Sex differences in diet-induced regulation of non-exercise activity thermogenesis in overweight and obese adults

A thesis submitted for the degree of Masters by Research (MbR)

by

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Declaration

Candidate's declarations:

I, Matevz Arcon, hereby certify that this thesis submitted in partial fulfilment of the requirements for the award of Masters by Research (MbR), Abertay University, is wholly my own work unless otherwise referenced or acknowledged. This work has not been submitted for any other qualification at any other academic institution.

Signed [candidates signature]…….
Date……..

Supervisor's declaration:

I, [insert Principal Supervisors name] hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of [XXXXXXX] in Abertay University and that the candidate is qualified to submit this thesis in application for that degree.

Signed [Principal Supervisors signature]……...
Date……..

Certificate of Approval

I certify that this is a true and accurate version of the thesis approved by the examiners, and that all relevant ordinance regulations have been fulfilled.

Supervisor……..…….
Date……..

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Dedication

(if required)

Abstract

Introduction: Reductions in physical activity energy expenditure (PAEE) and basal metabolic rate (BMR) have been proposed as factors that may hinder diet-induced body mass loss. Although diet-mediated changes in PAEE and BMR are subject to large inter-individual variability, research investigating the impact of sex on diet-induced modulation of PAEE and BMR is lacking. Therefore, this study examined the effect of a diet-induced energy restriction on PAEE and BMR in non-exercising individuals with overweight and obesity. **Methods**: Eleven women (Age: 25 ± 7 yr; BMI: 29.7 ± 4.2 kg/m^2) and eight men (Age 29.6 ± 4.0 yr; BMI: 29.7 ± 4.0 kg/m^2) completed a 29-day investigation. Assessment of PA (PAEE and step count), BMR, body mass, systolic blood pressure (SBP), diastolic blood pressure (DBP) and fasting blood glucose (BG) occurred on days 1, 8, 15, 22 and 29. Between day 15 and day 22, participants consumed a liquid diet formula equivalent to 50% of their total daily energy expenditure. The effects of time, sex and their interaction on all variables were assessed through a two-way mixed model ANOVA. **Results:** Both men and women achieved a modest 3% body mass loss at the end of the intervention week. An effect of time was detected for body mass ($p<0.001$), BMI ($p<0.001$), body fat % ($p=0.001$), SBP ($p=0.007$), DBP ($p=0.033$) and BG ($p<0.001$). There was a time and sex interaction in both males and females for body mass ($p=0.002$), BMI ($p=0.002$) and body fat % ($p=0.043$). Sex differences were only present for body fat % ($p=0.001$) and BMR ($p<0.001$). No main or interaction effects were present for PAEE and step count. **Conclusion:** A 7-day diet-induced energy restriction of 50% may be a viable short-term strategy to produce initial reductions in body mass and body fat %, and improvements in fasting blood glucose and resting blood pressure with no compensatory changes on PA.

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Definitions

BMR – basal metabolic rate

BG – fasting blood glucose

BF – body fat %

DBP – diastolic blood pressure

EAT – exercise activity thermogenesis

EE – energy expenditure

EI – energy intake

LED – low-energy diet

LBM – lean body mass

NEAT – non-exercise activity thermogenesis

PAEE – physical activity energy expenditure

PP – pulse pressure

SBP – systolic blood pressure

TDEE – total daily energy expenditure

TEF – thermic effect of food

VLED – very-low energy diet

1. Introduction

Obesity is a complex and multifactorial disease characterised by abnormal or excess body fatness which is associated with several pathological conditions such as hypertension, type 2 diabetes and certain forms of cancer (Hruby and Hu, 2014). In addition, the increasing worldwide prevalence of obesity is now recognised to be a major economic burden to national health care systems due to its close association with other comorbidities (Ng et al. 2014). Although the aetiology of obesity is not entirely understood, genetic and environmental factors contribute considerably to the increasing incidence of obesity in Western society (Castillo et al. 2017; Hall 2017). For example, genetic differences account for 50-70% of the divergence in body mass within the population (Locke et al. 2015). However, because our genome has remained relatively unchanged in the last few decades (Castillo et al. 2017), it has been suggested that modernisation has led to the creation of an obesogenic environment which favours physical inactivity and consumption of energy dense food (Hall 2017). Put simply, body mass gain and obesity are caused by a prolonged state of positive energy balance, where energy intake (EI) exceeds energy expenditure (EE) (Romieu et al. 2017). By contrast, to induce body mass loss, a state of negative energy balance (i.e. EE exceeds EI) must be elicited over weeks or even months (Hall et al. 2012). Importantly, within people with overweight or obesity populations a clinically significant 5% reduction in body mass is known to elicit positive effects such as reduced hypertension, improved blood glucose control and lower risk of cardiovascular disease and certain cancers (Rueda-Clausen, Ogunleye and Sharma 2015).

1.1 Formula based diets and changes in body composition

At energy balance, variations in body mass are largely attributable to changes in water content of fat-free mass (Bhutani et al. 2017). By contrast, energy restriction (ER) results in further changes in body stores which are largely responsible for the rapid decrease in body mass (Yoo 2018). For example, very-low energy diets (VLED) and low energy diets (LED) are formula based liquid diets that provide less than 800kcal/day and 800-1200kcal/day, respectively, and are enriched with micronutrients, essential fats and fibre (Lean 2011). These

types of diets are believed to be efficacious as a result of several interconnected mechanisms. First, they involve the substitution of conventional/solid food with defined portions, therefore, a higher degree of compliance can be ensured for those in which portion control is problematic (Harper et al. 2018). Second, in comparison to solid/conventional foods, greater energy deficits can be induced using enriched formulas whilst avoiding potential nutrient deficiencies (Lean 2011).

During the first phase of ER (up to 28 days), the rapid increase of insulin sensitivity coupled with decreased hepatic glucose output induces natriuresis and reduction in extracellular water, which results in a rapid decrease of body mass (Yoo 2018). Noteworthy, during this initial phase of body mass loss, a greater decrease of fat-free mass than fat mass is found (Müller et al. 2015). This loss of body protein occurs due to negative nitrogen balance, but initially results more prominently from gastrointestinal tract and liver proteins involved in nutrient processing (Hopkins and Blundell 2016; Carbone, McClung and Pasiakos 2012). Subsequently, proteolysis occurs in the skeletal muscle and other visceral organs (Yoo 2018). Ultimately, the combined effect of glycogen, body protein and fluid loss largely contribute to the rapid reduction in body mass which is often observed in the first phase of VLED or LED (Bhutani et al. 2017).

1.2 Energy restriction and metabolic adaptations to body mass loss

Total daily energy expenditure (TDEE) is comprised of several components namely basal metabolic rate (BMR), thermic effect of food (TEF) and physical activity energy expenditure (PAEE) (Müller et al. 2016). In inactive individuals, BMR accounts for 60-70% of TDEE and can be defined as the EE required for homeostatic processes (Villablanca et al. 2015), whereas TEF is the EE related to the digestion and storage of food which accounts for approximately 10% of TDEE (Hall et al. 2012). PAEE can be furtherly divided into two subgroups namely exercise activity thermogenesis (EAT) and non-exercise activity thermogenesis (NEAT). The former, EAT, represents the energy expenditure yielded from volitional exercise and is estimated to account for between 15-30% of the TDEE (Westerterp 2017). By contrast, NEAT is the energy cost of non-volitional exercise related activities such as spontaneous physical activity (SPA), but also other habitual activities like maintenance of body posture, ambulation and

fidgeting, and can account for up to 50% of the TDEE in highly-active individuals (Westerterp 2016; Levine 2004; Levine 2002).

When EAT is not accounted for due to lack of volitional exercise, NEAT accounts entirely for the changes in PAEE. Often, however, interventions adopting diet-induced ER alone report body mass losses 12-44% lower than predicted (Yoo 2018). This is because energy balance is a non-linear (dynamic) process, and an attempt to reduce EI often results in an unintended change in one or more components of TDEE to prevent starvation, and potentially death, thus ultimately altering the rate of body mass loss (Romieu et al. 2017). Indeed, when ER is induced, the initial body mass loss is accompanied by metabolic and behavioural changes that manifest primarily in BMR and PAEE (Piaggi 2019). For example, Doucet, Pomerleau and Harper (2004) showed that 4 days of diet-induced ER (800kcal/day) caused a decrease in BMR (-43kcal/day) in 15 overweight men. Similar findings were reported in a more recent study on obese men and women by Nymo et al. (2018), where similar reductions in BMR (-48kcal/day) were observed after 3 days of ER using a LED which provided 670kcal and 550kcal/day for men and women, respectively.

Disproportionate changes have been observed when BMR was separately analysed for biological sex. In fact, after adjusting for fat-free mass (FFM) and fat mass (FM), 3 days of ER produced a 24kcal/day greater decrease of BMR in women than in men. Moreover, an in-patient study on non-obese men, showed a significant decrease in BMR (-70kcal/day) after just 3 days of 50% diet-induced ER (Müller et al. 2015). They also observed further decreases in BMR after 7 days of sustained ER. Nevertheless, this drop in BMR started to plateau after 21-days, when a 5% reduction in body mass was achieved. Potentially, the greater drop in BMR at the 3-day mark could be explained by study design, which involved 7 days of overfeeding at 50% above maintenance calories prior to dieting. Interestingly, a systematic review by Schwartz and Doucet (2010) examined changes in BMR during diet-induced ER. Although they reported similar decreases in BMR for both sexes, their findings might not be applicable to the first phase of ER as only trials that lasted more than 2 weeks were included in their analysis.

In addition to its effects on BMR, diet-induced ER also appears to have an impact on PAEE. For example, de Groot et al. (1989) showed that 4 weeks of

50% ER though diet resulted in a 4-11% reduction in BMR and was accompanied by concomitant decreases in PAEE, which almost entirely explained the collective drop in TDEE (-274kcal/day). Similarly, Heyman et al. (1992) induced a 20% ER via diet, which after 20 days resulted in reduced BMR and PAEE of 99kcal/day and 198kcal/day, respectively. Comparable findings were observed in longer term trials. For example, Camps, Verhoef and Westerterp (2013) reported a 220kcal/day decrease in PAEE after 8 weeks of LED (500kcal/day) in obese men and women. Nonetheless, PAEE returned to baseline when energy balance was re-established.

In a randomised control trial of 105 adults, Martin et al. (1985) used doubly-labelled water and accelerometery, a gold-standard combination technique, to assess the effects of 10% and 30% ER on PAEE. Although biological sex and age were not found to be associated with the effect of ER on PAEE, a 200kcal/day reduction in PAEE was observed after 12 weeks of 30% ER. Similar findings were reported by Weigle et al. (1988) where they showed that body mass loss elicited a 29% reduction in TDEE. Although BMR accounted for only 8% of the total reduction, PAEE explained the remaining 21% drop in TDEE. Moreover, in their 'weight clamping' experiment, Liebel, Rosenbaum and Hirsch (1995) observed a 15% decrease in TDEE with underfeeding in obese women, and participants were not taking part in any volitional exercise, changes in TDEE were believed to be largely attributed to changes in PAEE. Similarly, Racette and colleagues (1995) showed reductions in BMR and PAEE which accounted for a 350kcal/day drop of TDEE after 12 weeks of diet-induced ER.

Even when compared to exercise, diet-induced ER provoked the greatest compensation in PAEE. In a study where ER was induced either via diet alone, diet plus low-intensity exercise or diet plus moderate intensity exercise, the greatest compensation in PAEE (-30%) was observed in the diet-only group (Wang et al. 2008). Moreover, the magnitude of body mass regain in this study was greater in participants who experienced larger reductions in TDEE during ER. Decreases in PAEE were also reported in a study by Martin et al. (2007), where 12 weeks of 25% diet-induced ER and LED (890kcal/day) yielded 12% and 20% drops in PAEE, respectively. Taken together, components of TDEE, particularly BMR and PAEE, change in response to perturbations of energy

balance. However, these changes were more pronounced during the first phase of ER (Yoo 2018).

Adaptations to ER, dictated by hormonal and metabolic changes, result in reduced sympathetic nervous activity tone, decreased leptin and triiodothyronine (T3) production and increased skeletal muscle efficiency, which in turn limit body mass loss and predispose for future body mass regain (Rosenbaum and Leibel 2010). The magnitude of these hormonal and metabolic adaptations seems to depend on the phenotypic profile of an individual (Piaggi 2019). For example, ‘spendthrift’ phenotypes show greater increases in TDEE during overfeeding and have smaller reductions in TDEE while fasting. By contrast, ‘thrifty’ phenotypes are more resilient to increases in TDEE during overfeeding but are more likely to reduce TDEE in response to fasting (Piaggi 2019). This is very significant, as an increasing body of evidence suggests that faster rates of initial body mass loss, and hence higher sustained EE, is positively associated with successful body mass loss and long-term maintenance (Nackers, Ross and Perri 2013). Moreover, men have been shown to display greater reductions in body mass than women, even after adjustment for differences in body mass percentage (Fogelholm et al. 2017). Therefore, this may suggest a greater prevalence of thriftiness in women than in men. To the knowledge of the investigator, there is a paucity of research examining the sex-mediated differences of PAEE and BMR in response to diet-induced ER (Yoo 2018).

1.3 Aims and hypothesis

The aim of this study was to investigate whether 7 days of diet-induced ER had an effect on PAEE, and BMR in overweight and obese men and women. It was hypothesised that acute diet-induced ER would result in lowered BMR and PAEE, and that this compensatory response would be relatively greater in women than in men.

2. Methods

2.1 Experimental design

This study used a time series design, with an initial 2-week control period to investigate the effect of 1-week of diet-induced ER (50% of TDEE) on PAEE and BMR in overweight and obese men and women. Participants visited the institution's laboratory on five separate occasions over four weeks (i.e. 2-week control period before the intervention, 1-week of intervention and 1-week after the intervention). The five visits to the lab occurred on study days 1, 8, 15, 22 and 29 for assessment of body composition, fasting blood glucose, blood pressure and BMR. The 7-day time period between each visit to the laboratory was selected to match the time frame of the liquid-based diet intervention and the usual time used to assess physical activity in the free-living (Levine 2002). Consequently, this study used hip and wrist-worn tri-axial accelerometers to determine total amount of steps and PAEE that were measured in 7-day blocks between study days 1 and 29 and 8 and 29, respectively (see Figure 1).

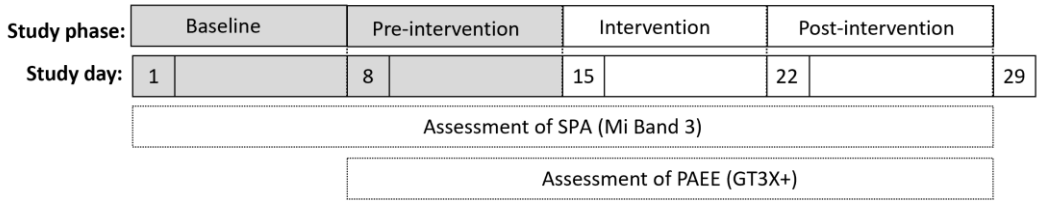

Figure 1: Study timeline overview. Grey shaded area represents the control period used to assess PAEE Study days 1, 8 and 15 were used to determine baseline values before the intervention.

2.2 Participants

A total of 25 adults with overweight or obesity volunteered to participate in the study. Six participants (4 men and 2 women) withdrew from the study prior to completion. Two men dropped out of the study because they were unable to visit the laboratory on the designated assessment days. Two women and two men withdrew from the study due to nausea from the liquid diet. Therefore, a total of 11 women and 8 men completed the study and were included in the data analysis.

The recruitment process was carried out via social media, word of mouth and leaflets, which were placed on notice boards of the Abertay University campus. Participants were eligible to participate if they satisfied the following criteria: 1) aged between 18 and 40 years; 2) body mass index (BMI) between 25 and 40 kg/m^2; 3) engage in ≤ 1 session of volitional exercise per week. The inclusion criteria were determined to limit potential confounding factors. Total daily energy expenditure (TDEE) and PAEE both increase during the first two decades of life, however, plateau between 17 and 40 years of age and tend to decline after the age of 40 (Manini 2010). Second, there seems to be a divergent response in regulation of PAEE due to ERbetween normal weight and overweight/obese, but not between overweight and obese (Ravussin 2005). Furthermore, because negative energy balance can transiently alter BMR and PAEE, volunteers who had attempted volitional body mass loss via reduction of EI and/or increased weekly bouts of structured exercise (more than one bout of exercise per week) one month prior to taking part in the study were excluded. This was verbally verified on the first laboratory visit via self-reporting.

Although the target sample size (10 men and 10 women), which reflected group sizes in previous trials, was not achieved due to drop-outs, the final sample size was set based upon the logistical limitations of the research project, and comparable to other trials investigating changes in PAEE in the free living (Leibel, Rosenbaum and Hirsch 1995; Levine 1999). Prior to commencing the study, all participants were fully informed both verbally and in writing about the study and then given a period of 7 days to decide whether to participate in the study. A written informed consent and completed a Physical Activity Readiness Questionnaire (PARQ) to ensure there were no underlying health issues. The study was approved by the Abertay University School of Health and Social Sciences Ethics Committee (EMS1014) and all experimental procedures were carried out in accordance with the World Medical Association Declaration of Helsinki (Emanuel 2011). Participants' personal information was kept confined within the study by using coded aliases and password protected files to ensure compliance with GDPR policies (Voigt and Von dem Bussche, 2017).

2.3 Preparation for laboratory visits

To ensure consistency between measurements, participants visited the laboratory between 7am – 9am following an overnight fast. The overnight fast consisted of avoiding consumption of food, caffeine, nicotine and any caloric beverage 12 hours prior to testing but allowed water *ad libitum* (Lopes, Chaia and de Brito 2013). Furthermore, participants were asked to avoid taking part in vigorous exercise 24 hours prior to testing to ensure accurate measurement of BMR (Edwadson and Gorely, 2010). Upon arrival, participants were asked to void prior to commencing testing (Fernández-Verdejo, Aguirre and Galgani 2019). Compliance was verified by the investigator on the testing day by participants' verbal self-reporting.

These criteria were required to gather an accurate analysis of BMR, body composition, blood pressure and fasting blood glucose (Hunt and Hellwig 2018; Kesavadev et al. 2014). For instance, analysis of BMR via indirect calorimetry is subject to change in reflection to nutritional status (Fernández-Verdejo, Aguirre and Galgani 2019). In fact, the impact which TEF has on BMR is largely variable and depends on the magnitude of the EI macronutrient ratio (Westerterp 2004).

Other potential confounding variables such as caffeine and nicotine intake can also affect BMR and blood pressure via augmented catecholamine release, which may translate in raised heart rate and increased fatty acid mobilisation (Lafontan and Langin 2009; Versmold 1991). Vigorous exercise performed 24-hours prior to analysis can also transiently increase BMR. In fact, BMR has been shown to increase transiently by up to approximately 300kcal/day following a bout of resistance exercise training mainly due changes in body temperature, lactate clearance and muscle protein synthesis (Laforgia, Withers and Gore 2006). This acute exercise-induced increase in BMR is due to excess post-exercise oxygen consumption (EPOC) and is mainly driven by intensity of exercise (Borsheim and Bahr 2003).

2.4 Anthropometry

During day 1 only, stature was measured to the nearest 0.1 cm using a stadiometer (SECA 216, SECA, Hamburg, Germany). Participants stood without shoes with their heels together, back against the plastic cover and arms by their side. The head was erect and in the Frankfort horizontal plane) (Bryant and Green

2012). The stadiometer moveable head plate was then lowered to the top of the head at which point the participant was asked to breathe in deeply. The measurement was recorded just before the participant exhaled (Bryant and Green 2012).

The remaining anthropometric variables were measured during all laboratory visits. To ensure an accurate and consistent reading of body composition via the leg-to-leg bioelectrical impedance scale (SC-330ST, Tanita Europe, Amsterdam, the Netherlands), participants were asked to void and then remove excess clothing (i.e. jacket, jumper) just prior to recording body mass (kg) and body fat (%) (Fernández-Verdejo, Aguirre and Galgani 2019). The participant stepped barefoot on the analyser's footpads and minimised movement until analysis was completed. In line with the Tanita guidelines for body type assessment, which due to the weekly activity level deemed the participants as non-athletic, body type analysis was set to standard adult mode (Franco-Villoria et al. 2016). Participants' chronological age and stature were entered and output values for body mass (kg) and body fat (%) measured to the nearest 0.1 respective unit. Ultimately, body fat (%) was determined using two electrodes located on the analyser's footpads which send an imperceptible electrical current through the body (50 kHz alternating current of 800μA between electrodes). The impedance value (Z) reflects the resistance and reactance which the electrical signal encounters when passing through the body; and because the ionised fluid in lean body mass acts as a conductor, differentiation between lean body mass and fat mass can be determined (Lemos and Gallagher 2017). In addition, accurate analysis of body composition using a two-compartment model technique (i.e. bioelectrical impedance) is highly dependent on changes in hydration level and nutrition status (glycogen content and presence of food in the digestive system) (Lemos and Gallagher 2017). This is because bioelectrical impedance predicts body composition based on the electrically conductive proprieties of fat free mass and fat mass. The latter is less conductive, however, due to its higher content of water and electrolytes, fat free mass is more conductive which in turn allows differentiation between tissues (Vasold et al. 2019).

2.5 Blood pressure

Resting blood pressure as systolic blood pressure, diastolic blood pressure and pulse pressure, were recorded twice on the participant's non-dominant arm using an automated oscillomettric blood pressure monitor (DSK-1031, Nissei Healthcare, Henfield, UK). Prior to commencing the test, all clothing covering the location of cuff placement was removed to prevent blood flow restriction. The first blood pressure measurement was taken after the participant was seated comfortably for 5 min, with the back supported, feet on the floor, arm supported in the horizontal position on a table, with the middle of the blood pressure cuff on the participant's upper arm at the heart level. Both the participant and the investigator remained silent during the entire procedure. The second measurement was taken immediately after the first was completed, and the measurement with the lower pulse pressure was then used for analysis (Hunt and Hellwig 2018).

2.6 Fasting blood glucose

Fasting blood glucose was determined via fingertip blood samples (Freestyle Lite, Abbott Diabetes Care Inc., Alameda, USA). After the puncture site (tip of the index finger) had been wiped with alcohol swabs, the skin was punctured using an Accu-check single use lancet (Roche Diagnostics, UK) and pressure applied to the finger to draw the blood (Pickering and Marsden 2014). As recommended by the World Health Organisation (Dhingra 2010), the initial drop was discarded, and the second drop was taken for analysis.

2.7 Basal metabolic rate

In this study, BMR was determined by breath-by-breath analysis using an open-circuit indirect calorimetry (MetaMax 3B, Cortex Biophysik, Leipzig, Germany) (Compher et al. 2006). The participant had to rest in a comfortable supine position, minimise movement and remain silent in quiet environment with soft lighting for 30 min. A silicone mask was fitted on the participant's face and tightened to avoid air leakage. Whilst the participant was resting, full calibration of the metabolic cart was carried out in accordance to the manufacturer's guidelines by the investigator. This comprised of volume calibration using a 3-litre

calibration syringe, pressure calibration with a digital barometer (Barometer GA690, Castle Group, UK) and gas calibration using a 1.2 litre bottle with 15% oxygen (O_2), 5% and carbon dioxide (CO_2), in nitrogen (N_2). After the metabolic cart had been calibrated, BMR was measured for 15-20 minutes and the average VO_2 and VCO_2 values from the last 10 minutes were used for analysis (Popp et al. 2016; Psota and Che 2013). This method was based on the systematic review by Compher et al. (2006) which determined the optimal conditions for obtaining reliable measures of BMR by indirect calorimetry. Due to its high prevalence in human studies, Weir's equation with dismissed protein oxidation was used to calculate BMR (Fernández-Verdejo, Aguirre and Galgani 2019).

BMR (kcal/day) = (3.941 x VO_2 (ml/min) + (1.106 x VCO_2 (ml/min)) x 1.44)(Popp et al. 2016)

2.8 Physical Activity Indices

Step count, was measured from day 1 to day 29 of the study using a tri-axial accelerometer (Mi Band 3, Xiaomi, Beijing, China) which had an embedded heart rate sensor, and was worn on the wrist of the dominant arm The first iteration of this activity tracker has been shown to be an accurate and valid alternative to more costly accelerometers which are already validated in clinical research (El-Amrawy and Nounou 2015). For this study, heart rate sampling frequency (Mi Band 3, Xiaomi, Beijing, China) was set to 60 s via the Android application Mi Fit (Xiaomi, Beijing, China).

To measure PAEE, participants wore a second tri-axial accelerometer (ActiGraph GT3X+, Florida, USA) on the right hip from study day 8 to study day 29 (Sasaki, John and Freedson 2011). Participants were advised to wear the accelerometers continuously except during their sleep and activities which would submerge the accelerometers in water. A day was considered valid only when the accelerometers were worn for at least 10 h between 0700 and 2300 (LeCheminant et al. 2017). In addition, a phase (week) was considered valid only when it was comprised of four or more valid days (Migueles et al. 2017). The ActiGraph accelerometer was set-up with 60 s sampling epochs which were collected at a 30 Hz sample rate, and the Freedson VM3 combination algorithm

was used to estimate PAEE from the vector magnitude counts per min of the three axis (Brown et al. 2017). PAEE data from each phase was averaged and presented as kcal/day. The method for the ActiGraph GT3X+ accelerometer was based on the systematic review by Migueles et al. (2017) which provided practical considerations such as optimal placement, sampling frequency, epoch length and day/week validity for adults.

2.9 Diet-induced energy restriction

The methods to assess TDEE require quantification of all its components, namely BMR, TEF and PAEE. To calculate participant's average pre-intervention TDEE, BMR and TEF from study days 1, 8 and 15 were averaged and summed with daily average of PAEE from the Pre-intervention phase. TEF was assumed as a generic 10% value of TDEE (Kinabo and Durnin 1990):

$$\mathrm{TEF(kcal/day)} = (\mathrm{BMR} + \mathrm{PAEE})\ \mathrm{x}\ 0.1$$

$$TDEE(kcal/day) = \mathrm{BMR} + \mathrm{TEF} + \mathrm{PAEE}$$

During the intervention period (study days 15 - 21), participants were given a 7-day supply of the formula-based liquid diet (Meal Replacement, MyProtein, UK) which was purchased by the investigator and provided a macronutrient breakdown of 38% protein, 38% carbohydrates, 15% fats and 9% fibre (Nymo et al. 2018). Participants were asked to use the formula-based liquid diet as their only source of energy intake which resulted in a 50% energy restriction in relation to participants' mean pre-intervention TDEE. The formula-based diet was weighed by the investigator using a commercially available kitchen scale (Salter, HoMedics Group Ltd, Kent, UK) and individually packaged to provide the exact energy value each day during the intervention period. No recommendation was given in regards to meal pattern or meal frequency. Participants could consume any very-low or non-caloric beverage such as black coffee, green tea or soft drinks with no added sugar (i.e. diet soda).

2.10 Study compliance survey

To gather insights regarding the rate of compliance to the study procedures, at the end of data collection an anonymous survey was sent to participants. The survey comprised of 4 closed questions which served to evaluate the dietary compliance to the liquid diet and participant's conditions on each testing day (See Appendix 2).

2.11 Statistical analyses

All statistical analyses were performed using the statistical package for the social sciences software for windows (SPSS 24.0, IBM, Chicago, IL, USA). All data were checked for normality using histograms and the Shapiro-Wilk test. Levene's and Mauchley's test were respectively used to check for homogeneity of variance and sphericity. When the latter was violated ($p \geq 0.05$) the Greenhouse Geiser correction was used (Field 2018). A two-way (Time x Sex) mixed model ANOVA was used to assess the effects of time, sex and their interaction on all variables during the control period (except PAEE assessed by ActiGraph which only included 1 week of control) as well as across all periods (i.e. control, intervention and post-intervention). Pairwise comparisons were performed using the Bonferroni correction (Jackson 2015). As BMI was not normally distributed, an adjusted rank-transformation was applied to these data (Leys and Schumann 2010). All data are presented as M±SD (95% confidence intervals: lower, upper) unless specified, and mean differences (MD) provided when significant main effects were found. Partial eta squared (η_p^2) effect sizes were interpreted as 0.01 small, 0.06 moderate and 0.14 as large whilst Cohen's effect size (*d*) was defined as 0.2 small, 0.5 moderate, 0.8 large. Significance level was set at $p < 0.05$.

3. Results

3.1 Control period

Of the 25 initially recruited volunteers, 8 men (age: 26 ± 4; BMI: 29.6±3.7) and 11 women (age: 25 ± 7; BMI: 29.7 ± 4.1) completed the study. The control period served to evaluate the consistency of the measurements and to compare the physical characteristics between men and women (Table 1).

The key findings are that there was a significant difference between men and women for body fat ($F(1,17) = 16.007$; MD =-10.833; 95% CI:-16.55 to -5.120; $p < 0.001$; $d = 2.0$) and BMR ($F(1,17) = 13.633$; MD =-347.594; CI: -546.139 to -148.959 ; $p < 0.002$; $d = 1.6$). In addition, there was a main effect of time on body fat ($F(2,34) = 4.674$; $p=0.027$; $\eta_p2=0.562$) between day 1 and day 8 of the study (MD: -0.618; p=0.004; CI:-0.194 to 1.043). There was a significant main effect of time on lean body mass ($F(2,34)=4.290$; $p=0.022$; $\eta_p2=0.201$) between day 8 and day 15 of the study (MD:0.477; p=0.038; CI: 0.23 to 0.930). Moreover, significance was detected for the interaction between time and sex for lean body mass ($F(2,34) = 1.134$; $p = 0.026$; $\eta_p2 = 0.194$). Lastly, significance in lean body mass was also detected for sex ($F(1,17) = 73.715$; $p < 0.001$; MD =16.212; CI: 12.228 to 20.196; $d = 3.8$). No other main or interaction effects were observed in the remaining variables during the control period.

Table 1. Anthropometric, metabolic and physiological measures during control period

	Day 1	Day 8	Day 15	Statistical significance of effect of:		
Variable	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Time**	**Sex**	**Time x Sex**
Body mass (kg)	**84.0 ± 13.3**	**83.8 ± 13.5**	**83.7 ± 13.5**	**0.373**	**0.061**	**0.504**
Men	*90.7 ± 13.5*	*90.6 ± 14.1*	*90.2 ± 14.3*	/	/	/
Women	*79 ± 11.4*	*78.9 ± 11.1*	*78.9 ± 11.1*	/	/	/
BMI (kg/m2)	**29.6 ± 3.7**	**29.6 ± 4.0**	**29.6 ± 3.9**	**0.912**	**0.726**	**0.390**
Men	*29.6 ± 3.7*	*29.6 ± 4.1*	*29.5 ± 4.2*	/	/	/
Women	*29.7 ± 4.1*	*29.6 ± 4.6*	*29.6 ± 3.9*	/	/	/
Body fat (%)	**33.6 *± 7.9***	**33.0 *± 8.0***	**33.4 *± 7.8***	**0.027***	**0.001***	**0.290**
Men	*27.4 ± 5.4*	*26.6 ± 5.7*	*27.3 ± 6*	/	/	/
Women	*38.1 ± 6.3*	*37.8 ± 6*	*37.9 ± 5.5*	/	/	/
LBM (kg)	**52.7 *± 9.1***	**53.1 *± 9.4***	***52.6 ± 8.9***	**0.022***	**<0.001***	**0.026***
Men	62.2 *± 5.7*	62.7 *± 5.9*	*61.8 ± 5.9*	/	/	/
Women	45.9 *± 2.1*	46.1 *± 2.3*	*46.1 ± 2.3*	/	/	/
BMR (kcal/24h)	**1489 *± 293***	**1429 *± 257***	***1476 ± 284***	**0.232**	**0.002***	**0.935**
Men	*1685 ± 317*	*1627 ± 235*	*1686 ± 237*	/	/	/
Women	*1347 ± 176*	*1285 ± 163*	*1323 ± 211*	/	/	/
BMR/kg(kcal/kg/24h)	**17.8 *± 2.0***	**17.1 *± 2.0***	***17.7 ± 2.4***	**0.245**	**0.081**	**0.807**
Men	*18.5 ± 0.9*	*18 ± 1.1*	*18.8 ± 1.4*	/	/	/
Women	*17.2 ± 2.5*	*16.5 ± 2.3*	*16.9 ± 2.8*	/	/	/
SBP (mm Hg)	**138 *± 23***	**135 *± 16***	**131 *± 18***	**0.068**	**0.108**	**0.252**
Men	*148 ± 16*	*140 ± 13*	*138 ± 21*	/	/	/
Women	*130 ± 25*	*132 ± 17*	*125 ± 15*	/	/	/
DBP (mm Hg)	**82 *± 14***	**81 *± 11***	**28 *± 13***	**0.323**	**0.675**	**0.594**
Men	*83 ± 9*	*80 ± 8*	*81 ± 18*	/	/	/
Women	*81 ± 17*	*81 ± 13*	*76 ± 9*	/	/	/
PP (mm Hg)	**71 *± 12***	**68 *± 8***	**69 *± 9***	**0.493**	**0.739**	**0.556**
Men	*71 ± 11*	*69 ± 8*	*71 ± 8*	/	/	/
Women	*71 ± 13*	*68 ± 8*	*67 ± 8*	/	/	/
BG (mmol/L)	**4.5 *± 0.4***	**4.6 *± 0.4***	**4.5 *± 0.5***	**0.584**	**0.108**	**0.493**
Men	*4.6 ± 0.5*	*4.7 ± 0.5*	*4.7 ± 0.5*	/	/	/
Women	*4.4 ± 0.3*	*4.6 ± 0.4*	*4.3 ± 0.4*	/	/	/

BMI = body mass index, BMR = basal metabolic rate, BMR/kg = basal metabolic rate per kilogram of body mass, SBP = systolic blood pressure, DBP = diastolic blood pressure, LBM = lean body mass PP = pulse pressure, BG = fasting blood glucose. p≤0.05.

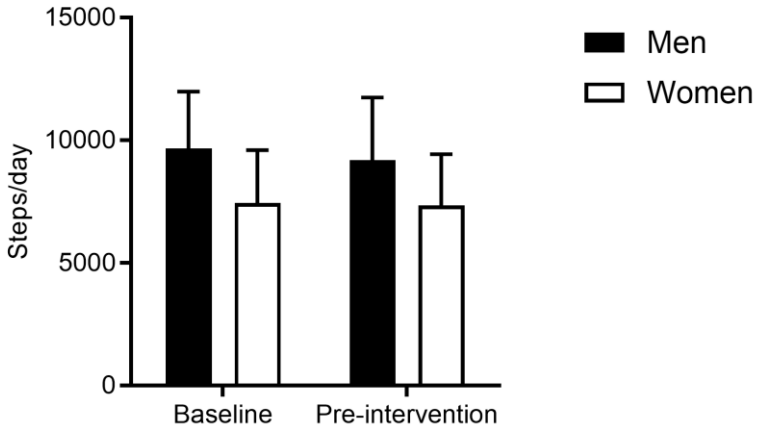

Figure 2. Step count during control period

Step count did not significantly change during the control period (F(1,15) = 0.376; p = 0.549; MD = 227; CI:-564 to 1019; η_p2 = 0.024). Moreover, no interaction between time and sex was detected for steps (F(2,34) = 0.407; p = 0.533; η_p2 = 0.026). Lastly, there was no significant difference in the number of steps between men (9656 ± 2332 steps count) and women (7434 ± 2157 step count) (F(1,15) = 2.985; p = 0.105; MD = 1984; CI:-463 to 4432; d = 1.2) at baseline.

3.2 Entire study period

3.2.1 Anthropometric and metabolic parameters with VLCD

Due to the VLCD, there was a significant main effect of time on body mass (F(2,34) = 60.686; p < 0.001; η_p2 = 0.781) which resulted in a 3% body mass loss in both men and women. Significance was reached between control and day 22 (MD = 2.855; p < 0.001; CI:2.190 to 3.520), control and day 29 (MD = 1.681; p < 0.001; CI: 0.997 to 2.366), as well as between day 22 and day 29 (MD= -1.174; p = 0.001; CI: -1.897 to -0.450). There was a significant interaction between time and sex on body mass (F(2,34) = 7.368; p = 0.002; η_p2 = 0.302), however, no sex difference was detected ((F(1,17) = 3.646; MD = 10.725; CI: -1.125 to 22.575; p < 0.073; d = 0.87). For BMI, a significant main effect of time (F(2,34), p<0.001, η_p2=0.827) was reached between day control and day 22 (MD = 0.992; CI: 0.818 to 1.167; p < 0.001), control and day 29 (MD = 0.562; CI: 0.333 to 0.792; p < 0.001) as well as between day 22 and day 29 (MD = -0.430; CI: -0.645 to -0.215;

$p < 0.001$). Furthermore, an interaction effect between time and sex was present for BMI ($F(2,34) = 7.684$; $p = 0.002$; $\eta_p2 = 0.827$). Significance for time was also achieved for body fat ($F(2,34) = 23.683$; $p < 0.001$, $\eta_p2 = 0.582$). This significance was achieved between control and day 22 (MD = 1.204; CI: 0.750 to 1.658; $p<0.001$), control and day 29 (MD = 1.111; $p = 0.001$; CI: 0.469 to 1.1754), but no significance was observed between day 22 and day 29 (MD: -0.093; $p = 1.000$; CI: -0.520 to 0.335). Moreover, significance on body fat % was achieved for the interaction between time and sex ($F(2,34) = 3.460$; $p = 0.043$; $\eta_p2 = 0.169$) and for main effect of sex on body fat % (MD = 11.386; $p = 0.001$; CI: 5.512 to 17.260; d=1.9). A significant main effect of time was also observed for lean body mass ($F(2,30) = 9.030$; $p = 0.001$; $\eta_p2 = 0.376$) between control and day 22 of the study (MD = 1.030; $p = 0.001$; CI: 0.445 to 1.615) as well as between day 22 and day 29 of the study (MD = -0.665; $p = 0.028$; CI: -1.265 to –0.065). Additionally, a sex difference for lean body mass was detected ($F(1,15) = 63.325$; MD = 15.053; $p < 0.001$; CI: 11.021 to 19.068; d = 4.0), which is typical biological distinction between men and women.

Significant main effect of sex was achieved for BMR (MD = 366; $p < 0.001$; CI: 191.111 to 540.831, d = 1.6). A main effect of time was also observed for SBP ($F(2,34) = 5.727$; $p = 0.007$; $\eta_p2 = 0.252$), however, only between control and day 22 (MD = 8.835; $p = 0.007$; CI: 2.275). A significant effect of time was also detected for DBP ($F(2,34) = 3.788$; $p = 0.033$; $\eta_p2 = 0.182$), but only between control and day 29 of the study (MD = 4.780; $p = 0.046$; CI: 0.065 and 9.496). For pulse pressure, significant effect for interaction between time and sex was achieved ($F(2,34) = 3.991$; $p = 0.028$; $\eta_p2 = 0.190$).

Lastly, a significant main effect of time of blood glucose ($F(2,34) = 11.755$; $p < 0.001$; $\eta_p2 = 0.409$) was reached between control and day 22 (MD = 0.358; $p = 0.002$: CI: 0.129 to 0.587) and day 22 and day 29 (MD = -0.586; $p = 0.001$; CI: -.0945 to -0.227). No other main or interaction effects were observed in the remaining variables during the control period.

Table 2. Anthropometric, metabolic and physiological measures during entire study period.

	Control	Day 22	Day 29	Statistical significance of effect of:		
Variable	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Time**	**Time x Sex**	**Sex**
Body mass (kg)	**83.9 ± 13.4**	**81.0 ± 12.9**	**82.3 ± 12.6**	**<0.001***	**0.073**	**0.002***
Men	*90.5 ± 14.0*	*87.4 ± 13.3*	*87.9 ± 13.6*	/	/	/
Women	*78.9 ± 11.2*	*76.4 ± 10.9*	*78.2 ± 10.6*	/	/	/
BMI (kg/m2)	**29.7 ± 4.0**	**28.6 ± 3.8**	**29.1 ± 3.7**	**<0.001***	**0.873**	**0.002***
Men	*29.6 ± 4.0*	*28.6 ± 4.0*	*28.7 ± 3.9*	/	/	/
Women	*29.7 ± 4.2*	*28.7 ± 4.0*	*29.4 ± 3.8*	/	/	/
Body fat (%)	**33.4 ± 7.9**	**32.2 ± 8.3**	**32.3 ± 8.5**	**0.001***	**0.001***	**0.043**
Men	*27.1 ± 5.7*	*25.6 ± 6.2*	*25.5 ± 6.7*	/	/	/
Women	*37.9 ± 5.9*	*37.0 ± 5.9*	*37.3 ± 5.8*	/	/	/
LBM (kg)	***52.3 ± 8.8***	***51.3 ± 8.6***	***52.1 ± 8.2***	**0.001***	**<0.001***	**0.125**
Men	*61.5 ± 5.8*	*60.3 ± 5.3*	*60.6 ± 5.1*	/	/	/
Women	*45.9 ± 2.3*	*45.1 ± 2.4*	*46.1 ± 2.5*	/	/	/
BMR (kcal/24h)	**1465 ± 264**	**1403 ± 265**	**1499 ± 312**	**0.164**	**<0.001***	**0.828**
Men	*1666 ± 263*	*1610 ± 206*	*1727 ± 259*	/	/	/
Women	*1318 ± 183*	*1252 ± 193*	*1334 ± 238*	/	/	/
BMR/kg(kcal/kg/24h)	**17.5 ± 1.9**	**17.4 ± 2.8**	**18.4 ± 3.7**	**0.211**	**0.067**	**0.573**
Men	*18.4 ± 1.1*	*18.6 ± 2.2*	*20.0 ± 3.7*	/	/	/
Women	*16.9 ± 2.5*	*16.6 ± 3.0*	*17.2 ± 3.4*	/	/	/
SBP (mm Hg)	**135 ± 17**	**126 ± 16**	**130 ± 14**	**0.007***	**0.086**	**0.737**
Men	*142 ± 17*	*133 ± 19*	*135 ± 15*	/	/	/
Women	*129 ± 19*	*120 ± 11*	*126 ± 14*	/	/	/
DBP (mm Hg)	**80 ± 11**	**75 ± 8**	**75 ± 10**	**0.033***	**0.225**	**0.183**
Men	*82 ± 12*	*81 ± 10*	*76 ± 13*	/	/	/
Women	*79 ± 13*	*71 ± 5*	*74 ± 8*	/	/	/
PP (mm Hg)	**69 ± 7**	**69 ± 9**	**66 ± 9.6**	**0.183**	**0.058**	**0.028***
Men	*70 ± 9*	*73 ± 6*	*72 ± 5*	/	/	/
Women	*69 ± 10*	*67 ± 10*	*61 ± 9*	/	/	/
BG (mmol/L)	**4.5 ± 0.3**	**4.1 ± 0.3**	**4.7 ± 0.6**	**<0.001***	**0.113**	**0.651**
Men	*4.7 ± 0.5*	*4.3 ± 0.4*	*5.0 ± 0.7*	/	/	/
Women	*4.4 ± 0.4*	*4.2 ± 0.3*	*4.6 ± 0.6*	/	/	/

BMI = body mass index, BMR = basal metabolic rate, BMR/kg = basal metabolic rate per kilogram of body mass, SBP = systolic blood pressure, DBP = diastolic blood pressure, LBM=lean body mass PP = pulse pressure, BG = fasting blood glucose. p≤0.05.

3.2.2 Measures of PA

Step count did not significantly change during the course of the study period ($F(2,34) = 1.089$; $p = 0.348$; $\eta_p2=0.060$). Moreover, no sex differences ($F(1,17) = 2.374$; $p = 0.142$; $d = 0.6$), or interaction between sex and time ($F(2,34) = 1.554$; $p = 0.226$; $\eta_p2 = 0.060$) were detected.

Similarly, PAEE did not reach significance for either time ($F(2,34) = 1.528$; $p = 0.231$; $\eta_p2=0.082$), sex($F(1,17) = 1.771$; $p = 0.201$; $d = 0.6$), or their interaction ($F(2,34) = 0.396$; $p = 0.676$; $\eta_p2 = 0.023$).

Table 3. PA measures during entire study period

	Pre-Intervention	Intervention	Follow-up	Statistical significance of effect of:		
Variable	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Time**	**Sex**	**Time x Sex**
Step count	**8212 ± 2411**	**7539 ± 2677**	**8246 ± 2985**	**0.348**	**0.142**	**0.226**
Men	*9423 ± 2435*	*8824 ± 2330*	*8629 ± 3322*	/	/	/
Women	*7332 ± 2070*	*6605 ± 2611*	*7968 ± 2848*	/	/	/
PAEE (kcal)	**387 ± 166**	**343 ± 192**	**370 ± 213**	**0.231**	**0.201**	**0.676**
Men	*453 ± 196*	*418 ± 241*	*420 ± 279*	/	/	/
Women	*339 ± 129*	*289 ± 134*	*333 ± 154*	/	/	/

PAEE=physical activity energy expenditure. p≤0.05.

3.2.3 Dietary compliance survey

Out of the 19 participants who successfully finished the study, 14 of them completed the study compliance survey. The remaining 5 participants did not respond to our invitation to take part in the survey. This survey served to gather insights regarding participants' compliance to the study procedures. The results showed that only one participant consumed and/or ingested energy dense foods or beverages, nicotine and/or alcohol prior to all 5 visits to the laboratory two participants reported that they were not able to consume all the given liquid diet formula. Two participants reported consumption of energy dense food and/or beverages which were not part of the liquid diet. It is noteworthy, however, that no statistical analysis was conducted.

4. Discussion

This study shows that a 7-day diet-induced ER of 50% of TDEE can reduce body mass and decrease fasting blood glucose levels and systolic blood pressure without eliciting detrimental changes in BMR, steps count and PAEE in both men and women.

Significant body mass losses were observed after 7 days of diet-induced ER in both men (-3.1kg) and women (-2.5kg). Moreover, after adjusting for total body mass, body mass losses were identical and accounted for 3% of total body mass in both groups. These findings are in line with previous studies investigating body mass change in response to a similar diet (Nymo et al. 2018; Muller et al. 2015; Muller et al. 2016). Interestingly, at follow-up (i.e. 7 days after *ad-libitum* energy intake resumed), body mass changes became more divergent between men and women. In men, body mass regain was only 0.5kg whereas women experienced a threefold greater body mass regain, which amounted to 1.8kg. Because PAEE and steps count did not significantly differ between the two groups, a divergent response in post-starvation hyperphagia may explain the greater body mass gain observed in women (Dulloo, Jacquet and Girardier 1997). Indeed, volitional body mass loss and its maintenance seem to result in augmented fasting and post-prandial appetite, which is mainly driven by alterations in hormonal regulators of appetite and can contribute to body mass gain (Perry and Wang 2012). Notably, in a recent review article investigating appetite changes following successful body mass loss, Hintze et al. (2017) suggested that although body mass loss results in increased fasting and post-prandial appetite, these changes do not seem to differ between men and women. Similar findings were reported in a short-term trial by Alajmi et al. (2016) where no divergent responses in appetite regulation between men and women were found after a single day of 800kcal ER. It is important to highlight, however, that Hintze et al. (2017) analysed only longer-term trials (3+ weeks of ER) whereas Alajmi et al. (2016) investigated a single day of ER. This is paramount to consider because compensatory responses to alterations in energy intake may start to manifest with a 3 to 4-day lag (Bray et al. 2008; De Castro 1998).

Although in the present study, there were no significant differences in PAEE and step count, both PAEE (-44kcal) and step count (-673 steps) decreased during

the diet-intervention period. Interestingly, after 7 days of *ad-libitum* energy intake, only women recovered PAEE (-6kcal) and step count (+663 steps) to baseline values. By contrast, in men, PAEE (-33kcal) and step count (-793 steps) did not return to baseline values but rather, reduction continued during the 7 days of ad-libitum energy intake. This divergent response between men and women in PAEE and step count during the post-intervention period may be partly explained by the different time period in which data collection was carried out; most of the female participants were recruited in spring, whereas most male participants were recruited in the beginning of summer. This may be an important confounding factor to consider as some evidence suggests that seasonal variations in physical activity, self-weighing behaviour and body mass management may have served as an incentive in the men's group to maintain the body mass reduced state (Westerterp 2019; Fahey et al. 2019). If seasonal variation played a key role in body mass change after the diet intervention then analysis of BMR at follow-up (day 29) should have also shown a trend for decrease, however, that was not the case. In comparison to baseline, after 7 days of energy intake ad-libitum (day 29), men still had a greater BMR per kg of total body mass than women (+1.6kcal/kg vs +0.3kcal/kg) despite maintaining a state of reduced body mass (+0.5kg vs +1.8kg). Another possible explanation could be attributed to the protective role of a higher energy flux in men. Mounting evidence suggests that coupling a high energy intake to a high energy expenditure can aid maintenance of a reduced body mass state by fine-tuning appetite at higher energy expenditures (Melby et al. 2017). In the study, men had a consistently higher step count than women during control (+22%), diet-intervention (+25%) and post-intervention periods (+7%), which in turn might have resulted in a better appetite regulation after the diet-intervention (Hägele et al. 2019).

In the present study, changes in BMR were assessed using indirect calorimetry before participants commenced the diet intervention (control period), immediately after the diet (day 22) and at follow-up (day 29). We observed a significant difference in BMR between men and women during the control period, but no difference was detected when BMR was adjusted per kilogram of body mass. Although BMR is determined by all metabolically active tissue, including the liver, brain and kidney, skeletal muscle has been shown to be main factors contributing to the metabolic discrepancy between men and women. In fact, this divergence

in BMR seems to furtherly dissipate after BMR is adjusted in proportion to LBM only (Bucholz, Rafii and Pencharz 2001). After the diet-induced ER (day 22), BMR decreased in both men and women by 56kcal and 66kcal, respectively. The observation that reductions in body mass led to a decrease in BMR has been extensively investigated (Melby et al. 2017). Schwartz and Doucet (2010) reported that for every kilogram of body mass that is lost, BMR decreases by approximately 15kcal, which largely explains the BMR drop observed in the study. This metabolic adaptation often persists after body mass loss, and in some cases can become permanent thus eliciting an energy gap (Melby et al. 2017). The latter can be defined as the discrepancy between energy requirements and appetite following successful body mass loss, which in turn promotes body mass regain (Fothgeril 2016; Camps, Verhoef and Westerterp 2013, Rosenbaum et al. 2008). It is important to highlight that manifestation of the energy gap is predominantly observed in longer-term studies where losses in body mass are more than 5% (Maclean et al. 2011). Interestingly, however, metabolic adaptations seem to be greater in magnitude during the first days of ER, mainly due to reduced insulin and leptin concentrations, intracellular water and glycogen content of skeletal muscle (Muller, Enderle and Westphal 2016). Nonetheless, in this study, BMR returned to baseline values after the final 7 days of *ad-libitum* energy intake (day 29), which is in line with the findings of other studies, and therefore suggest a high degree of plasticity of BMR in response to changes in energy intake (Nymo et al. 2018; Enderle and Westphal 2016; Doucet, Pomerleau and Harper 2004). In addition to body mass, BMR, PAEE and step count, the study measured changes in systolic blood pressure, diastolic blood pressure and fasting blood glucose, which all showed significant improvements with the diet-induced intervention. Positive changes in these markers of hypertension and insulin sensitivity are often reported in interventions where body mass loss is at least 3% of the initial body mass, which significantly reduces the risk of obesity-related diseases and mortality (Magkos et al. 2016).

4.1 Study Limitations

The present study had several limitations. Firstly, the study did not control for the menstrual cycle, which might have impacted energy expenditure and/or energy intake depending on the phase the participant was in (Sims and Heather 2018).

For example, in the first commercial diet tailored around the menstrual cycle, the Menstralean diet, showed that increasing energy intake during the luteal phase can result in better adherence to the dietary intervention and greater body mass losses (Geiken et al. 2016). Secondly, the gas exchange accuracy, variability in room temperature, the equation used, and the protein oxidation assumptions are also important considerations to have when measuring and reporting BMR (Fernández-Verdejo, Aguirre and Galgani 2019). The conditions under which the indirect calorimeter was used included the use of the Weir's equation coupled with the dismissed protein oxidation and testing in a laboratory where strict temperature control was not possible, therefore this may have affected intra-individual variance BMR (Fernández-Verdejo, Aguirre and Galgani 2019). Notably, our survey found that results might have been partially skewed by 5 participants who were not fully compliant with the study. Due to several drop-outs, the target number of participants was not reached. Therefore, the smaller sample size and uneven group sizes must be considered when interpreting the findings of this study (Jackson 2015). The original study design was intended to have identical time-frames for wearing the wrist- and hip-worn accelerometers, however, the latter was implemented with a 7-day delay due to firmware issues.
The main strengths of our study were the implementation of a diet-induced ER that was proportional to the individual's TDEE, and the frequent assessment of BMR via indirect calorimetry. This study therefore provided insights on how mild (3%) body mass loss can improve the measured health markers in both men and women. In practical applications, similar diet-induced ER can be potential implemented over the short term in specific contexts where rapid body mass loss is paramount (i.e. pre-surgery body mass loss), and without conferring detrimental effects in BMR and LBM. In future research, we would like to emphasise the potential refinement of such studies by implementing doubly-labelled water to asses TDEE and four compartment models to asses body composition (DXA, MRI).

4.2 Conclusions

The present study demonstrates that 7 days of diet-induced ER at 50% of TDEE can translate in favourable changes in body mass, body composition, blood

fasting glucose, systolic blood pressure and diastolic blood pressure in overweight and obese adults. These rapid changes occurred concomitantly and in the absence of significant detrimental effects in BMR, PAEE and step count in both men and women. Findings suggest that men may also be more likely to maintain a state of reduced body mass than women.

5.Appendices

5.1 Compliance survey

Q1 - Prior to assessments of basal metabolic rate, did you consume food/caffeine/nicotine?

A1 – Yes.

A2 – No.

A3 – I do not remember.

Q2 - If the answer to the previous question was YES, do you remember which study day was that? (Multiple choice)

A1 – Study day 1.

A2 – Study day 8.

A3 – Study day 15.

A4 – Study day 22.

A5 – Study day 29.

A6 – I do not remember

Q3 - During the 7-day diet intervention, did you manage to finish all of your liquid diet?

A1 – Yes.

A2 – No.

A3 – I do not remember.

Q4 - During the 7-day diet phase of the study, did you consume any other caloric food/beverage outside of the liquid diet?

A1 – Yes.

A2 – No.

A3 – I do not remember.

5.3 Participant information sheet

PARTICIPANT INFORMATION SHEET

Research Title: Effect of dieting on energy expenditure in overweight and obese adults.

Research Aim:

The aim of this study is to clarify if losing weight through dieting has an effect on different types of energy expenditure and if sex plays a role in this. Some evidence suggests that attempting weight loss through diet may lead to compensatory effect on some types of energy expenditure, however, this is still not clear.

PART 1

1. Invitation

You have been invited to take part in this study because you fulfil the following criteria: BMI ≥ 25 and ≤ 40, aged between 18 and 40 years, untrained (i.e. not engaging in any structured exercise) and with no chronic disease.

2. What is the purpose of the study?

The purpose of this study is to examine the effect that initial diet-induced weight loss has on different types of energy expenditure and determine if there are differences between men and women.

3. Do I have to take part?

No, you do not have to take part. It is up to you to decide if you would like to volunteer as a participant in this study. In addition, you can withdraw from the study at any point without needing to provide any explanation.

4. What will happen to me if I take part?

If you take part in this study, you will be asked to carry on with your normal life for 3 weeks and change your diet for one week. In total, the study will involve five morning visits to the laboratory over a 28-day period. You will be asked to fast for at least 10 hours and refrain from smoking for at least 2 hours before the visit. During these visits we will measure your height, body mass, blood pressure, resting heart rate, blood glucose and your energy expenditure at rest. The latter test will require you to lay on your back in a comfortable position, stay in a silent environment for 30 minutes while wearing a silicone mask for gas analysis. Following this measurement, an activity tracker will be given to you to be worn on your left or right wrist throughout the whole period of this investigation (i.e. 28 days). This device is water and dust resistant, and it should be worn throughout the day to estimate your energy expenditure. You will also be provided with 7-day food and physical activity diaries to complete during each week. Collectively, each visit will take approximately 45 minutes of your time. On the third visit to the lab, you will receive a very-low liquid-based energy diet for you to follow for 7 days (i.e. you will only follow this diet from day 15 to day 21 of the study). The liquid diet will provide 50% of your energy needs and should therefore induce some weight loss.

5. What are the possible risks of taking part?

The risks associated with this study are the same as if you were undertaking any other substantial change to your diet. These include potentially experiencing constipation or diarrhoea. However, due to the short-time of this diet (i.e. 7-days duration) the risks of these occurring are low.

6. What happens when the research study stops?

You will be allowed to see your results, if you wish to do so. You will also be able to request to see the results of the entire study after this has been completed and written up (If that is the case please ensure you send an e-mail to the researcher requesting this). It is expected that results will be published in a scientific journal. Any presented data will be anonymous.

7. What if there is a problem?

If there is any question, doubt or problem regarding any step of the study, ask the investigator for additional explanation or clarification. If matter is still not dealt satisfactorily you should contact the research supervisor.

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

8. What will happen if I don't want to carry on with the study?

If you do not wish to continue with the study, you can withdraw at any time without having to give a reason.

9. Will my part in this study be kept confidential?

Yes, any information will be kept confidential. Furthermore, to ensure anonymity and confidentiality, data will not associated with a non-identifiable code and be stored on a password-protected file on a protected University network server. Due to university's regulation, data will be kept for 10 years.

10. What will happen to the results of this study?

The results of this study will be used for a masters research project and it is expected that it will also get published in a scientific journal and/or presented at a scientific conference. In all cases, presented data will be anonymous.

11. Who is organising and funding this study?

This study is led by the Abertay University and no external funding body is associated.

12. Contact for further information

If you have any question regarding this study, do not hesitate and contact the investigator Matevz Arcon (email: 1401312@abertaty.ac.uk) or the supervisor Joel Rocha (email: j.rocha@abertay.ac.uk).

This project has been reviewed and approved by the Research Ethics Committee of the School of Social and Health Science

5.4 Participant Informed consent form

Abertay University

PARTICIPANT INFORMED CONSENT FORM

Research title: Effect of dieting on energy expenditure in overweight and obese adults.

The purpose and expected process of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that Abertay University has approved all procedures.

□ **I confirm that I have read and understand the participant information sheet provided.**

□ **I have had the opportunity to consider the information, ask questions about my participation and have had these answered satisfactorily.**

□ **I understand that my participation is voluntary and that I can stop taking part at any time without giving reason.**

□ **I also understand that if this happens, my relationship(s) with the investigator and Abertay University and my legal rights will not be affected.**

□ **I understand that my information will be used for reporting purposes but I will not be identified.**

□ **I understand that researchers are obliged to retain research data for up to 10 years' post-publication, however your anonymised research data may be retained indefinitely (e.g., so that researchers engage in open practice and other researchers can access their data to confirm the conclusions of published work). Researchers retain consent forms for as long as we continue to hold information about the participant and for 10 years for published research (including Research Degree thesis).**

□ **I agree to participate in this 28-day study conducted by Matevz Arcon who intends to use my data for further examining the effect of a 7-day liquid diet on energy expenditure in overweight and obese adults.**

Participant Name: _____

Participant Signature: _____

Date: _____

Person taking consent: _____

Signature: _____

Date: _____

5.5 Screening questionnaire

	SCREENING QUESTIONNAIRE. Participant No……	YES	NO
Q1.	HAS A DOCTOR OR NURSE EVER TOLD YOU THAT YOU HAVE HIGH BLOOD PRESSURE? IF YES GIVE SOME DETAILS		
Q2.	HAVE YOU HAD ANY HISTORY OF HEART TROUBLE? IF YES GIVE SOME DETAILS		
Q3.	DO YOU SUFFER FROM ASTHMA?		
Q4.	DO YOU SUFFER FROM A WHEEZY CHEST?		
Q5.	DO YOU FREQUENTLY HAVE PAINS IN YOUR HEART OR CHEST?		
Q6.	DO YOU FREQUENTLY FEEL FAINT OR HAVE SPELLS OF DIZZINESS?		
Q7.	HAS A DOCTOR TOLD YOU THAT YOU HAVE A BONE OR JOINT PROBLEM WHICH COULD BE MADE WORSE BY EXERCISE?		
Q8.	DO YOU HAVE ANY PHYSICAL DISABILITIES OF ANY KIND? IF YES GIVE DETAILS		
Q9.	ARE YOU, OR DO YOU THINK YOU MIGHT BE, PREGNANT AT THE MOMENT? IF YES, HOW MANY WEEKS?		
Q10	HAVE YOU BEEN IN HOSPITAL IN THE LAST TWO YEARS? IF YES GIVE REASON AND OUTCOME		
Q11	HAVE YOU HAD ANY OPERATIONS OR MAJOR ILLNESS IN THE PAST 6 MONTHS? IF YES GIVE DETAILS		
Q12	ARE YOU UNDERGOING TREATMENT OR HAVING ANY REGULAR CHECKS MADE FOR ANYTHING AT THE DOCTORS, OR A HOSPITAL OR A CLINIC AT THE MOMENT? IF YES GIVE SOME DETAILS		
Q13	ARE YOU TAKING MEDICATION REGULARLY? IF YES GIVE DETAILS, INCLUDING NAME OF MEDICATION		
Q14	ARE YOU CURRENTLY SUFFERING FROM A COLD OR VIRUS? IF YES GIVE DETAILS		

Name:……..

Signature:…….

Witness:……..

Date:……

Permission to undertake protocol: Yes / No Tutor's signature:……..

5.6 Food declaration

Food Declaration

The following label includes nutritional values and ingredients of each flavour for the VLCD Meal Replacement Shake. Please, read carefully the ingredients of each flavour to avoid ingestion of potential allergens.

Salted caramel (nutritional values):

Per/ Pour / Pro / Por Cada / Per 100g 51g

Energy / Énergie / Energie / Valores Energéticos /Energia 1663 kJ/392 kcal 848 kJ/200 kcal

Fat/ Lipides / Fett/ Grasas / Grassi 13 g 6 . 7 g of which saturates / dont saturés / davon gesättigt /de las cuales saturadas / di cui saturi 6 . 2 g 3 . 1 g

Carbohydrates / Glucides / Kohlenhydrate /Hidratos de Carbono / Carboidrati 34 g 17 g of which sugars / dont sucres / davon Zucker/ de los cuales azúcares / di cui zuccheri 27 g 14 g

Fibre / Fibres alimentaires / Ballaststoffe / Fibra alimentaria / Fibre 8 . 0 g 4 . 1 g

Protein / Proteines / Protein / Proteínas / Proteine 34 g 18 g

Salt/ Sel / Salz / Sal / Sale 1 . 8 g 0 . 90 g

Vitamin A / Vitamine A / Vitamin A /

Vitamina A / Vitamina A 550µg RE (69% RI*) 280mg (35%RI*)

Vitamin D / Vitamine D / Vitamin D / Vitamina D / Vitamina D 4 . 3µg (86% RI*) 2 . 2µg (44% RI*)

Vitamin E / Vitamine E / Vitamin E /

Vitamina E / Vitamina E 9 . 4mg a-TE (78% RI*)4 . 8mg a-TE (40%RI*)

Vitamin K / Vitamine K / Vitamin K /

Vitamina K / Vitamina K 58µg (77% RI*) 30µg (40% RI*)

Vitamin C / Vitamine C / Vitamin C /

Vitamina C / Vitamina C 64mg (81% RI*) 33mg (45% RI*)

Thiamin / Thiamine / Thiamin /

Tiamina / Tiamina 0 . 9mg (82% RI*) 0 . 5mg (45 % RI*)

Riboflavin / Riboflavine / Riboflavin /

Riboflavina / Riboflavina 1 . 9mg (136% RI*) 1 . 0mg (71% RI*)

Niacin / Niacine / Niacin /

Niacina / Niacina 15mg NE (94% RI*) 7 . 4mg NE (46% RI*)
Vitamin B6 / Vitamine B6 / Vitamin B6 /
Vitamina B6 / Vitamina B6 1 . 3mg (93% RI*) 0 . 7mg (50% RI*)
Per/ Pour / Pro / Por Cada / Per 100g 51g
Folic Acid / Acide Folique / Folsäure /
Ácido Fólico / Acido Folico 166µg (93% RI*) 84 . 6µg (42% RI*)
Vitamin B12 / Vitamine B12 / Vitamin B12 /
Vitamina B12 / Vitamina B12 2 . 5µg (100% RI*) 1 . 3µg (52% RI*)
Biotin / Biotine / Biotin / Biotina / Biotina 41µg (82% RI*) 21µg (42% RI*)
Pantothenic Acid / Acide Pantothénique /
Pantothensäure / Ácido Pantoténico / Acido Pantotenico 5 . 2mg (87% RI*) 2 . 6mg (43% RI*)
Potassium / Potassium / Kalium /
Potasio / Potassio 2057mg (102% RI*) 1049mg (52% RI*)
Chloride / Chlorure / Chloride / Cloruro / Cloruro 1016mg (127% RI*) 518mg (65% RI*)
Calcium / Calcium / Kalzium / Calcio / Calcio 758mg (95% RI*) 387mg (48% RI*)
Phosphorous / Phosphore / Phosphor /
Fósforo / Fosforo 523mg (75% RI*) 267mg (38% RI*)
Magnesium / Magnesium / Magnesium /
Magnesio / Magnesio 265mg (70%RI*) 135mg (36%RI*)
Iron / Fer / Eisen / Hierro / Ferro 15mg (107%RI*) 7 . 4mg (53%RI*)
Zinc / Zinc / Zink / Zinc / Zinco 8 . 7mg (87%RI*) 4 . 4mg(44%RI*)
Copper / Cuivre / Kupfer/ Cobre / Rame 0 . 7mg (70%RI*) 0 . 4mg (40%RI*)
Manganese/ Manganèse / Mangan /
Manganeso / Manganese 1 . 6mg (80%RI*) 0 . 8mg (40%RI*)
Fluoride / Fluor / Fluorid / Floruro / Fluoruro 2 . 8mg (80%RI*) 1 . 4mg (40%RI*)
Selenium / Sélénium / Sélénium / Selenio / Selenio 47µg (85%RI*) 24µg(43%RI*)
Chromium / Chrome / Chrom / Cromo / Cromo 31µg (78%RI*) 16µg(40%RI*)
Molybdenum / Molybdène / Molybden /
Molibdeno / Molibdeno 53µg (107%RI*) 27µg (54%RI*)
Iodine / Iode / Jod / Yodo / Iodio 114µg (76%RI*) 58µg(39%RI*)

Salted Caramel (Ingredients):

Skimmed Milk Powder (41%), Dietary Fat Concentrate (Refined Coconut Oil, Refined Soybean Oil, Milk Proteins, Whey Powder (Milk), Maltodextrin, Emulsifier (Lecithins [Contains Soy]), Stabiliser (Triphosphates), Antioxidants (Fatty Acid Esters of Ascorbic Acid, Alpha-Tocopherol), Anti Caking Agent (Calcium Phosphates), Whey Protein Concentrate (Milk, Soy Protein Isolate (Soy]) , Flavouring, Inulin Powder, Potassium Citrate, Thickener (Xanthan Gum) , Di Potassium Phosphate Powder, Salt, Calcium Chloride, Colour (E150c), Sweetener (Sucralose), Vitamin and Mineral Premix (Folic Acid, Vitamin C, Niacin, Iron, Vitamin E, Zinc, Pantothenic Acid, Fluoride, Manganese, Vitamin B6, Vitamin B2, Vitamin B1, Copper, Vitamin A, Iodine, Vitamin K, Selenium, Molybdenum, Biotin, Chromium, Vitamin D, Vitamin B12), Magnesium Oxide.

Banana (Nutritional Values):

Per/ Pour / Pro / Por Cada / Per 100g 51g

Energy / Énergie / Energie / Valores Energéticos /Energia 1663 kJ/392 kcal 848 kJ/200 kcal

Fat/ Lipides / Fett/ Grasas / Grassi 13 g 6 . 7 g of which saturates / dont saturés / davon gesättigt / de las cuales saturadas / di cui saturi 6 . 2 g 3 . 1 g

Carbohydrates / Glucides / Kohlenhydrate /Hidratos de Carbono / Carboidrati 34 g 17 g of which sugars / dont sucres / davon Zucker/de los cuales azúcares / di cui zuccheri 27 g 14 g

Fibre / Fibres alimentaires / Ballaststoffe / Fibra alimentaria / Fibre 8 . 0 g 4 . 1 g

Protein / Proteines / Protein / Proteínas / Proteine 34 g 18 g

Salt/ Sel / Salz / Sal / Sale 1 . 8 g 0 . 90 g

Vitamin A / Vitamine A / Vitamin A /

Vitamina A / Vitamina A 550µg RE (69% RI*) 280mg (35%RI*)

Vitamin D / Vitamine D / Vitamin D / Vitamina D / Vitamina D 4 . 3µg (86% RI*) 2 . 2µg (44% RI*)

Vitamin E / Vitamine E / Vitamin E /

Vitamina E / Vitamina E 9 . 4mg a-TE (78% RI*)4 . 8mg a-TE (40%RI*)

Vitamin K / Vitamine K / Vitamin K /

Vitamina K / Vitamina K 58µg (77% RI*) 30µg (40% RI*)

Vitamin C / Vitamine C / Vitamin C /
Vitamina C / Vitamina C 64mg (81% RI*) 33mg (45% RI*)
Thiamin / Thiamine / Thiamin /
Tiamina / Tiamina 0 . 9mg (82% RI*) 0 . 5mg (45 % RI*)
Riboflavin / Riboflavine / Riboflavin /
Riboflavina / Riboflavina 1 . 9mg (136% RI*) 1 . 0mg (71% RI*)
Niacin / Niacine / Niacin /
Niacina / Niacina 15mg NE (94% RI*) 7 . 4mg NE (46% RI*)
Vitamin B6 / Vitamine B6 / Vitamin B6 /
Vitamina B6 / Vitamina B6 1 . 3mg (93% RI*) 0 . 7mg (50% RI*)
Per/ Pour / Pro / Por Cada / Per 100g 51g
Folic Acid / Acide Folique / Folsäure /
Ácido Fólico / Acido Folico 166µg (93% RI*) 84 . 6µg (42% RI*)
Vitamin B12 / Vitamine B12 / Vitamin B12 /
Vitamina B12 / Vitamina B12 2 . 5µg (100% RI*) 1 . 3µg (52% RI*)
Biotin / Biotine / Biotin / Biotina / Biotina 41µg (82% RI*) 21µg (42% RI*)
Pantothenic Acid / Acide Pantothénique /
Pantothensäure / Ácido Pantoténico / Acido Pantotenico 5 . 2mg (87% RI*) 2 . 6mg (43% RI*)
Potassium / Potassium / Kalium /
Potasio / Potassio 2057mg (102% RI*) 1049mg (52% RI*)
Chloride / Chlorure / Chloride / Cloruro / Cloruro 1016mg (127% RI*) 518mg (65% RI*)
Calcium / Calcium / Kalzium / Calcio / Calcio 758mg (95% RI*) 387mg (48% RI*)
Phosphorous / Phosphore / Phosphor /
Fósforo / Fosforo 523mg (75% RI*) 267mg (38% RI*)
Magnesium / Magnesium / Magnesium /
Magnesio / Magnesio 265mg (70%RI*) 135mg (36%RI*)
Iron / Fer / Eisen / Hierro / Ferro 15mg (107%RI*) 7 . 4mg (53%RI*)
Zinc / Zinc / Zink / Zinc / Zinco 8 . 7mg (87%RI*) 4 . 4mg(44%RI*)
Copper / Cuivre / Kupfer/ Cobre / Rame 0 . 7mg (70%RI*) 0 . 4mg (40%RI*)
Manganese/ Manganèse / Mangan /
Manganeso / Manganese 1 . 6mg (80%RI*) 0 . 8mg (40%RI*)

Fluoride / Fluor / Fluorid / Floruro / Fluoruro 2 . 8mg (80%RI*) 1 . 4mg (40%RI*)
Selenium / Sélénium / Sélénium / Selenio / Selenio 47µg (85%RI*)
24µg(43%RI*)
Chromium / Chrome / Chrom / Cromo / Cromo 31µg (78%RI*) 16µg(40%RI*)
Molybdenum / Molybdène / Molybden /
Molibdeno / Molibdeno 53µg (107%RI*) 27µg (54%RI*)
Iodine / Iode / Jod / Yodo / Iodio 114µg (76%RI*) 58µg(39%RI*

Banana (Ingredients) :

Skimmed Milk Powder (41%), Dietary Fat Concentrate (Refined Coconut Oil, Refined Soybean Oil, Milk Proteins, Whey Powder (Milk), Maltodextrin, Emulsifier (Lecithins [Contains Soy]), Stabiliser (Triphosphates), Antioxidants (Fatty Acid Esters of Ascorbic Acid, Alpha-Tocopherol), Anti Caking Agent (Calcium Phosphates), Whey Protein Concentrate (Milk, Soy Protein Isolate (Soy]), Flavouring, Inulin Powder, Potassium Citrate, Thickener (Xanthan Gum) , Di Potassium Phosphate Powder, Salt, Calcium Chloride, Colour (Curcumin), Sweetener (Sucralose), Vitamin and Mineral Premix (Folic Acid, Vitamin C, Niacin, Iron, Vitamin E, Zinc, Pantothenic Acid, Fluoride, Manganese, Vitamin B6, Vitamin B2, Vitamin B1, Copper, Vitamin A, Iodine, Vitamin K, Selenium, Molybdenum, Biotin, Chromium, Vitamin D, Vitamin B12), Magnesium Oxide.

Chocolate (nutritional values):

Per/ Pour / Pro / Por Cada / Per 100g 51g
Energy / Énergie / Energie / Valores Energéticos /Energia 1662 kJ/392 kcal 848 kJ/200 kcal
Fat/ Lipides / Fett/ Grasas / Grassi 13 g 6 . 7 g of which saturates / dont saturés / davon gesättigt / de las cuales saturadas / di cui saturi 6 . 3 g 3 . 2 g
Carbohydrates / Glucides / Kohlenhydrate /Hidratos de Carbono / Carboidrati 34 g 17 g of which sugars / dont sucres / davon Zucker/de los cuales azúcares / di cui zuccheri 28 g 14 g
Fibre / Fibres alimentaires / Ballaststoffe / Fibra alimentaria / Fibre 8 . 5 g 4 . 3 g
Protein / Proteines / Protein / Proteínas / Proteine 34 g 17 g
Salt/ Sel / Salz / Sal / Sale 1 . 8 g 0 . 90 g
Vitamin A / Vitamine A / Vitamin A /

Vitamina A / Vitamina A 550µg RE (69% RI*) 280mg (35%RI*)
Vitamin D / Vitamine D / Vitamin D / Vitamina D / Vitamina D 4 . 3µg (86% RI*) 2 . 2µg (44% RI*)
Vitamin E / Vitamine E / Vitamin E /
Vitamina E / Vitamina E 9 . 4mg a-TE (78% RI*)4 . 8mg a-TE (40%RI*)
Vitamin K / Vitamine K / Vitamin K /
Vitamina K / Vitamina K 58µg (77% RI*) 30µg (40% RI*)
Vitamin C / Vitamine C / Vitamin C /
Vitamina C / Vitamina C 64mg (81% RI*) 33mg (45% RI*)
Thiamin / Thiamine / Thiamin / Tiamina / Tiamina 0 . 9mg (82% RI*) 0 . 5mg (45 % RI*)
Riboflavin / Riboflavine / Riboflavin /
Riboflavina / Riboflavina 1 . 9mg (136% RI*) 1 . 0mg (71% RI*)
Niacin / Niacine / Niacin /
Niacina / Niacina 15mg NE (94% RI*) 7 . 4mg NE (46% RI*)
Vitamin B6 / Vitamine B6 / Vitamin B6 /
Vitamina B6 / Vitamina B6 1 . 3mg (93% RI*) 0 . 7mg (50% RI*)
Per/ Pour / Pro / Por Cada / Per 100g 51g
Folic Acid / Acide Folique / Folsäure /
Ácido Fólico / Acido Folico 166µg (93% RI*) 84 . 6µg (42% RI*)
Vitamin B12 / Vitamine B12 / Vitamin B12 /
Vitamina B12 / Vitamina B12 2 . 5µg (100% RI*) 1 . 3µg (52% RI*)
Biotin / Biotine / Biotin / Biotina / Biotina 41µg (82% RI*) 21µg (42% RI*)
Pantothenic Acid / Acide Pantothénique /
Pantothensäure / Ácido Pantoténico / Acido Pantotenico 5 . 2mg (87% RI*) 2 . 6mg (43% RI*)
Potassium / Potassium / Kalium /
Potasio / Potassio 2057mg (102% RI*) 1049mg (52% RI*)
Chloride / Chlorure / Chloride / Cloruro / Cloruro 1016mg (127% RI*) 518mg (65% RI*)
Calcium / Calcium / Kalzium / Calcio / Calcio 758mg (95% RI*) 387mg (48% RI*)
Phosphorous / Phosphore / Phosphor /
Fósforo / Fosforo 523mg (75% RI*) 267mg (38% RI*)

Magnesium / Magnesium / Magnesium /
Magnesio / Magnesio 265mg (70%RI*) 135mg (36%RI*)
Iron / Fer / Eisen / Hierro / Ferro 15mg (107%RI*) 7 . 4mg (53%RI*)
Zinc / Zinc / Zink / Zinc / Zinco 8 . 7mg (87%RI*) 4 . 4mg(44%RI*)
Copper / Cuivre / Kupfer/ Cobre / Rame 0 . 7mg (70%RI*) 0 . 4mg (40%RI*)
Manganese/ Manganèse / Mangan /
Manganeso / Manganese 1 . 6mg (80%RI*) 0 . 8mg (40%RI*)
Fluoride / Fluor / Fluorid / Floruro / Fluoruro 2 . 8mg (80%RI*) 1 . 4mg (40%RI*)
Selenium / Sélénium / Sélénium / Selenio / Selenio 47µg (85%RI*)
24µg(43%RI*)
Chromium / Chrome / Chrom / Cromo / Cromo 31µg (78%RI*) 16µg(40%RI*)
Molybdenum / Molybdène / Molybden /
Molibdeno / Molibdeno 53µg (107%RI*) 27µg (54%RI*)
Iodine / Iode / Jod / Yodo / Iodio 114µg (76%RI*) 58µg(39%RI*)
Chocolate (Ingredients) : Skimmed Milk Powder (41%), Dietary Fat Concentrate (Refined Coconut Oil, Refined Soybean Oil, Milk Proteins, Whey Powder (Milk), Maltodextrin, Emulsifier (Lecithins [Contains Soy]), Stabiliser (Triphosphates), Antioxidants (Fatty Acid Esters of Ascorbic Acid, Alpha-Tocopherol), Anti Caking Agent (Calcium Phosphates), Whey Protein Concentrate (Milk, Soy Protein Isolate (Soy]), Flavouring, Inulin Powder, Potassium Citrate, Thickener (Xanthan Gum) , Di Potassium Phosphate Powder, Salt, Calcium Chloride, Cocoa Powder, Sweetener (Sucralose), Vitamin and Mineral Premix (Folic Acid, Vitamin C, Niacin, Iron, Vitamin E, Zinc, Pantothenic Acid, Fluoride, Manganese, Vitamin B6, Vitamin B2, Vitamin B1, Copper, Vitamin A, Iodine, Vitamin K, Selenium, Molybdenum, Biotin, Chromium, Vitamin D, Vitamin B12), Magnesium Oxide.

Strawberry (Nutritional values):

Per/ Pour / Pro / Por Cada / Per 100g 51g
Energy / Énergie / Energie / Valores Energéticos /Energia 1660 kJ/391 kcal 847 kJ/199 kcal
Fat/ Lipides / Fett/ Grasas / Grassi 13 g 6 . 7 g of which saturates / dont saturés / davon gesättigt / de las cuales saturadas / di cui saturi 6 . 2 g 3 . 1 g
Carbohydrates / Glucides / Kohlenhydrate / Hidratos de Carbono / Carboidrati

33 g 17 g of which sugars / dont sucres / davon Zucker/ de los cuales azúcares / di cui zuccheri 27 g 14 g
Fibre / Fibres alimentaires / Ballaststoffe / Fibra alimentaria / Fibre 8 . 1 g 4 . 1 g
Protein / Proteines / Protein / Proteínas / Proteine 35 g 18 g
Salt/ Sel / Salz / Sal / Sale 1 . 8 g 0 . 90 g
Vitamin A / Vitamine A / Vitamin A /
Vitamina A / Vitamina A 550µg RE (69% RI*) 280mg (35%RI*)
Vitamin D / Vitamine D / Vitamin D / Vitamina D / Vitamina D 4 . 3µg (86% RI*) 2 . 2µg (44% RI*)
Vitamin E / Vitamine E / Vitamin E /
Vitamina E / Vitamina E 9 . 4mg a-TE (78% RI*)4 . 8mg a-TE (40%RI*)
Vitamin K / Vitamine K / Vitamin K /
Vitamina K / Vitamina K 58µg (77% RI*) 30µg (40% RI*)
Vitamin C / Vitamine C / Vitamin C /
Vitamina C / Vitamina C 64mg (81% RI*) 33mg (45% RI*)
Thiamin / Thiamine / Thiamin /
Tiamina / Tiamina 0 . 9mg (82% RI*) 0 . 5mg (45 % RI*)
Riboflavin / Riboflavine / Riboflavin /
Riboflavina / Riboflavina 1 . 9mg (136% RI*) 1 . 0mg (71% RI*)
Niacin / Niacine / Niacin /
Niacina / Niacina 15mg NE (94% RI*) 7 . 4mg NE (46% RI*)
Vitamin B6 / Vitamine B6 / Vitamin B6 /
Vitamina B6 / Vitamina B6 1 . 3mg (93% RI*) 0 . 7mg (50% RI*)
Per/ Pour / Pro / Por Cada / Per 100g 51g
Folic Acid / Acide Folique / Folsäure /
Ácido Fólico / Acido Folico 166µg (93% RI*) 84 . 6µg (42% RI*)
Vitamin B12 / Vitamine B12 / Vitamin B12 /
Vitamina B12 / Vitamina B12 2 . 5µg (100% RI*) 1 . 3µg (52% RI*)
Biotin / Biotine / Biotin / Biotina / Biotina 41µg (82% RI*) 21µg (42% RI*)
Pantothenic Acid / Acide Pantothénique /
Pantothensäure / Ácido Pantoténico / Acido Pantotenico 5 . 2mg (87% RI*) 2 . 6mg (43% RI*)
Potassium / Potassium / Kalium /
Potasio / Potassio 2057mg (102% RI*) 1049mg (52% RI*)

Chloride / Chlorure / Chloride / Cloruro / Cloruro 1016mg (127% RI*) 518mg (65% RI*)
Calcium / Calcium / Kalzium / Calcio / Calcio 758mg (95% RI*) 387mg (48% RI*)
Phosphorous / Phosphore / Phosphor /
Fósforo / Fosforo 523mg (75% RI*) 267mg (38% RI*)
Magnesium / Magnesium / Magnesium /
Magnesio / Magnesio 265mg (70%RI*) 135mg (36%RI*)
Iron / Fer / Eisen / Hierro / Ferro 15mg (107%RI*) 7 . 4mg (53%RI*)
Zinc / Zinc / Zink / Zinc / Zinco 8 . 7mg (87%RI*) 4 . 4mg(44%RI*)
Copper / Cuivre / Kupfer/ Cobre / Rame 0 . 7mg (70%RI*) 0 . 4mg (40%RI*)
Manganese/ Manganèse / Mangan /
Manganeso / Manganese 1 . 6mg (80%RI*) 0 . 8mg (40%RI*)
Fluoride / Fluor / Fluorid / Floruro / Fluoruro 2 . 8mg (80%RI*) 1 . 4mg (40%RI*)
Selenium / Sélénium / Sélénium / Selenio / Selenio 47µg (85%RI*)
24µg(43%RI*)
Chromium / Chrome / Chrom / Cromo / Cromo 31µg (78%RI*) 16µg(40%RI*)
Molybdenum / Molybdène / Molybden /
Molibdeno / Molibdeno 53µg (107%RI*) 27µg (54%RI*)
Iodine / Iode / Jod / Yodo / Iodio 114µg (76%RI*) 58µg(39%RI*)

Strawberry (Ingredients) : Skimmed Milk Powder (41%), Dietary Fat Concentrate (Refined Coconut Oil, Refined Soybean Oil, Milk Proteins, Whey Powder (Milk), Maltodextrin, Emulsifier (Lecithins [Contains Soy]), Stabiliser (Triphosphates), Antioxidants (Fatty Acid Esters of Ascorbic Acid, Alpha-Tocopherol), Anti Caking Agent (Calcium Phosphates), Whey Protein Concentrate (Milk, Soy Protein Isolate (Soy]), Flavouring, Inulin Powder, Potassium Citrate, Thickener (Xanthan Gum) , Di Potassium Phosphate Powder, Salt, Calcium Chloride, Colour (Beetroot Powder), Sweetener (Sucralose), Vitamin and Mineral Premix (Folic Acid, Vitamin C, Niacin, Iron, Vitamin E, Zinc, Pantothenic Acid, Fluoride, Manganese, Vitamin B6, Vitamin B2, Vitamin B1, Copper, Vitamin A, Iodine, Vitamin K, Selenium, Molybdenum, Biotin, Chromium, Vitamin D, Vitamin B12), Magnesium Oxide.

Vanilla (nutritional values):

Per/ Pour / Pro / Por Cada / Per 100g 51g

Energy / Énergie / Energie / Valores Energéticos /Energia 1663 kJ/392 kcal 848 kJ/200 kcal

Fat/ Lipides / Fett/ Grasas / Grassi 13 g 6 . 7 g of which saturates / dont saturés / davon gesättigt / de las cuales saturadas / di cui saturi 6 . 2 g 3 . 1 g

Carbohydrates / Glucides / Kohlenhydrate / Hidratos de Carbono / Carboidrati 34 g 17 g of which sugars / dont sucres / davon Zucker/ de los cuales azúcares / di cui zuccheri 28 g 14 g

Fibre / Fibres alimentaires / Ballaststoffe / Fibra alimentaria / Fibre 8 . 0 g 4 . 1 g

Protein / Proteines / Protein / Proteínas / Proteine 34 g 18 g

Salt/ Sel / Salz / Sal / Sale 1 . 8 g 0 . 90 g

Vitamin A / Vitamine A / Vitamin A /

Vitamina A / Vitamina A 550µg RE (69% RI*) 280mg (35%RI*)

Vitamin D / Vitamine D / Vitamin D / Vitamina D / Vitamina D 4 . 3µg (86% RI*) 2 . 2µg (44% RI*)

Vitamin E / Vitamine E / Vitamin E /

Vitamina E / Vitamina E 9 . 4mg a-TE (78% RI*)4 . 8mg a-TE (40%RI*)

Vitamin K / Vitamine K / Vitamin K /

Vitamina K / Vitamina K 58µg (77% RI*) 30µg (40% RI*)

Vitamin C / Vitamine C / Vitamin C /

Vitamina C / Vitamina C 64mg (81% RI*) 33mg (45% RI*)

Thiamin / Thiamine / Thiamin /

Tiamina / Tiamina 0 . 9mg (82% RI*) 0 . 5mg (45 % RI*)

Riboflavin / Riboflavine / Riboflavin /

Riboflavina / Riboflavina 1 . 9mg (136% RI*) 1 . 0mg (71% RI*)

Niacin / Niacine / Niacin /

Niacina / Niacina 15mg NE (94% RI*) 7 . 4mg NE (46% RI*)

Vitamin B6 / Vitamine B6 / Vitamin B6 /

Vitamina B6 / Vitamina B6 1 . 3mg (93% RI*) 0 . 7mg (50% RI*)

Per/ Pour / Pro / Por Cada / Per 100g 51g

Folic Acid / Acide Folique / Folsäure /

Ácido Fólico / Acido Folico 166µg (93% RI*) 84 . 6µg (42% RI*)

Vitamin B12 / Vitamine B12 / Vitamin B12 /

Vitamina B12 / Vitamina B12 2 . 5µg (100% RI*) 1 . 3µg (52% RI*)
Biotin / Biotine / Biotin / Biotina / Biotina 41µg (82% RI*) 21µg (42% RI*)
Pantothenic Acid / Acide Pantothénique /
Pantothensäure / Ácido Pantoténico / Acido Pantotenico 5 . 2mg (87% RI*) 2 . 6mg (43% RI*)
Potassium / Potassium / Kalium /
Potasio / Potassio 2057mg (102% RI*) 1049mg (52% RI*)
Chloride / Chlorure / Chloride / Cloruro / Cloruro 1016mg (127% RI*) 518mg (65% RI*)
Calcium / Calcium / Kalzium / Calcio / Calcio 758mg (95% RI*) 387mg (48% RI*)
Phosphorous / Phosphore / Phosphor /
Fósforo / Fosforo 523mg (75% RI*) 267mg (38% RI*)
Magnesium / Magnesium / Magnesium /
Magnesio / Magnesio 265mg (70%RI*) 135mg (36%RI*)
Iron / Fer / Eisen / Hierro / Ferro 15mg (107%RI*) 7 . 4mg (53%RI*)
Zinc / Zinc / Zink / Zinc / Zinco 8 . 7mg (87%RI*) 4 . 4mg(44%RI*)
Copper / Cuivre / Kupfer/ Cobre / Rame 0 . 7mg (70%RI*) 0 . 4mg (40%RI*)
Manganese/ Manganèse / Mangan /
Manganeso / Manganese 1 . 6mg (80%RI*) 0 . 8mg (40%RI*)
Fluoride / Fluor / Fluorid / Floruro / Fluoruro 2 . 8mg (80%RI*) 1 . 4mg (40%RI*)
Selenium / Sélénium / Sélénium / Selenio / Selenio 47µg (85%RI*) 24µg(43%RI*)
Chromium / Chrome / Chrom / Cromo / Cromo 31µg (78%RI*) 16µg(40%RI*)
Molybdenum / Molybdène / Molybden /
Molibdeno / Molibdeno 53µg (107%RI*) 27µg (54%RI*)
Iodine / Iode / Jod / Yodo / Iodio 114µg (76%RI*) 58µg(39%RI*

Vanilla Flavour(ingredients): Skimmed Milk Powder (41%), Dietary Fat Concentrate (Refined Coconut Oil, Refined Soybean Oil, Milk Proteins, Whey Powder (Milk), Maltodextrin, Emulsifier (Lecithins [Contains Soy]), Stabiliser (Triphosphates), Antioxidants (Fatty Acid Esters of Ascorbic Acid, Alpha-Tocopherol), Anti Caking Agent (Calcium Phosphates), Whey Protein Concentrate (Milk, Soy Protein Isolate (Soy]), Flavouring, Inulin Powder,

Potassium Citrate, Thickener (Xanthan Gum) , Di Potassium Phosphate Powder, Salt, Calcium Chloride, Sweetener (Sucralose), Vitamin and Mineral Premix (Folic Acid, Vitamin C, Niacin, Iron, Vitamin E, Zinc, Pantothenic Acid, Fluoride, Manganese, Vitamin B6, Vitamin B2, Vitamin B1, Copper, Vitamin A, Iodine, Vitamin K, Selenium, Molybdenum, Biotin, Chromium, Vitamin D, Vitamin B12), Magnesium Oxide.

I have carefully read the nutritional values and ingredient lists for each flavour and can confirm that I am not allergic to any of ingredients listed: _____ (signature)

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