total PYY, active GLP-1 and CCK after WL (49, 84), while others have reported a significant reduction in basal and postprandial total and active PYY (79, 80).

Contradictory results are also found for hunger feelings; some studies found no significant change (80, 94), others an increase in fasting and a reduction in the postprandial state (49).

A WL $\geq$ 10 % induced by LEDs has systematically been shown to increase basal active and total ghrelin plasma concentrations (50, 82, 89, 95-97). Regarding satiety peptides, one study found a reduction in basal active GLP-1 (97), while another showed increased postprandial total GLP-1 concentration (82). No change in basal total PYY or CCK was reported in some studies (89, 97), while basal PYY_{3-36} was reported to be reduced in another study (82). Regarding subjective feelings of appetite, hunger and DTE have been reported to be increased in one study (89), while another study showed no significant changes in subjective feelings of appetite after WL (97).

Changes in appetite with ketogenic VLED interventions

WL interventions with VLEDs have reported contradictory findings regarding appetite after 2-5 days of ER (85 %), with some studies showing increased postprandial total ghrelin

concentration (98), while others report no difference in postprandial total/active ghrelin concentration (87, 99), or in basal or postprandial GLP-1 concentration (99).

With a WL of 5 - 10 % under ketosis, no significant change has been described for active ghrelin, either in the fasting or postprandial state (49). Changes in the plasma concentration of satiety peptides are contradictory, with a reduction in postprandial active GLP-1 in one study (100), and no differences in basal or postprandial active GLP-1 or CCK and a reduction in basal total PYY in another (49). Similarly, no significant change in fasting hunger or fullness feelings were seen in some studies (101-103), with contradictory results in postprandial hunger; one study showing a reduction (104), another no difference (105). On the other hand, postprandial fullness feelings have been reported to increase (100, 105).

Studies on the impact of a large WL (> 10 %) induced by VLEDs on appetite markers while under ketosis are limited. No changes in either basal or postprandial active ghrelin (AG) concentration were described compared to baseline in two studies (49, 54). Regarding satiety peptides, one study reported a reduction in basal active GLP-1, despite no change in postprandial concentration and a reduction in both basal and postprandial total PYY concentration (54). Results regarding CCK are contradictory, with one study reporting reduction in basal and no change in postprandial state (54), while another showed no change in fasting and a reduction in postprandial state (106). Regarding subjective feelings of appetite, one study reported no change either in the fasting or postprandial states (54). In another study, fasting and postprandial hunger were significantly reduced, while fullness was shown to be increased in both fasting and postprandial state after WL (106).

Gibson et al. (2015) has shown, in a systematic review and meta-analysis, that ketogenic diets prevent the expected increase in appetite otherwise seen with WL, with participants reporting less hunger and greater fullness after the intervention (51). It is a paradox that despite severe ER and rapid WL, ketogenic VLEDs do not lead to an increase in appetite (52, 107). After 2 weeks of refeeding and out of ketosis, studies have shown that both total and active ghrelin, both basal and postprandial concentrations, are significantly increased compared with baseline (54, 95). On the other hand, the concentration of several satiety peptides, has been shown to be reduced after refeeding (with a reduction in both basal and postprandial active GLP-1 and CCK concentrations and a reduction on postprandial total PYY concentration)

(54). Meanwhile, hunger and PFC ratings, both in the fasting state and after a meal, have been reported to increase after refeeding and no ketosis, compared with pre-WL levels (54).

Chearskul et al. (2008), on the other hand, with only 1-week stabilisation phase, reported no differences in CCK, hunger or satiety in fasting or postprandial state compared to pre-WL (106). In a study where individuals with obesity underwent 4 weeks of a VLED followed by 4 weeks of refeeding and weight stabilization, only fasting hunger feelings were increased compared to baseline, with no difference in other appetite feelings or plasma concentration of appetite hormones being observed (49).

Overall, plasma ghrelin concentration seems to be increased with VLEDs, when measurements are taken after a period of 1-4 weeks of refeeding. Results regarding satiety peptides are contradictory and few. However, fasting hunger ratings seems to be increased with refeeding.

Long-term sustainability of changes in appetite with WL

Only a few studies have examined long-term changes in objective and subjective appetite markers in the post obese state (81, 82, 90, 108).

In a landmark paper by Sumithran et al. (2011), after an initial 14 % WL, with partial weight regain (50 %) at 1-year follow up, AG plasma concentrations, both in the fasting state and after a meal, were reported to be increased (81). Moreover, a reduction in the postprandial release of active GLP-1, total PYY and CCK was also found and postprandial hunger and DTE were still increased after 1-year follow-up (81).

In another study by Iepsen et al. (2016), after an initial 13 % WL, followed by weight maintenance at 1-year follow-up, total ghrelin (basal and postprandial) was also found to be increased (82). However, contrary to Sumithran's findings, the postprandial secretion of total GLP-1 and PYY_{3-36} were increased after 1-year (82). Unfortunately, they did not investigate subjective feelings of appetite.

Two other studies have looked into changes in single objective appetite markers after respectively 3 and 6 months WL maintenance (90, 108). Adam et al. (2006), after initial 8 % WL, did not see any significant change in active GLP-1 after 3 months sustained weight

maintenance, nor changes in hunger or fullness feelings compared to baseline (108). In an RCT (CR or IER) by Catenacci et al, (2016) (initial 6 and 8 % WL, respectively), increased AG concentration in the fasting state was reported after 6 months (partial weight regains) of unsupervised follow-up in the CR group only (90).

Overall, sustained WL maintenance is associated with increased hunger, likely driven by increased plasma ghrelin concentration. The impact of sustained WL on fullness feelings and plasma concentration of satiety peptides is unfortunately less clear. Further investigation of the long-term sustainability of changes in subjective feelings of appetite and the plasma concentration of appetite hormones is need.

Moreover, the studies on the impact of ketogenic diets on appetite have mainly been done in females (51), so more studies need to be done to evaluate if males respond in a similar way.

1.5.2 Adaptation to the weight-reduced state at the level of EE

The decrease in TEE observed with WL is due to a reduction in each one of its components: RMR (109), DIT and activity thermogenesis (77).

Changes in RMR

The reduction seen in TEE with WL is mainly driven by reduction in RMR, given that RMR is the largest contributor to TEE. RMR has been shown to be significantly reduced after acute ER, even in the absence of WL or change in body composition (110). However, the evidence is controversial, as no significant changes in RMR have been reported after 6 days of total fast (111).

Even though most of the studies, where a WL of < 10 % was achieved through diet, report a significant reduction in RMR (49, 110, 112, 113), others have reported no change after 4 weeks refeeding, in either total or adjusted RMR (49, 112, 114). Kreitzman et al. (1992) showed a significant reduction in RMR (14 %) after 2 weeks on a VLED, which subsequently stabilized despite continued WL, up to 18 kg WL. However, RMR returned to baseline levels after 5 weeks refeeding (113). Müller et al. (2015), in a WL intervention in normal weight males, found a significant reduction in RMR already at day-3 of ER, with no further

significant reduction during progressive ER up to a total of 7.5 % WL (both for absolute and RMR adjusted for FM and FFM). After 2 weeks of refeeding and partly weight regain (4.5 %), RMR was no longer different from baseline (112).

Studies where a large WL was achieved (≥ 10 % of baseline weight) through diet alone tend also to report a significant reduction in RMR (97, 115), but some studies report surprisingly no significant changes (97, 116).

The controversial results previously reported can probably be accounted for by differences in study population, sample size and timing of measurements (acute WL vs. after a refeeding period). Even though some studies show that the reduction in RMR, both absolute and adjusted, seen with WL is sustained after 10-14 days of refeeding (15, 113, 115), others show no differences from baseline after 4-6 weeks of refeeding (49, 112). The results of studies on short-term sustainability of changes in RMR in post-obese state are therefore diverse.

Studies on changes in RMR in the longer-term in post-obese state after refeeding are limited. RMR was shown to be reduced still after 9 months and the reduction was present even 6 years after initial WL, despite partly weight regain (117). Moreover, in another study, RMR was still reduced after 1-year follow-up, even though weight was stable (118).

The time-course and magnitude of adaptation in RMR with progressive WL, in participants with obesity, remains controversial and deserves further investigation.

Changes in exercise induced energy expenditure

The reduction in exercise induced energy expenditure (EIEE) seen with WL could potentially be due to a combination of reduced PA levels, even though the evidence is minimal (119, 120), or more probably due to increased exercise efficiency (ExEff) (75).

A 10 - 20 % WL has been shown to lead to a significant reduction in EIEE, particularly at lower PA intensity levels (15, 74, 75, 97). However, one study reported no change in ExEff after a 19 % WL (121).

In another study, after 2 weeks of refeeding and weight regain, a significant increase in non-resting energy expenditure (NREE) was seen compared to after initial WL (-6 kg), but after refeeding there were no changes compared to baseline (-0.7 kg) (112). In another study, after

a 9 % WL and 4 weeks refeeding, and weight maintenance, a significant reduction in EIEE was reported (49).

Only a couple of studies (inducing 10-18 % WL) have examined the long-term sustainability of changes in NREE and EIEE with WL, and they have found a reduction in both EIEE and NREE compared to baseline after 1-year (118, 122). However, another study, with an initial 10 % WL and 10 months follow-up (50 % weight regain) found that EIEE returned to baseline levels (123). Again, the evidence is controversial and more studies are needed regarding how progressive WL and WL maintenance impact on NREE and EIEE. It is the reduction in TEE below predicted levels seen with WL (adaptive thermogenesis) that seems to come mainly from NREE.

Adaptive thermogenesis (AT)

AT is the reduction in TEE, or any of its components that cannot be explained by changes in body weight and /or body composition (FM and/ or FFM) (124). This might be viewed as a survival mechanism in case of starvation or poor access to food (125). AT is present when measured EE is lower than predicted. Unsuccessful WL interventions and reduced body weight maintenance could partly be due to AT (124, 126).

Several studies have shown that a WL ≥ 10 % achieved by a VLED results in a reduction in TEE larger than expected (15, 110, 118, 127).

In a recent study by Müller et al. (2015), AT at the level of RMR in non-obese males was reported at day-3 and after 1 week with a LED intervention (1 and 4 % WL, respectively), but not thereafter with progressive WL up to 5 % (112).

AT at the level of RMR has been reported for WL between 10 and 20 % in obese individuals (15). The evidence of AT at the level of RMR is, however, inconsistent, as other studies have not found AT at the level of RMR immediately after a 10 % WL (111, 114, 128).

The long-term sustainability of AT at the level of RMR has, to my knowledge, only been evaluated in a few studies. Sustainability in AT was seen even 6 years from baseline and with partial weight regain (117). In a large study by Camps et al. (2013) in obese males and females, after a 10 % initial WL induced by a VLED, they found AT at the level of RMR, which was sustained up to 1-year with 50 % weight regain (129).

A reduction in EIEE larger than predicted, after WL, has also been found in several studies (15, 130, 131). Moreover, Leibel et al. (1995) showed that AT at the level of EIEE was positively correlated with the magnitude of WL (between 10-20 %) (15). However, the evidence regarding the existence of AT is controversial, some studies show AT at the level of EIEE (130), but others not (121, 122).

The practical relevance of AT is not fully understood, and needs therefore further investigation. AT may increase the risk of relapse and reduce WL maintenance, however the evidence remains inconclusive (77, 118).

1.6 Impact of sex on adaptive physiological mechanisms

Sex has been shown to modulate appetite sensations and the secretion of several appetite-related hormones (132, 133). Moreover, given that a greater FFM loss occurs during energy restriction in males, compared with females (134), this may suggest that the changes in EE variables with progressive WL may be modulated by sex.

The influence of sex on adaptive thermogenesis remains to be established (135). Therefore, if sex has an impact on the adaptive physiological responses with WL remains to be explored.

1.7 Overall remarks

WL leads to compensatory responses on both sides of EB equation, which try to bring body weight back to its original state (78, 125). WL and the postobese state are characterized by increased hunger and drive to eat (73), despite a significant reduction in TEE (15, 75), in many cases below predicted values (AT) (15, 75, 122, 127). These mechanisms can reduce WL rate and increases the risk of relapse (77).

Evidence regarding the activation of the above-mentioned mechanisms is unfortunately controversial. Differences in the magnitude of WL, EB (measurements done under energy imbalance – acutely after WL, or in EB after a period of refeeding or a longer period of WL maintenance), dietary interventions (ketogenic vs. non-ketogenic diets) and study population (gender, BMI, age) are likely to play a role. The increase in hunger seen with WL seems to be absent when ketogenic diets are used (51). Given that ketogenic VLEDs are seen as a valid tool in obesity management, associated with similar changes in body composition (49) and

long-term WL maintenance as LEDs (50), more research is needed regarding how WL induced by VLEDs impacts on the several compensatory mechanisms activated with weight reduction.

To my knowledge, no study has investigated the timeline over which adaptive metabolic responses are activated with progressive WL achieved with a VLED, in people with obesity. How long does it take for its inhibitor effects on appetite to be seen? How does progressive WL impacts on appetite and EE variables under ketosis? What happens after a period of refeeding and sustained WL maintenance? Do males and females respond similarly?

2 Aims

The general aim of this PhD research is to increase the knowledge on how WL, under and outside ketosis, impacts on the physiological adaptations to weight reduction seen in individuals with obesity, using a repeated measures design. To answer the general aim, three specific aims were addressed in the three articles that form the basis of this thesis.
The specific aims are outlined below:

2.1 Primary aims

- ✓ To determine the timeline over which changes in appetite (both subjective feelings of appetite, and plasma concentration of appetite-related hormones) occur during progressive WL with a VLED in individuals with obesity (Paper 1)
- ✓ To determine the timeline over which EE variables (RMR, EIEE and AT) change during progressive WL with a VLED (Paper 2)
- ✓ To investigate the long-term sustainability (1-year follow up) of the changes in appetite seen with WL (Paper 3)

2.1.1 Secondary aims

- ✓ To assess if sex impacts on the timeline of adaptive physiological responses seen with progressive WL and sustained WL maintenance.

3 Method

3.1 Study design

This was a longitudinal clinical intervention study, with a repeated measurement design, aimed at tracking down the timeline of activation of adaptive physiological responses to progressive WL with a VLED. The study was conducted between 2013 and 2017 at the Norwegian University of Science and Technology (NTNU), in collaboration with St Olavs University Hospital in Trondheim, Norway.

Healthy individuals with obesity underwent an initial WL phase induced through an 8-week VLED, followed by a 4-week refeeding phase and a 1-year weight maintenance phase. Participants were asked not to change their PA levels up to week 13.

A sub-study with the study population, but including additional assessment time points was also performed. A summary of the assessment time points used in the main study and sub-study can be seen in Figure 3.

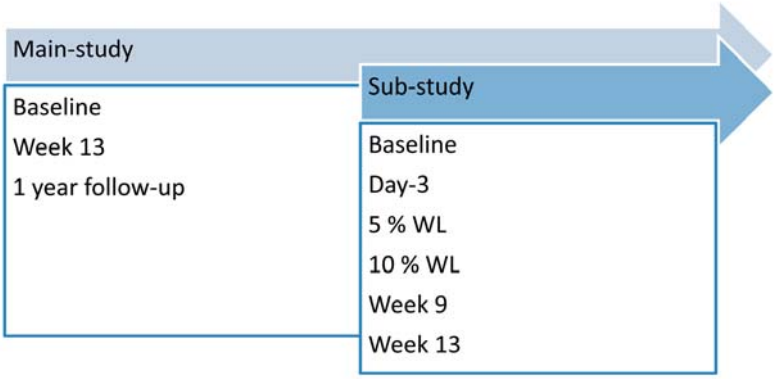
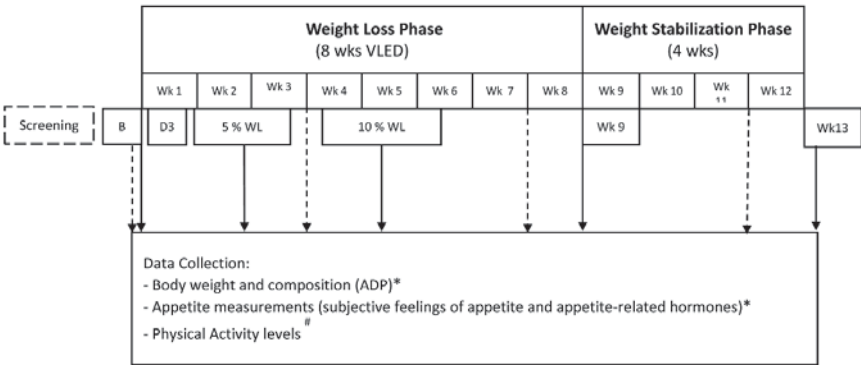


Figure 3. Overview of assessment time-points for participants taking part in the main study and sub-study. WL: weight loss.

For a detailed overview of the design of the studies included in this thesis, assessment timepoints and main outcome variables (see Figure 4).



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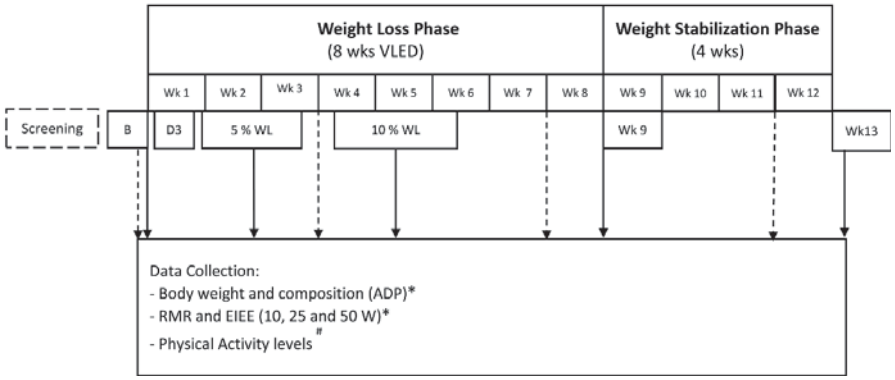

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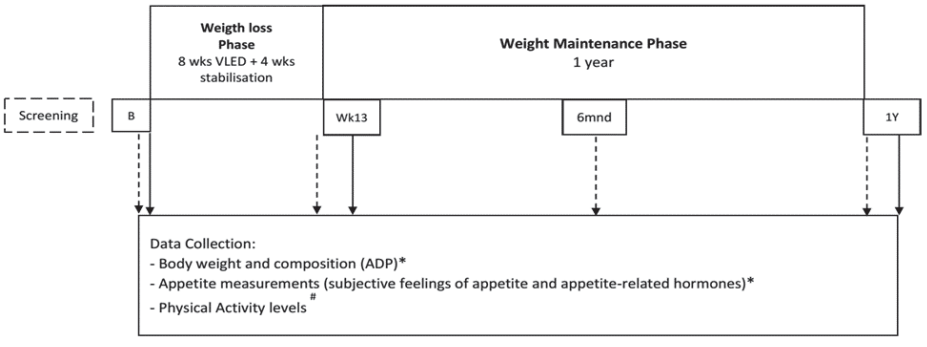

C.

Figure 4. Overview of Paper I (Sub-study) (A), II (Sub-study) (B) and III (The Main Study) (C) Arrows show data collection time points. *See solid arrows; # See dashed arrows. VLED: Very low energy diet. Wk: week. B: baseline. Y: year. Mnd: months. ADP: air displacement plethysmography. β-HB: β-hydroxybutyric acid. RMR: resting metabolic rate. RQ: respiratory quotient. EIEE: exercise induced energy expenditure.

3.2 Participants

Healthy adults (18-65 years) with obesity ((30 < BMI < 50 kg/m^2) were recruited from the local community around Trondheim, Norway, via newspapers and internet advertisements. Participants were required to be weight stable (<2 kg body weight change over the last 3 months), and not currently dieting to lose weight. Participants should also be sedentary (not engaged in strenuous work or in regular brisk leisure time exercise more than once a week or in light exercise for more than 20 minutes/day in more than 3 times/week).

Due to the known effect of phase of menstrual cycle on appetite (136) and RMR (137), for the main study females had to have a regular menstrual cycle (average 28 days) (or be post-menopausal) and for the sub-study (papers I and II), females had to be post-menopausal or taking hormonal contraceptives.

Exclusion criteria were pregnancy, breast-feeding, and clinically significant illness, including diabetes, previous WL surgery and/or medication known to affect appetite/metabolism or induce WL.

3.3 Detailed protocol

Weight loss phase.

The participants followed a ketogenic VLED (Allevo, Karo Pharma AS, Stockholm, Sweden) containing 550 vs. 660 kcal/day for males and females, respectively, for 8 weeks. The macronutrient composition was 42 E % carbohydrates, 36 E % protein, 18 E % fat and 4 E % fibre. They were advised to drink 2.5 L/day of non-energy liquids and could eat a maximum of 100 g/day of low starch vegetables in addition to the diet (see Figure 5).

Figure 5. Example of 50 g low starch vegetables. Photo Siren Nymo.

Compliance with the VLED was checked on a weekly basis throughout the intervention. Food diary and side effects were reviewed weekly as well. Participants were also told not to change their PA levels during this phase.

Refeeding phase

During weeks 9 and 10 the participants were gradually reintroduced to regular food, while VLED products were withdrawn (two and three products/day for females and males on week 9, respectively, and one product/day on week 10). During the WL intervention and the 4 weeks follow-up the participants met for a 20 minutes individual consultation with a dietician for weighing.

A tailored diet plan aimed at weight stabilisation was provided to the participants at week 10 according to their measured RMR at week 9 and PA-levels (PAL) at week 8 (measured RMR X PAL). The recommended diet followed the Nordic Nutrition Recommendations (25-40 E % fat, 10-20 E % protein and 45-60 E % carbohydrates) (138).

Participants were also told not to change their PA levels during this phase.

Weight maintenance phase

At week 13, dietary recommendations were adjusted (based on RMR measured at week 13 and PA levels at week 12) and participants were asked to increase their PA levels. All participants were offered a multidisciplinary 1-year follow up program, aimed at WL maintenance. The program included regular individual and group based sessions, where the participants received education on diet and lifestyle changes and were encouraged to follow the "Nordic Nutritional Recommendations" (138). The focus of the program was on a balanced energy restricted diet, increased PA-levels and cognitive behaviour therapy.

3.4 Outcome variables

All measurements were performed after an overnight fast. On the evening before each test day, participants were asked to have their dinner no later than 8 pm and not to eat or drink

anything after that, except water. They were also asked not to exercise on the day before, to abstain from tobacco and caffeine (12 hours) before testing (139) and to use public transportation or car to get to the test site.

3.4.1 Body weight and composition

Body weight was measured by Seca 877 digital weight (SECA, Hamburg, Germany), without shoes and in underwear. Height was measured with Seca 217 stadiometer (SECA, Hamburg, Germany) without shoes. Weight was measured to the nearest 0.1 kg, and height to 0.5 cm, and the participants were asked to empty their bladder beforehand.

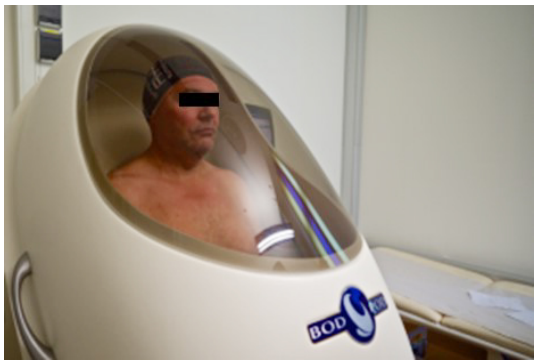

Figure 6. Participant getting his body composition measured in the BodPod. Photo Tryggve Andreassen.

Following this initial procedure, air-displacement plethysmography (ADP) (BodPod, COSMED, Rome, Italy) was used to measure body composition (see Figure 6). Participants had to remove jewellery and wear a head-cap to minimise air-volume in the hair when entering the BodPod in their underwear (see Figure 6). A two-step calibration was performed every morning and a single calibration between participants. Measurements were repeated twice for each participant and the BodPod software (Cylinder Volume 50.187 L S/N 3757) adjusted for the individual thoracic gas volume and body surface. The Brozek equation were used to calculate the volume in this Caucasian obese population (140). ADP is a reliable (within 1 %) (141) and valid method for the measurement of FM (142).

3.4.2 Appetite

Subjective appetite feelings (hunger, fullness, DTE and PFC) were assessed using a validated 10 cm visual analogue scale (VAS), pen and paper (143), and blood samples were collected in the fasting state and every 30 minutes (0, 30, 60, 90, 120 and 150 min) after a standardised breakfast (600 kcal: 35 E % fat, 17 E % protein and 48 E % carbohydrates), for a period of 2.5 hours. The breakfast consisted of 75 oat bread (whole grain), 5 g butter, 40 g strawberry jam, 35 g cheese (38 E % fat) and 250 ml low fat (1.2 E % fat) milk.

Blood samples were collected through an intravenous line into EDTA-coated tubes (3ml). 1ml of full blood was transferred into a micro tube and 20µl mixture of inhibitors (10µl of Pefabloc (Roche Diagnostic, Germany) + 10µl DPP-IV (Merck Millipore, Germany)) was added. The samples were then centrifuged at 3200 rpm for 10 minutes at 18 °C. The resulting plasma was then placed in another micro tube and frozen at -80 °C for later analysis. For CCK analysis, aprotinin (DSM, Coatech AB, Kaiseraugst, Switzerland) was added to the EDTA tube in order to achieve 500KIU/ml blood, before centrifugation. The generated plasma was frozen at -80 °C until analysis.

Plasma samples were analysed for AG, total PYY, active GLP-1 and insulin using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore, St Louis, MO, USA) and CCK using an "in-house'' RIA method (144) (intra- and inter-assay coefficient of variation (CV) were <10 % and <20 % for AG, GLP-1 and PYY; <10 % and <15 % for insulin and <5 % and <15 % for CCK, respectively).

3.4.3 RMR

RMR was measured by indirect calorimetry (Vmax Encore 29N, Care Fusion, Hoechberg, Germany). While calibration of the equipment was performed, the participants rested for 10 min on a chair. After that, a ventilation hood was placed over the persons head, and O_2 consumption and CO_2 production were measured for 15-20 min (or longer if required) until "steady state" was reached. The first 5 minutes were excluded, and 10 min of stable data (CV for volume of oxygen (VO_2) and volume of carbon dioxide (VCO_2) < 10 %) were used (139).

3.4.4 Exercise-induced energy expenditure

Exercise induced energy expenditure was measured on a graded cycle ergometer (Monark exercise AB, Eromedic bicycle 839E, GIH, Vansbro, Sweden) using indirect calorimetry (Vmax Encore 29N, Care Fusion, Hoechberg, Germany). The participants cycled at 60 rpm for 16 minutes with a mouthpiece and a nose clip during the whole test. After an initial 5 minutes of acclimatization to the bike, participants cycled at 10, 25 and 50 W in consecutive 4-minutes stages. EIEE was determined from the last 2 minutes of steady state for each level of resistance (75). Net EIEE was calculated by subtracting RMR (kcal/min) from the gross EIEE (130).

3.4.5 Compliance measurements

Ketosis:

Compliance with the VLED was checked on a weekly basis during the first 8 weeks of the intervention, through the measurement of urine acetoacetic acid concentration (AcAc) (Ketostix reagent strips, Bayer Corp, Elkhart, IN, USA). Participants with negative or traces values for (AcAc) (≤0.5 mmol/L) were educated as to follow the dietary protocol. If negative (AcAc<0.5) concentrations were measured more than once, the participant was excluded from the study. In addition, plasma concentration of ß-hydroxybutyrate (ß-HB) was measured using Ketone Body Assay Kit (Mark134, Sigma-Aldrich, St Louis, MO, USA) in the fasting state on all assessment time points in the sub-study.

Physical activity:

Armbands (SenseWear, BodyMedia, Pittsburgh, PA, USA) were used, for a one-week period, to monitor changes in PA-levels during the study. Participants were asked to wear the armband over their triceps on the upper part of their non-dominant arm. Data was considered valid if the device had been worn for ≥ 4 days, including at least 1 weekend day, and more than 95 % (22.8 hours) of the time (145). The following variables were analysed: time spent on sedentary (< 1.5 metabolic equivalents (METs)), light- (1.5- 3.0 METs), moderate- (3-6.0

METs) and vigorous- to very vigorous activities (> 6 METs), total PA duration (> 1.5 METs) and steps/day (146, 147).

3.5 Power calculation

The power calculations were done with Stata13 (StataCorp LLC, Collage Station, Texas, USA).

3.5.1 Paper I

Sample size estimation for paper I was based on expected changes (from baseline) in basal AG plasma concentration overtime (day 3: 27 pmol/L; 5 and 10 % WL: 0 pmol/L; week 9: 4 pmol/L and week 13: 53 pmol/L). Taking into consideration that no previous study had looked into changes in AG with progressive WL with a VLED ketogenic diet, data from Sumithran et al. (2013) (54) was used for weeks 9 and 13 and it was hypothesised that there would be an increase in AG on day 3 (approximately half of that seen on week 13) and no changes at 5 and 10 % WL (given that participants would be under ketosis). For a repeated measures design with 6 time-points, assuming a standard deviation (SD) of 89 pmol/ L for AG (54), at a power of 80 %, a significance level of 5 %, and assuming a low correlation between time points (r=0.3), 32 participants would be necessary.

3.5.2 Paper II

Sample size estimation in paper II was based on expected changes from baseline in RMR (day 3: -50; 5 % WL: -100, 10 % WL: -130, Wk9: -160 and Wk13: -90 kcal/day) (85, 116, 127, 129) and assuming an SD of 229 kcal/day (129). Design, power, significance level, and correlation between time points was the same as in paper I, and also here 32 participants were deemed to be necessary.

3.5.3 Paper III

This study was powered to look at changes overtime in the plasma concentration of AG in the fasting state. The expected changes from baseline were 57 and 26 pg/ml at W13 and 1Y, respectively, and the SD 110 pg/ml (based on Sumithran et al. 2011 study) (81). For a power of 90% and a significance level of 0.05, 67 participants would be needed. Assuming a drop

out rate of 40% (Sumithran et al. 2011 reported a drop out rate of 32%), 94 participants were deemed necessary and 100 were, therefore, recruited.

3.6 Statistical analysis

3.6.1 General

Statistical analysis for all papers was performed with SPSS version 22 (SPSS Inc., Chicago, IL, USA). Baseline characteristics and time to achieve 5 and 10 % WL are presented as mean ± SD. All other data are presented as estimated marginal mean ± standard error of the mean (SEM), unless otherwise stated. Statistical significance level was set at $P<0.05$. Data was analysed by linear mixed-effects models (LMM), with restricted maximum-likelihood estimation, including fixed effects for time and sex, and their interaction. Bonferroni correction was used for post hoc pairwise comparisons. The Benjamini-Hochberg method, which controls for the false discovery rate (148) was used to adjust for the large number of outcome variables in all papers.

Analysis was performed in completers only. Participants with data for at least 3 of 6 time-points were considered completers for paper I and II, while for paper III, participants with data at 1-year were considered completers and kept in the analysis. In paper III, an intention to treat analysis with baseline values carried forward was also performed for changes in body weight overtime.

Total area under the curve (tAUC) for subjective feelings of appetite and appetite hormones was calculated from 0 to 150 minutes using the trapezoid rule.

In paper II, RMR was adjusted for FM and FFM (RMRadj), by using these variables as co-variates in the LMM analysis. The presence of AT was tested by paired t-tests, comparing measured and predicted variables for both RMR and EIEE, and a $P<0.003$ was considered significant after correcting for multiple comparisons. Correlation analysis was performed between WL and AT at the level of RMR and AT at the level of EIEE.

3.7 Ethics

3.7.1 Health Risk

The intervention was non-invasive. Testing procedures were non-invasive except for venepuncture for blood sampling.

3.7.2 Approval

The study was approved by the Regional Committee for Medical Research Ethics, Central Norway (REK nr. 2012/1901). The study was registered in ClinicalTrial.gov (NCT01834859), and conducted according to the guidelines laid down in the Declaration of Helsinki. All participants received oral and written information about the study, and provided written informed consent (Appendix 1. Consent form for main study and Appendix 2. Consent form for the sub-study), before entering the study. They were also informed that participation was voluntary and that they could withdraw from the study at any time without the need to provide a reason.

4 Results

The results are described in detail in the three papers attached, and presented in a summary way below.

4.1 Paper I

The VLED led to a transient increase in subjective drive to eat up to 5 % WL (maximum of 3 weeks). Thereafter, while ketotic and up to 17 % reduction in weight, no significant change in subjective feelings of appetite was seen. Postprandial concentrations of active GLP-1 was significantly increased at 5 % WL only. Basal PYY concentration was significantly reduced only at 10 % WL. CCK concentration after a meal was significantly reduced for the first time at 5 % WL and that was sustained until week 9.

With refeeding, hunger feelings in the fasting state, and AG, both in fasting state and after a meal, were significantly increased compared with baseline. The plasma concentration of satiety peptides was unchanged after refeeding compared to baseline. A summary of the results can be seen in Figure 7.

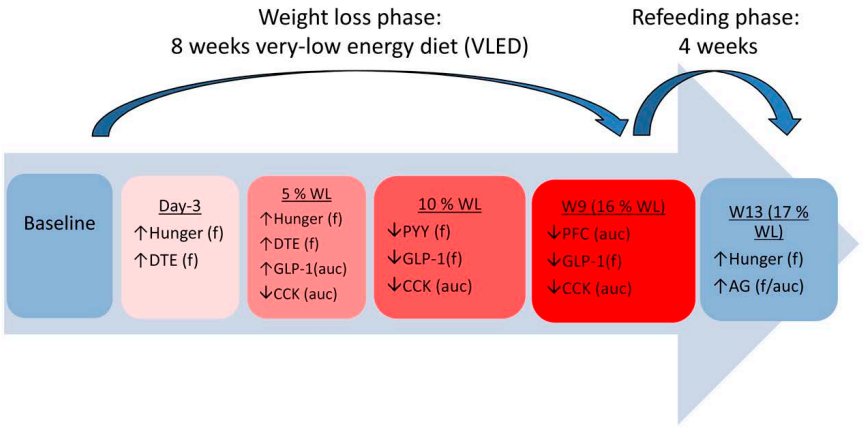

Figure 7. Timeline of changes in appetite with progressive weight loss (in comparison with baseline) (n=31). Blue colour indicates weight stability and no ketosis. Stronger shades of red indicate increasing weight loss. auc: area under the curve. DTE: desire to eat. WL: weight loss. W9: week 9. GLP-1: glucagon-like peptide-1. CCK: cholecystokinin. PYY: peptide YY. PFC: prospective food consumption. © Nymo, S.

FM (kg) was significantly reduced for the first time at 5 % WL in all groups and was lower than baseline at all time-points thereafter. FFM (kg) was significantly reduced for the first time at 16 % WL, but did not change in females compared to baseline at any time-point throughout the study.

Males, opposite females, did not at any time-point report increased hunger feelings. On the other hand, males, opposite females, experienced a significant increase in DTE until 5 % WL. A significantly reduction in PFC aft a meal was only observed in females at week 9. Moreover, females, opposite males, experienced an increase in postprandial concentration of GLP-1 both at 5 % WL and week 9 (16 % WL) and PYY at week 9.

4.2 Paper II

The VLED resulted in a significant reduction in RMR for the first time when WL reached 5 % and in EIEE when WL reached 10 %. With further WL, RMR did not change significantly, but all time-points were significantly lower than baseline. AT the level of RMR was only seen transiently with 10 % reduction in body weight (- 111±165 kcal/day, P<0.01). A summary of the results is shown in Figure 8.

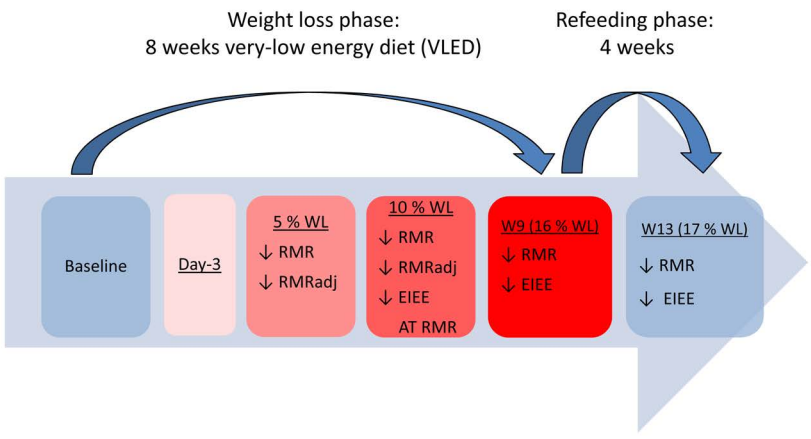

Figure 8. Timeline of changes in energy expenditure variables with progressive weight loss (compared with baseline) (n=31). Blue colour indicates weight stability no ketoses. Stronger shades of red indicate increasing weight loss and ketoses. WL: weight loss. RMR: resting metabolic rate. adj:

adjusted. AT: adaptive thermogenesis. EIEE: exercise induced energy expenditure. W: week. © Nymo, S.

A significant reduction in Net EIEE was only seen at the power of 10 W at week 13 (16 % WL) in females, differently from males where Net EIEE was seen at all powers after 10 % WL. AT was only present at the level of RMR in males at 10 % WL and at week 9. No AT was found neither at the level of RMR or EIEE at any time-point in females.

4.3 Paper III

One-hundred (45 males) started the intervention and seventy-one came to the 1-year follow up. Reasons for dropout are presented in Figure 9.

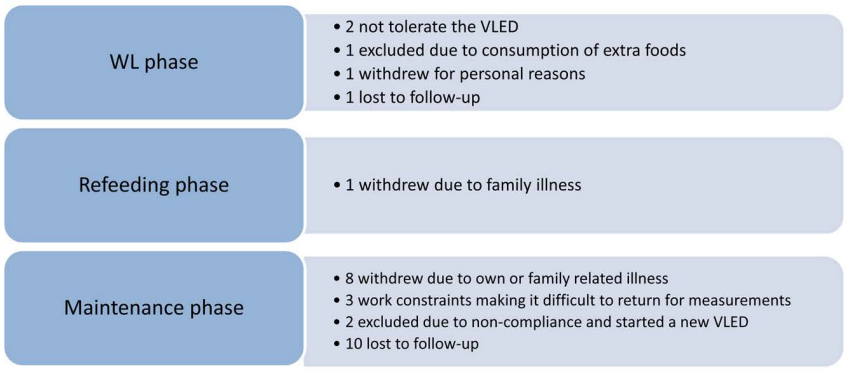

Figure 9. Reasons for dropouts in study III.

At week 13, with a 16 % WL (-18±1 kg, P<0.001) and without ketosis, hunger feelings were significantly increased, both in the fasting state and after a meal. In addition, postprandial fullness feelings were significantly increased, and postprandial feelings of PFC were reduced. This was accompanied by a significant increase in AG plasma concentration, both in the fasting state and after a meal. CCK concentration was significantly reduced after a meal at this time-point.

At 1-year follow-up, and with a 15 % sustained WL, fasting hunger and postprandial fullness feelings remained significantly increased. In addition, PFC was still reduced after a meal and AG plasma concentration, both in the fating state and after a meal, remained also significantly

elevated. Surprisingly, postprandial CCK plasma concentration was significantly increased compared with baseline, while PYY was reduced. A summary of the results can be seen in Figure 10.

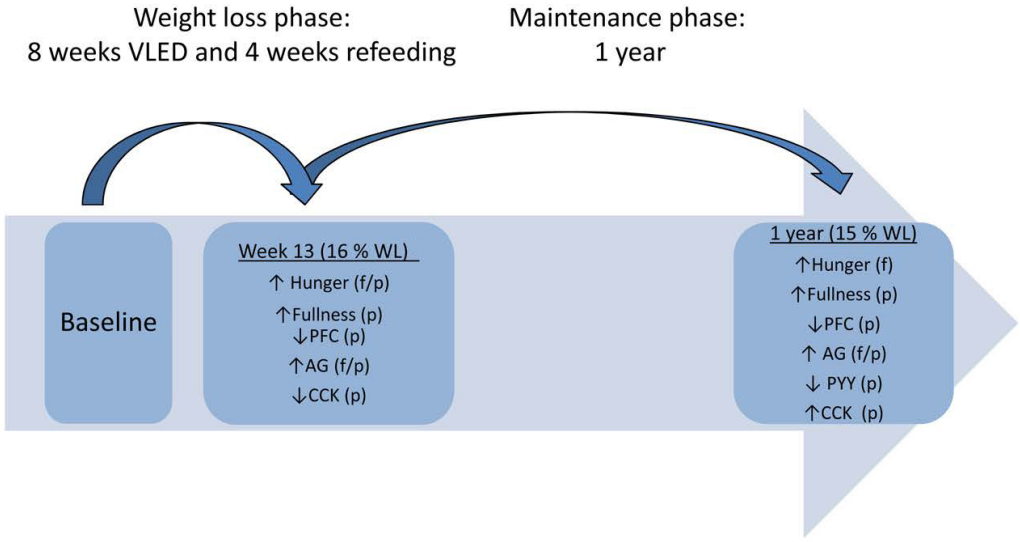

Figure 10. Long-term sustainability of changes in appetite (n=71). Blue colour indicates stable weight and no ketoses. WL: weight loss. f: fasting. p: postprandial. AG: active ghrelin. PFC: prospective food consumption. CCK: cholecystokinin. © Nymo, S.

Only females experienced increased hunger at week 13. Males had increased postprandial hunger and fullness feelings, and reduced PFC at week 13. Increased fullness feelings were only seen at 1-year in females. Postprandial CCK plasma concentration did not change in females, but was significantly increased postprandially in males at 1-year.

5 Discussion

The aim of my PhD thesis was to determine the timeline over which compensatory mechanisms to weight reduction are activated, on both sides of the EB equation, and to investigate if sex modulated these mechanisms. Figure 11 gives an overview of the main findings.

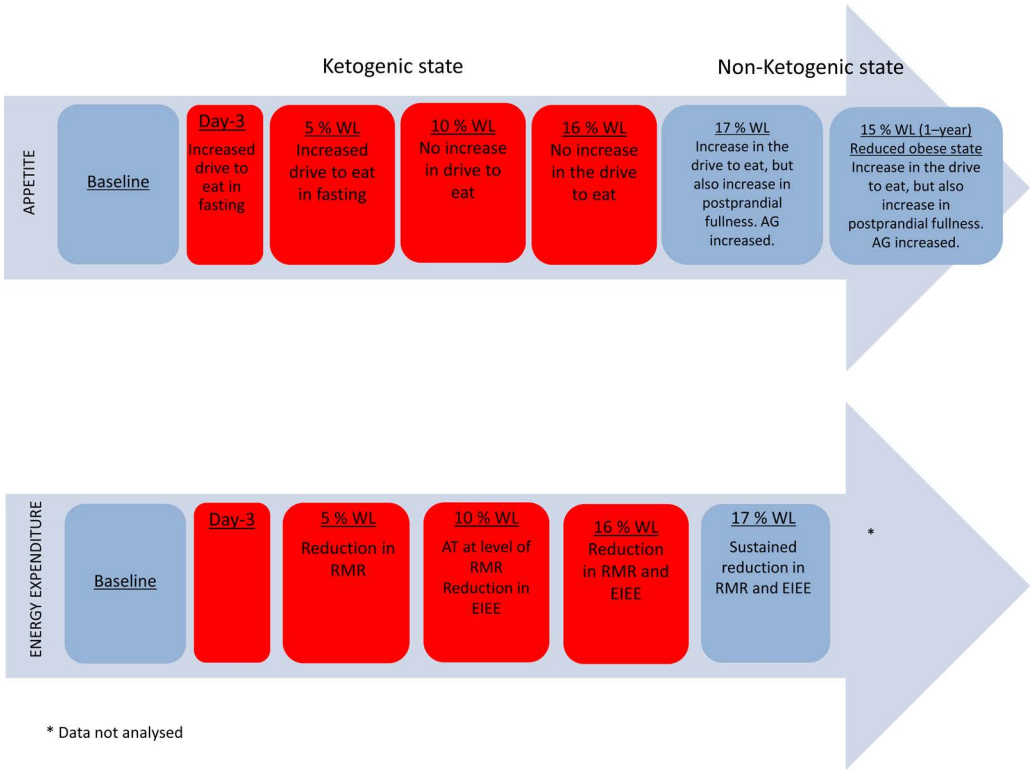

Figure 11. Overview of the main findings regarding the timeline of changes in appetite- and EE-variables with progressive weight loss. Blue colour: no ketoses. Red colour: in ketoses. WL: weight loss. AG: active ghrelin. RMR: Resting metabolic rate. AT: adaptive thermogenesis. EIEE: energy induced energy expenditure. © Nymo, S.

The drive to eat in fasting was increased up to 5 % WL (2-3 weeks) despite no change in AG concentrations while participants were ketotic. After that, and for a WL between 10-17 %, no increase in subjective feelings of hunger or AG were observed, despite a reduction in satiety peptides. With refeeding (17 % WL), there was an increase in hunger feelings in fasting and AG concentration, but also in postprandial fullness feelings. One year later with 15 %

sustained WL, hunger feelings in fasting and AG concentration were still increased. Postprandially fullness feelings were also increased, while the findings were inconsistent in the postprandial plasma concentration of satiety hormones (increased CCK and a reduction in PYY).

On the other side of the EB equation, with 5 % WL, there was a reduction in RMR, and at 10 % WL a reduction in EIEE at all levels of power was observed. Furthermore, transient AT at the level of RMR was found at 10 % WL. The reduction in both RMR and EIEE was sustained after refeeding with 16 % WL.

5.1 Changes in appetite with weight loss

Papers I and III describe the impact of WL on appetite. Paper I is the first to identify a timeline of changes in both subjective and objective appetite markers with progressive WL achieved with a ketogenic VLED, in an population with obesity. Paper III is the largest longitudinal study investigating the sustainability of changes in appetite with long-term WL maintenance, and the only study that investigates the potential impact of sex.

In paper I, fasting hunger feelings and desire to eat were increased up to 5 % WL (2-3 weeks) despite no change in AG concentration, while a 10-17 % WL was associated with no changes in subjective feelings of appetite (despite a reduction in satiety peptides). Refeeding (17 % maintained WL) was associated with increased hunger feelings and AG concentration. These results go partly against some of the other research that is highly cited in many other papers and discussed at conferences all around the world. The idea that appetite is supressed or not changed while under ketosis (54, 80, 92, 101-103, 105, 149) is becoming entrenched in the field of obesity management and among slimmer's in general. These ideas gained ground mainly from the results obtained by the landmark paper by Sumithran et al. (2013) (54) the systematic review and meta-analysis by Gibson et al. (2015) (51). However, as shown in paper I, it may take up to 3 weeks for the inhibitory effects of ketosis on appetite to be seen. The increase in appetite (subjective feelings of hunger and AG plasma concentration) seen with refeeding is in line with previous studies (54, 90).

In paper III, a 15% WL, both in the short-term and at *one year follow up,* was associated with

increased hunger feelings in fasting and basal AG concentrations, increased postprandial fullness feelings and reduced postprandial PFC feelings. At 1-year, postprandial concentrations of AG, CCK were increased, while PYY was reduced. Increased postprandial fullness ratings were seen at week 13 in this paper, differently from my first Paper, probably due to lack of power in the sub-study for subjective appetite ratings (n=31 vs. n=71, respectively for paper I and III). Study III is the largest to evaluate changes in both subjective and objective appetite markers with sustained WL.

Sumithran et al. (2011) (81), using a similar study design (but 2 instead of 4 refeeding weeks) report increased hunger and AG plasma concentration both with short-term and sustained WL at 1-year follow up. However, they did not report an increase in postprandial fullness. The inconsistencies between studies may be explained by differences in the magnitude of WL (14 vs. 18 kg) (81), baseline characteristics of the participants (BMI 34 vs. 37 kg/m^2), sample size (50 vs. 100 participants), energy load of the test meal (550 vs. 600 kcal) and WL maintenance (50 % regain vs. sustained WL maintenance). These factors may explain the differences between Sumithrans et al.s vs. our study. Given that a higher BMI has been found to be associated with decreased postprandial fullness (150), the larger WL and BMI reduction seen in paper III could be a potential explanation for the findings. The increase in postprandial fullness seen in paper III, both at week 13 and 1-year is unlikely to be explained by increased postprandial secretion of satiety peptides, as at week 13 CCK was reduced, while at 1-year it was increased, active GLP-1 did not change and total PYY was reduced. Even though the impact of BMI on gastric capacity has not been systematically studied, a reduction in fasting gastric capacity has been reported after 5 % WL in people with obesity (151). This could potentially explain why participants felt fuller after a 15 % WL. Unfortunately, neither, Iepsen et al. (2016) (82), nor Adam et al. (2006) (108), looked at changes in subjective feelings of appetite with sustained WL maintenance. Regarding satiety peptides, Sumithran et al. (2011) (81) did find a reduction in active GLP-1, no change in total PYY and a reduction in CCK , while Iepsen et al. (2016) (82) found an increase in both total GLP-1 and PYY_{3-36} and Adams et al. (2006) (108), did see a reduction in active GLP-1 both after WL and WL-maintenance. Thus, our results may be influenced by the hormone fraction analysed (152), and it remains to be elucidated which fraction of satiety peptides are best reflecting their actions (153, 154).

5.1.1 Sex differences on the impact of weight loss on appetite

Sex differences were identified regarding the impact of progressive WL (paper I) and WL-maintenance (paper II) on appetite. In my first paper, males, unlike females, reported no changes in hunger feelings throughout the study. This was surprising, since both sexes experienced an increase in AG concentration after the refeeding-phase (17 % WL). With short and long-term WL maintenance in my third paper, sex differences were seen both in the WL-phase (week 13) and in the WL maintenance-phase (1-year). Like in paper I, fasting ratings of hunger were only increased in females at week 13, but, on the other hand, only males experienced an increase in postprandial ratings of fullness and a reduction in PFC at this time-point. In the long-term (1-year), only females experienced increased fullness postprandially, while not experiencing any change in CCK concentration throughout the study, while males had an increase in CCK concentration at 1-year compared to baseline.

The increased hunger while participants were ketotic up to 3 weeks in the VLED was only seen in females (paper I). Moreover, increased hunger after the refeeding was also only seen in females, both in papers I and III.

It needs to be acknowledged, nevertheless, that with the VLED used in this study, males had a much larger energy deficit/day compared with females (660 vs. 550 kcal/day, respectively), relative to their energy requirements, which resulted in a larger overall absolute WL in males. However, if anything this would have led to more hunger in response to WL in males, not the opposite as seen in papers I and II.

It was also surprisingly that only females experienced increased postprandial fullness at 1-year follow-up, however this may reflect the fact that in males there was a trend towards weight regain during the maintenance phase, while females continued to lose weight over time. Another explanation, could be that the same standard breakfast was served throughout the study independently of baseline body weight, therefore females did consume a relatively higher energy load than males. The standard breakfast, also representing a larger energy load with progressive WL in females, which could have had an impact of postprandial fullness ratings. Anyway, females have been found to feel fuller than males after a meal (132).

A review article by Hintze et al. (2017) concluded that sex did not play a major role in variations of appetite in the post obese state. However, it needs to be mentioned that the review included different WL interventions (diets, exercise, drugs and combined interventions) (155).

5.2 Changes in energy expenditure with weight loss

The study presented in paper II is the first to identify the timeline of physiological adaptations at the level of EE in an obese population. These adaptations can make progressive WL harder, and may contribute to relapse during and after diet interventions are completed.

A reduction in RMR and FM was first seen at 5 % WL (2-3 weeks), and in EIEE at 10 % WL (4-5 weeks) for all levels of power and these changes were sustained with refeeding. AT_{RMR} was transiently present at 10 % WL only.

The reduction in RMR at 5 % WL is in line with previous studies (15, 76, 109). No further reduction in RMR was seen after 5 % WL. Even after refeeding no change was seen, which has also been reported by other studies (15, 129). This indicates that a larger weight reduction up to 16 %, which may be needed to induce health benefits in patients with severe obesity (156), will not necessarily lead to a further decline in RMR . This findings is supported by another study where a reduction in RMR was seen after 10 days of a diet intervention (VLED), and thereafter stabilized despite further WL (113).

A systematic review by Schwartz et al. (2010), showed that there was a greater fall in RMR in studies with a short rather than longer duration (≤ vs.> 6 weeks) (109), which is in line with the findings from paper II. Ballor et al. (1996) found a significant reduction in RMR with 10 % WL, both absolute and adjusted values (76). Contrarily, Leibel et al. (1995) did not report a reduction in RMR at 10 % WL, but with 20 % WL a reduction in RMR adjusted for FFM was seen (15). Inconsistent findings may be caused by diverse ways of presenting data, some with absolute values, others adjusted by FFM, or both FFM and FM.

There was a reduction in EIEE after 10 % WL and this remained lower than baseline throughout the study, which is in line with most of the previous evidence (74-76), except from one study where no change was seen after a 19 % diet induced WL and 2 weeks of refeeding

(121). Similarly, Rosenbaum et al. (2003) did see a decrease in muscle efficiency, also in a mixed sample of men and women with 10 % WL, induced with a VLED intervention (75). However, Amati et al. (2008) did not find any change in gross EIEE (at an average power 38±2 W) with a 19 % WL induced by diet alone, followed by 2 weeks of weight stabilisation (121).

Regarding AT, our study revealed AT_{RMR} only at 10 % WL in all participants. Several other studies have reported AT_{RMR} after a 10% WL (125, 127, 129, 157). Moreover, Rosenbaum and Leibel (2016) did not find AT_{RMR} with a 20 % WL (157). Contrary to paper II, however, they observed AT at the level of NREE at 10 and 20 % WL (157). This was also confirmed by Goldsmith et al. (2010) who found an increase in ExEff greater than what could be accounted for by reduction in body weight (10 % WL) and composition (74). Camps et al. (2013) found sustained AT_{RMR} both after 12 weeks and at 1-year after an initial 9 % WL (129).

The transient AT_{RMR} may slow down WL rate at this specific timepoint. It is possible that different results for AT have been obtained with different protocols (cycling vs. treadmill), as treadmill protocols also were different both with (131) and without (130) elevation, different workloads (powers and speed) and also according to how the results are reported (net (15) or gross). In a review by Astrup et al. (1999), a 3-5 % lower RMR was reported in post-obese compared with never-obese, even after adjusting for differences in body weight and composition (158).

5.2.1 Impact of sex on changes in energy expenditure during weight loss

Regarding sex differences, changes in EE with progressive WL were less pronounced in females than in males. Compared to baseline, females did not experience a significant reduction in either absolute or adjusted RMR after 16 % WL and out of ketosis, opposite males. Similarly, an increase in EIEE at 50W was not seen in females after 16 % WL and out of ketosis, opposite males. Moreover, AT_{RMR} or AT_{EIEE} was never seen in females, while in males AT_{RMR} was present both at 10 % WL and week 9.

The no reduction in RMR in females after refeeding is in line with Doucet et al. (2000), who reported a reduction in RMR in males only after 10 % WL (159). Contrarily, a systematic review by Schwartz et al. (2010) on diet induced WL reported an equal reduction in RMR in both sexes (109). Differences in changes of body composition between sexes during WL could have contributed to these differences in compensatory responses at the level of EE between sexes. This is supported by a study showing that the proportion of FFM lost during WL is larger in males (134).

The reduction in EIEE at 50 W was not seen in females after refeeding and out of ketoses. Another study in females only, did show a reduction in EIEE only after refeeding, it should be elucidated that different from use they used a treadmill protocol (3.2 km/h and 10 % elevation) (122).

The potential modulating effect of sex on AT remains controversial. Doucet et al. (2001) reported AT_{RMR} only in males after 5 % WL with a LED (127). Camps et al. (2013), on the other hand, found no sex differences in AT_{RMR} induced by a VLED intervention and 10 % initial WL while in ketoses, but also with refeeding and out of ketoses (9 % WL) and after partial weight regain (7 % WL) (129). The mechanisms behind sex differences in AT are speculative (135), but leptin may be involved (160). AT was found to be positively correlated with the reduction in leptin seen with WL (160). Given that males have a lower plasma concentration of leptin, compared with females (161), WL may lead to a reduction in leptin below a certain threshold in males, and therefore potentially explain why AT may be more pronounced in males compared with females.

5.3 Methodological considerations

5.3.1 Body weight and composition

The WL seen during the first days on a ketogenic diet is known to be partly accounted for by loss of body water since glycogen is stored with three times its own weight in water (162, 163). As such, and due to the fact that a 2 compartment model, which does not account for changes in total body water (TBW), was used in the studies reported in this thesis, part of the reduction in FFM seen with WL and part of the increase in FFM seen with refeeding were

due to changes in TBW. This means that our estimation of AT may be biased because ADP does not take into account FFM.

However, ADP is, in general, a reliable and valid technique that can evaluate body composition in a quick and safe way (141). It is very user-friendly, which is important in large clinical intervention studies. Another limitation of the method is that it does not adjusted for air in the gut. The interpretation of changes in body composition with WL is challenging, differences in composition of reduction in FFM (bone mass and organ mass) could have an impact on the outcome of compensatory metabolic changes in RMR as seen in the review by Dulloo et al. (2012) (164).

5.3.2 Appetite markers

The fraction of hormones measured, and methods used to quantify them in the plasma vary among studies and may explain some of the inconsistencies seen.

There are two different fractions of ghrelin; acetylated (or active) and des-acylated (or inactive) (165). In studies I and III, AG was measured, because it has been shown that this is the fraction that has a physiological role on appetite and the ability to cross the blood brain barrier and act at the level of the hypothalamus (166). It has been suggested that AG stimulates food intake independently of des-acylated ghrelin (167). However, both fractions of ghrelin seem to be physiologically relevant, and should be analysed to detect mechanisms behind the blunted effect of ghrelin during WL with future research (168). WL has been shown to increase both active (Sumithran et al. 2011) and total ghrelin (Iepsen et al. 2016).

Iepsen et al. (2016) (82) and Verdich et al. (2001) (83) reported an increase in total GLP-1 postprandially with WL. On the other hand, Sumithran et al. (2011) (81), which measured active GLP-1 did not find any changes with WL, which is in line with the results of study I and III. Differences between studies may be due to differences in hormone fractions and methods of analysis. The accurate measurement of GLP-1 is challenging since plasma concentration of active GLP-1 is low, and this is further degraded with splitting into several fractions (total GLP-1), making comparisons between studies difficult (154). Also, various

methods of GLP-1 analysis have been compered, showing different results, suggesting again that comparisons between studies should be done with caution (153).

The multiplex assay used to analyse AG, active GLP-1 , total PYY and insulin is likely to result in less accurate and precise measurements compared with optimized assays for each individual hormone, since they have pronounced different sensitivity, specificity and precision (152). The in-house RIA assay used to measure CCK plasma concentrations was found to be reliable and have a good detection limit, specificity, between- and within-assay reproducibility, and accuracy (144).

Regarding subjective feelings of appetite, VAS are considered the best method when looking at subjective ratings of appetite (143, 169).

5.3.3 Energy expenditure

Indirect calorimetry was used to measure both RMR and EIEE. The measurements of RMR was done by the following standardised procedures, therefore, the participants were asked to fast over night (12 hours), not to drink caffeine for at least 6 hours, be nicotine abstinent over the last 2 hours and not perform moderate intensity PA for 2 hours before test (139). The Vmax Encore 29N is one of the most valid (with in subjects' CV < 10 %) gas analysing systems for the measurement of RMR (170).

5.4 Strengths and limitations

The studies, which are part of this thesis, contain several strengths. First, its design is longitudinal with repeated measurements. The assessment time-points chosen allowed to evaluate the impact of minimal, but significant WL at day-3, 5-10 % WL, known to have significant health benefits (39, 156), a larger WL 15-17 %, acutely under ketosis and after a period of refeeding and weight stabilisation, and long-term WL maintenance (1-year) on compensatory responses to weight reduction. Second, compliance with the 8-week VLED was excellent and controlled by objective measurements (weighing, urine AcAc and PA levels). The dropouts during the WL maintenance phase are in line with other similar long-term WL studies (171). The fact that body weight was kept stable during refeeding (weeks 9

and 13) and follow-up (1-year) means that conclusions can be made regarding the impact of refeeding and the long-term WL on appetite, without the potential confounding effect of weight fluctuations. Third, several outcome variables were assessed, both at the level of appetite and energy expenditure, allowing a broader overview of the timeline over which compensatory responses to weight reduction are activated. Moreover, both subjective and objective measurements of appetite were taken, in the fasting state and after a standardized breakfast. Fourth, adjustment for multiple testing was performed by using Bonferroni adjustments for multiple time comparisons, and the Benjamini-Hochberg method (false discovery rate) (148) for multiple outcome variables. Last, but not least, given that no significant changes in PA levels were seen during the whole study, the changes in both appetite, RMR and EIEE with progressive WL are likely to be a true reflection of WL.

Unfortunately, the studies that are part of this thesis also suffer some limitations. First, we did not have a control group and it is possible that appetite and EE variables are affected by seasonality, habituation, etc. Second, the studies were not powered to look at sex differences and therefore, the results regarding potential sex effects need to be interpreted with caution. Third, it is possible that WL could have altered central sensitivity to the hormones assessed in this thesis, which was not taken into consideration. Fourth, the multiplex assay used for measurements of appetite related hormones (except for CCK) may be less sensitive compared to optimized assays for each individual hormone. Finally, the test meal was not adjusted according to energy requirements (sex and changes over time due to a lower body weight). This could have affected the sex differences found. However, adjustments would mean a lower nutritional stimulus in females and over time which would result in a blunted secretion of satiety hormones, independently of the real effect of sex differences and WL.

5.5 Generalizability

The results from paper I and II cannot be generalized to other WL-strategies since the WL intervention was a ketogenic VLED. The results of Paper III, looking at the long-term sustainability of changes in appetite with WL maintenance, may be generalizable to all diet-WL interventions, given that week 13 measures were taken outside of ketosis. Moreover, the

large BMI range (30 > BMI < 50 kg/m^2) of the participants allows the results to be generalised to the whole obese population.

5.6 Practical implications

Knowledge regarding the timeline of changes in appetite and EE with progressive WL with a ketogenic VLED is of important practical relevance. Both patients and clinicians need to be aware that it may take up to 3 weeks before appetite is supressed with a VLED. It is also necessary to be aware that hunger feelings are expected to increase with refeeding, both compared to baseline and to while being in ketoses. This means that patients may need extra follow-up and support during these phases. Moreover, they need to be aware that hunger feelings will still be increased after 1-year, even though postprandial fullness seems to be also increased. This knowledge about when to intervene, and when the adaptation is stronger, makes clinicians able to prepare patients to meet these challenging phases.

A WL of between 5-16 % will not always translate into further significant decrease in RMR. This is of immense importance for clinicians when tailoring dietary prescriptions for progressive WL or WL maintenance. After 10 % WL or more, if aiming for progressive WL, a larger reduction in EI and/or increase in PAL may be necessary to counteract the reduction in EIEE seen at this time-point. Moreover, a reduction WL rate may be explained by the transient AT_{RMR} seen here, and not necessarily a result of non-compliance with the diet. This knowledge about when changes in energy requirements (both at rest and during exercise), occur with progressive WL may help clinicians and patients to understand the resistance to further WL and relapse after diet interventions, which can be useful to discuss with the patients for a better understanding alongside their expectations on their WL journey.

It is useful for patients and clinicians to know which phases of the WL intervention are the most challenging. This information may also be useful in influencing patients' adherence and expectations during WL and motivate for long-term WL maintenance.

5.7 Future research

Several aspects deserve further investigation. The sex differences found in the studies that are part of this thesis need to be further investigated in well-powered studies. Moreover, the dietary intervention used (VLED) was very restricted, both in terms of energy and carbohydrates. It would be interesting to identify the highest threshold of carbohydrate intake still associated with ketosis and appetite suppression. This would allow the design of more liberal ketogenic diets, more in line with the dietary recommendations (138).

The lack of association between the changes in appetite seen with WL and long-term relapse reported in study III go against the "compensatory theory" and may suggest that the changes in appetite seen with WL reflect a normalization towards a lower body weight. More research is needed to investigate this further.

6 Conclusion

With a ketogenic VLED the drive to eat is increased up to 2-3 weeks, even though participants are ketotic and AG is unchanged. After that is achieved and up to a 17 % WL under ketosis, appetite feelings remain at baseline levels. Refeeding results in increased hunger feelings and AG plasma concentration and this is sustained in the long term (1-year). This may, nevertheless, be balanced out by increased postprandial fullness feelings observed at these two last time points.

A fall in RMR should be anticipated at ≥5 % WL and a reduction in EIEE at ≥10 % WL. Transient AT_{RMR} can be expected at 10 % WL. All these physiological adaptations may make progressive WL difficult and need to be taken into consideration by both patients and clinicians.

Sex seems to modulate some of the adaptive physiological responses seen with progressive WL and long-term WL maintenance, but more studies, with enough power, are needed to clearly investigate this important aspect.

7 Epilogue

Weight loss maintenance following conservative approaches is possible. In the public health system and hospital wards WL management should aim at maintaining a healthy body weight to prevent comorbidity in the long term, for everyone. Today, all programs are supposed to fit all, but we know that there are many challenges that makes it difficult to fit into a WL regime. I believe that a VLED alone may be a valid option to induce a very large WL in a short period of time if a multidisciplinary WL maintenance program is in place afterwards.

During my work with my PhD, I have been lucky to present my findings at European and World conferences both in Prague; Czech Republic, Vancouver; Canada and Porto; Portugal and Vienna; Austria with oral and poster presentations. It has been a pleasure to share my findings at these scientific arenas. Additionally, sharing my study and its practical implications with the larger population, struggling with WL, such as the Trøndelag area through the regional broad casting NRK has been useful. National (NRK) TV also followed one of our participants during his WL journey in the television program “Schrödinger’s cat”.

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Paper I

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ORIGINAL ARTICLE

Timeline of changes in appetite during weight loss with a ketogenic diet

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BACKGROUND/OBJECTIVE: Diet-induced weight loss (WL) leads to increased hunger and reduced fullness feelings, increased ghrelin and reduced satiety peptides concentration (glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK) and peptide YY (PYY)). Ketogenic diets seem to minimise or supress some of these responses. The aim of this study was to determine the timeline over which changes in appetite occur during progressive WL with a ketogenic very-low-energy diet (VLED).
SUBJECTS/METHODS: Thirty-one sedentary adults (18 men), with obesity (body mass index: 37 ± 4.5 kg m^{-2}) underwent 8 weeks (wks) of a VLED followed by 4 wks of weight maintenance. Body weight and composition, subjective feelings of appetite and appetite-related hormones (insulin, active ghrelin (AG), active GLP-1, total PYY and CCK) were measured in fasting and postprandially, at baseline, on day 3 of the diet, 5 and 10% WL, and at wks 9 and 13. Data are shown as mean ± s.d.
RESULTS: A significant increase in fasting hunger was observed by day 3 (2 ± 1% WL), ($P < 0.01$), 5% WL (12 ± 8 days) ($P < 0.05$) and wk 13 (17 ± 2% WL) ($P < 0.05$). Increased desire to eat was observed by day 3 ($P < 0.01$) and 5% WL ($P < 0.05$). Postprandial prospective food consumption was significantly reduced at wk 9 (16 ± 2% WL) ($P < 0.01$). Basal total PYY was significantly reduced at 10% WL (32 ± 8 days) ($P < 0.05$). Postprandial active GLP-1 was increased at 5% WL ($P < 0.01$) and CCK reduced at 5 and 10% WL ($P < 0.01$, for both) and wk 9 ($P < 0.001$). Basal and postprandial AG were significantly increased at wk 13 ($P < 0.001$, both).
CONCLUSIONS: WL with a ketogenic VLED transiently increases the drive to eat up to 3 weeks (5% WL). After that, and while participants are ketotic, a 10–17% WL is not associated with increased appetite. However, hunger feelings and AG concentrations increase significantly from baseline, once refeeding occurs.

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INTRODUCTION

Obesity has reached epidemic proportions worldwide and is associated with negative public health consequences and large socioeconomic costs.[1] A weight loss (WL) between 5 and 10% of baseline weight, if sustained, can have large health benefits by preventing or improving several obesity-related risk factors and comorbidities.[2] Even though this can be achieved in the short-term with lifestyle interventions,[3] most adults will experience weight regain in the long term.[3–5]

Maintaining lost weight is physiologically challenging, as diet-induced WL is associated with compensatory responses on both sides of the energy balance equation.[6] These responses are driven by a cross talk between the gut and the brain, leading to an increase in appetite and reduction in satiety,[7–9] concomitant with an overall reduction in energy expenditure.[10–12] These mechanisms can reduce WL rate and increases the risk of relapse.[9] An increase in ghrelin plasma concentration[13–15] and a reduction in the concentration of several satiety hormones, such as peptide YY (PYY), glucagon-like peptide-1 (GLP-1)[13,16–18] and cholecystokinin (CCK)[13,19] has also been reported with diet-induced WL.

To our knowledge, no studies to date have determined how appetite is affected with progressive WL. With a WL of only 1–2 kg being shown to increase subjective feelings of hunger,[20] and a WL of 0.8 kg associated with an increase in postprandial total ghrelin and a reduction in subjective feelings of fullness,[21] it is not surprising that adults find WL maintenance extremely hard. However, when carbohydrates and/or energy (using a very-low-energy diet (VLED)) are restricted sufficiently to induce ketosis, the increase in appetite seen with WL appears to be absent.[12,22] This has contributed to the generalised idea that ketogenic diets are easy to follow. Most of the studies looking at the impact of WL induced with ketogenic diets on appetite have, nevertheless, been done in females[22] and none has investigated how long time does it take for appetite suppression to occur under ketogenic diets. Therefore, the primary aim of this study was to assess the timeline over which changes in both subjective feelings of appetite and appetite-related hormones are activated during WL with a ketogenic VLED. A secondary aim was to assess if males and females respond differently.

MATERIALS AND METHODS

Participants

Healthy adults with obesity ($30 <$ BMI < 45 kg m^{-2}) were recruited via newspaper advertising serving the community of Trondheim, Norway. The study was approved by the regional ethics committee (Ref., 2012/1901). The study was registered in ClinicalTrial.gov (NCT01834859), and conducted according to the guidelines laid down in the Declaration of Helsinki. All participants provided written informed consent before commencement.

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Participants were required to be weight stable (< 2 kg body weight change over the last 3 months), not currently dieting to lose weight, and with a sedentary lifestyle (not engaged in strenuous work or in regular brisk leisure time exercise more than once a week or in light exercise for more than 20 min per day in more than 3 times per week). Due to the known effect of phase of menstrual cycle on appetite[23] females had to be postmenopausal or taking hormonal contraceptives. Exclusion criteria were pregnancy, breastfeeding and clinically significant illness including diabetes, previous WL surgery and/or medication known to affect appetite/metabolism or induce WL.

Study design

This was a longitudinal intervention study with repeated measurements. Participants were provided with an 8-week ketogenic VLED, followed by 4 weeks of weight stabilisation, and were requested not to change their physical activity levels throughout the study (Supplementary Figure I).

Detailed protocol

Weight loss phase. Participants followed for 8 weeks a ketogenic VLED (Allevo, Karo Pharma AS, Sweden) with 550/660 kcal per day for females and males, respectively (macronutrient composition: carbohydrates 42%, protein 36%, fat 18% and fibre 4%). No energy fluids were allowed *ad libitum*. Intake of low-starch vegetables (max 100 g per day) was encouraged, to provide dietary fibre.

Weight stabilisation phase

At week 9, participants were gradually introduced to normal food, while reducing intake of the VLED products. An individual diet plan was prescribed by a trained dietician tailored to individual energy requirements (measured resting metabolic rate (RMR) × physical activity level (PAL) (extracted from individual physical activity monitors (BodyMedia, SenseWear, Pittsburgh, PA, USA) at week 8)), with 15–20% protein, 20–30% fat and 50–60% carbohydrates, aimed at weight stabilisation.[24]

Objective measures of compliance

Diet: Participants met every week for an individual 20 min consultation with a dietician, to review their food records. Urine acetoacetic acid concentration was also measured weekly, using Ketostix reagent strips. Participants who were not ketotic on more than one occasion were considered not compliant and were excluded from the analysis. Concentration of plasma β-hydroxybutyric acid (β-HB) in the fasting state was also measured with a Ketone Body Assay Kit (Mark134, Sigma-Aldrich, St Louis, MO, USA) at baseline, day 3, 5 and 10% WL and weeks 9 and 13.

Physical activity: Armbands were used for 7 days at baseline, weeks 4, 8 and 12. Data were considered valid if participants wore the device for ⩾4days, including at least 1 weekend day, on more than 95% (22.8 h per day) of the time.[25]

Data collection

The following measurements were performed in fasting at baseline, day 3 of the VLED, when each individual participant reached 5 and 10% WL, and at weeks 9 (the day immediately after the end of the VLED) and 13.

Body weight and composition. Air displacement plethysmography (BodPod, COSMED, Rome, Italy) was used.

Appetite measures. Subjective appetite feelings (hunger, fullness, desire to eat and prospective food consumption (PFC)) were measured using a validated 10 cm visual analogue scale[26] and blood samples were collected in fasting and every 30 min (0, 30, 60, 90, 120 and 150 min) after a standardised breakfast (600 kcal: 17% protein, 35% fat and 48% carbohydrates), for a period of 2.5 h. Plasma samples were analysed for active ghrelin (AG), total PYY, active GLP-1 and insulin using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore, St Louis, MO, USA) and CCK using an 'in-house'' RIA method[27] (intra- and inter-assay coefficient of variation were < 10% and < 20% for AG, GLP-1 and PYY; < 10% and < 15% for insulin and < 5% and < 15% for CCK, respectively).

Power calculation

Sample size estimation was based on expected changes (from baseline) in fasting AG plasma concentration overtime (day 3: 27; 5 and 10%: 0; week 9: 4 and week 13: 53 pmol l^{-1}). Having into consideration that no previous studies have been done in this area, we used data from Sumithran *et al.*[12] for weeks 9 and 13 and hypothesised that there would be an increase in AG on day 3 (approximately half of that seen on week 13) and no changes at 5 and 10% WL (given that participants would be under ketosis). For a SD of 89 pmol l^{-1},[12] a power of 80%, a significance level of 5% and assuming a low correlation between time points ($r = 0.3$), 32 participants would be necessary.

Statistical analysis

Statistical analysis was performed with SPSS version 22 (SPSS Inc., Chicago, IL, USA), and data presented as estimated marginal means ± s.e.m., with the exception of BMI, age, average time to achieve 5 and 10% WL and WL (%) at day 3, weeks 9 and 13, where means ± s.d. are given. Statistical significance was set at $P < 0.05$. Data were analysed using linear mixed-effects models, with restricted maximum likelihood estimation, including fixed effects for time and sex, and their interaction. Bonferroni correction was used for *post hoc* pairwise comparisons. The Benjamini—Hochberg method, which controls for the false discovery rate was used to adjust for the fact that we have looked at a large number of outcome variables.[28] Analyses of fasting and 2.5-h postprandial hormone profile for AG and subjective feelings of hunger were also carried out by linear mixed-effects models (fixed effects for sampling time points (0, 30, 60, 90, 120 and 150 min), time (baseline, day 3, 5 and 10% WL and wks 9 and 13), sex and interactions).

Weight at baseline was used as a covariate in the linear mixed-effects models when looking at changes in subjective and objective measures of appetite. Given that this did not change the significance of the results; the unadjusted values are presented. Total area under the curve for subjective feelings of appetite and appetite hormones was calculated from 0 to 150 min using the trapezoid rule. Participants with available data on at least three out of the six time points were considered completers.

RESULTS

Participants

Thirty-three participants met study entry criteria and 31 (13 females, 6 postmenopausal) were included in the analysis (one female withdrew due to personal reasons and one male due to not tolerating the VLED (verified incidence of vomiting, dizziness and fatigue)). Completers had an average BMI of 36.7 ± 4.5 kg m^{-2} and a mean age of 43 ± 10 years. Males were heavier and with greater FFM (kg) than females ($P < 0.01$ and $P < 0.001$, respectively), but there were no significant differences in age or BMI between sexes (Supplementary Table I).

Objective measures of compliance

Diet: Compliance with the VLED was excellent and no participant was excluded based on not being compliant. Participants were already ketotic at day 3 (0.60 ± 0.13mmol l^{-1} of β-HB), even though β-HB plasma concentrations were only significantly increased, compared with baseline, at 5% WL (12 ± 8 days) ($P < 0.001$ for all, males and females), and continued increased up to week 9 (16 ± 2% WL) ($P < 0.001$ for all, males and females) (Figure 1).

Physical activity: All participants were sedentary at baseline and there were no significant changes over time in any PA variable studied (Supplementary Table II).

Body weight and composition

A significant main effect of time, sex and interaction ($P < 0.001$, $P < 0.05$ and $P < 0.001$, respectively) was found for body weight. WL was already significant ($P < 0.001$) on day 3 in all participants and males (2 ± 1% WL for both) and body weight continued decreasing progressively, but with no significant differences between week 9 and 13 (16 ± 2% and 17 ± 2% WL, respectively) (Figure 2a). About 5% WL was achieved on day 12 ± 6 (11 ± 5 and

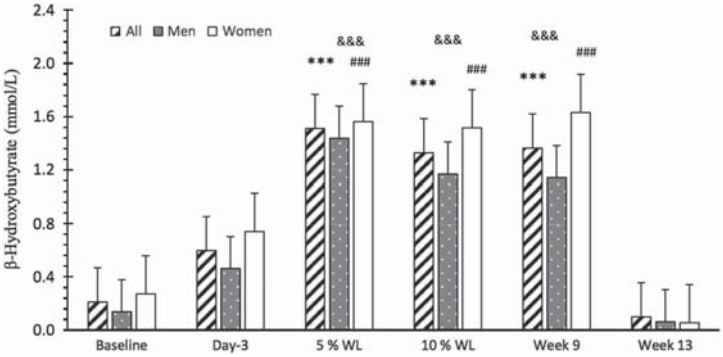

Figure 1. Basal plasma concentration of β-hydroxybutyric acid over time in all participants, males and females. Results presented as estimated marginal means ± s.e.m. A significant main effect of time was found for β-hydroxybutyrate ($P < 0.001$). Symbols denote significant differences from baseline in all participants $^{***}P < 0.001$, males $^{\&\&\&}P < 0.001$ and females $^{\#\#\#}P < 0.001$. WL, weight loss

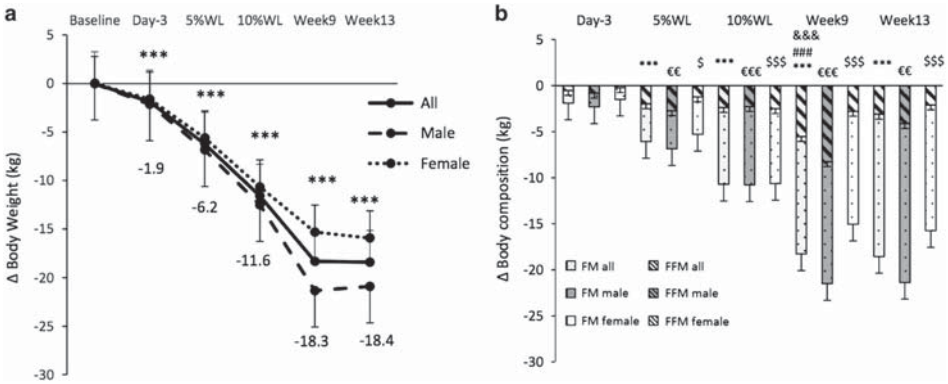

Figure 2. Changes in body weight (**a**) and body composition (**b**) over time in all participants, males and females. Results are presented as estimated marginal means ± s.e.m. Symbols denote significant changes from baseline (all participants: $^{***}P < 0.001$ for body weight and FM and $^{\#\#\#}P < 0.001$ for FFM; males: $^{\&\&\&}P < 0.001$ for FFM and $^{€€€}P < 0.001$, and $^{€€}P < 0.01$ for FM and females: $^{\$\$\$}P < 0.001$ and $^{\$}P < 0.05$ for FM). FFM, fat-free mass; FM, fat mass; WL, weight loss.

15 ± 7, for males and females, respectively) and 10% WL on day 32 ± 8 (28 ± 7 and 37 ± 6 for males and females, respectively). Overall WL (kg) was significantly larger in males compared with females ($P < 0.001$), and also at weeks 9 and 13 ($P < 0.001$ for both). When WL was expressed in % there were no overall significant sex differences ($P = 0.053$), but at week 9 ($P < 0.01$) males had a significantly larger WL than females.

FM (kg) decreased significantly for the first time after 5% WL (12 ± 8 days) ($P < 0.001$ for all participants, $P < 0.01$ for males and $P < 0.05$ for females) and was significantly lower than baseline at all the other subsequent time points (Figure 2b).

There was a significant decrease in FFM (kg) for the first time at week 9 (16 ± 2% WL), in all participants and in males (5.8 ± 1.0 kg and 8.3 ± 1.4 kg, respectively, $P < 0.001$ for both). FFM (kg) was never significantly lower than baseline in females (Figure 2b).

Appetite feelings

Fasting feelings of appetite in all participants and by sex are reported in Figure 3.

A significant main effect of time, but no main effect of sex or interaction, was found for hunger and desire to eat in fasting ($P < 0.01$, for both). Feelings of hunger in fasting were significantly increased, compared to baseline, at day 3 (2 ± 1% WL) ($P < 0.01$), after 5% WL (12 ± 8 days) ($P < 0.05$) and at week 13 (17 ± 2% WL) ($P < 0.05$) in all participants. In women, fasting hunger was significantly increased on day 3 and week 13 ($P < 0.01$ and $P < 0.05$, respectively). In men, no significant changes were seen at any time point. Feelings of desire to eat were significantly increased at day 3 and after 5% WL in all participants ($P < 0.01$ and $P < 0.05$, respectively) and males ($P < 0.05$ for both). For feelings of PFC in fasting, there was a significant main effect of time only ($P < 0.05$). However, no significant differences were found between baseline and any other time point. Men reported greater feelings of PFC in fasting overall (6.8 ± 0.4 vs 5.5 ± 0.4 cm, $P = 0.05$). No significant main effect of time, sex or interaction was found for feelings of fullness in fasting.

AUC for subjective feelings of appetite overtime is reported in Figure 4. A significant main effect of sex, but no main effect of time or interaction was found for hunger AUC ($P < 0.01$). A significant main effect of time and sex was found for fullness AUC ($P < 0.01$ and $P < 0.001$, respectively), desire to eat ($P < 0.05$ for both) and PFC ($P < 0.001$ and $P < 0.01$, respectively). No significant differences were found between baseline and any other time point for hunger AUC, desire to eat or fullness. PFC AUC was significantly lower at week 9 (16 ± 2% WL), compared with baseline in all participants and females ($P < 0.01$ and $P < 0.05$, respectively). Males had significantly

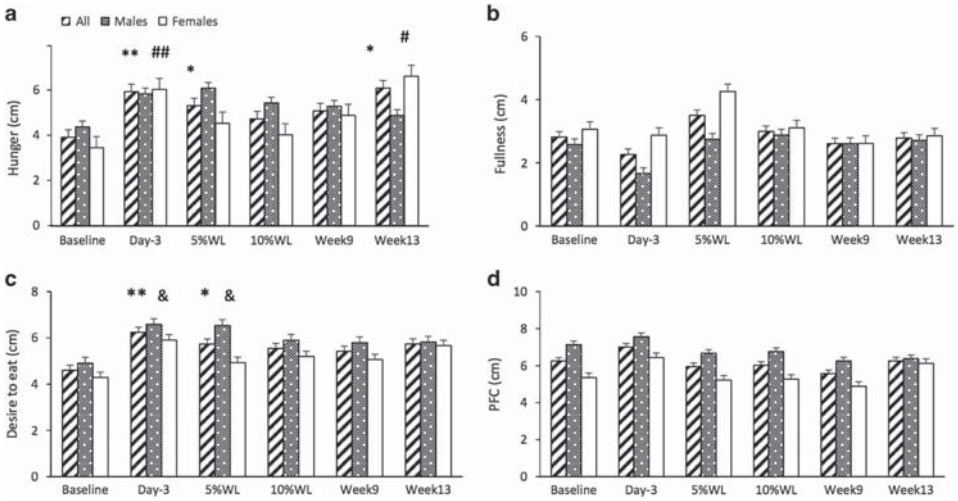

Figure 3. Subjective feelings of appetite (**a**: hunger, **b**: fullness, **c**: desire to eat and **d**: PFC) in fasting, over time, in all participants, males and females. Results presented as estimated marginal means ± s.e.m. Symbols denote significant differences from baseline in all participants: $^{**}P < 0.01$ and $^{*}P < 0.05$, males: $^{\&}P < 0.05$ and females: $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$. PFC, prospective food consumption; WL, weight loss.

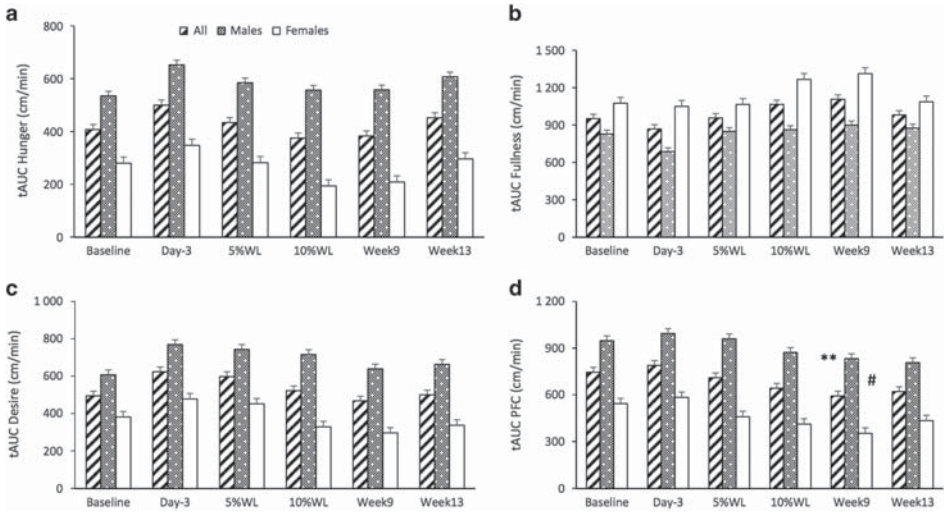

Figure 4. AUC for subjective feelings of appetite (**a**: hunger, **b**: fullness, **c**: desire to eat and **d**: PFC) over time in all participants, males and females. Results presented as estimated marginal means ± s.e.m. Symbols denote significant differences from baseline in all participants: $^{**}P < 0.01$ and females: $^{\#}P < 0.05$. AUC, total area under the curve; PFC, prospective food consumption; WL, weight loss.

higher overall hunger AUC (582.2 ± 52.4 vs 267.18 ± 61.8 cm min^{-1}, respectively), PFC AUC (904.1 ± 80.9 vs 459.8 ± 95.2 cm min^{-1}, respectively) and desire to eat (689.1 ± 72.1 vs 380.1 ± 84.8 cm min^{-1}, respectively), compared to females. Females had significantly higher fullness AUC (832.6 ± 35.9 vs 1149.7 ± 42.3 cm min^{-1}, respectively), compared to males.

Fasting and 2.5-h postprandial hunger feelings can be seen in Supplementary Figure II.

Appetite regulating hormones

Basal plasma concentration of appetite-related hormones over-time is reported in Figure 5. There was a significant main effect of time, sex and interaction ($P < 0.001$, $P < 0.01$, $P < 0.05$, respectively) for basal AG. There was a significant increase in basal AG compared to baseline only on week 13 (16 ± 2% WL) in all participants, females ($P < 0.001$ for both) and males ($P < 0.05$). Basal AG concentration was significantly lower overall in males compared with females (69.2 ± 8.3 vs 110.3 ± 10.2 pmol l^{-1}, respectively). No significant main effects of time, sex or interaction were found for basal active GLP-1 or CCK. A significant main effect of time ($P < 0.05$), but no main effect of sex or interaction was found for basal total PYY. A significant decrease in basal active GLP-1 was seen at 10% WL (32 ± 8 days) and at week 9 (16% WL), compared with baseline, in males only ($P < 0.05$ for both).

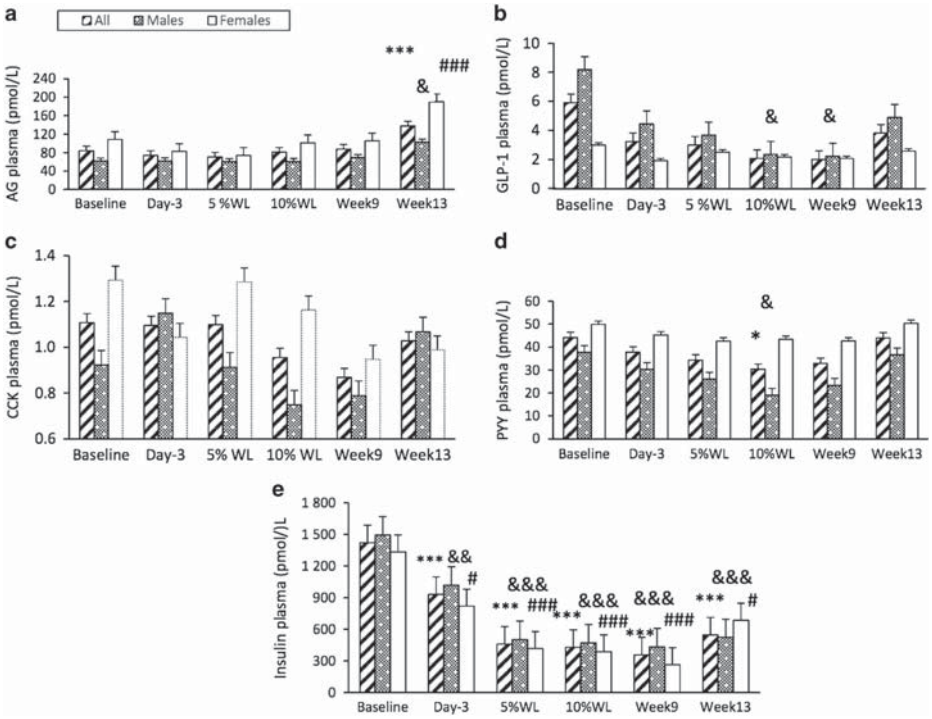

Figure 5. Basal plasma concentrations of appetite-related hormones (**a**: active ghrelin (AG), **b**: GLP-1, **c**: CCK, **d**: PYY and **e**: insulin) over time in all participants, males and females. Results presented as estimated marginal means ± s.e.m. Symbols denote significant differences from baseline in all participants: $^{***}P < 0.001$ and $^{*}P < 0.05$, males: $^{\&\&\&}P < 0.001$, $^{\&\&}P < 0.01$ and $^{\&}P < 0.05$, and females: $^{\#\#\#}P < 0.001$ and $^{\#}P < 0.05$. CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PYY, total peptide YY; WL, weight loss.

A significant reduction in basal PYY was present only after 10% WL (32 ± 8 days), when compared with baseline, in all participants and males ($P < 0.05$ for both). A significant main effect of time ($P < 0.001$), but no effect of sex or interaction was found for basal insulin. There was a significant reduction in basal insulin, compared to baseline, for the first time at day 3, in all participants, males and females ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively), which persisted thereafter.

AUC for appetite-related hormones overtime is reported in Figure 6. A significant main effect of time was found for AG AUC ($P < 0.001$). AG AUC was significantly increased at week 13 (17 ± 2% WL) in all participants, males and females ($P < 0.001$ for all participants and $P < 0.05$ for males and females). A significant main effect of time, but no sex or interaction, was seen for active GLP-1, insulin and CCK AUC ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively). Active GLP-1 AUC was only significantly increased after 5% WL (12 ± 8 days) in all participants ($P < 0.01$). In females, GLP-1 AUC was significantly higher than baseline at 5% and week 9 (16 ± 2% WL) ($P < 0.05$, for both). CCK AUC was significantly reduced at 5 and 10% WL (32 ± 8 days) ($P < 0.01$ for both), and week 9 ($P < 0.001$) in all participants and 5% WL and week 9 in males ($P < 0.01$ for both). A significant main effect of time ($P < 0.05$), but no main effect of sex or interaction, was found for PYY AUC. There was only a significant increase between baseline and week 9 ($P < 0.05$) in females. Insulin AUC was significantly reduced at all time points except day 3 in all participants and males, ($P < 0.001$ for all) and in females at 5 and 10% WL and week 9 ($P < 0.05$ for all).

Fasting and 2.5-h postprandial AG concentrations can be seen in Supplementary Figure III.

DISCUSSION

In this novel study investigating progressive changes in appetite in adults during a ketogenic WL diet, both subjective feelings of hunger and desire to eat were significantly increased at day 3 (2 ± 1% WL), but were not accompanied by significant changes in physiological appetite hormones except for insulin. This occurred despite participants being already ketotic (β-HB plasma concentration 0.60 ± 0.13 mmol l^{-1}). Previous studies looking at the impact of an acute period of energy restriction on appetite in people with normal weight and overweight (ranging from 1 to 4 days; between 60 and 85% energy restriction and inducing a WL between 0.8 and 2.4 kg) report an increase in feelings of hunger (desire to eat and PFC), both in the fasting and postprandial state.[29–32] Interestingly, these were not accompanied by changes in either AG,[29] total ghrelin[31,32] or GLP-1,[29] which is in line with our findings. However, the study from Pasiakos *et al.*[21] showed a significant increase in postprandial total ghrelin after 2 days on a 321 kcal diet in individuals with normal weight (12 males and 1 female). The different hormone fraction, lower BMI, lower energy intake and different sex distribution compared to our study probably accounts for some of these differences. However, in our study sex responses were apparent. Males (which would be more easily comparable with Pasiakos and colleagues study[33]), displayed no changes in AG.[21] Unfortunately, none of the previously mentioned studies measured ketosis during WL.

After 5% WL (12 ± 8 days), feelings of hunger and desire to eat in fasting remained elevated, GLP-1 AUC had increased significantly and CCK AUC was significantly reduced. After 10% WL (32 ± 8 days), no significant changes in subjective feelings of

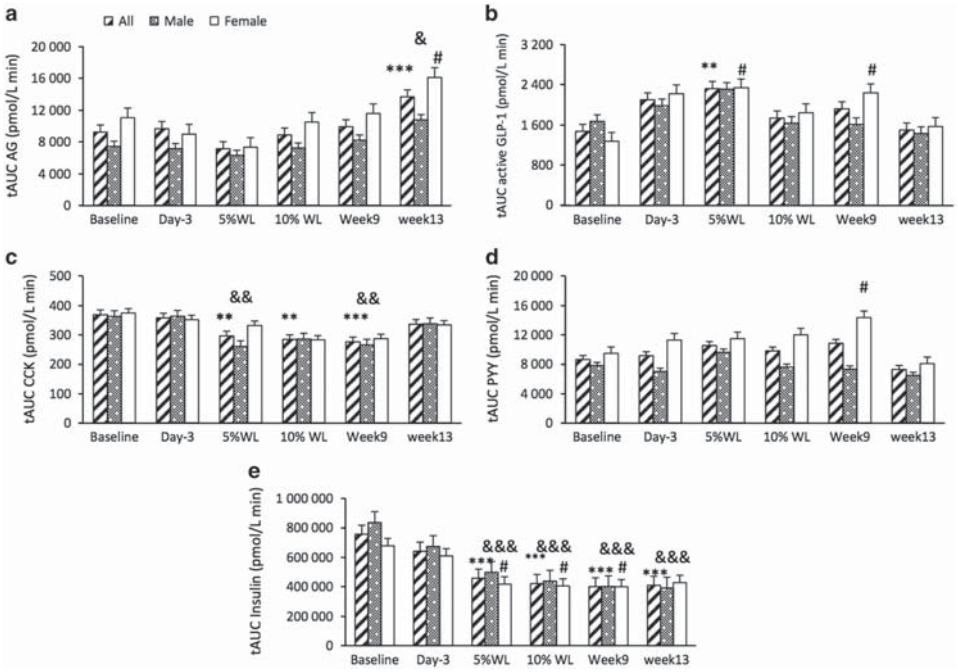

Figure 6. AUC for appetite-related hormones (**a**: active ghrelin (AG), **b**: GLP-1, **c**: CCK, **d**: PYY and **e**: insulin) over time in all participants, males and females. Results presented as estimated marginal means ± s.e.m. Symbols denote significant differences from baseline in all participants: $^{***}P < 0.001$, $^{**}P < 0.01$, males: $^{\&\&\&}P < 0.001$, $^{\&\&}P < 0.01$ and $^{\&}P < 0.05$ and females: $^{\#}P < 0.05$. CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PYY, total peptide YY; tAUC, total area under the curve; WL, weight loss.

appetite were reported, while basal GLP-1 (in males only), basal PYY (in all and males) and CCK AUC (in all) were significantly reduced. Previous studies using ketogenic diets and inducing a similar WL (6–8%) have systematically shown no change in hunger feelings.[18,34–39] Even though some of these studies report a significant increase in fullness feelings (both fasting and postprandial),[36,38] others report no changes in feelings of fullness in the fasting state after WL.[34,35,37]

A study by Adams *et al.*,[16] where participants with obesity (males and females) underwent 6 weeks of a VLED (7% WL), reported a reduction in hunger at 90 and 120 min, an increase in fullness at 120 min postprandial, as well as a decrease in postprandial total GLP-1 (but no changes in fasting). Diepvens *et al.*[39], where females with overweight experienced an 11% WL with a VLED, reported a significant reduction in basal concentration of AG and no changes in CCK or GLP-1 concentration (either fasting or postprandial). Similar to us, Soenen *et al.*[18] reported a significant reduction in basal concentration of PYY after a 6% WL (4 weeks VLED).

A recent meta-analysis by Gibson *et al.* reported that losing weight with a ketogenic diet is associated with a reduction in hunger and increase in fullness feelings. All but one of the studies included in the analyses had average β-HB plasma concentration around 0.50 mmol l^{-1}.[22] It is surprising that hunger feelings in fasting in our study were increased on day 3 and 5% WL, but not at 10% WL, even though β-HB concentration did not differ from 5% WL. The reasons for this mismatch between β-HB concentration and subjective appetite feelings are unknown. However, it can be that participants got used to feeling hungry and that this perception therefore was attenuated overtime. Moreover, a recent review has suggested that other factors such as free fatty acids, reactive oxygen species and microbiota may also play a role.[40]

At week 9, with a 16 ± 2% WL and ketosis, no changes in subjective feelings of appetite were seen, with the exception of PFC AUC, which was reduced compared to baseline in all participants and females. Basal active GLP-1 was significantly reduced in males and GLP-1 AUC was increased in females, while CCK AUC was reduced in all participants and males. Only a few studies have looked at the impact of a WL > 10% under ketosis on appetite.[12] In the systematic review and meta-analysis from Gibson *et al.*[22], a significant increase in fullness, and decrease in hunger feelings were reported while ketotic, with a WL ranging from 5 to 12.5 kg. After 4 weeks of weight stabilisation and without ketosis, the only significant changes observed were increased hunger feelings in fasting (in all participants and females) and increased basal and postprandial concentration of AG.

Sumithran *et al.*[12], in a similar WL intervention, reported no changes in subjective feelings of appetite and a significant decrease in basal active GLP-1, but different from our study a significant decrease in basal PYY and CCK, and a significant decrease in postprandial concentration of PYY at week 9 under ketosis. After 2 weeks of weight stabilisation and no ketosis, appetite feelings were increased. Both basal and postprandial concentration of AG were also increased, which is in line with our findings. Opposite to our findings, both basal active GLP-1 and CCK plasma concentration, and postprandial PYY and CCK concentration were reduced.[10] This discrepancy may be due to differences in the duration of the weight stabilisation period and/or lack of power. It is possible that more than 2 weeks of weight stabilisation are needed for the concentration of satiety hormones

to normalise. Another study by Chearskul *et al.*[19] with an 8-week VLED (429 kcal per day) in 12 males with obesity who experienced a 15% WL, did not report any changes in hunger or satiety feelings, or CCK concentration, either fasting or postprandial, immediately after the intervention, while in ketosis. However, 1 week later and without ketosis, a significant reduction in postprandial CCK was described (opposite to our study where no changes were seen). Again, differences in the duration of the stabilisation phase might have impacted on the results. In a recently published study, Iepsen *et al.*[41] reported a significant increase in fasting and postprandial total ghrelin, and PYY_{3-36} and postprandial concentration of total GLP-1 after a 17% WL induced with an 810 kcal per day diet over 8 weeks when participants were out of ketosis. Differences in the hormones' fractions measured may account for some of the differences.

The increased hunger feelings and AG plasma concentrations seen after refeeding (week 13) in the present study may have important implications regarding WL outcomes. A recent study has reported that the increase in appetite seen with WL was three times larger than the corresponding reduction in total energy expenditure, highlighting the important role the feedback control of energy intake may have on long-term maintenance of a reduced body weight.[33]

Throughout the study period, a mismatch was apparent between subjective appetite feelings and anticipated relevant appetite-related hormones. Among others, there was an increase in hunger on day 3 ($2 \pm 1\%$ WL), despite no changes in appetite hormones except for insulin, and 5% WL, despite an increase in GLP-1 AUC and a reduction in CCK. Even though this mismatch can derive from lack of power, it is well known that the appetite control system is extremely complex and subjective feelings of appetite are not always correlated with the concentration of appetite-related hormones.[42] Pre-lunch plasma concentrations of total ghrelin and PYY were also found not to be associated with lunch energy intake in healthy women.[43] Moreover, a recent review by Holt *et al.*[44] concluded that self-reported appetite ratings of appetite cannot reliably predict subsequent energy intake.

As previously described, some sex differences were noted in both subjective feelings of appetite and appetite hormones with progressive WL. The fact that males, as opposed to females, reported no increase in hunger throughout the study (despite increased AG at week 13 ($16 \pm 2\%$ WL)) is surprising. Females were found to have a more sensitive neuronal response to food-related visual cues, which can contribute to the sex differences reported in the present study. Moreover, in our study, females had overall higher fullness AUC, and overall higher basal and AUC for AG, while males had overall higher hunger, desire to eat and PFC AUC. This is in line with previous findings showing that females have a higher satiating response to meals[45] and lower ratings of hunger and PFC.[46] It needs to be acknowledged, nevertheless, that with the VLED used in this study, males had a much larger energy deficit per day compared with females, which resulted in a larger overall absolute WL, and this might have contributed to the sex differences reported.

A strength of this study is its design; the fact that multiple measurements were undertaken during progressive WL under ketosis, and after a period of weight stabilisation and no ketosis. This is important, given that ketosis is thought to modulate appetite.[12,22] Moreover, subjective and objective aspects of appetite were assessed, both in fasting and after a meal. Compliance with the intervention was monitored throughout and was excellent. The choice of time points is also a strength, given that it allowed to evaluate the impact of minimal, but significant WL (achieved at day 3), 5 and 10% WL, considered necessary to achieved health benefits,[2] and large WL (16–17%) under ketosis vs no ketosis, on appetite outcomes. It is also a strength that we have adjusted for the increased risk of finding statistically significant differences by chance alone (by using Bonferroni adjustment for multiple time comparisons and the Benjamini—Hochberg method for multiple outcome variables).[28] A limitation of this study is that it may be underpowered to examine sex differences.

This study has important practical implications for patients, clinicians and researchers alike. Opposite to previous reports that the drive to eat is suppressed while under a ketogenic diet,[19,22] we have shown a transitory increase in hunger from baseline up to 3 weeks on a ketogenic VLED, which then disappear and only come back after refeeding (and no ketosis). This information is of importance because it can influence patients' expectations and adherence to VLEDs.

CONCLUSIONS

Progressive WL with a ketogenic VLED induces a transient increase in fasting hunger feelings up to 5% WL (3 weeks), despite no changes in AG and an increase in active GLP-1 AUC. A WL between 10 and 17% under ketosis is not associated with increased appetite feelings or AG plasma concentration, despite reduced fasting concentration of total PYY and CCK AUC. Increased hunger and AG plasma concentration return with refeeding (no ketosis) and weight stabilisation. Sex seems to modulate some of the changes in appetite seen with WL, however, larger studies powered to detect sex differences are required to confirm these findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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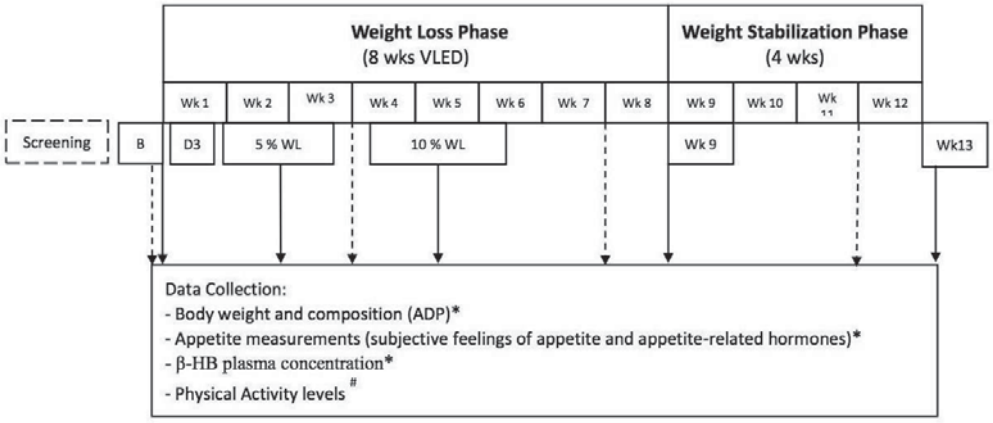
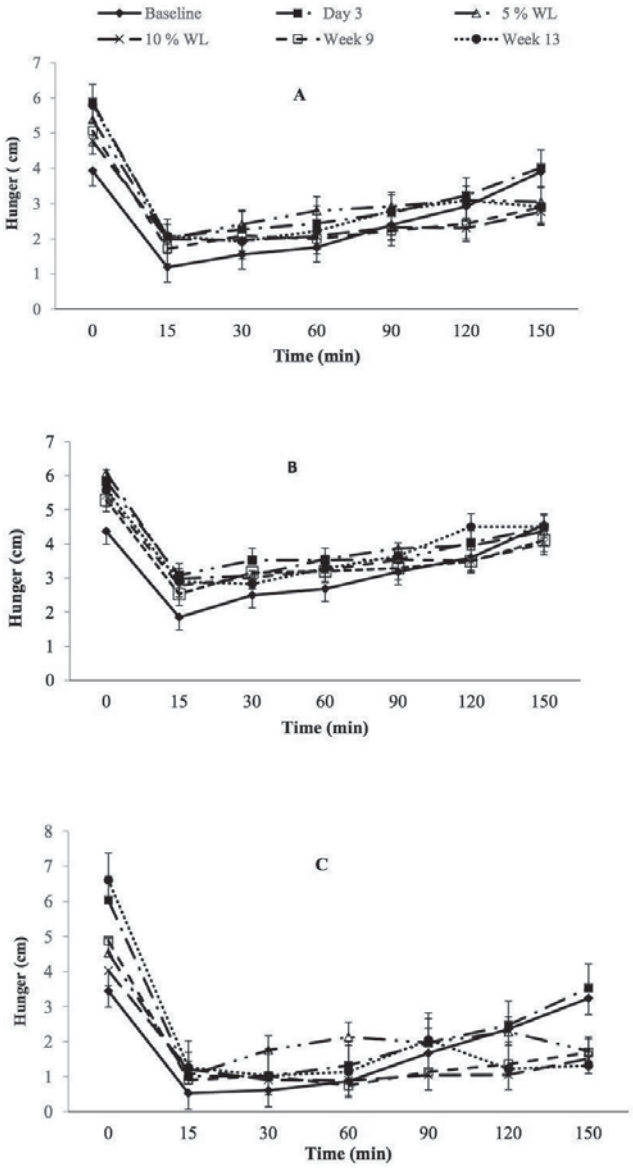
Supplementary Information accompanies this paper on *International Journal of Obesity* website (http://www.nature.com/ijo)

Supplementary figure I. Study-diagram**.** Data collection was done see arrows. VLED: very low energy diet, ADP: Air-displacement plethysmography. β-HB : β-hydroxybutyric acid. * See solid arrows; # See dashed arrows.



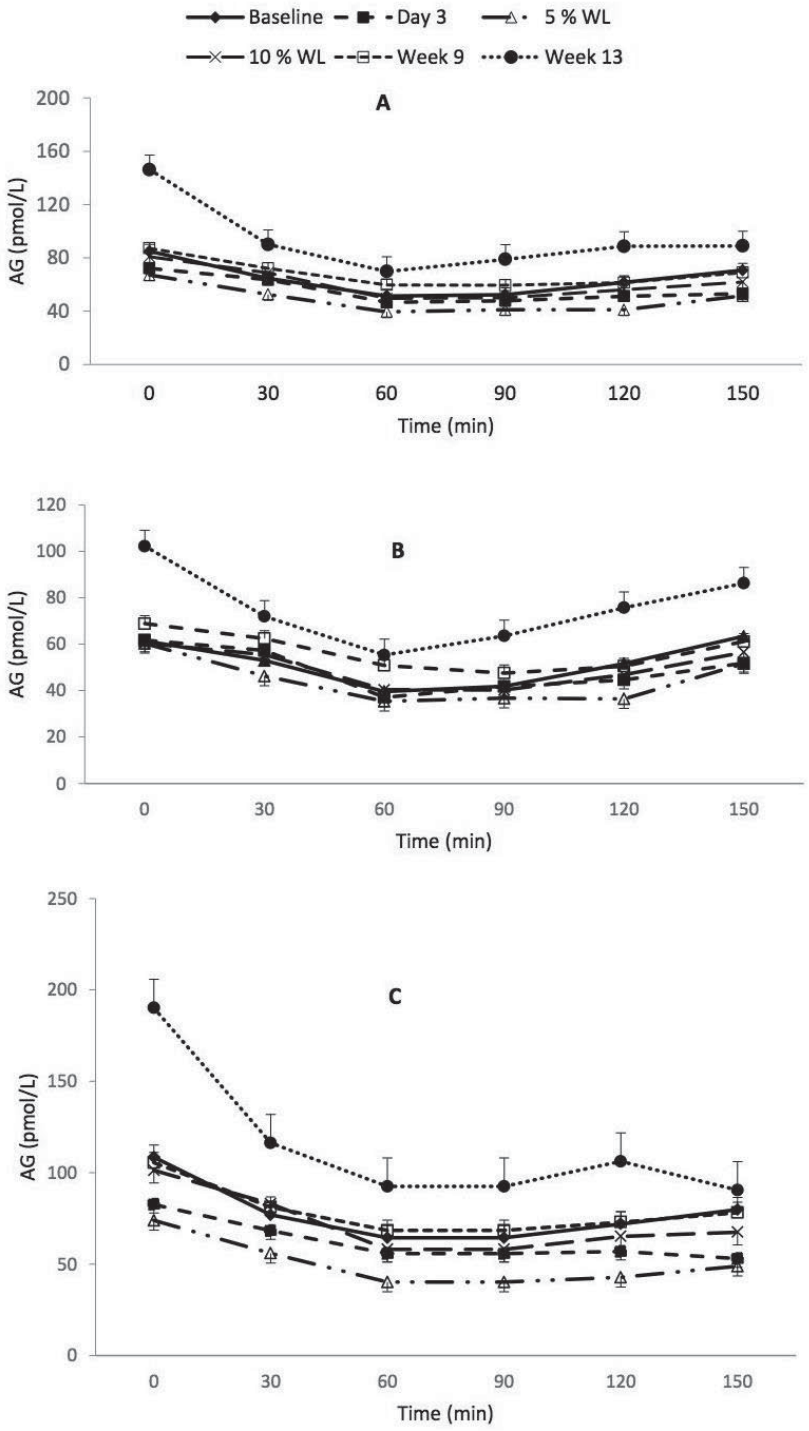
Supplementary figure II: Mean fasting and postprandial ratings of hunger feelings in all participants (A), males (B) and females (C) at baseline, day 3, 5 and 10 % WL, weeks 9 and 13. WL: weight loss. LMM revealed a significant main effect of sampling time, assessment time and sex (P<0.001 for all), but no significant interactions. Postprandial hunger feelings were significantly reduced 15 min after breakfast, with no further significant reductions (and hunger was lower at all time points compared with fasting levels, P<0.001 for all) Mean hunger values were significantly increased at day 3 (P<0.001) and 5 % WL (P<0.01) only. Males had an overall hunger feeling compared with females (3.73±0.29 cm vs. 1.97±0.34 cm, respectively).



Supplementary figure III: Mean fasting and postprandial plasma concentrations of AG for all participants (A), males (B) and females (C) at baseline, day 3, 5 and 10 % WL, weeks 9 and 13. AG: active ghrelin. WL: weight loss. LMM revealed a significant main effect of sampling time, assessment time ($P<0.001$ for both), sex ($P<0.05$) and an interaction between sex and assessment time ($P<0.001$). Postprandial AG concentrations were significantly reduced at 30 min postprandially, with a further reduction at 60 min ($P<0.001$, for both) and no further reductions afterwards (AG was lower at all-time points compared to basal concentration ($P<0.001$ for all)). AG concentrations were significantly increased at week 13 compared with baseline at 30 min and 90 min ($P<0.01$ for both), at 120 min between baseline and 5 % WL and week 13 ($P<0.05$ and $P<0.01$, respectively) and 150 min between baseline and 5 % WL ($P<0.05$).

Mean AG concentrations were significantly reduced compared with baseline at day 3 ($P<0.01$), 5 % WL ($P<0.01$), and significantly increased at week 13 ($P<0.001$) for all participants and females, but only an increase at week 13 in males ($P<0.001$).

Mean AG concentrations were significantly higher in females compared to males (76.4±8.0 pmol/L vs.54.8±6.5 pmol/L, respectively).


Baseline
Day 3
5 % WL
10 % WL
Week 9
Week 13
A
AG (pmol/L)
Time (min)
B
AG (pmol/L)
Time (min)
C
AG (pmol/L)
Time (min)

Supplementary table I. Baseline characteristics of all participants, males and females

	All	♂	♀
	(N=31)	**(n=18)**	**(n=13)**
Age (years)	43.1±10.2	40.5±7.9	46.8±12.1
Weight (kg) *	114.8±20.0	123.4±18.0	102.7±16.3
BMI (kg/m^2)	36.7±4.5	36.8±4.8	36.5±4.4
FM (kg)	47.8±10.8	46.2.5±10.9	50.0±10.7
FFM (kg) **	65.3±13.5	75.1±7.9	52.5±6.3

Results are expressed as mean ± SD. BMI: Body Mass Index. FM: Fat Mass, FFM: Fat Free Mass. Symbols denote significant gender differences: **P<0.001, *P<0.01

Supplementary table II. Time spent on different activity (min/d and steps/d) over time in all participants, males and females.

	Baseline			Week4			Week8			Week12		
	All	Men	Women	All	Men	Women	All	Men	Women	All	Men	Women
Average MET	1.23±0.03	1.32±0.05	1.15±0.04	1.19±0.03	1.23±0.54	1.15±0.04	1.22±0.04	1.34±0.06	1.10±0.05	1.29±0.07	1.31±0.11	1.26±0.06
Sed Time	1612±413	1123±675	2100±477	1153±430	1155±675	1150±534	1150±487	1133±758	1167±616	1136±872	1171±1510	1100±871
Light Act	207±21	207±34	206±24	214±21	198±33	230±24	202±23	201±36	202±29	215±37	183±65	247±37
Mod Act	79±11	98±18	59±13	56±11	60±18	52±14	75±12	103±19	47±15	84±20	78±35	90±20
Vig Act	1.7±0.6	3.3±1.0	0.1±0.7	0.7±0.6	1.3±1.0	0.2±0.8	2.0±0.7	3.4±1.1	0.7±0.9	0.2±1.2	-2.3±2.1	1.9±1.2
Total PA	287±26	309±42	266±31	271±27	259±42	283±33	278±29	307±45	250±36	299±48	258±84	339±47
Steps/day	7727±1016	7811±1632	7642±1210	6630±1027	5583±1620	7678±1264	7527±1091	8393±1715	6661±1350	8537±1637	7887±1715	9187±1637

Results are expressed as estimated marginal means±SEM. MET: metabolic equivalent. Sed: sedentary. PA: physical activity. No significant main effect of time or time*sex interaction were found. Main effect of sex showed that males had higher MET (1.3±0.04 min/d vs. 1.16±0.03min/d, $P<0.05$) and spent more time on vigorous activity (2.36±0.64 min/d vs. 0.53±0.49 min/d ($P<0.05$).

Paper II

British Journal of Nutrition, page 1 of 9 doi:10.1017/S0007114518000922

Timeline of changes in adaptive physiological responses, at the level of energy expenditure, with progressive weight loss

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Abstract

Diet-induced weight loss (WL) is associated with reduced resting and non-resting energy expenditure (EE), driven not only by changes in body composition but also potentially by adaptive thermogenesis (AT). When exactly this happens, during progressive WL, remains unknown. The aim of this study was to determine the timeline of changes in RMR and exercise-induced EE (EIEE), stemming from changes in body composition *v.* the presence of AT, during WL with a very-low-energy diet (VLED). In all, thirty-one adults (eighteen men) with obesity (BMI: 37 (SEM 4·5) kg/m^2; age: 43 (SEM 10) years) underwent 8 weeks of a VLED, followed by 4 weeks of weight maintenance. Body weight and composition, RMR, net EIEE (10, 25 and 50 W) and AT (for RMR (AT_{RMR}) and EIEE (AT_{EIEE})) were measured at baseline, day 3 (2 (SEM 1) % WL), after 5 and 10 % WL and at weeks 9 (16 (SEM 2) %) and 13 (16 (SEM 1) %). RMR and fat mass were significantly reduced for the first time at 5 % WL (12 (SEM 8) d) ($P<0·01$ and $P<0·001$, respectively) and EIEE at 10 % WL (32 (SEM 8) d), for all levels of power ($P<0·05$), and sustained up to week 13. AT_{RMR} was transiently present at 10 % WL (−460 (SEM 690) kJ/d, $P<0·01$). A fall in RMR should be anticipated at ≥5 % WL and a reduction in EIEE at ≥10 % WL. Transient AT_{RMR} can be expected at 10 % WL. These physiological adaptations may make progressive WL difficult and will probably contribute to relapse.

Key words: Adaptive thermogenesis: RMR: Exercise-induced energy expenditure

Obesity, owing to its high prevalence, associated co-morbidities and large socio-economic costs[1], is probably one of the largest public health problems of the 21st century. Even though a modest weight loss (WL) of 5–10 % is sufficient to induce health benefits[2] and can be achieved in the short term (3–6 months), 80 % will experience relapse, with weight regain apparent after 6–12 months[3,4], making WL maintenance a substantial unresolved issue.

The reduced obese state is associated with increased appetite[5–7] that fuels the desire to consume more energy, despite an overall reduction in total energy expenditure (EE), attributable to a reduction in both resting and non-resting EE, mainly driven by the loss of metabolic active tissue[8,9]. The reduction in non-resting EE seen with WL seems to be accounted for mainly by a reduction in exercise-induced EE (EIEE)[8,9], probably owing to increased efficiency[10], given that physical activity (PA) levels have been shown to increase or not to change with sustained WL[11,12]. Increased skeletal muscle work efficiency means that less energy is used to perform the same volume of exercise[10]. Moreover, some[8,10,13,14], but not all, studies[15,16] report a reduction in total EE and its components (resting and non-resting EE) in excess of what would be predicted, given the measured alterations in fat mass (FM) and fat-free mass (FFM), a mechanism known as adaptive thermogenesis (AT). Therefore, AT can account for a small proportion on the reduction in EE seen with WL. The extent to which these different, but inter-related, physiological mechanisms are important remains controversial. However, combined, these mechanisms may act to reduce WL rate and increase the risk of weight re-gain[7].

AT, which is induced by conditions of negative energy balance, has been shown to be under the influence of several hormones and the sympathetic nervous system. Thyroid hormones, insulin and leptin, as well as sympathetic activity, are likely to be involved in the greater than predicted reduction in both resting and non-resting EE observed with WL[17]. At a cellular level, mitochondrial adenosine triphosphate synthesis efficiency and uncoupling proteins are likely to be involved[17,18].

Abbreviations: AT, adaptive thermogenesis; EE, energy expenditure; EIEE, exercise-induced energy expenditure; FM, fat mass; FFM, fat-free mass; PA, physical activity; VLED, very-low-energy diet; Wk9, week 9; Wk13, week 13; WL, weight loss.

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British Journal of Nutrition

To our knowledge, no studies have determined the timeline over which EE, both at rest and during exercise, changes with progressive WL in the obese population. A minimal, but significant, WL (1–2 kg) has been shown to reduce RMR, even below predicted values (AT) in some studies[13], whereas others report no change[19]. A reduction in EIEE has been reported after 5 % and 10 % WL (10–13 kg)[10,20,21], in some cases below predicted values (AT)[21], whereas others have reported no change even after a 19 % WL[22]. The results are clearly controversial and more research is needed. Moreover, the greater FFM content of WL during energy restriction in men, compared with women[23], may suggest that the changes in EE variables with progressive WL are modulated by sex. Therefore, the primary aim of this study was to determine the timeline over which changes in EE variables (RMR, EIEE and AT) occur during progressive WL with a very-low-energy diet (VLED). A secondary aim was to assess whether this timeline was modulated by sex.

Methods

Participants

Healthy adults (18–65 years of age) with obesity (30 ≤ BMI <45 kg/m^2) were recruited from the local community by means of newspaper and internet advertising. This study nests within a large WL intervention (*n* 100), where individuals with obesity undertook 8 weeks of a VLED and were followed up to 1 year.

Inclusion criteria were as follows: weight stability (<2 kg change over the last 3 months), not dieting to lose weight and an inactive lifestyle (defined as <150 min of PA of at least moderate intensity[24], which was corroborated via data from the SenseWare activity data collected at baseline; see more details below). Owing to the known effect of phase of menstrual cycle on RMR[25], women had to be post-menopausal or taking hormonal contraceptives. Exclusion criteria were pregnancy, breast-feeding, clinical significant illness, including diabetes, previous WL surgery and medication known to affect appetite/metabolism or induce WL.

Ethical statement

The study was approved by the regional ethical committee (reference 2012/1901), registered in ClinicalTrial.gov (NCT01834859) and conducted according to the Declaration of Helsinki, with all participants providing informed written consent.

Study design

This was a clinical intervention study with repeated measurements. All participants underwent a supervised VLED for 8 weeks, followed by 4 weeks of weight stabilisation, and were asked not to change their PA levels throughout the study (see Fig. 1).

Weight-loss phase

Participants followed for 8 weeks a VLED (Allévo; Karo Pharma AS) with 2·3/2·8 MJ/d, for women and men, respectively (carbohydrates 42 %, protein 36 %, fat 18 % and fibre 4 %), as well as no-energy fluids and low-starch vegetables (max 100 g/d).

Weight stabilisation phase

At week 9 (Wk9), participants were gradually introduced to normal food, and an individual diet plan was prescribed by a trained dietitian based on estimated energy requirements (measured RMR × PAL (from individual SenseWear data at week 8)), with 15–20 % energy provided by protein, 20–30 by E % fat and 50–60 E% by carbohydrates, tailored to achieve weight stabilisation[26].

Objective measures of compliance

Diet. Participants received a weekly follow-up face-to-face consultation with a dietitian, which included measuring body weight, review of daily food records and monitoring of side effects. Urine acetoacetic acid concentration was measured weekly, using Ketostix reagent strips®. Negative ketones (<0·5 mmol/l) more than once were the reasons for exclusion from the analysis.

Physical activity. Armbands (BodyMedia®; SenseWare) were used for 7 d at baseline, and at weeks 4, 8 and 12. The data were considered valid if the participants wore the device for ≥4 d, including at least 1 weekend day, >95 % of the time[27]. The following variables were analysed: average metabolic equivalent

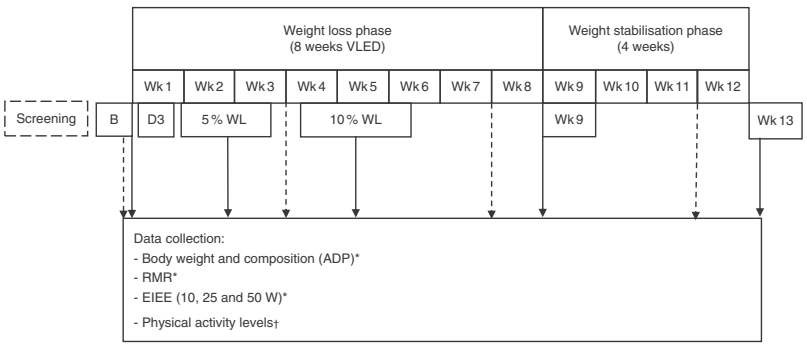

Fig. 1. Study diagram. For data collection points, see arrows. Wk, weeks; VLED, very-low-energy diet; ADP, air-displacement plethysmography; EIEE, exercise-induced energy expenditure. * ⟶; † - - - -►.

British Journal of Nutrition

of task (MET), time spent on sedentary, light, moderate and vigorous activities, total PA duration and steps/d.

Data collection

The following measurements were conducted at baseline, day 3, when each individual participant reached 5 and 10 % WL, and at week 9 (the day immediately after the end of the VLED) and week 13 (Wk13).

Body weight and body composition. Air-displacement plethysmography (ADP) (BodPod; COSMED) was used while participants were in fasting state and in accordance with standard operating procedures.

RMR. RMR was measured in fasting state by indirect calorimetry (V_{max} Encore 29N; CareFusion) using a canopy system and following standard procedures[28]. Participants were asked to fast for 12 h, not to drink caffeine for at least 6 h, be nicotine abstinent over the last 2 h and not to perform moderate-intensity PA for 2 h before test. Although calibration of the equipment was performed, the participants rested for 10 min on a chair. Thereafter, a ventilation hood was placed over the person's head, and VO_2 and CO_2 production (VCO_2) were measured for 15–20 min (or longer if required) until 'steady state' was reached. The first 5 min were excluded, and 10 min of stable data (CV for VO_2 and $VCO_2 < 10$ %) were used[28].

Exercise-induced energy expenditure. EIEE was measured by graded cycle ergometry (Eromedic 839E, GIH; Monark), 3 h after a standardised meal (2·5 MJ: 17 % protein, 35 % fat and 48 % carbohydrates). Participants pedalled at 60 rpm against graded resistance to generate 10, 25 and 50 W of power in sequential 4-min intervals. Gas exchange was measured continuously using a face mask by indirect calorimetry (V_{max} Encore 29 N), and the average of the last 2 min at each stage was used for analysis. Net EIEE was calculated by subtracting RMR (kJ/min) from the gross EIEE[21].

Adaptive thermogenesis

AT was present when measured EE (RMR or EIEE) was lower than predicted, given the body composition (FM and FFM) measured at each time point.

Regression analysis was performed to develop equations to predict both RMR (RMRp) and net EIEE (EIEEp) at each time point, using body composition (FM and FFM (kg)), sex, age and height as predictors. Equations to predict RMR and net EIEE were derived from a data set of ninety-nine participants (forty-four male, aged 43 (SEM 10) years with a BMI of 36 (SEM 4) kg/m^2), which this study is a part of (the participants included in this study were part of the data set):

$$\text{RMRp(kJ/d)} = 975{\cdot}712 + (33{\cdot}764 \times \text{FM (kg)}) + (63{\cdot}604 \times \text{FFM (kg)}) + (731{\cdot}538 \times \text{sex}) + (11{\cdot}080 \times \text{age (years)}) - (905{\cdot}169 \times \text{height (m)}).$$

$R^2 = 0{\cdot}78$, SEM = 591 kJ/d and $P < 0{\cdot}001$.

$$\text{Net EIEEp 10 W (kJ/min)} = 35{\cdot}141 + (0{\cdot}029 \times \text{age (years)}) + (0{\cdot}118 \times \text{FM (kg)}) + (0{\cdot}185 \times \text{FFM (kg)}) + (1{\cdot}651 \times \text{sex}) - (25{\cdot}691 \times \text{height (m)}).$$

$R^2 = 0{\cdot}47$; SEM = 2·10 kJ/min and $P < 0{\cdot}001$.

$$\text{Net EIEEp 25 W (kJ/min)} = 36{\cdot}595 + (0{\cdot}013 \times \text{age (years)}) + (0{\cdot}122 \times \text{FM (kg)}) + (0{\cdot}168 \times \text{FFM (kg)}) + (1{\cdot}399 \times \text{sex}) - (23{\cdot}822 \times \text{height (m)}).$$

$R^2 = 0{\cdot}45$; SEM = 2·19 kJ/min and $P < 0{\cdot}001$.

$$\text{Net EIEEp 50 W (kJ/min)} = 40{\cdot}904 + (0{\cdot}029 \times \text{age (years)}) + (0{\cdot}118 \times \text{FM (kg)}) + (0{\cdot}155 \times \text{FFM (kg)}) + (1{\cdot}663 \times \text{sex}) - (23{\cdot}008 \times \text{height (m)}).$$

$R^2 = 0{\cdot}36$; SEM = 2·41 kJ/min and $P < 0{\cdot}001$.

Power calculation

Sample size estimation was based on expected changes (from baseline) in RMR (day 3: −209; 5 % WL: −419, 10 % WL: −544, Wk9: −670 and Wk13: −377 kJ/d)[13,19,29,30] for a repeated-measures design. For an SD of 958 kJ/d[30], at a power of 80 %, a significance level of 5 % and assuming a 30 % correlation between time points, thirty-two participants were needed.

Statistical analysis

Statistical analysis was performed with SPSS version 22 (SPSS Inc.), and data were presented as means with their standard errors, except for baseline anthropometric data, time to achieve 5 and 10 % WL and WL (%) at day 3, Wk9 and Wk13, where means and standard deviations are presented. Statistical significance was set at $P < 0{\cdot}05$. Data were analysed using linear mixed-effects models, with restricted maximum-likelihood estimation, including fixed effects for time and sex, and their interaction. Bonferroni correction was used for post-hoc pairwise comparisons. RMR was also adjusted for FM and FFM (RMR_{adj}) and analysis was performed by linear mixed-effects models (LMM). Participants with at least three time points were considered completers and kept in the analysis. The Benjamini–Hochberg method, which controls for the false discovery rate[31], was used to adjust for the number of outcome variables.

The presence of AT was tested by paired *t* tests, comparing measured and predicted variables (RMR and EIEE), and a

British Journal of Nutrition

$P < 0{\cdot}003$ was considered significant after correcting for multiple comparisons. Correlation analysis was performed between WL and AT_{RMR} and AT_{EIEE}.

The data sets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Results

Participants

A total of thirty-three Caucasian participants started the study and thirty-one (eighteen males) were included in the analysis (one woman withdrew owing to personal reasons and one man owing to not tolerating the VLED). Completers had a BMI of 36·7 (SEM 4·5) kg/m^2 and were 43 (SEM 10) years of age. Women had significantly lower body weight (102·7 (SEM 16·3) *v.* 124·1 (SEM 18·1) kg, $P < 0{\cdot}01$) and FFM (55·6 (SEM 9·1) *v.* 74·2 (SEM 11·6) kg $P < 0{\cdot}001$) compared with men, but there were no significant differences in BMI between sexes.

Compliance

Diet. Compliance with the VLED was excellent, with no participant being excluded on the basis of not being ketotic.

Physical activity. No significant changes in any of the PA variables analysed were found[32].

Body weight and composition

Changes in body weight/composition are reported in Fig. 2. Significant WL (kg) occurred by day 3 ($P < 0{\cdot}001$) in all participants and in males (1·9 (SEM 0·9) and 2·1 (SEM 1·1) kg, respectively), which continued until Wk9 in all participants (18·7 (SEM 4·1) kg, $P < 0{\cdot}001$), and then stabilised (19·2 (SEM 3·4) kg, $P < 0{\cdot}001$). On average, participants achieved 5 % WL in 12 (SEM 6) d (11 (SEM 5) and 15 (SEM 7) d, for men and women, respectively, NS) and 10 % WL in 32 (SEM 8) d (28 (SEM 7) and 37 (SEM 6) d, for men and women, $P < 0{\cdot}01$). Men lost significantly more weight than women overall (12·8 (SEM 0·4) *v.* 10·0 (SEM 0·4) kg, $P < 0{\cdot}05$).

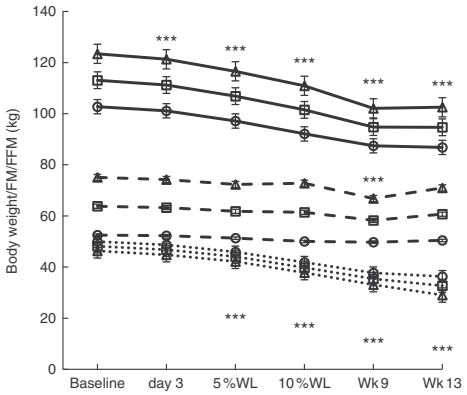

Fig. 2. Body weight and composition over time in all participants, men and women, with progressive weight loss. Values are estimated marginal means with their standard errors. Wk9, week 9; Wk13, week 13; WL, weight loss; FM, fat mass; FFM, fat-free mass; □, all participants; Δ, males; ○, females; ——, body weight; – – –, FFM; ·····, FM. Significant differences from baseline in all participants: ***$P < 0{\cdot}001$ for body weight, FFM and FM.

FM (kg) was significantly reduced by 5 % WL in all participants, men ($P < 0{\cdot}001$ for both) and women ($P < 0{\cdot}01$), and continued to decrease with progressive WL, being lower than baseline at all time points from WL ≥ 5 %. FM loss at Wk9 (16 % WL) was significant in all groups (12·8 (SEM 0·8), 13·2 (SEM 1·1) and 12·3 (SEM 1·2) kg, $P < 0{\cdot}001$ for all). A significant loss of FFM was seen at Wk9 only (16 % WL) in all participants and in males (5·2 (SEM 1·0) and 8·3 (SEM 1·4) kg, $P < 0{\cdot}001$ for both) (no significant changes were seen in females at any time point). Women had a significantly lower overall FFM compared with men (51·0 (SEM 1·9) *v.* 72·1 (SEM 1·6) kg, $P < 0{\cdot}001$).

RMR

RMR (kJ/d) was significantly reduced after 5 % WL in all participants (674 (SEM 121) kJ/d, $P < 0{\cdot}001$), men (770 (SEM 159) kJ/d, $P < 0{\cdot}001$) and women (574 (SEM 188) kJ/d, $P < 0{\cdot}05$), and further WL did not alter it significantly (see Table 1). RMR was lower than baseline at all time points, except for women at Wk13, where RMR was no longer different from baseline. No significant changes in absolute RMR were seen between Wk9 and Wk13, except in males where an increase was seen ($P < 0{\cdot}01$), even though values at Wk13 were still below baseline ($P < 0{\cdot}05$). RMR was significantly higher in men overall (7046 (SEM 197) *v.* 5347 (SEM 230) kJ/d, respectively). Adjusted RMR (kJ/d) was only significantly lower than baseline at 5 and 10 % WL in all participants ($P < 0{\cdot}01$, for both), and 10 % and Wk9 (16 % WL) in men ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively). A significant increase in adjusted RMR was seen between Wk9 and Wk13 ($P < 0{\cdot}01$) in men only.

Adjusted RMR was significantly higher in men overall (6703 (SEM 155) *v.* 5690 (SEM 180) kJ/d, respectively, $P < 0{\cdot}001$).

Net exercise-induced energy expenditure

Net EIEE at 10 W was significantly reduced, compared with baseline, after 10 % WL in all participants ($P < 0{\cdot}01$) and in males ($P < 0{\cdot}05$), and remained significantly lower than baseline at Wk9 ($P < 0{\cdot}001$ and $P < 0{\cdot}01$, respectively) and Wk13 ($P < 0{\cdot}001$ for both) (see Table 2). In women, a significant reduction in net EIEE at 10 W was seen at Wk13 ($P < 0{\cdot}01$). Net EIEE at 25 W was significantly reduced for the first time at 10 % WL in all participants ($P < 0{\cdot}01$), and in men at Wk9 ($P < 0{\cdot}01$) and continued to be lower afterwards ($P < 0{\cdot}001$, for both all and males). Net EIEE at 50 W was significantly reduced at 10 % WL in all participants and in males ($P < 0{\cdot}05$ for both), and remained lower than baseline at Wk9 ($P < 0{\cdot}01$ for both) and Wk13 ($P < 0{\cdot}001$ for both), but no differences between Wk9 and Wk13 were seen for any groups.

Adaptive thermogenesis

AT_{RMR} was only significantly reduced after 10 % WL for all participants (−465 (SEM 691) kJ/d, $P < 0{\cdot}01$) and after 10 % WL

Table 1. RMR over time in all participants, men and women
(Mean values with their standard errors)

	Baseline		Day 3		5 %WL		10 %WL		Wk9		Wk13	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
RMR (MJ/d)												
All	6·8	0·2	6·6	0·2	6·1***	0·2	5·9***	0·2	5·8***	0·2	6·1***	0·2
Men	7·7	0·2	7·6	0·2	6·9***	0·2	6·6***	0·2	6·3***	0·2	7·1*	0·2
Women	5·8	0·3	5·6	0·3	5·2*	0·3	5·1**	0·3	5·2*	0·3	5·1	0·3
RMR_{adj} (MJ/d)												
All	6·5	0·1	6·4	0·1	6·0**	0·1	5·9**	0·1	6·0	0·1	6·4	0·2
Men	7·0	0·2	7·0	0·2	6·5	0·2	6·3**	0·2	6·3*	0·2	7·1	0·2
Women	5·9	0·2	5·8	0·2	5·5	0·2	5·5	0·2	5·8	0·2	5·7	0·3

WL, weight loss; Wk9, week 9; Wk13, week 13; RMR_{adj}, RMR adjusted for fat-free mass and fat mass as covariates in LMM.
Significant differences from baseline: * $P<0·05$, ** $P<0·01$, *** $P<0·001$.

Table 2. Net exercise-induced energy expenditure (EIEE) over time in all participants, men and women†
(Mean values with their standard errors)

	Baseline		Day 3		5 %WL		10 %WL		Wk9		Wk13	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Net EIEE 10 W (kJ/min)												
All	11·93	0·50	11·30	0·50	10·93	0·50	10·47**	0·50	10·22***	0·50	9·21***	0·54
Men	12·48	0·63	11·68	0·63	11·47	0·63	10·97*	0·67	10·38**	0·67	9·67***	0·71
Women	11·35	0·75	10·89	0·75	10·34	0·75	10·01	0·75	10·01	0·75	8·71**	0·88
Net EIEE 25 W (kJ/min)												
All	14·82	0·54	14·03	0·54	13·82	0·54	13·36**	0·54	13·10**	0·54	12·31***	0·59
Men	15·41	0·67	14·44	0·64	14·49	0·67	13·86	0·67	13·40**	0·71	12·73***	0·75
Women	14·24	0·80	13·61	0·80	13·60	0·80	12·90	0·80	12·81	0·80	11·93	0·96
Net EIEE 50 W (kJ/min)												
All	20·22	0·54	19·68	0·54	19·85	0·54	18·71*	0·54	18·51**	0·54	17·50***	0·59
Men	20·72	0·67	20·10	0·71	20·47	0·67	18·92*	0·71	18·55**	0·71	17·79***	0·75
Women	19·72	0·84	19·22	0·84	19·05	0·80	18·15	0·80	18·51	0·84	17·25	1·00

WL, weight loss; Wk9, week 9; Wk13, week13.
Significant differences from baseline: * $P<0·05$, ** $P<0·01$, *** $P<0·001$.
† No differences between time points were seen.

and at Wk9 in men (−716 (SEM 670) and −553 (SEM 582) kJ/d, respectively, $P<0·01$ for both) (See Fig. 3(a)). AT_{RMR} was significantly higher in men compared with women at 5 and 10 % WL, and Wk9 ($P<0·05$, $P<0·01$ and $P<0·05$, respectively).

A negative correlation was found between magnitude of WL (kg) and AT_{RMR} at 5 % WL (n 30, r −0·491 and $P<0·01$), 10 % WL (n 29, r −0·391 and $P<0·05$) and Wk9 (n 29, r −0·224 and $P<0·01$), with a higher WL being associated with a larger AT_{RMR} ($RMR_m < RMR_p$) (Fig. 3(b)).

No evidence of AT_{EIEE} was found (Fig. 4) and AT_{EIEE} was not correlated with WL.

Discussion

This study is the first to repeatedly measure RMR and EIEE, using a longitudinal design, to explore physiological adaptions to progressive WL. Despite significant WL by day 3 (2 (SEM 1) kg WL), there was no significant drop in RMR, which is in line with previous research[19]. Our findings show a significant reduction in RMR (10 (SEM 2) %) by the time 5 % WL (12 (SEM 8) d) was reached, with no further reduction up to 16 % WL, which is in agreement with previous research[33]. Moreover, we found that the reduction in RMR was sustained even after a period of weight stabilisation, which again is in line with previous studies[8,30].

A greater fall in RMR has been reported in studies with a shorter *v.* longer duration (≤ *v.* >6 weeks)[34], suggesting that the reduction in RMR seen with WL is more pronounced during the 1st week of energy restriction, which supports our findings. When we adjusted RMR for FM and FFM, only a transient reduction was seen at 5 and 10 % WL in all participants and 10 and 16 % in men. This concurs with Ballor *et al.*[35], who reported a significant reduction in RMR (both absolute and adjusted values) with a 10 % WL induced by diet alone. Contrary to our findings, Leibel *et al.*[8] reported a reduction in RMR adjusted for FFM at 20 %, but not 10 % WL, in a mixed sample of men and women with obesity. Overall, our results show that a WL ≥ 5 %, seen as the minimum required to achieve health benefits[2], already leads to a significant reduction in RMR, but further WL up to 16 % does not induce a further significant decline in RMR.

The fact that we did not detect a significant change in EIEE until the WL was ≥10 % (12 (SEM 2) kg) for all levels of power is in line with most of the available evidence[10,20,35]. However, Amati *et al.*[22] did not find any change in gross EIEE (at an

British Journal of Nutrition

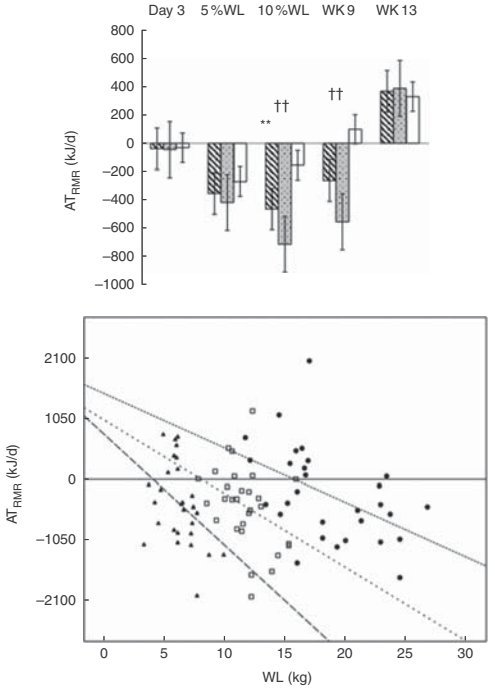

Fig. 3. (a). Adaptive thermogenesis (AT) at the level of RMR with progressive weight loss (WL), in all participants (◥), men (▩) and women (□). Values are means with their standard errors. Wk9, week 9; Wk13, week 13. $RMR_{measured} < RMR_{predicted}$: $^{**}P < 0{\cdot}01$ for all, $\dagger\dagger P < 0{\cdot}01$ for males. (b). Correlation of AT_{RMR} against WL at 5 %, 10 % and week 9 in all participants was investigated by using Spearman's ρ correlation coefficient; a larger AT_{RMR} was associated with a larger WL. The equation for the regression lines: 5 % WL; $Y = -210 \times X + 962$, 10 % WL; $Y = -126 \times X + 1025$, and week 9; $Y = -92 \times X + 1483$. – – – –, AT_{RMR} 5 % WL, $R^2 = 0{\cdot}241$ ($P < 0{\cdot}01$); ·····, AT_{RMR} 10 % WL, $R^2 = 0{\cdot}153$ ($P < 0{\cdot}05$); ------, AT_{RMR} W9, $R^2 = 0{\cdot}285$ ($P < 0{\cdot}01$).

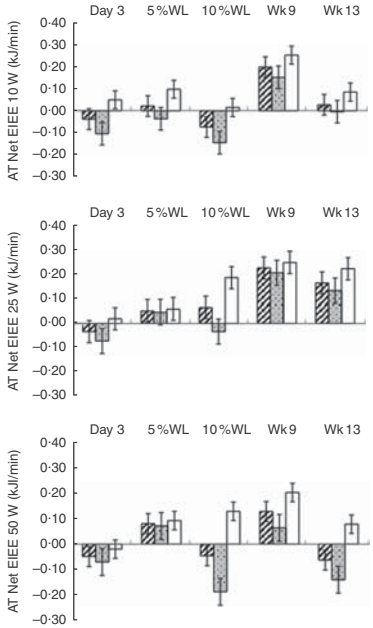

Fig. 4. Adaptive thermogenesis (AT) at the level of net exercise-induced energy expenditure (EIEE) (10, 25 and 50 W) with progressive weight loss (WL) in all participants (◥), men (▩) and women (□). Values are means with their standard errors. Wk9, week 9; Wk13, week 13. No significant differences were found between net EIEE measured and predicted at any time point.

average power 38 ± 2 W) with a 19 % WL induced by diet alone, followed by 2 weeks of weight stabilisation. Conflicting results may be explained by different protocols used to measure EIEE and the fact that this is sometimes expressed as gross and others as net EIEE.

AT_{RMR} was only present at 10 % WL in all participants and at 10 % WL and Wk9 (16 % WL) in men, which is similar to other studies[30,36,37]. Rosenbaum & Leibel[37] reported AT_{RMR} after 10 %, but not 20 %, WL induced by diet alone. Camps *et al.*[30] also found AT_{RMR} with 10 % WL achieved with a VLED, but opposite to us, that was sustained after 12 weeks of follow-up. On the other hand, other studies do not support the existence of AT_{RMR} after 5 or 10 % WL[38,39]. Differences in compliance, follow-up and sample size may account for some of these discrepancies. More studies are needed to confirm whether AT_{RMR} is indeed a transient phenomenon, and whether women are protected from it. In the present study, a larger AT_{RMR} was associated with a larger WL, up to 22 % WL, but not after a period of weight stabilisation. Camps *et al.*[30] also found a correlation between AT_{RMR} and magnitude of WL, up to 25 % WL. In an in-patient, well-controlled study, Muller *et al.*[40] showed in eight normal-weight men a significant reduction in RMR, and the presence of $AT_{RMR,}$ after only 3 d on a 50 % energy-restricted diet (WL, approximately 1·7 kg). They also showed a significant reduction in RMR and the presence of AT_{RMR} after 1 week (WL, approximately 2·2 kg) in 32 non-obese men, with no further significant changes with progressive WL up to 3 weeks (WL, approximately 4 kg (5 % WL)). Inconsistencies in outcomes between this and the present study may be owing to differences in sex distribution (males *v.* mixed sex), participant's characteristics (non-obese *v.* obese), dietary intervention and magnitude of WL. Even though the accuracy of our RMRp was not perfect, it is in line with that seen with WHO equation[41] and we were unable to find any established equation that would result in a better accuracy.

No AT_{EIEE} was found at any time point or level of power in the present study. Other studies have reported AT_{EIEE} to be present after a WL between 10 and 20 %, followed by 2–3 weeks of weight stabilisation[8,9]. Differences in outcomes among studies can probably be explained by diverse sample sizes, participants' characteristics, magnitude of WL, WL intervention and protocols used to measure and predict EIEE (stationary bike *v.* treadmill, different resistances, speeds and inclinations). Moreover, non-resting EE is not the same as EIEE, and thus comparisons between studies need to be done carefully. When adjusting RMR for body composition, and assessing the presence of AT_{RMR}, it was assumed that the composition of

British Journal of Nutrition

FFM was constant during WL. However, FFM hydration probably changed, given that ketogenic diets lead to a large loss of total body water, owing to glycogen depletion, during the 1st days of the diet[42,43]. This might have biased body composition results and affected our outcomes, particularly those taken at day 3. These results need to be interpreted with caution, given that the accuracy of EIEEp was not optimal. However, we are not aware of any established equation that could be used to improve the accuracy of EIEEp.

This study revealed some important sex differences. In women, there was no significant change from baseline in neither absolute nor adjusted RMR, after a 16 % sustained WL. This is in line with Doucet *et al.*[44], who found a sustained reduction in RMR after an average 10 % WL only in men. On the other hand, Schwartz & Doucet[34], in a systematic review on the effects of diet-induced WL on RMR, reported a similar decrease in RMR for both sexes. A reduction in net EIEE in women was only seen for 10 W at Wk13 (16 (SEM 2) %), which is in line with a previous study[14]. Some of the sex differences seen in this study may be attributed to differences in energy and protein deficit, which lead to a larger overall WL in men and might have also contributed to the fact that FFM did not change in women, whereas in men there was a significant reduction at 16 % WL. This is supported by literature, which suggests that FFM reduction during WL is proportionally greater in men[23]. AT_{RMR} was not seen in women at any time point. This is in line with Doucet *et al.*[13], who reported AT_{RMR} after 8 weeks on a diet in men only[13]. On the other hand, Camps *et al.*[30] reported AT_{RMR} in both sexes after a 9·6 (SEM 4·1) kg WL induced with a VLED. Leptin has been suggested as a potential mediator to explain the differences in AT between sexes[45]. Owing to their relatively higher percentage of FM compared with men, women have a higher leptin plasma concentration, and the reduction seen with WL may translate in leptin plasma concentration falling below a threshold level in men, but not in women[46]. Given that AT_{RMR} has been shown to be positively correlated with the reduction in leptin seen with WL[47], it could potentially explain why AT_{RMR} was only seen in men in this study.

This study has several strengths. First, its longitudinal design is unique, with multiple measurements undertaken during progressive WL. This allowed us to evaluate the effect of minimal, but significant WL (day 3), WL known to induce health benefits (5–10 %)[2] and a larger WL (16 %), before and after weight stabilisation, on the different outcome variables. Second, compliance was objectively monitored and was excellent. Third, we adjusted for multiple comparisons and multiple outcome variables. However, this study has also limitations. The fact that body composition was measured by ADP, and as such did not take into account the level of FFM hydration, may have affected the absolute values, particularly regarding adjusted RMR and AT at rest. Moreover, the best regression model to predict EIEE had a relatively modest R^2, with <47 % of the variation in EIEE being explained by the model, which could have an impact on our estimation of AT_{EIEE}. It needs also to be acknowledged that measured baseline RMR values in this study were on average 20 % lower than predicted by the Mifflin equation[48], with 80 % of the participants presenting RMR values below predicted (difference between measured and predicted >10 %). This has been previously described. Weijs[49] showed in an adult Dutch population with overweight and obesity (average BMI 30 kg/m^2) that 50 % of the individuals had a measured RMR (measured with the same equipment as in the present study) lower than that predicted by the Mifflins equation. The reasons for the lower percentage of individuals with accurate values in the present study compared with Weijs remains speculative, but the fact that our population was substantially more obese (BMI 36·7 (SEM 4·5) *v.* 30·8 (SEM 3·6) kg/m^2), had a body composition with a high % of non-metabolically active body fat and also with different genetic background (Norwegian) might all have had an impact. Moreover, our sample comprises individuals with obesity who had sought treatment, and it is possible that at least some of the participants presented with AT as a result of previous weigh loss–regain cycles[17]. As the main aim of this study was to look at changes over time, even if RMR values are underestimated compared with a standard predictive equation, the longitudinal nature of the study methods and statistical analysis takes into consideration baseline values, and thus the overall findings of the study are still valid. Finally, the study may be underpowered to examine sex differences.

This study has several practical implications. Patients need to be assured that a WL >5 %, and up to 16 %, will not necessarily translate into further significant reductions in their basal energy needs. This knowledge is important for practitioners when reformulating dietary prescriptions for progressive WL and WL maintenance. When aiming for progressive weight reduction after ≥10 % WL, a larger dietary energy restriction and/or an increase in PA levels are essential to counteract the decrease in EIEE seen at this time point. A slowdown in WL rate after ≥10 % WL can, at least partially, be explained by the transient AT_{RMR} seen at this time point. Practitioners need to be aware of these physiological adaptations and not assume that non-compliance with the diet is the sole explanation. Knowing when changes in EE, at rest and during exercise, occur with progressive WL is important to understand resistance with progressive WL and relapse after treatment, and should be discussed alongside patients' expectations of their WL journey.

In conclusion, a fall in RMR should be anticipated at ≥5 % WL, a reduction in EIEE at ≥10 % WL and transient ATRMR at 10 % WL. These metabolic compensatory responses can make further WL difficult and increase the risk of relapse. Sex seems to modulate some of these responses, but larger long-term longitudinal studies are needed.

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C. M., B. K., H. T. and S. N. formulated the research questions and designed the study. S.N., S. R. C., L.C. H. T., O. J. B. and I. H. carried out the study, S. N. and S. R. C. analysed the data and all authors were involved in the writing of the article.

The authors declare that there are no conflicts of interest.

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Paper III

Title: Investigation of the long-term sustainability of changes in appetite after weight loss

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Keywords: hunger; fullness; ghrelin; desire to eat; prospective food consumption; GLP-1; PYY; CCK; sustainability; weight maintenance

Running head: Long-term changes in appetite with weight loss

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Abstract (295 words)

Background/Objective: Diet-induced weight loss (WL) leads to a compensatory increase in appetite and changes in the plasma concentration of appetite-regulating hormones are likely to play a role. Whether these changes are transient or sustained remains unclear. This study aimed to assess if changes in subjective and objective appetite markers observed with WL are sustained after 1 year (1Y).

Subjects/Methods: 100 (45 males) individuals with obesity (BMI: 37±4 kg/m^2, age: 43±10 years) underwent 8 weeks (wks) of a very-low energy diet (VLED), followed by 4 wks refeeding, and a 1Y maintenance program. Fasting/postprandial subjective ratings of hunger, fullness, desire to eat, and prospective food consumption (PFC) were assessed, and plasma concentration of active ghrelin (AG), total peptide YY (PYY), active glucagon-like peptide 1, cholecystokinin (CCK) and insulin measured, at baseline, week 13 (Wk13) and 1Y.

Results: At Wk13, 16 % WL (-18±1 kg, P<0.001) was associated with a significant increase in fasting and postprandial hunger ratings (P<0.01 and P<0.05, respectively), and postprandial fullness (P<0.01) combined with a reduction in PFC (P<0.001). These were accompanied by a significant rise in basal and postprandial AG concentrations (P<0.001, for both), a reduction in postprandial CCK (P<0.01) and in basal and postprandial insulin (P<0.001). At 1Y follow-up, with sustained WL (15%; -16±1 kg, P<0.001), fasting hunger and postprandial fullness ratings remained increased (P<0.05 for both), and postprandial PFC reduced (P<0.001). Basal and postprandial AG remained elevated and insulin reduced (P<0.001, for all), while postprandial CCK was increased (P<0.01) and PYY decreased (P<0.001).

Conclusion: With a 15 % sustained WL at 1Y, the drive to eat in the fasting state is increased, but this may be balanced out by raised postprandial feelings of fullness. To assist with WL maintenance, new strategies are required to manage increased hunger and drive to eat.

Introduction

Worldwide more than 40 % of all adults have attempted to control their body weight by weight loss strategies (1). Weight loss (WL) maintenance is now the most challenging part within obesity management as relapse is common and only 10-20 % succeed in maintaining their lower body weight long-term (2, 3). Reasons for recidivism are poorly understood and likely to be complex, involving a combination of reduced motivation and compliance to restrict energy intake by dieting and increase energy expenditure via exercise regimens, (4-7), together with metabolic, neuroendocrine and autonomic adaptive responses that oppose the reduced obese state (8-11).

The landmark papers by Leibel et al. (1995) (10) and Dulloo et al. (1997) (11) showed that WL is followed by a reduction in total energy expenditure (TEE) larger than predicted and, despite that, an increase in hunger and hyperphagia. Since then, reviews by Cornier et al. (2011) (8), Rosenbaum et al. (2010) (9) and Doucet et al. (2007) (12), among others, have described the compensatory mechanisms activated with WL, on both sides of the energy balance equation, which may contribute to weight re-gain.

A recent study has estimated that the increased appetite seen with WL is probably threefold larger than the corresponding reduction in TEE and likely the main driver of weight re-gain (13). Several studies, that use visual analogue scales (VAS) to measure appetite, demonstrate that diet-induced WL, outside of ketosis, is associated with increased hunger feelings in the fasted state (14-16), likely mediated via an increase in plasma ghrelin concentration (15-17), even though some studies report no change (18, 19). The impact of WL on fullness feelings (15, 16, 20) and secretion of peptides signalling satiety is, unfortunately, less clear (15, 16, 20, 21).

Whether the changes in appetite associated with WL are sustained in the long-term remains a point of debate, as the available research is inconsistent in its findings (15, 20, 21), with

different methods to measure appetite being employed and different hormonal fractions being measured. Sumithran et al (2011) reported a sustained increase in subjects feelings of hunger using VAS, plus an increase in ghrelin plasma concentration at 1 year follow up, as well as no change in postprandial concentration of active glucagon-like peptide 1 (GLP-1) and a reduction in total peptide YY (PYY), after an initial 12% WL followed by 50% regain (15). However, another study reported a sustained increase in both total GLP-1 and PYY_{3-36} postprandial secretion after a 12% sustained WL at 1 year follow up (21), while another showed no change in active GLP-1 postprandial secretion after 6 months of sustained WL maintenance (20). Moreover, from our knowledge, no study has determined if sex modulates the changes in appetite seen with WL. This is of great importance given that sex has been shown to modulate appetite sensations and the secretion of several appetite-related hormones (22, 23). The primary aim of this study was to determine if changes in appetite (both subjective feelings of appetite using VAS and objective plasma concentrations of appetite related hormones) seen after WL, are sustained at 1-year (1Y) follow-up. A secondary aim was to determine if sex modulates any changes in appetite.

Methods

Participants

Healthy adults with obesity ($30 < BMI < 50$ kg/m^2) from the local community of Trondheim, Norway, were recruited for this study by social media and articles in the local newspaper. The study was approved by the regional ethics committee (Ref., 2012/1901), registered in ClinicalTrial.gov (NCT01834859) and conducted according to the Declaration of Helsinki. All participants signed informed consent before participation.

Participants had to be weight stable (<2 kg change over the last 3 months), not dieting to lose weight and with a sedentary lifestyle. Exclusion criteria were pregnancy, breast-feeding, clinical significant illness, including diabetes, previous WL surgery and/ medication known to affect appetite/metabolism or induce WL.

Study design

This was a longitudinal intervention study with repeated measurements. Participants underwent an 8-week supervised very-low energy diet (VLED), followed by a 4-week refeeding phase, and a 1-year weight maintenance program (study diagram, Supplementary Figure I).

- Weight loss phase

Participants followed for 8 weeks a VLED (Allévo, Karo Pharma AS, Sweden) with 550/660 kcal/day, for females and males respectively (carbohydrate 42 %, protein 36 %, fat 18 % and fibre 4 %), plus no-energy fluids and low starch vegetables (max 100 g/day) (16).

At week 9, participants were gradually reintroduced to normal foods, while withdrawing from the VLED products. An individual diet plan (estimated energy requirements (Ereq) at Wk9: 1690±407 kcal/day) was prescribed by a trained dietician tailored to individual energy requirements (measured resting metabolic rate (RMR; Wk9 (1339±252 kcal/ day) x (physical activity level (PAL)) (extracted from individual physical activity monitors (BodyMedia®, SenseWear, Pittsburgh, USA), with 15-20 % protein, 20-30 % fat, and 50-60 % carbohydrate, aimed at weight stabilization (24). VLED products ceased at the end of week 10.

Participants were asked not to change their PA levels during this phase of the study. To check for compliance, participants wore SenseWear armbands for 7-days at baseline and at weeks 9 and 13 (Wk9 and Wk13). Data were considered valid if the participants wore the device for ≥ 4 days, including at least 1 weekend day and >95 % of the time (25).

- Weight maintenance phase

A 1Y follow-up program aimed at WL maintenance was offered from Wk13. The diet plan provided at week 9 was revised at week 13 by a trained dietician having into account individual energy requirements (measured RMR (1584±285 kcal/day) x PAL at Wk13) and designed for weight maintenance (Ereq at Wk13: 2088±520 kcal/day). The multidisciplinary follow-up program included regular individual and group based sessions, focusing on nutritional counselling, increased PA levels and cognitive behavioural therapy. A dietitian was present in all group meetings and participants had an individual consultation with a dietitian (1 hour) every other month. Participants were also asked to wear SenseWear armbands at 6 and at 12 months to record PA levels.

Data Collection

The following measurements were conducted at baseline, Wk13 and 1Y follow-up.

- *Body weight and composition*

Air-displacement plethysmography (BodPod, COSMED, Italy) was used, while participants were in the fasting state.

- *Appetite measurements*

Subjective appetite feelings (hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC)) were measured with a 10-cm visual analogue scale (VAS) (26), and blood samples for the analysis of appetite related hormones (active ghrelin (AG), active glucagon-like peptide 1 (GLP-1), total peptide YY (PYY), cholecystokinin (CCK) and insulin) collected in fasting and every 30 min after a standardised breakfast (600 kcal: 17 % protein, 35 % fat, and 48 % carbohydrate) for 2.5 hours. The breakfast consisted of 75 oat-bread (whole grain), 5 g butter, 40 g strawberry jam, 35 g cheese (38 E % fat) and 250 ml low fat (1.2 E % fat) milk.

The satiety quotient (SQ) for each appetite sensation, as well as an average estimate, was calculated at each assessment time point (27).

Plasma samples were analysed for AG, active GLP-1, total PYY, CCK and insulin using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore) and CCK using an" in-house" RIA method (28) (intra-and inter-assay CV were for AG, active GLP-1 and PYY <10 % and <20 %; insulin <10 % and <15 % and CCK <5 % and <15 %, respectively. Blood was collected into EDTA-coated tubes. One ml of full blood was then transferred into a micro tube and 20µl mixture of inhibitors (10µl of Pefabloc (Roche Diagnostic, Germany) + 10µl DPP-IV (Merck Millipore, Germany)) added. For CCK analysis, aprotinin (DSM, Coatech AB, Kaiseraugst, Switzerland) (500 KIU/ml whole blood) was added to the EDTA tube. Samples were then centrifuged at 3200 rpm for 10 minutes at 18 °C and the plasma frozen at -80 °C until further analysis. Samples were analysed when all time points were available within the same participant (average 1 year). The analysis was performed by the same technician, except for CCK which was analysed at Prof. Rehfelds lab (Rigshospitalet, University of Copenhagen, Copenhagen, Denmark).

Statistical analysis

Statistical analysis was performed with SPSS version 22 (SPSS Inc., Chicago, IL), and data presented as mean±SEM, except for baseline anthropometric data at baseline, where mean±SD was used. Statistical significance was set at $P<0.05$. Data was analysed using linear mixed-effects models, with restricted maximum-likelihood estimation, including fixed effects for time and sex, and their interaction. Bonferroni correction was used for post hoc pairwise comparisons. Average values for appetite ratings and plasma concentrations of appetite-related hormones refer to the average of all time points (fasting and postprandial). Participants with data at 1Y were considered completers and kept in the analysis. All the analysis was done for

completers, except for changes in body weight where an intention to treat analysis with baseline values carried forward was used. The Benjamini-Hochberg method, which controls for the false discovery rate (29) was used to adjust for the large number of outcome variables. The association between changes in subjective and objective appetite markers (both at Wk13 and 1Y), between the magnitude of WL (both at Wk13 and 1Y) and the respective changes in both subjective and objective appetite markers and between changes in appetite at Wk13 and WL maintenance at 1Y were investigated with Pearson or Spearmen correlation.

Results

Participants

One-hundred (55 females) participants fulfilled the study entry criteria and started the study. Of those, 95 participants completed the 8-week VLED (2 did not tolerate the VLED, 1 was excluded due to consumption of extra foods, 1 withdrew for personal reasons and 1 was lost to follow-up), 94 completed Wk 13 measurements (1 withdrew due to family illness) and 71 (41 females) completed the full 1Y (8 withdrew due to own or family related illness, 3 due to work constraints making it difficult to return for measurements, 2 were excluded due to non-compliance as they had started a new VLED, and 10 were lost to follow-up).

Baseline characteristics of the participants who started and completed the study are presented in Table 1. There were no significant differences in any baseline measurement between those who completed and those who did not complete the study. Moreover, no differences were seen between completers and non-completers regarding changes in appetite with WL (Wk13), even though completers lost more weight at Wk13 (20.0±5.0 vs 16.8±4.4 kg, $P<0.05$). Females were older ($P<0.05$), lighter ($P<0.001$) and had a higher FM (%) than males ($P<0.001$), but there was no difference in BMI between sexes.

No changes in total PA duration or time spent in light, moderate or vigorous activities were measured during the WL phase. Time spent in vigorous activities was significantly increased at 6 months in all participants and females ($P<0.01$ and $P<0.05$, respectively), but returned to baseline levels at 1Y for all groups. Steps/day were significantly increased at 6 months in all participants ($P<0.01$) and at 1Y in males only ($P<0.05$). See Supplementary Table I.

Body weight and composition

Changes in body weight are reported in Figure 1. Mean WL at Wk13 was 16 % (-18±1 kg), and this was maintained at 1Y follow-up in completers: 15 % WL (-16±1 kg, $P<0.001$). The intention to treat analysis revealed an increase in body weight from Wk13 to 1Y in all participants (6±1 kg, $P<0.001$), but body weight at 1Y was still significant lower than baseline (-11±1 kg, $P<0.001$).

FM (%) was significantly decreased at Wk13 in all participants, males and females (-9±1 %, -11±1 % and -7±1 %, respectively, $P<0.001$ for all groups) and remained lower than baseline at 1Y (-9±1 %, -9±1 % and -8±1 %, respectively, $P<0.001$ for all groups). The changes in absolute FM over time were not statistical significant different between sexes.

Appetite feelings

Hunger ratings in fasting were increased at Wk13 in all participants (38 %) and females ($P<0.01$ and $P<0.05$, respectively), and this was sustained at 1Y follow-up in all participants (22 %) ($P<0.05$). No significant change overtime were found for rating of fullness, DTE or PFC in fasting (see Fig. 2). Females had overall significant lower ratings of PFC in fasting than males (5.4±0.2 vs.6.1±0.2 cm, $P<0.05$).

Mean ratings of hunger were significantly increased in all participants and males at Wk13 ($P<0.01$ and $P<0.05$, respectively) compared to baseline, but this was not sustained at 1Y

follow-up. Mean fullness ratings were significantly increased in all participants (10 %) and males at Wk13 ($P<0.01$ and $P<0.05$, respectively), and this was sustained at 1Y follow-up in all participants (6 %) ($P<0.01$), but not in males. Females, had a significant increase in mean rating of fullness at 1Y follow-up only ($P<0.05$). Mean ratings of PFC were significantly reduced in all participants and males at Wk13 ($P<0.001$ and $P<0.001$, respectively) and this was sustained at 1Y follow-up ($P<0.001$, for both) (see Fig. 3). Females experienced a significant reduction in ratings of PFC at 1Y only ($P<0.05$), and had significant lower overall rating of DTE (2.3±0.2 vs. 3.3±0.3 cm, $P<0.05$) and PFC (3.4±0.3 vs. 4.6±0.3 cm, $P<0.01$), compared to males.

A significant increase in SQ hunger was seen at Wk13 and this was sustained at 1Y in all participants ($P<0.01$ and $P<0.05$, respectively) and females ($P<0.05$, for both). See Supplementary Table III.

Appetite related Hormones

Basal concentration of AG was significantly increased in all participants, males and females at Wk13 ($P<0.001$, $P<0.001$ and $P<0.01$ respectively) and this was sustained at 1Y follow-up ($P<0.001$, $P<0.01$ and $P<0.001$, respectively). Basal insulin concentration was significantly reduced in all participants, males and females at Wk13 ($P<0.01$, $P<0.001$ and $P<0.01$, respectively) and this was sustained at 1Y ($P<0.001$, for all groups). Females had significant overall lower basal concentration of insulin (485±66 vs. 803±69 pg/mL, $P<0.01$) than males (see Fig. 4).

Mean AG plasma concentration was significantly increased at Wk13 in all participants, males and females ($P<0.001$, for all groups) and this was sustained at 1Y follow-up ($P<0.001$, for all groups). Mean total PYY concentration did not change significantly by Wk13, but was significantly lower than baseline at 1Y follow-up in all participants, males and females

(P<0.001, P<0.001 and P<0.01, respectively). There was a significant reduction in mean CCK concentrations in all participants at Wk13 (P<0.01), but an increase at 1Y follow-up, compared with baseline, in all participants and males (P<0.01, for both). There was a significant reduction in mean insulin concentration in all participants, males and females at Wk13 (P<0.001, for all groups), which was sustained at 1Y (P<0.001, for all groups) (see Fig. 5).

Correlation analysis

No significant correlation was found between changes in subjective appetite feelings and changes in the plasma concentrations of appetite-related hormones at any time point (either at W13 or 1Y). The larger the WL at 1Y, the larger was the increase seen in basal and postprandial concentration of AG (r=-0.455 and r=-0.566, respectively, P<0.001, for both). The opposite was seen for insulin, with a larger reduction in basal and postprandial insulin plasma concentrations as the magnitude of WL increased at 1Y (r=0.282 and r=0.277, respectively, P<0.05 for both). No significant association was found between the changes in appetite (namely increased hunger and AG plasma concentrations) seen with WL (Wk13) and WL maintenance/relapse at 1Y.

Discussion

In this longitudinal study, a 16 % WL at Wk13 was sufficient to induce significant health benefits (30, 31), and was associated with an increase in hunger, while postprandial feelings of fullness and SQ hunger were increased and PFC reduced. There was also a significant increase in basal and postprandial AG concentrations, a reduction in postprandial CCK and in basal and postprandial insulin. At 1Y, and with sustained WL (15 %), fasting hunger, SQ hunger and postprandial fullness ratings were still increased and postprandial PFC reduced. Basal and postprandial AG remained increased and insulin reduced, while postprandial CCK was increased and PYY decreased at 1Y follow-up, compared to baseline.

Few studies have been performed on the long-term sustainability of changes in appetite with WL and, to date, the results have been contradictory (15, 20, 21). Sumithran et al (2011) (15), in a similar longitudinal study, reported a sustained increase in postprandial hunger and DTE ratings, and AG plasma concentrations and a reduction in the postprandial concentration of total PYY and CCK at 1Y follow-up (15). However, the sample was composed of predominately post-menopausal women (68%) and participants experienced an average 50% weight regain, after the initial 14% WL. Iepsen et al (2016), using a similar study design, reported an increase in the plasma concentration of total ghrelin (both in the fasting and postprandial states), but surprisingly, an increase in total GLP-1 and PYY_{3-36} postprandial concentrations with a sustained 13 % WL at 1Y follow-up (21). Unfortunately, they did not measure changes in subjective feelings of appetite. Adam et al (2006), on the other hand, reported an increase in the postprandial concentrations of active GLP-1 after an 8 % WL, which was not sustained after a 12-week WL maintenance period (20). The present study is the largest evaluating the impact of sustained WL maintenance, employing both subjective and objective appetite markers, and offers a perspective of sex being a mediator of outcome.

Even though ours and other studies tend to consistently show a sustained increase in subjective feelings of hunger, when using VAS, combined with an increase in ghrelin (either active or total) with long-term WL (15, 21), changes in postprandial fullness ratings and plasma concentrations of satiety peptides remain controversial (15, 21). Differences in the magnitude of WL and its sustainability, hormonal fractions measured and methods of analysis of gut peptides (32-34) are likely to contribute to this inconsistent picture alongside most studies having mixed gender. Moreover, SQ hunger increased with WL, indicating a larger reduction in hunger after the same test meal, which is likely to reflect more accurate appetite sensation responses (35).

The increase in postprandial fullness with WL and WL maintenance is a novel. Sumithran et al (2011) reported no change in fullness either with a 14 % WL at Wk 10 or with a 7 % WL maintenance at 1Y follow-up in 34 overweight and obese individuals (15). Adam et al (2006) reported a similar outcome after an 8 % WL, both acutely and after 12 weeks of WL maintenance in 32 overweight and obese individuals (20). Inconsistencies may be due to differences in the magnitude of WL, baseline participant characteristics and sample size. In our study, an initial 16 % WL was achieved, and this was sustained at 1Y (15 % WL) in 71 obese individuals, which is a much larger WL and sample size compared with the other available studies (15, 20). In a study by Delgado-Aros et al. (2004) (36), a higher BMI was associated with decreased postprandial fullness, which may explain why a so large WL, and concomitant BMI reduction, in our study led to increased fullness, when compared with baseline ratings. Even though studies on the impact of BMI on gastric capacity have not been consistent (37), a reduction in fasting gastric capacity has been reported after ≥ 5 % WL in individuals with obesity (38). It remains speculative if changes in gastric capacity with WL contributed to increased postprandial fullness in the present study. Increased fullness after acute and sustained WL is unlikely to be explained by changes in CCK secretion, given that a reduction in CCK

secretion was measured at Wk13, while an increase was seen with sustained WL at 1Y follow-up. Another explanation for the increased fullness with WL may be an increased postprandial secretion GLP-1 and PYY. Even though we saw no change in active GLP-1, and a reduction in total PYY plasma concentrations, others have shown that a large WL (14-15 %) leads to an increase in the secretion of both total GLP-1 (21, 39) and PYY_{3-36} (21). Finally, it is possible that increased fullness reflects the larger relative energy load of the test meal after WL.

To date, there is a dearth of information on the impact of WL on CCK plasma concentrations. The available evidence suggests that acute and rapid WL results in a reduction in postprandial concentrations of CCK (15, 40), which is consistent with our results, and probably reflects a lower stimulation due to less food (and fat) intake. It remains speculative why in the present study an increase in postprandial concentrations of CCK was seen with sustained WL at 1Y follow up, while in Sumithran's study (15), with a similar design (8 weeks of VLED followed by refeeding and 1 year follow up) and methodology (same RIA protocol for CCK analysis), a reduction was reported. However, due to continued dietetic support we achieved a 15 % sustained WL at 1Y follow-up, while Sumithran had a 50 % weight regain, with only a 7 % WL at 1Y follow-up. This could have had an impact on CCK concentrations, and the increased postprandial CCK secretion may reflect a long-term adaptation to substantial WL, but that requires further substantiation.

The lack of association between subjective appetite feelings and the plasma concentration of appetite-related hormones seen in this study is not new (41, 42) and probably reflects the complexity of the appetite control system and the fact that changes in appetite feelings are unlikely to be attributable to alterations in a single hormone.

It has long been suggested that the increased hunger and reduced satiety seen after WL are part of a compensatory response that tries to bring body weight back to its set point (8, 43-45). However, the findings from the present study: increased fasting hunger and basal AG plasma

concentrations, as well as increased postprandial fullness, AG and CCK in response to large sustained WL may suggest otherwise. It is well known that obese individuals have lower plasma concentration of ghrelin in fasting (46) and a blunted postprandial secretion of total GLP-1 (39, 47), active GLP-1 (48), total PYY (48, 49) and CCK (50) and lower ghrelin postprandial suppression (46, 48). Therefore, our overall findings, with the exception of PYY, could reflect a normalization of appetite markers towards those seen in healthy-weight individuals. This is supported by Verdich et al. (2001), who showed that postprandial total GLP-1 concentration increased after a 19 kg WL toward levels seen in a control normal-weight group (39). WL also leads to a reduction in TEE proportional to the new reduced body weight, even though some individuals may experience a larger than predicted reduction – a mechanism known as adaptive thermogenesis (AT) (10). With, the exception of AT, which seems to occur in only some individuals, the changes in appetite and energy expenditure seen with WL could, therefore, be seen as a normalisation towards a lower body weight and not a compensatory mechanism that drives relapse. This new hypothesis is supported by the fact that neither us, nor Sumithran et al, 2011 (15) have reported any association between the changes in appetite seen with WL and long-term relapse at 1 year follow up.

This study revealed several sex differences in the changes in appetite seen overtime with WL and WL maintenance. Hunger ratings in fasting were increased at Wk13 in females only, while mean postprandial ratings of hunger and fullness were significantly increased, and PFC reduced, in males only at Wk13, and postprandial fullness was increased in females only at 1Y follow-up. Moreover, mean postprandial CCK plasma concentrations did not change at any time point in females, while in males there was an increase at 1Y follow-up, compared to baseline. The fact that postprandial fullness was increased at 1Y follow-up in females only may reflect the fact that in males there was a tendency towards weight regain from Wk13 to 1Y, while females continued to lose weight over time. More studies, with larger sample sizes and

equal sex distribution, are needed to fully ascertain the potential modulating effect of sex on the changes in appetite seen with WL and WL maintenance and the explanatory mechanisms behind it.

This study has several strengths. First, it is the largest longitudinal study to examine changes in appetite with sustained WL. Second, it included both objective and subjective markers of appetite. Third, the participants were able to maintain their body weight at 1Y follow-up (compared with Wk13), probably due to on-going and tailored advice provided by dieticians. Finally, both males and females were included in the study in similar numbers. There are also some limitations. The multiplex assay used for the measurements of appetite hormones (except for CCK) is likely to result in less accurate and precise measurements compared with optimized assays for each individual hormone. The fact that the same type of test meal was given to all participants, regardless of their Ereq, constitutes a limitation. Females consumed a larger relative energy load compared with males and the same test meal represented a larger energy load with progressive WL. However, if we had adjusted the test meal accordion to Ereq (smaller meals in females compared with females and after WL) the appetite response would be blunted, because the nutrient stimuli would also be reduced, independently of the effect of sex or WL on appetite. This study was not powered to examine sex differences *per se*, so we are unable to draw firm conclusions about sex differences in responses to WL.

Our findings have some important practical implications. Patients with obesity who have lost and maintained significant amounts of weight via dieting, and benefited in terms of metabolic and overall health markers (30, 31), should expect a sustained increase in hunger feelings in the fasting state and to be prepared for these feelings to occur. This increased drive to eat may lead to overconsumption and increase the risk of weight regain. However, this may be balanced out by increased postprandial fullness. Health professionals working with this patient group, should be aware of the sustained increase in the drive to eat in the fasting state and help individuals

develop management strategies to reduce the risk of overeating. However, the changes in appetite seen with WL (increased hunger and AG) were not associated with long-term relapse, which likely reflects the complexity of body weight regulation (51).

Conclusions

With a 15 % sustained WL at 1Y follow-up, the drive to eat in the fasted state is increased, but this might be balanced out by an increased postprandial fullness. Some sex differences were revealed, but larger studies are needed to support these findings. Future studies should evaluate if changes in appetite markers with WL are part of a compensatory response or a simple normalization towards healthy-weight values, its relationship with actual food intake and its real impact on long-term WL maintenance.

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Figure 1: Body weight in all participants (intention to treat analysis) and completers over time. Results presented as mean±SEM.

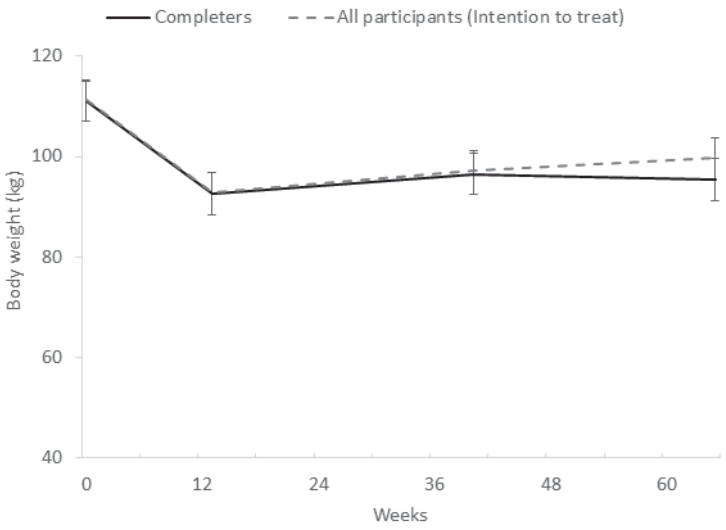


Figure 2. Subjective feelings of hunger (a), fullness (b), desire to eat (c) and prospective food consumption (PFC) (d) in fasting, over time, in all participants, males and females. Results presented as estimated marginal means±SEM. Symbols denote significant differences from baseline in all participants: $^{**}P<0.01$ and $^{*}P<0.05$ and females: $^{¥}P<0.05$.

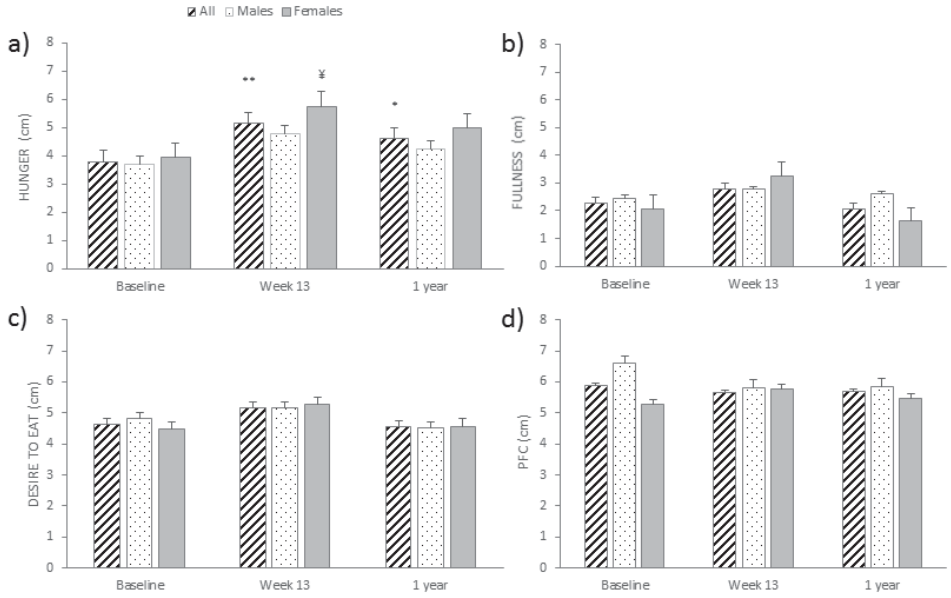


Figure 3. Mean fasting and postprandial ratings of hunger a), fullness (b), desire to eat (c) and prospective food consumption (PFC) (d) in all participants over time. Results presented as estimated marginal means±SEM.

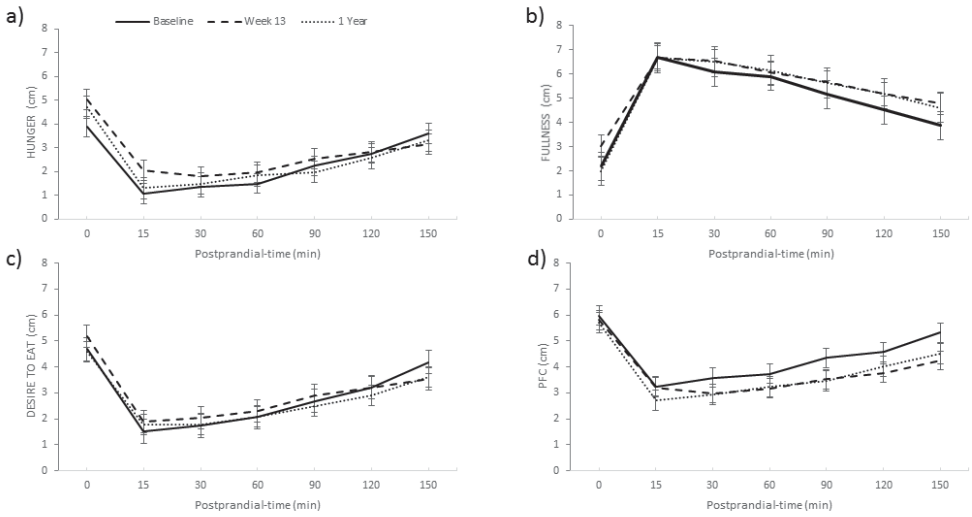


Figure 4. Basal plasma concentrations of appetite related hormones: AG (a), active GLP-1 (b), total PYY (c), CCK (d) and insulin (e), over time in all participants, males and females. Results presented as estimated marginal means±SEM. AG: active ghrelin. GLP-1: glucagon-like peptide-1. CCK: cholecystokinin. PYY: total peptide YY. Symbols denote significant differences from baseline in all participants: ***P<0.001, males: ^{ǂǂ}P<0.001 and ^{ǂ}P<0.01 and females: $^{¥¥¥}$P<0.001 and $^{¥¥}$P<0.05.

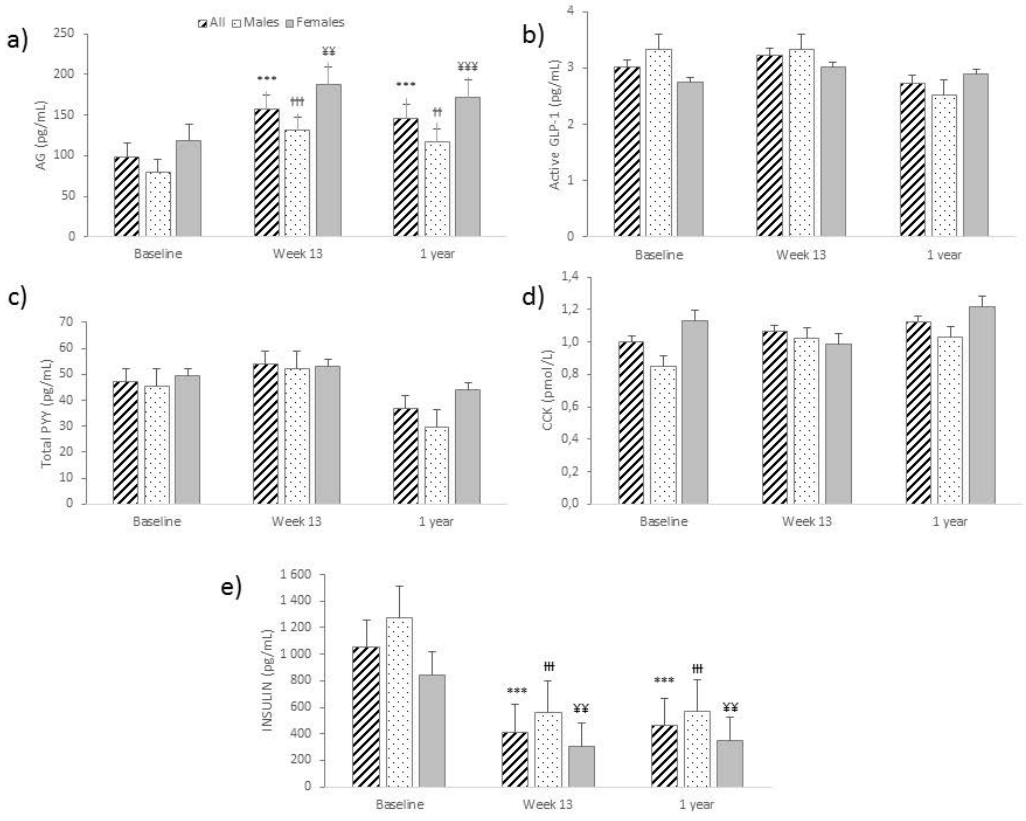
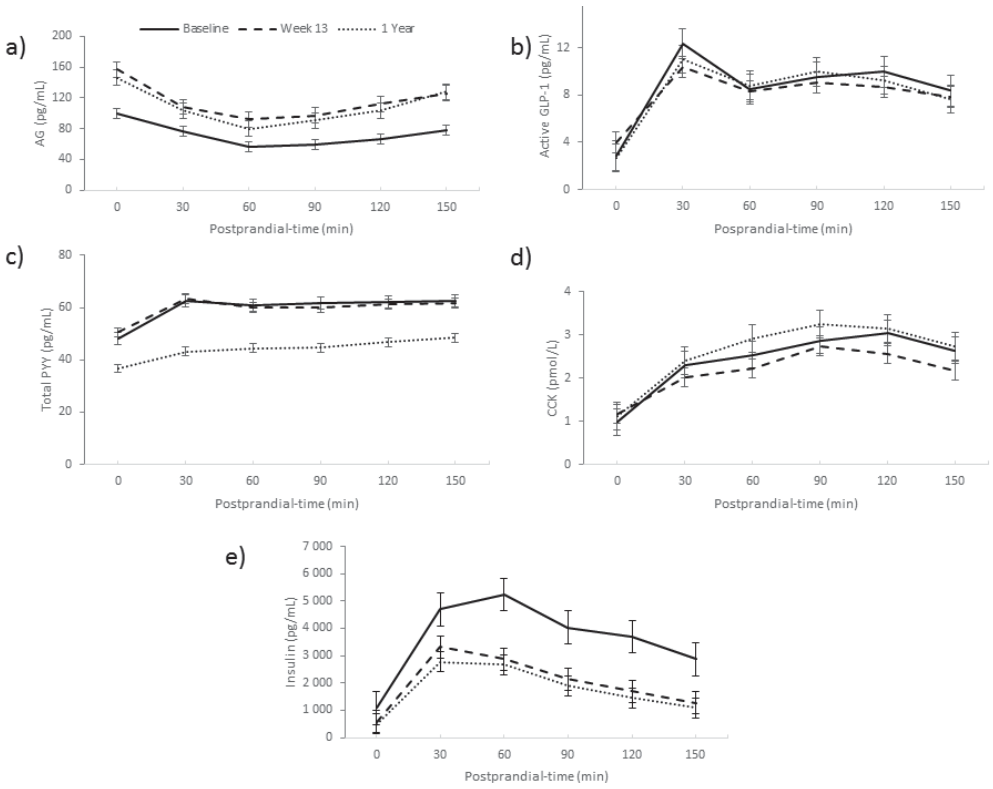


Figure 5: Mean basal and postprandial plasma concentrations of appetite related hormones; a) active ghrelin, b) GLP-1, c) PYY, d) CCK and e) insulin, for all participants over time. AG: active ghrelin. GLP-1: glucagon-like peptide-1. CCK: cholecystokinin. PYY: total peptide YY. Results presented as estimated marginal means±SEM



Supplementary figure I. Study diagram. Arrows show data collection time points. VLED: Very low energy diet. Wk: week. B: baseline. Y: year. Mnd: months. ADP: air displacement plethysmography. ADP: Air-displacement plethysmography. * See solid arrows; # See dashed arrows.

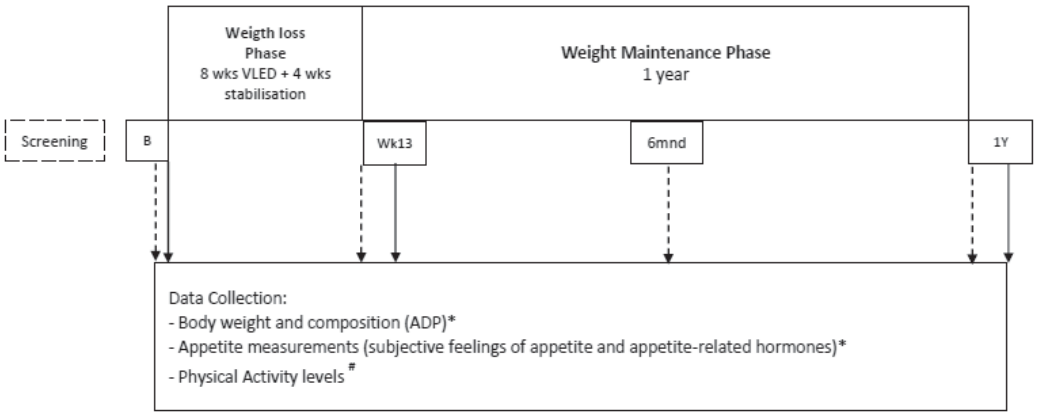


Table 1. Baseline characteristics of the participants

	All (N=100)			Completers (N=71)			P-value
	All (N=100)	Males (n=45)	Females (n=55)	All (N=71)	Males (n=30)	Females (n=41)	
Age (year)	42.5±9.7	39.7±9.1	44.8±9.7**	43.4±9.4	40.3±9.2	45.6±9.0*	0.140
Weight (kg)	110.3±18.4	120.1±19.6***	102.3±12.8	109.1±18.7	120.4±19.7***	100.9±12.9	0.313
BMI (kg/m)	36.7±4.2	36.6±4.9	36.7±3.5	36.4±4.0	36.6±4.7	36.2±3.5	0.274
FM %	44.1±6.4	39.3±5.7	47.9±3.9***	44.0±6.4	39.2±6.2	47.3±4.0***	0.849

Values are mean ± SD. BMI: body-mass index (calculated as the weight in kg divided by the square of the height in meters). FM: Fat mass. P-values are for comparisons between all participants and completers. Symbols denote significant differences between sexes in each group, *P<0.05, **P<0.01 and ***P<0.001.

Supplementary table I. Physical activity levels over time in all participants, males and females

	Baseline	Week 13	6 months	1 year
Average MET (kcal/kg/hour)				
All	1.20 ± 0.02	1.33 ± 0.030***	1.34 ± 0.030***	1.29 ± 0.02**
Males	1.28 ± 0.04	1.42 ± 0.04$^{¤}$	1.42 ± 0.04$^{¤}$	1.37 ± 0.04
Females	1.13 ± 0.03	1.23 ± 0.05	1.26 ± 0.05$^{¥}$	1.21 ± 0.03$^{¥¥}$
SedTime (min/day)				
All	1134.98 ± 15.43	1111.33 ± 18.68	1105.94 ± 18.14	1119.15 ± 15.25
Males	1113.23 ± 23.66	1078.46 ± 24.29	1096.95 ± 24.60	1101.46 ± 23.46
Females	1156.05 ± 20.01	1163.07 ± 31.53	1109.06 ± 27.67	1137.60 ± 19.74
LightAct (min/day)				
All	216.07 ± 12.29	241.84 ± 15.14	232.29 ± 14.67	230.90 ± 12.13
Males	212.60 ± 18.79	250.81 ± 19.34	22965 ± 19.61	214.17 ± 18.61
Females	219.96 ± 15.86	210.36 ± 25.80	230.80 ± 22.50	243.34 ± 15.62
ModAct (min/day)				
All	69.90 ± 7.42	74.73 ± 9.44	96.06 ± 9.10	82.82 ± 7.30
Males	92.18 ± 11.44	95.32 ± 11.83	126.94 ± 12.03	112.23 ± 11.30
Females	46.49 ± 9.62	61.74 ± 16.55	62.37 ± 14.27	54.54 ± 9.45
VigAct (min/day)				
All	0.81 ± 1.16	4.43 ± 1.52	6.71 ± 1.46**	3.48 ± 1.14
Males	1.18 ± 1.80	6.44 ± 1.86	6.22 ± 1.90	4.97 ± 1.77
Females	0.31 ± 1.51	0.84 ± 2.69	7.95 ± 2.30$^{¥}$	2.20 ± 1.48
TotalPA (min/day)				
All	288.17 ± 13.50	315.35 ± 16.28	326.87 ± 15.82	292.61 ± 13.35
Males	305.13 ± 20.70	349.85 ± 21.25	350.11 ± 21.51	322.21 ± 20.53
Females	268.76 ± 17.51	266.80 ± 27.47	303.51 ± 24.14	264.10 ± 17.29
StepsDay				
All	6650.35 ± 389.73	7111.89 ± 468.70	8600.74 ± 455.75**	7883.77 ± 385.49
Males	6198.86 ± 598.77	6915.94 ± 614.45	8553.78 ± 621.87	7820.95 ± 593.96^{ł}
Females	7026.84 ± 506.58	7276.78 ± 791.01	8528.31 ± 695.72	7984.04 ± 500.14

Results presented as estimated marginal means±SEM. MET; Metabolic Equivalent of Task. Act; activity. Mod; moderate. Vig; vigorous/very vigorous. PA; physical activity. Min; minutes. Symbols denote significant differences from baseline (for all participants ***P<0.001 and **P<0.01, males $^{¤}$P<0.01 and ^{ł}P<0.05, and females $^{¥¥}$P<0.01 and $^{¥}$P<0.05).

Supplementary table 2 A. Subjective feelings of appetite feelings variables at baseline and change over time in all participants.

	Baseline	Δ B to W13	P-value (B to W13)	Δ B to 1 Y	P-value (B to 1 Y)
Fasting					
Hunger	3.7 ± 0.2	1.4 ± 0.4	**<0.01**	0.8 ± 0.3	**<0.05**
Fullness	2.3 ± 0.2	0.8 ± 0.3	0.081	-0.2 ± 0.3	0.179
DTE	4.6 ± 0.3	0.6 ± 0.4	0.442	-0.1 ± 0.3	0.240
PFC	5.9 ± 0.3	-0.3 ± 0.4	0.252	-0.2 ± 0.4	0.141
2.5-hour AUC					
Hunger	347.5 ± 28.3	51.0 ± 35.2	0.452	6.5 ± 27.2	0.202
Fullness	872.5 ± 31.0	74.2 ± 38.6	0.171	61.2 ± 29.8	0.126
DTE	424.5 ± 32.3	22.4 ± 37.6	0.240	-15.5 ± 28.4	0.244
PFC	683.4 ± 39.0	-125.8 ± 54.7	0.07	-99.5 ± 39.4	<0.05
Average					
Hunger	2.4 ± 0.2	0.4 ± 0.2	**<0.05**	0.1 ± 0.1	0.990
Fullness	4.9 ± 0.2	0.5 ± 0.2	**<0.01**	0.3 ± 0.1	**<0.01**
DTE	2.9 ± 0.2	0.1 ± 0.1	0.951	-0.1 ± 0.1	0.562
PFC	4.4 ± 0.2	-0.6 ± 0.2	**<0.001**	-0.6 ± 0.1	**<0.001**

Results presented as estimated marginal means±SEM. DTE: desire to eat. PFC: Prospective food consumption.
AUC: total area under the curve. Symbols denote significant differences from baseline ***P<0.001, **P<0.01 and *P<0.05.

Supplementary table 2B. Appetite related hormones at baseline and changes over time in all participants.

	Baseline	Δ B to W13	P-value (B to W13)	Δ B to 1Y	P-value (B to 1Y)
Fasting					
Ghrelin	98.1 ± 11.4	59.0 ± 10.5	<0.001	47.1 ± 8.2	<0.001
GLP-1	3.0 ± 0.5	0.2 ± 0.6	0.240	-0.3 ± 0.5	0.164
PYY	47.1 ± 6.5	6.8 ± 6.9	0.978	-10.1 ± 5.4	0.194
CCK	1.0 ± 0.1	0.1 ± 0.2	0.313	0.1 ± 0.1	0.106
Insulin	1053.5 ± 57.2	-640.0 ± 77.4	<0.001	-588.1 ± 60.3	<0.001
2.5-hour AUC					
Ghrelin	10390 ± 1188	5889 ± 962	<0.001	4935 ± 752	<0.001
GLP-1	1375 ± 92	-133 ± 127	0.891	-61 ± 99	0.193
PYY	8993 ± 962	15 ± 941	0.327	-2274 ± 732	<0.01
CCK	375 ± 17	-41 ± 19	0.111	31 ± 15	0.132
Insulin	586992 ± 29253	-281129 ± 37189	<0.001	-299002 ± 28885	<0.001
Average					
Ghrelin	72.6 ± 8.0	42.7 ± 3.6	<0.001	35.9 ± 2.5	<0.001
GLP-1	8.6 ± 0.5	-0.6 ± 0.5	0.802	-0.4 ± 0.4	0.900
PYY	59.7 ± 5,9	0.2 ± 2,7	0.315	-15.7 ± 2.0	<0.001
CCK	2.4 ± 0.1	-0.3 ± 0.1	<0.01	0.2 ± 0.1	<0.01
Insulin	3599.2 ± 164.0	-1620.3 ± 146.6	<0.001	-1872.3 ± 100.4	<0.001

Results presented as estimated marginal means±SEM. B: baseline. W13: week 13. 1Y: 1 year. AG: active ghrelin. GLP-1: glucagon-like peptide-1. CCK: cholecystokinin. PYY: total peptide YY. AUC: total area under the curve. Symbols denote significant differences from baseline ***P<0.001, **P<0.01 and *P<0.05.

Supplementary Table 3. Satiety quotient (SQ, mm/kcal) overtime with progressive WL in participants, males and females.

		All groups	Males	Females
SQ Hunger	B	2,4 ± 0,3	1,7 ± 0,5	3,2 ± 0,5*
	W13	4,2 ± 0,5##	2,8 ± 0,6	6,0 ± 0,9**#
	1Y	3,6 ± 0,3#	2,4 ± 0,5	4,7 ± 0,5**#
SQ Fullness	B	4,9 ± 0,4	3,8 ± 0,6	6,1 ± 0,5**
	W13	4,6 ± 0,5	3,8 ± 0,7	4,7 ± 1,0
	1Y	5,2 ± 0,4	3,9 ± 0,6	6,5 ± 0,5**
SQDTE	B	2,9 ± 0,3	2,5 ± 0,5	3,4 ± 0,4
	W13	3,8 ± 0,4	2,9 ± 0,5	5,1 ± 0,8*
	1Y	2,9 ± 0,3	2,3 ± 0,5	3,6 ± 0,4*
SQ PFC	B	2,7 ± 0,5	2,9 ± 0,7	2,8 ± 0,6
	W13	3,5 ± 0,7	2,6 ± 0,8	5,2 ± 1,2
	1Y	3,1 ± 0,5	2,5 ± 0,7	3,6 ± 0,6
Average SQ				
SQB		3,2 ± 0,3	2,7 ± 0,4	3,9 ± 0,4*
SQW13		4,0 ± 0,4	3,0 ± 0,4	5,3 ± 0,7**
SQ1Y		3,7 ± 0,3	2,8 ± 0,4	4,6 ± 0,4**

Results expressed as estimated marginal means±SEM. PFC: prospective food consumption. DTE: desire to eat. Significant change overtime $^{\#}P<0.05$ and $^{\#\#}P<0.01$. Significant difference between sex *P<0.05 and *P<0.01.

Appendix

Forespørsel om deltakelse i forskningsprosjektet

Hvordan holde vekten etter diettindusert vekttap?

Bakgrunn og hensikt
Dette er et spørsmål til deg om å delta i en forskningsstudie med utgangspunkt i en 8-ukers streng diett etterfulgt av ett års oppfølging med sikte på å stabilisere vekten. Problemsstillinger i studie er:

- Er de ulike oppfølgingsprogrammene like gode?
- Hvordan påvirkes hormonene som regulerer appetitt i diettens aktive fase?
- Hvordan påvirker tarmens bakterieflora kroppsvekten etter en vektreduksjons fase?
- Hvordan påvirker fedme og vektreduksjon immunologiske og homeostatiske mekanismer i relasjon til aterosklerose
- Hvordan påvirker vekttap beintetthet og markører for beinomsetning

Det er St. Olavs Hospital som er ansvarlig for studien.

Hva innebærer studien?
Studien er delt i to faser. Den første fasen er en 8-ukers diettperioden, etterfulgt av 4 uker med vekt vedlikehold, som vil være den samme for alle som deltar. En slik lavkalorikur kan gi noen bivirkninger (beskrevet senere). Når dietten er overstått vil du gå over i studiens andre fase som dreier seg om oppfølging med sikte på å opprettholde vekttapet.

Halvparten av pasientene vil få oppfølging i Fedmepoliklinikken ved St. Olavs Hospital, mens den andre halvparten får oppfølging ved Røros Rehabilitering. Hvilken oppfølging du får er avhengig av hvor det er kapasitet for oppfølging på tidspunktet du inkluderes i studien. Oppfølgingen varer i ett år og du kan lese mer om den på neste side.

Undersøkelsene er de samme uansett hvilket oppfølgingsprogram du følger og innebærer blodprøver, blodtrykk, målinger av energibehov, kroppsmasse, DXA (dual X-ray absorbiometry) skanning til å måle beintetthet og oksygenopptak, samt ulike former for spørreskjema.

Mulige fordeler, ulemper og bivirkninger
Fordelen med studiedeltakelse kan være at man går ned i vekt og oppnår bedre helse uten kirurgisk behandling. Deltakelse kan også gjøre at du blir bedre kjent med mekanismene i din egen kropp som påvirker appetitten. Dessuten vil du spare kostnader til mat i studiens diettfase (diettproduktene får du gratis ved sykehuset). Behandlingen anses ikke som risikabel. Undersøkelsene innebærer noen blodprøver.

Lavkalorikurer kan ha flere bivirkninger. Omfanget av disse varierer fra person til person og kan være enten helt fraværende eller temmelig plagsomme. Bivirkninger er forbigående. Rapporterte bivirkninger er:

- slapphet
- svimmelhet
- forstoppelse
- hårtap
- tørr hud
- neglene kan bli sprøere
- kvalme

- diaré
- forstyrret menstruasjonssyklus
- økt kuldefornemmelse

Beintetthet i rygg og hofte vil måles med DXA (dual X-ray absorbiometry) skanning. Dette er en smertefri og rask lavdose røntgenundersøkelse. Mengden stråling ved DXA er mindre enn en tiendedel av dose ved et standard røntgenbilde av brystet, og mindre enn mengden av naturlig stråling du blir utsatt for.

Hva skjer med prøvene og informasjonen om deg?
Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Noen helseopplysninger vil også lagres i din pasientjournal, og disse vil være knyttet til ditt personnummer.

Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte studiekoordinator Hege Bjøru, telefon 40 87 34 24.

Studien er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk REK Midt-Norge.

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer*.
Ytterligere informasjon om personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring*.

Samtykkeerklæring følger etter kapittel B.

Kapittel A – Utdypende forklaring av hva studien innebærer

Kriterier for deltakelse
De som kan delta i denne studien må

1. ha BMI mellom 35 og 45 kg/m^2,
2. være mellom 18 og 65 år,
3. ha et ønske om å gå ned i vekt ved hjelp av diett,
4. være relativt vektstabil siste tre måneder

Kvinner må dessuten enten være over menstruerende alder eller benytte p-piller.

Mange kan ha forsøkt dietter tidligere og du bør derfor tenke deg godt om hvorvidt dette er en behandling som er verdt å forsøke igjen. Hvis dette føles galt, så bør du ikke ta del i studien.

Bakgrunn for studien
Lavkaloridietter (< 800 kcal/dag) er en relativt sikker metode for å gå ned i vekt og gir også et raskt vekttap. Slike dietter kan gi vekttap i størrelse 10-15 % og med det også bedring i overvektsrelaterte sykdommer og risikofaktorer. Langtidseffektene er imidlertid usikre og særlige utfordringer er knyttet til opprettholdelse av vekttap på sikt. Det er behov for mer kunnskap om diettens vedlikeholdsfase, spesielt knyttet til tidspunktet man går over fra diettprodukter til mer normal, energiredusert kost.

Hovedhensikt med denne studien er å sammenligne opprettholdelse av vekttap etter 8-ukers lavkaloridiett hos pasienter som deltar i to ulike oppfølgingsprogram. Oppfølgingen varer i ett år.

Vi vil også se nærmere på hvordan den hormonelle appetittreguleringen endres i diettens aktive fase. Appetitten er et komplisert samspill av blant annet hormoner som både stimulerer og reduserer matlysten og vi vil følge utviklingen i disse i løpet av de ukene dietten varer. Det er hittil gjort lite forskning på dette.

Undersøkelser
Som del av studien vil du gjennomgå ulike undersøkelser.

- Veiing og kroppsmassemåling
- Blodtrykksmåling
- Blodprøver
    - Måling av appetitthormoner og ketone kropper (72ml)
    - Testing for kjente gener som disponerer for fedme (3ml)
    - Måling av immun og homeostatiske mekanismer (25ml)
    - Måling av markører for beindannelse og beinnedbrytning (3ml)
- Indirekte kalorimetri (måling av energibehov)
- Måling av oksygenopptak
- Spørreskjema
- Avføringsprøver
- Måling av beintetthet i hofte og ryggrad med DXA (dual X-ray absorbiometry) skanning

Undersøkelsene finner sted ved studiens start, ved avslutning av dietten (uke 9), etter fire uker av vekt holdning (uke 12) og ved avslutning av oppfølgingen (etter ett år).

Tidsskjema for intervensjonperioden (12 uker) - felles for alle
Du vil få utdelt et variert utvalg av diettprodukter (milkshakes, smoothies, supper) tilsvarende et daglig energiinntak på 550 kcal (kvinner) og 660 kcal (menn). Du skal utelukkende spise disse produktene

mens du er i diettens aktive fase (8 uker) (standardisert for alle), men du oppfordres til å drikke rikelig (minst 2,5 liter) vann og evt kalorifri drikke i tillegg. Du vil så få time hos en sykepleier i Fedmepoliklinikken hver uke. Kostdagbok, veiing og urinprøver er del av denne fasen og bivirkninger rapporteres systematisk. I studieuke 9 får du time hos klinisk ernæringsfysiolog som vil foreskrive en ny diett av normalkost som du skal følge i året som kommer med sikte på å opprettholde vekttapet. Overgangen fra diettprodukter til normalkost skjer gradvis i løpet av studieuke 9 og 10.

Tidsskjema for deltakere ved Røros Rehabilitering (1 år) - halvparten av deltakerne
For de som trekkes ut til å delta på Røros Rehabilitering, innebærer deltakelse tre opphold ved Røros. Hvert opphold varer i tre uker og gjøres unna i løpet av ett år. Oppholdene innebærer mye fysisk aktivitet, oppfølging av helsepersonell både individuelt og i grupper, samt matlaging i fellesskap. Mer informasjon og tidsplan for oppholdene vil bli distribuert senere.

Tidsskjema for deltakere ved Fedmepoliklinikken (1 år) - halvparten av deltakerne
For de som trekkes ut til å delta i Obesitaspoliklinikkens program, innebærer det en individuell konsultasjon hos klinisk ernæringsfysiolog og senere gruppemøter med ulike helsepersonell. Gruppemøtene finner sted 3, 6, 9 og 12 mnd etter dietten og fokuserer mye på ernæring og fysisk aktivitet.

Studiedeltakerens ansvar
Det er studiedeltakerens ansvar å møte til avtalt tid. For de som deltar ved Røros Rehabilitering, må de påregne å være der gjennom hele treukersperiodene.

Kompensasjon og egenandel
Det gies ingen premiering for å delta i studien, men du vil få diettproduktene i diettens aktive fase gratis. Det er viktig å standardisere dietten slik at alle spiser det samme.

For deltakere ved Røros Rehabilitering vil fastlegen gi sykmelding for perioden oppholdene varer. NAV innvilger i de aller fleste kommunenes tilfelle også fritak for arbeidsgiverperioden, men det er også noen kommuner som ikke gjør dette pr i dag.

For deltakere ved Røros Rehabilitering vil det også tilkomme egenandel. Denne dekker behandling, kost og losji og betales inntil man når beløpsgrensen for Frikort 2. (Beløpsgrense fastsettes av myndighetene fra år til år.)

Kapittel B – Personvern, biobank, økonomi og forsikring

Personvern
Ulike opplysninger vil registreres om deg som del av dette prosjektet. Prøvesvar og innledende screeningnotat vil legges i din pasientjournal og er derfor personidentifiserbart. Opplysninger på bakgrunn av testene du gjennomgår og intervjuet vil lagres på sykehusets server og vil være avidentifiserte så lenge studien pågår (det vil si at et unikt ID-nummer erstatter navnet ditt). Kodenøkkelen som knytter navn til nummer makuleres når studien er slutt, slik at data da anonymiseres. Alle som jobber med data fra studien har taushetsplikt.

Vi vil benytte et internettbasert system for å samle spørreskjemadata. Dette betinger at du har tilgang til en datamaskin eller smartphone. Rapporteringssystemet krypterer dine svar slik at det ivaretar kravene til personvern.

St. Olavs Hospital ved administrerende direktør er databehandlingsansvarlig.

Biobank
Blodprøvene for analyser av appetitthormoner og mulige fedmegener som blir tatt vil bli lagret i en forskningsbiobank ved St. Olavs Hospital. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Overlege Bård Kulseng er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi
Studien finansieres over driften ved St. Olavs Hospital og Røros Rehabilitering. Diettproduktene for deltakerne er gitt av produsenten.

Forsikring
Studiedeltakerne omfattes av Norsk pasientskadeforsikring, jf. pasientskadelovens §1.

Informasjon om utfallet av studien
Publikasjoner på bakgrunn av studien vil bli lagt ut på vår hjemmeside, www.stolav.no/overvekt .

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Forespørsel om deltakelse i *delstudie* knyttet til studien

Hvordan holde vekten etter diettindusert vekttap?

Bakgrunn og hensikt
Informasjonen du har lest for hovedstudien *Hvordan holde vekten etter diettindusert vekttap* gjelder også for denne studien. Bakgrunn for et eget informasjonsskriv for denne delstudien er at den innebærer tre ekstra måletidspunkt . Prøvene er de samme som de som blir tatt i hovedstudien.

Bakgrunn for hyppigere prøver er ønsket om å tidfeste mer presist hvordan kroppens kompensatoriske mekanismer mot å gå ned i vekt opptrer. Med andre ord, på hvilket tidspunkt de setter inn sterkest, og når de eventuelt avtar.

Hva innebærer delstudien?
Delstudien innebærer ekstra prøver på dag 3 etter studiestart, samt i det du har tapt henholdsvis 5 og 10 % av vekten din. De to siste prøvene er derfor ikke mulig for oss å tidfeste på forhånd.

Kroppssammensetning ville også gjøres med bioimpedance. Ut fra det, dette er samme type prøver som vi ellers tar av deg i hovedstudien.

Frivillig deltakelse
Det er frivillig å delta i delstudien. Du kan velge å si nei til denne delstudien, men samtidig være med i hovedstudien.

Samtykke til deltakelse i delstudien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)