Appetite xxx (2015) 1–8

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Response of appetite and potential appetite regulators following intake of high energy nutritional supplements

Sadia Fatima [b], Konstantinos Gerasimidis [a], Charlotte Wright [a], Melina Tsiountsioura [a], Eirini-Iro Arvanitidou [a], Dalia Malkova [a, *]

[a] School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Scotland, UK
[b] Khyber Medical University Peshawar, Pakistan

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form
5 June 2015
Accepted 15 June 2015
Available online xxx

Keywords:
High energy supplements
Appetite
Appetite hormones
Gastric emptying
Thermogenesis
Control of food intake

ABSTRACT

Background: The net clinical benefit of high-energy nutritional supplements (HENSDs) consumption is lower than expected.
Objectives: To investigate the extent to which consumption of oral HENSD in the fasted state reduces energy intake in slim females during consecutive breakfast and lunch, and whether this relates to changes in appetite and metabolic appetite regulators.
Design: Twenty three females of 24.4 ± 2.8 years with BMI of 18.2 ± 0.8 kg/m^2 consumed HENSD (2.5 MJ) or PLACEBO (0.4 MJ) in fasted state in a single blind randomized cross-over study. Appetite and metabolic rate measurements and blood collection were conducted prior to and during 240 min after the intake of the supplements. Energy intake was recorded during *ad libitum* buffet breakfast and lunch served 60 min and 240 min post supplementation respectively.
Results: Energy intake during breakfast was significantly ($P < 0.01$) lower in the HENSD trial but the net cumulative effect on energy intake was 1.07 ± 0.34 MJ higher in the HENSD compared to PLACEBO. Plasma concentration of CCK and PYY and insulin and were significantly ($P < 0.05$) higher in the HENSD trial while appetite measures were not significantly different between HENSD and PLACEBO trials. Correlations for the within participant relations between the responses of plasma hormones and appetite scores were significant ($P < 0.05$) for PYY and insulin but not CCK. The energy expended above resting metabolic rate was significantly ($P < 0.05$) higher in the HENDS trial but relative increase in energy expenditure was not significantly different between the two trials.
Conclusion: Oral high-energy nutritional supplements have a partial and relatively short lived suppressive action on energy intake and can be expected to increase net energy intake by approximately half the energy value of the supplement consumed.

1. Introduction

Systematic reviews and meta-analyses consistently suggest that oral, ready to drink high energy liquid supplements (HENSD) improve body weight and energy and nutritional intake, and thus may have various clinical and functional benefits in patients with an increased risk of becoming malnourished (Cawood, Elia, & Stratton, 2012; Stratton & Elia, 2010; Stratton, Hébuterne, & Elia, 2013). HENSD typically contain a mixture of macro and micronutrients, providing between 6·3 kJ/ml to 10·1 kJ/ml per typical serving of 125–220 ml (Stratton & Elia, 2010). Although HENSD should increase daily energy intake by an average of 1569 kJ/d, their net benefit is lower than anticipated (Milne, Avenell, & Potter, 2005; Poustie, Russell, Watling, Ashby, & Smyth, 2006), implying that HENSD consumption partially displaces food and energy intake during habitual meals. As another determinant of energy balance, energy expended during physical activity, may also play an important role. Indeed, a study investigating the impact of oral nutritional supplementation in depleted ambulatory patients with chronic obstructive pulmonary disease suggests that absence of body weight gain during three months of supplementation may be

Abbreviations: HENSDs, High energy nutritional supplement drinks; PYY, Peptide YY; CCK, Cholecystokinin; AUCs, areas under response vs. time curves.

* Corresponding author.
E-mail address: dalia.malkova@glasgow.ac.uk (D. Malkova).

http://dx.doi.org/10.1016/j.appet.2015.06.010
0195-6663/

explained by enhanced energy expenditure via physical activity (Goris, Vermeeren, Wouters, Schols, & Westerterp, 2003).

So far, the mechanisms mediating compensation of habitual energy intake in relation to malnutrition treatment have been investigated primarily in tube feeding studies and only considered the role of metabolic and hormonal appetite regulators (Stratton & Elia, 1999; Stratton, Stubbs, & Elia, 2008). Oral intake compensation for energy provided by 3 days of bolus tube feeding has been reported to be equivalent to 40% of the bolus feed energy content, with plasma concentration of ghrelin, leptin, insulin, and glucose being modified in the direction expected to reduce hunger and increase satiety (Stratton et al., 2008). It is important to note that tube feeding delivers nutrients directly into the stomach and thus bypasses the sensory aspects of oral consumption and the cephalic phase response (Stratton & Elia, 1999; Stratton et al., 2008). Thus, the interaction between appetite, food intake, and the wider spectrum of hormonal appetite and satiety regulators following oral supplementation remains to be investigated.

Appetite and energy intake following food or supplement consumption can also relate to their impact on gastric emptying and diet induced thermogenesis as reduced hunger and enhanced satiety were reported to be linked with delayed gastric emptying (Wang et al., 2008) and with higher diet induced thermogenesis (Crovetti, Porrini, Santangelo, & Testolin, 1998; Mansour et al., 2012). Therefore, the aim of this study was to investigate the level of energy intake compensation in lean adult women following HENSD consumption and find out how it relates to responses of a range of hormonal and metabolic appetite regulators as well as a measure of gastric emptying and diet induced thermogenesis.

2. Material and methods

2.1. Participants

Eligible participants of this study were slim (BMI < 20 kg/m^2), healthy, young women recruited by means of advertisement and word of mouth in the campus of the University of Glasgow and in other public places. Participants were non-smokers, with stable weight for one month prior to the study, not pregnant, had regular menstrual cycle, and were not on any medication, nutritional supplement or following a special diet. Before enrolling in the study, participants underwent a detailed health screen regarding participant's health to exclude chronic illness, eating disorders and past history of gastrointestinal operations which could interfere with the results of the study. All participants gave written informed consent. The study was approved by College of Medical, Veterinary and Life Sciences Ethics Committee of Glasgow University.

2.2. Study design

This study used a single blind crossover design with two randomly sequenced experimental trials, separated by four weeks. On the morning of the experiment trial, participants reported to the metabolic investigation room between 0800 and 0900 after 12 h fast. Height (Seca, Leicester, UK) body mass and body fat (TBF-300, TANITA, Cranlea, UK), and resting metabolic rate (RMR) were measured. A venous cannula was then inserted and, after an interval of 10 min, baseline blood sample was obtained. Subsequently, an appetite questionnaire was completed. Within 10 min, participants were then asked to consume 240 ml of either a HENSD (Scandishake, Chocolate, Nutricia) prepared with full fat milk (HENSD trial), supplying 2.5 MJ, or a low calorie drink made up with skimmed milk, cocoa and artificial sweeteners (PLACEBO trial), supplying 0.4 MJ (Table 1). Participants were blinded of the preload drink and they were not told which drink they would consume and

Table 1
Energy, carbohydrate (CHO), fat and protein provided by HENSD and PLACEBO drinks.

	HENSD	PLACEBO
Energy (MJ)	2.49	0.38
CHO (g)	68.8 g	11.3 g
Fat (g)	30.4 g	1.3 g
Protein (g)	11.9 g	9 g
Energy from CHO (%)	46	48
Energy from Fat (%)	46	13
Energy from Protein (%)	8	39

the order in which drinks were provided was randomly allocated. HENSD and PLACEBO drinks had the same colour, volume, flavour and texture and were not distinguishable, as reported in an independent pilot trial. Following supplement intake, 1000 mg of paracetamol with 100 ml of water were given to participants to measure a proxy of gastric emptying (Clements, Heading, Nimmo, & Prescott, 1978). Participants completed further appetite questionnaires and blood samples were collected at 30 and 60 min post supplement e whilst metabolic rate was measured every minute for the duration of 20 min after each blood sample. One hour after supplement consumption, an *ad libitum* buffet style breakfast was served. Appetite questionnaires and blood samples were collected at 120, 150, 180, 210, and 240 min. An *ad libitum* buffet lunch was presented 240 min after the baseline measurements (see Table 2). Q1

2.3. Ad libitum buffet meals

Ad libitum buffet meals consisted of a variety of standardised foods with total energy about three times what participants were expected to consume. They were served in the same setting, serving the same type of food in the same coloured dishes scheduling meals at the same time, and in the same table in order to avoid any bias in eating behaviour. The participants were given 30 min to consume their meal and were advised to eat according to their appetite until satisfied and comfortably full. Breakfast comprised of a variety of breakfast cereals, milk (semi-skimmed, skimmed and whole cream), croissants, jam, butter, banana and apples, apple and orange juice. Lunch comprised of two filled white bread sandwiches, two filled whole meal bread sandwiches, mixed leaf salad, yoghurt, apple, banana, grapes, and apple or orange juice. The food was cut into smaller pieces to eliminate portion related cues. All food offered and remaining after the intake was weighed by the researcher using an electronic kitchen scale (Salter Housewares Ltd., Tonbridge, U.K.). Water was available throughout the trial. Participants were given 30 min to consume their meal and were advised to eat according to their appetite until satisfied and comfortably full. The researchers were not present when the participants ate their meals to avoid any potential effect of the researcher on feeding behaviour. The participants were blinded to the actual purpose of buffet meals i.e. measurement of food intake.

3. Measurement of food and energy intake

The macronutrient and energy intake was calculated by dietary software Windiets 2005 (The Robert Gordon University, Aberdeen, Scotland, UK). The calculations on energy and macronutrient intake were independently conducted by two researchers and mean values of two calculations were used.

3.1. Appetite measurements

Participants were asked to mark their feeling of hunger, satiety, fullness, prospective food consumption and desire to eat on Visual

Please cite this article in press as: Fatima, S., et al., Response of appetite and potential appetite regulators following intake of high energy nutritional supplements, Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

Table 2
Energy, carbohydrate (CHO), protein and fat intake in HENSD and PLACEBO trials. Values are presented as Mean ± SE, (n = 23).

		HENSD	PLACEBO
Supplement	Energy (MJ)	2.49	0.38
Breakfast	Energy (MJ)	1.92 ± 0.17	2.89 ± 0.23**
	CHO (g)	80 ± 8	113 ± 1*
	Protein (g)	11 ± 1	16 ± 1*
	Fat (g)	12 ± 1	21 ± 2**
Supplement + Breakfast	Energy (MJ)	4.41 ± 0.17	3.27 ± 0.23**
	CHO (g)	149 ± 7.65	125 ± 9.70*
	Protein (g)	24 ± 1	25 ± 1
	Fat (g)	42 ± 1	23 ± 2**
Lunch	Energy (MJ)	2.16 ± 0.16	2.27 ± 0.20
	CHO (g)	69 ± 5	72 ± 7
	Protein (g)	22 ± 2	24 ± 2
	Fat (g)	18 ± 1.6	18 ± 1.9
Breakfast + Lunch	Energy (MJ)	4.09 ± 0.26	5.16 ± 0.32**
	CHO (g)	150 ± 11	186 ± 13**
	Protein (g)	33 ± 3	40 ± 3*
	Fat (g)	30 ± 2	40 ± 3**
Breakfast + Lunch + Supplement	Energy (MJ)	6.58 ± 0.26	5.54 ± 0.32**
	CHO (g)	219 ± 11	197 ± 13
	Protein (g)	45 ± 3	50 ± 3
	Fat (g)	60 ± 2	41 ± 3**

Significantly different (*$P < 0.05$, **$P < 0.01$) from HENSD trial.

Analogue Scale (VAS) line of 100 mm (A Flint, Raben, Blundell, & Astrup, 2000) by placing a vertical mark on the horizontal line at a point corresponding to their feelings at that time. The lines were anchored by negative feeling words (I am not hungry at all) on the left and by positive feeling words (never being hungrier) on the right. Quantification of the measurement was made by measuring distance from the left end of the line to the participant's mark.

3.2. Measurements of metabolic rate

Participants were rested in the supine position for 10 min before fasting expired air samples were collected using a ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). The rate of oxygen consumption ($\dot{V}O_2$) and rate of carbon dioxide production ($\dot{V}CO_2$) were recorded every minute for the duration of 20 min. These values were then used to calculate rate of energy expenditure by using indirect calorimetry equations described elsewhere (Frayn & Macdonald, 1997). Diet induced thermogenesis for the pre-breakfast period (0–60 min) was calculated by multiplying the duration of this period by the difference between the mean values of the metabolic rate measured for 60 min after supplement consumption and the metabolic rate measured at rest. Similarly, diet induced thermogenesis for the pre-lunch period (60–240 min) was calculated by multiplying the duration of this period and the difference between the mean values of the metabolic rate measured for 180 min after breakfast and mean value of the metabolic rate measured at rest. For the validation of the accuracy of Oxycon Pro system, an alcohol-burning test (Miodownik, Melendez, Carlon, & Burda, 1998) was included in the Standard Operating Procedure of the system and was conducted weekly with CV of 1.9%. In addition, prior to data collection, the reliability of Oxycon Pro system was determined by comparing resting $\dot{V}O_2$ and $\dot{V}CO_2$ values obtained for 20 min in nine healthy volunteers with normal body weight in the fasted state on two occasions, with an interval of seven-eight days. During the 2 days before the first measurement participants weighted and recorded all foods consumed and were asked to replicate this food intake during the 2 days preceding the second measurement. In addition, participants refrained from exercise and alcohol intake for 2 days prior each measurement. Mean $\dot{V}O_2$ and $\dot{V}CO_2$ values were 0.27 (SD 0.02) and 0.22 (SD 0.02) litres/min for the first measurements and 0.27 (SD 0.03) and 0.22 (SD 0.03) litres/min for the second measurement, respectively. The mean difference between measurements was 0.6 (SD 6.1) % for $\dot{V}O_2$ and 0.7 (SD 4.6) % for $\dot{V}CO_2$ and the Pearson correlation coefficients between measurements were 0.91 ($P < 0.0005$) for $\dot{V}O_2$ and 0.94 ($P < 0.0005$) for $\dot{V}CO_2$.

3.3. Metabolic appetite regulators

Venous blood samples were collected into ethylenediamine tetra-acetic acid (EDTA) Vacutainer tube (BD Vacutainer Systems, Plymouth, UK). Blood samples used for the analysis of insulin, glucose and for paracetamol concentrations were centrifuged at 4 °C, 3000 rpm for 15 min. After centrifugation the plasma supernatant was aspirated and frozen at −80 °C until analysed. The blood sample used for the determination of CCK and PYY was aliquoted into eppendorf tubes containing Aprotinin (0.6 TIU activity per ml, Sigma–Aldrich, UK) and then centrifuged at 14000 rpm for 4 min. Subsequently 300 μL of plasma was aliquoted into eppendorf tubes and kept until analysis at −80 °C. Plasma glucose was measured by enzymatic colorimetric method (Roche Diagnostics, Mannheim, Germany). Commercially available ELISA kits were used to measure concentration of plasma insulin (Mercodia, Uppsala, Sweden), PYY (Merck, Millipore, Bioscience Division, UK), and CCK (Phoenix, Pharmaceuticals, Inc., Burlingame, CA, USA). For assessment of gastric emptying, plasma paracetamol concentration was measured using the Acetaminophen Assay kits (Cambridge Life Sciences, Cambridge, U.K.). All samples for each participant were analysed in a single analyser run to minimise inter-assay variation. Coefficients of variation were <3% for all non-ELISA assays, <4% for the insulin ELISA and <8% and <7% for the PYY, and CCK respectively.

4. Statistical analysis

Two-way repeated measures ANOVA followed by post hoc Tukey tests was used to compare changes over time and between two trials. The total areas under the variable versus time curve (AUC), calculated using the trapezium rule, and the incremental AUC, calculated as the increment in AUC over baseline concentrations, were also used for the comparison of summary of the pre-breakfast (0–60 min) and pre-lunch (0–240 min) responses. Paired t-tests were used to compare summary data, fasting values,

Please cite this article in press as: Fatima, S., et al., Response of appetite and potential appetite regulators following intake of high energy nutritional supplements, Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

and energy intakes between the two trials. To assess the strength of within-subject relation between changes in appetite scores and changes in hormone and glucose concentrations, regression slopes and R^2 values for the regression of appetite score on hormone and glucose concentrations for the corresponding time points were calculated (Lemmens, Martens, Kester, & Westerterp-Plantenga, 2011). Student's one sample t-tests were used to test whether the means of the regression slopes were different from zero. Statistical analyses were performed using Statistica (version 6.0; StatSoft, Inc, Tulsa,OK) and Minitab (version 13.1; Minitab, Inc., State College,PA).

5. Results

Twenty-three healthy women with a mean (±SD) age of 24.4 ± 2.8 years (range: 18–35 years), body mass index (BMI) of 18.6 ± 0.9 kg/m^2 (range: 17–20 kg/m^2) and body fat mass of 9.1 ± 3.3 kg were recruited in this study. One participant dropped out and one participant developed vomiting on the day of trial thus 21 participants completed the study.

5.1. Energy intake

Data on energy and macronutrient intake for HENSD and PLACEBO trials are presented in Fig. 1 and Table. *Ad libitum* energy intake during breakfast was significantly ($P < 0.05$) lower in HENSD compared with PLACEBO trial but there was no significant difference between the two trials in the energy intake during lunch. For HENSD the suppression of energy intake during breakfast was equivalent to 57 ± 14% of energy provided by the supplement. Thus the total energy intake, calculated as a sum of energy intake during breakfast and lunch, and energy provided by supplement was by 1.07 ± 0.34 MJ higher ($P = 0.006$) in HENSD than the PLACEBO trial. Since the predicted daily energy requirement of our participants consisted of 6.72 ± 0.27 MJ, HENSD supplementation increased energy intake above the predicted daily energy requirement by 16.6 ± 5.3% with combined consumption of the supplement and breakfast providing 67 ± 5% and 59 ± 4% and combined consumption of the supplement, breakfast and lunch achieving 102 ± 8% and 83 ± 6% of daily energy requirement in HENSD and PLACEBP trials, respectively.

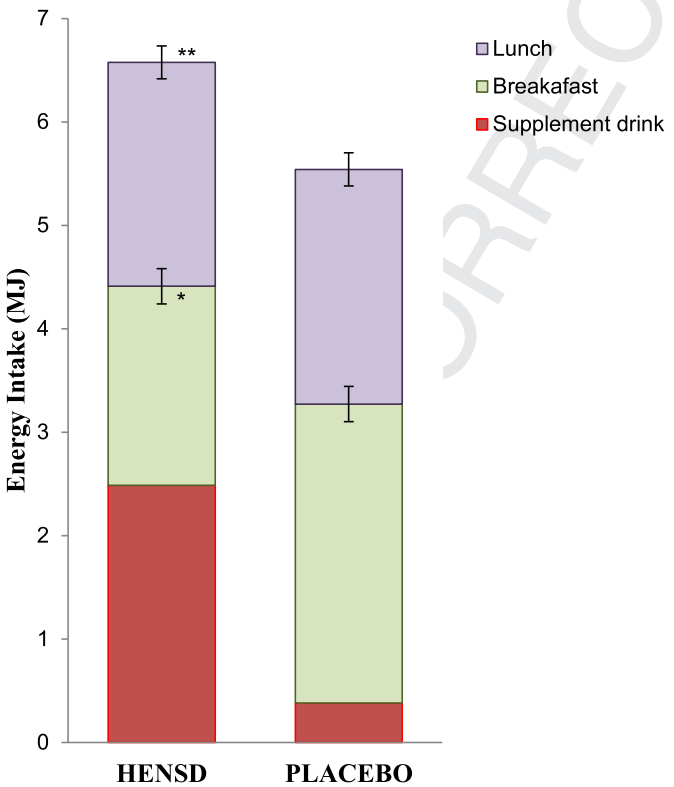

Fig. 1. Energy intake from supplements, breakfast and lunch in HENSD and PLACEBO trials. Values are presented as mean ± SE, n = 21. *Significantly ($P = 0.001$) different from energy intake during breakfast in PLACEBO trial; **Significantly ($P = 0.006$) different from total energy intake in PLACEBO trial.

5.2. Appetite responses

Responses of appetite measures during HENSD and PLACEBO trials are presented in Fig. 2. Analyses by two way ANOVA showed that all appetite measures were not significantly different between HENSD and PLACEBO trials ($P > 0.05$, trial effect). However, during pre-breakfast period (0–60 min) summary response (calculated as AUC) of hunger ($P = 0.03$) and desire to eat ($P = 0.007$) were significantly lower, and satiety ($P = 0.03$) and fullness ($P = 0.006$) were significantly higher in HENSD than PLACEBO trial. During the pre-lunch period (60–240 min) summary response of hunger, desire to eat, fullness, and satiety were not significantly different between HENSD and PLACEBO trails.

5.3. Metabolic rate and diet induced thermogenesis

Metabolic rate measured before and after supplement and breakfast intake is illustrated in Fig. 3. Resting metabolic rate (RMR) was not significantly ($P < 0.05$) different between HENSD and PLACEBO trials (HENSD, 3.1 ± 0.1 kJ/min; PLACEBO, 3.21 ± 0.1 kJ/min). In both trials, measured RMR expressed for 24 h (HENSD, 4.59 ± 0.12 MJ/day; PLACEBO 4.74 ± 0.15 MJ/day) was significantly lower ($P < 0.001$) than RMR calculated using Harris Benedict equation (Frankenfield, Muth, & Rowe, 1998) (HENSD, 5.48 ± 0.59 MJ/day; PLACEBO 5.48 ± 0.60 MJ/day). Differences between calculated RMR and measured RMR consisted of 0.90 ± 0.14 MJ/day and 0.74 ± 0.13 MJ/day in HENSD and PLACEBO trials, respectively and calculated-RMR was within 12–13% of the measured RMR in the participants of this study.

Analysis by repeated-measures ANOVA showed that the postprandial response of metabolic rate was significantly higher in the HENSD trial than in the PLACEBO trial ($P < 0.05$, trial effect). The amount of energy expended above resting metabolic rate (diet induced thermogenesis) during both pre-breakfast ($P = 0.04$) and pre-lunch ($P = 0.01$) was significantly higher in HENSD than PLACEBO with the difference in energy expended above the metabolic rate during the whole duration of the experimental trials being 0.124 ± 0.03 MJ (Table 3). After the supplement intake (0–60 min), the relative increase in energy expenditure (expressed as percentage of energy provided by the supplement) was not significantly different between two trials (HENSD, 1.1 ± 0.2%; PLACEBO, 2.7 ± 0.3%). Additionally, there was no significant difference in the relative increase in energy expenditure between trials following combined intake of the supplement and breakfast (0–240 min) (HENSD, 3.9 ± 0.5%; PLACEBO, 3.7 ± 0.6%).

5.4. Metabolic and hormonal responses

Responses of metabolic and hormonal appetite regulators are presented in Fig. 4. Analysis by repeated-measures ANOVA showed that plasma concentration glucose, insulin, CCK and PYY was significantly higher in the HENSD trial than in the PLACEBO trial ($P < 0.05$, trial effect). Summary responses of metabolic and hormonal appetite regulators, calculated as time-averaged AUC for pre-lunch and pre-breakfast periods, are presented in Table 4.

Please cite this article in press as: Fatima, S., et al., Response of appetite and potential appetite regulators following intake of high energy nutritional supplements, Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

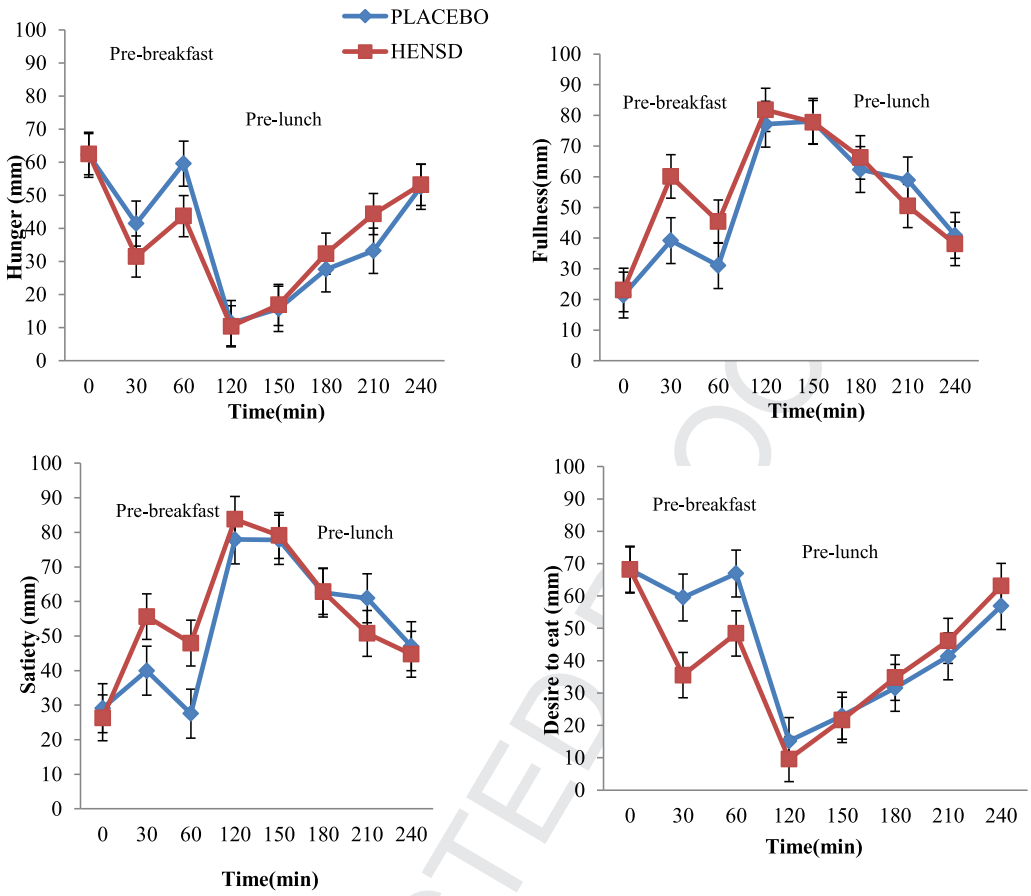

Fig. 2. Measures of hunger, satiety, fullness, and desire to eat in the fasted state (0 min), during pre-breakfast period (0–60 min) and pre-lunch periods (60–240 min) in HENSD and PLACEBO trials. Analysis by repeated-measures ANOVA showed that for all appetite measures differences were not significantly different between HENSD and PLACEBO trials ($P < 0.05$, trial effect). The supplements were consumed immediately after the fasting measurements. Values are presented as mean ± SE, n = 21.

During the pre-breakfast period, time-averaged AUC for plasma glucose was significantly ($P = 0.0001$) higher in HENSD than PLACEBO trial, whilst during the pre-lunch period there was no significant difference (P = 0.06) between trials for this measure.

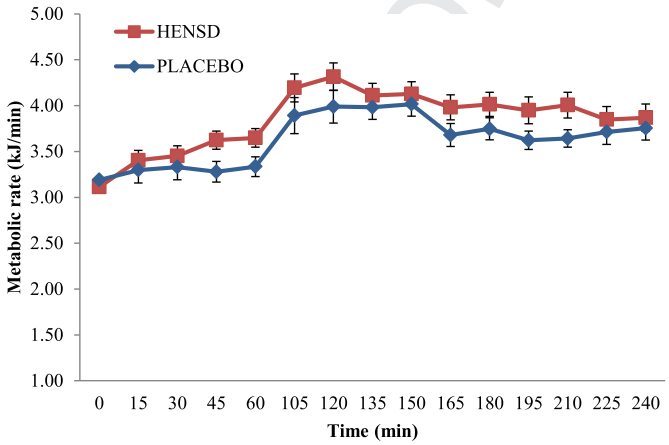

Fig. 3. Metabolic rate measured in the fasted state (0 min), during pre-breakfast period (0–60 min) and pre-lunch (60–240 min) periods in HENSD and PLACEBO trials. Analysis by repeated-measures ANOVA showed that metabolic rate was significantly higher in HENSD than in PLACEBO trials ($P < 0.05$, trial effect). The supplements were consumed immediately after the fasting measurements. Values are presented as mean ± SE, n = 21.

During the pre-breakfast period, time-averaged AUC for plasma insulin ($P = 0.006$), CCK ($P = 0.04$) and PYY ($P = 0.006$) were significantly higher in HENSD than PLACEBO trial. Time-averaged AUC for CCK ($P = 0.001$) and PYY ($P = 0.006$) were also significantly higher in the HENSD than PLACEBO trial during the pre-lunch period, Mean concentration of plasma paracetamol, a proxy measure of gastric emptying, during the pre-breakfast period (HENSD, 14 ± 4 μl/ml; PLACEBO, 26 ± 2 μl/ml) was significantly ($P = 0.01$) lower in HENSD trial than in PLACEBO trial, whilst during the pre-lunch period (HENSD, 68 ± 4 μl/ml; PLACEBO, 73 ± 5 μl/ml, $P = 0.30$), no significant difference was found between trials.

5.5. Regression of appetite measures on hormone and glucose responses

Regression slopes, 95% CIs and R^2 values for the regression of appetite scores on hormone and glucose concentrations for the

Table 3
Diet induced thermogenesis (kJ) after supplement (pre-breakfast) and breakfast (pre-lunch) consumption, and during the entire duration (0–240 min) of the HENSD and PLACEBO trials. Values are presented as Mean ± SE, (n = 21).

	HENSD	PLACEBO
Pre-breakfast	27.5 ± 5.2	10.7 ± 6.3*
Pre-lunch	143.0 ± 19.1	95.9 ± 16.1**
Over 240 min	296.6 ± 37.8	171.8 ± 40.0**

Significantly different (*$P < 0.05$, **$P < 0.001$) from HENSD trial.

Please cite this article in press as: Fatima, S., et al., Response of appetite and potential appetite regulators following intake of high energy nutritional supplements, Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

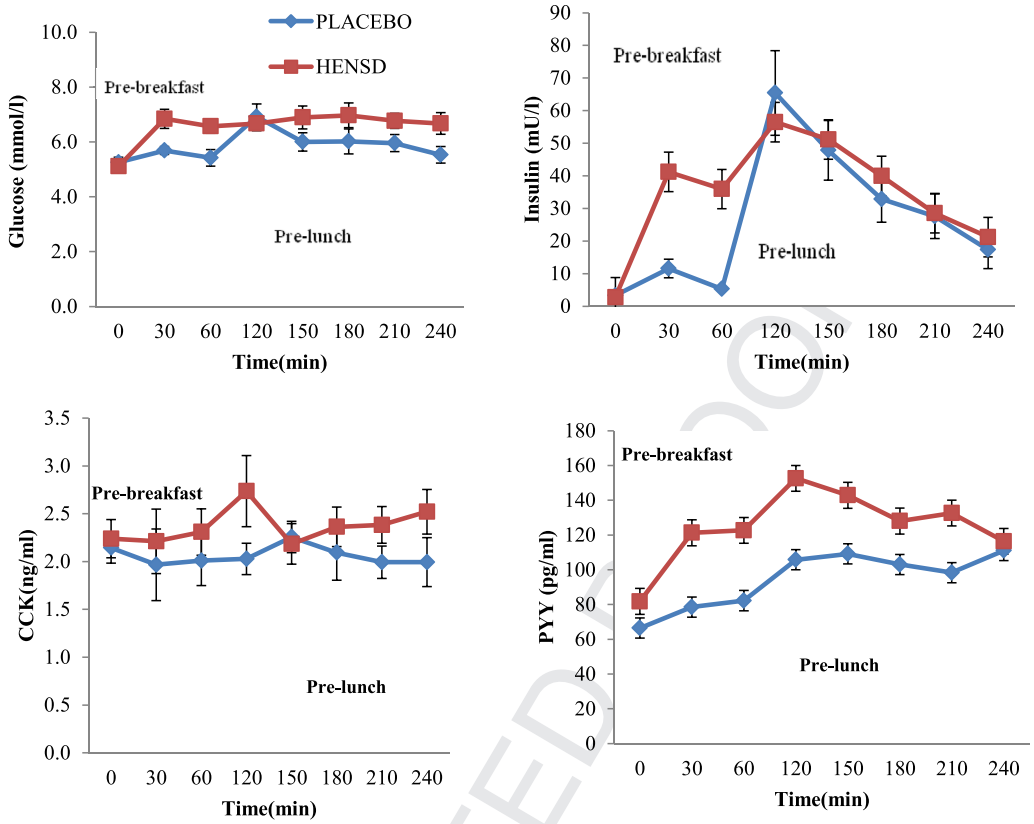

Fig. 4. Plasma concentration of glucose, insulin, cholecystokinin (CCK), peptide YY (PYY) in the fasted state (0 min), during pre-breakfast period (0–60 min) and pre-lunch periods (60–240 min) in HENSD and PLACEBO trials. Analysis by repeated-measures ANOVA showed that concentration glucose, insulin, CCK and PYY was significantly higher in the HENSD trial than in the PLACEBO trial ($P < 0.05$, trial effect). Values are presented as mean ± SE, n = 9.

corresponding time points are presented in Table 5. Relations between the responses of PYY and responses of appetite scores showed significant correlation (Satiety: $R^2 = 0.3 \pm 0.08$, $P < 0.01$; Fullness: $R^2 = 0.3 \pm 0.07$, $P < 0.01$; Hunger, $R^2 = 0.3 \pm 0.07$, $P < 0.05$; Desire to eat, $R^2 = 0.3 \pm 0.08$, $P < 0.01$). Correlations for the relations between responses of insulin and responses of appetite scores were also significant (Satiety: $R^2 = 0.4 \pm 0.08$, $P < 0.001$; Fullness: $R^2 = 0.4 \pm 0.08$, $P < 0.001$; Hunger, $R^2 = 0.4 \pm 0.06$, $P < 0.01$; Desire to eat, $R^2 = 0.5 \pm 0.09$, $P < 0.001$), while responses of glucose showed significant correlation only with responses of Fullness ($R^2 = 0.2 \pm 0.05$, $P < 0.05$) and Desire to Eat ($R^2 = 0.2 \pm 0.07$, $P < 0.01$). Correlations for the relations between responses of CCK and responses of appetite scores were not significant.

Table 4
Time-averaged AUC for the responses of glucose, insulin, peptide YY (PYY), cholecystokinin (CCK) during pre-breakfast, pre-lunch period and during the entire period (240 min) of the HENSD and PLACEBO trials. Values are presented as Mean ± SE, (n = 9).

	HENSD	PLACEBO
Glucose (mmol/L)		
Pre-breakfast	6.37 ± 0.25	5.51 ± 0.18**
Pre-lunch	6.76 ± 0.21	6.09 ± 0.29
Over 240 min	6.66 ± 0.17	5.95 ± 0.24*
Insulin (mU/L)		
Pre-breakfast	25.55 ± 6.00	7.94 ± 1.43**
Pre-lunch	33.80 ± 6.54	32.73 ± 5.69
Over 240 min	30.84 ± 5.74	26.41 ± 4.54*
PYY (pg/ml)		
Pre-breakfast	112 ± 10	76 ± 9**
Pre-lunch	136 ± 8	101 ± 11**
Over 240 min	130 ± 8	95 ± 10**
CCK (ng/ml)		
Pre-breakfast	2.2 ± 0.19	2.0 ± 0.22*
Pre-lunch	2.43 ± 0.17	2.06 ± 0.13**
Over 240 min	2.39 ± 0.17	2.05 ± 0.15**
Paracetamol (μl/ml)		
Pre-breakfast	14 ± 4	26 ± 2*
Pre-lunch	68 ± 4	73 ± 5
Over 240 min	55 ± 4	61 ± 4

Significantly different (*$P < 0.05$, **$P < 0.001$) from HENSD trial.

6. Discussion

The aim of this study was to provide insight into the appetite, metabolic, hormonal and gastric emptying responses to HENSD

Table 5
Means (±SE), 95 CIs for the means of the observed slopes and R^2 values for within-subject relations between appetite scores and hormone and glucose concentrations.

	Slope	95% CI	R^2
Satiety *vs* PYY	0.5 ± 0.1*	0.3, 0.8	0.3 ± 0.08
Fullness *vs* PYY	0.5 ± 0.1*	0.2, 0.8	0.3 ± 0.07
Hunger *vs* PYY	−0.3 ± 0.1*	−0.5, −0.1	0.3 ± 0.07
Desire to Eat *vs* PYY	−0.4 ± 0.3*	−0.7, −0.2	0.3 ± 0.08
Satiety *vs* CCK	3.3 ± 6.3	−11.6, 18.3	0.1 ± 0.03
Fullness *vs* CCK	2.8 ± 7.8	−15.5, 21.2	0.1 ± 0.05
Hunger *vs* CCK	−0.1 ± 5.3	−12.6, 12.4	0.1 ± 0.02
Desire to Eat *vs* CCK	−4.0 ± 6.9	−20.5, 12.4	0.1 ± 0.03
Satiety *vs* Insulin	0.9 ± 0.2*	0.5, 1.2	0.4 ± 0.08
Fullness *vs* Insulin	0.9 ± 0.2*	0.5, 1.3	0.4 ± 0.08
Hunger *vs* Insulin	−0.8 ± 0.2*	−1.1, −0.4	0.4 ± 0.06
Desire to Eat *vs* Insulin	−0.9 ± 0.2*	−1.3, −0.5	0.5 ± 0.09
Satiety *vs* Glucose	7.0 ± 4.0	−2.2, 16.1	0.3 ± 0.07
Fullness *vs* Glucose	7.7 ± 3.0*	0.8, 14.6	0.2 ± 0.05
Hunger *vs* Glucose	−8.7 ± 2.1	−13.6, −3.7	0.2 ± 0.07
Desire to Eat *vs* Glucose	−9.0 ± 2.4*	−14.5, −3.6	0.2 ± 0.07

PYY, Peptide YY; CCK, cholecystokinin. *Mean of the regression slopes significantly different from zero, $P < 0.05$ (Student's one sample t test).

Please cite this article in press as: Fatima, S., et al., Response of appetite and potential appetite regulators following intake of high energy nutritional supplements, Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

consumption in order to identify the mechanisms leading to the adjustment of energy intake commonly seen after taking HENSD. Primarily, we found that HENSD intake displaced some food subsequently eaten and thus increased net energy intake by only half the amount the energy supplied. The novel finding of this study is that energy compensation following consumption of HENDS is short lived and that intake of such supplement in the morning has no impact on energy intake later during the day.

We found that the net cumulative effect on energy intake (including supplement) was on average 1.07 MJ higher in the HENSD compared to PLACEBO. Thus, taking into consideration that predicted daily energy requirement of our participants consisted of approximately 6.72, our findings suggest that supplementation with HENSD applied to slightly undernourished healthy individuals should not be expected to enhance energy intake above daily energy requirements by more than 17%. Our data also suggests that overfeeding related to HENSD is not compromised by its effect on resting metabolic rate (diet induced thermogenesis). Indeed, the difference in energy expended above the metabolic rate during the whole 240 min of the experimental trials consisted only of approximately 0.124 MJ, whilst the net cumulative effect on energy intake (including supplement) was an increase of approximately 1.07 MJ. In addition, relative increase in energy expenditure, expressed as a percentage of the ingested energy, after the intake of the supplement alone and combined intake of the supplement and breakfast was relatively small and not significantly different between the HENSD and PLACEBO trials.

Although analyses by two way ANOVA showed that all appetite measures were not significantly different between HENSD and PLACEBO trials, comparison of summary responses of appetite measures (calculated as AUCs) showed that during 60 min post-supplementation, hunger suppression and enhancement in satiety was more profound after the HENSD than PLACEBO intake. This is in consistent with other studies reporting an effect of energy content of preload on appetite responses (Karl, Young, Rood, & Montain, 2013). Several mechanisms could have been responsible for these differences. Enhanced satiety and reduced hunger immediately after HENSD supplementation can be attributed to significantly higher plasma concentrations of PYY and CCK, which are known to elicit anorexigenic effects (Chaudhri, Field, & Bloom, 2008; Suzuki, Simpson, Minnion, Shillito, & Bloom, 2010) and to insulin, which plays a role in appetite regulation of lean individuals (A. Flint et al., 2007). Since delayed gastric emptying has been proposed to reduce hunger and enhance satiety by increasing gastric volume and stretch (Wang et al., 2008), the more profound appetite suppression found during immediate hour after supplementation with HENSD could also be related to slower gastric emptying. We found that concentration of plasma paracetamol, a proxy measure of gastric emptying (Näslund, 2000) was significantly lower after HENSD than PLACEBO intake.

Enhanced satiety and reduced hunger in HENSD trial, found during 60 min post supplementation (pre-lunch period), coincided with lower energy intake during *ad libitum* breakfast, but energy provided by combined intake of the supplement and breakfast was still significantly higher in the HENSD than the PLACEBO trial. Despite this overfeeding, subjective appetite measures during pre-lunch period and energy intake during *ad libitum* lunch did not differ between the HENSD and PLACEBO trials. Thus, HENSD may improve energy and nutritional intake in individuals with an increased risk of becoming malnourished (Cawood et al., 2012; R.J. Stratton & Elia, 2010; Rebecca J. Stratton et al., 2013) as the suppressive response of appetite and energy compensation following consumption of such supplements is short lived.

Even though the enhanced satiety and reduced hunger measured for 60 min (pre-breakfast period) after HENSD supplementation coincided with significantly higher plasma concentrations of PYY, CCK and insulin, during the pre-lunch period there was dissociation between appetite measures and expected action of hormonal appetite regulators, a phenomenon also found in some other studies (De Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004; Doucet et al., 2008; Gielkens, Verkijk, Lam, Lamers, & Masclee, 1998). For further understanding of relations between responses of appetite scores and hormone responses we used a statistical approach that focuses on within participant relations between changes in appetite scores and changes in hormone concentrations (Lemmens et al., 2011) and obtained regression slopes and R^2 values for the regression of appetite scores on each of the measured appetite hormones. We found that within participant relations between responses of appetite scores and responses of hormones showed significant correlations with regards to PYY and insulin, but not CCK. Thus, our findings contribute to the debate about whether appetite hormones act as biomarkers of appetite (De Graaf et al., 2004; A. Flint et al., 2007; Lemmens et al., 2011) and support the notion of the subjective appetite measures being better correlates of energy intake than circulating levels of individually considered appetite hormones (Doucet et al., 2008).

Some studies (Crovetti et al., 1998; Mansour et al., 2012; Westerterp-Plantenga et al.,1997) suggest that appetite and satiety are influenced by an increase in energy expenditure in the postprandial period due to absorbing, metabolizing, and storing ingested nutrients (Tappy, 1996), a phenomenon known as diet induced thermogenesis. Studies have found that higher diet induced thermogenesis (DIT) is correlated with reduced hunger and more satiety (Crovetti et al., 1998; Mansour et al., 2012; Westerterp-Plantenga et al.,1997). Thus, the current study also aimed to find out whether appetite responses following HENSD consumption were related to change in postprandial energy expenditure. We found that during 60 min post-supplementation energy expended above resting metabolic rate was significantly higher in the HENSD than PLACEBO trial, which coincided with lower hunger and higher satiety. The increase in diet induced thermogenesis persisted into the pre-lunch period, but this was not reflected in satiety and appetite differences. Thus, our data shows no consistent relationship between diet-induced thermogenesis and appetite and satiety regulation, which is in line with the findings from recent meta-analyses (Ravn et al., 2013). It has been suggested that satiety might be related to diet induced thermogenesis only on a high protein diet (Veldhorst et al., 2008). Thus, the protein content (11.9 g) of the HENSD supplement may be insufficient in terms of triggering a satiating effect through thermogenesis.

The study design included the use of preload paradigm which is an established methodology to assess the effects of food or drink on appetite and energy intake in the short term, under controlled conditions (Almiron-Roig et al., 2013). This design overcomes misreporting of food intake, allowing precise measurement of energy intake. To further optimize the quality of energy intake data, foods included in breakfast and lunch and leftovers of breakfast and lunch were weighed and analysed by two researchers. In contrast to most other studies, our study investigated the impact of HENSD consumption on energy compensation during two consecutive meals to explore shorter and longer term effect on food intake. This allowed us to find out that appetite suppressive responses and energy compensation is acute only, disappearing quite quickly. It should be noted however that the laboratory environment was experimental, and thus energy intake may have deviated from normal eating behaviours (Hetherington, Anderson, Norton, & Newson, 2006).

This study has some limitations. We recruited slim, but healthy young women in order to simulate the likely effect of HENSD in

Please cite this article in press as: Fatima, S., et al., Response of appetite and potential appetite regulators following intake of high energy nutritional supplements, Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

younger malnourished patients, since most previous research had been done in the elderly. Thus, results obtained in this study are more likely to be relevant to adolescents and younger adults, and cannot necessarily be generalized to sick people at risk of malnutrition where various other disease-associated factors impact energy intake and regulation. The responses of metabolic and hormonal appetite regulators were investigated in a subset of the study participants, as power calculations suggested that investigation of nine participants would allow identification of physiologically meaningful and significant differences in postprandial PYY, CCK, glucose, insulin, concentrations. Only medium term regulation of appetite and energy intake was measured which might not be applicable to clinical or home settings where HENSD are prescribed for long-term periods.

In summary, our study suggests that HENSD intake induces a partial and relatively short lived suppressive action on appetite and energy intake. In the short term these supplements increase net energy intake by about half the energy value of the supplement taken. This has an important implication if prescribing supplements to malnourished individuals.

Authors' contribution to manuscript

The authors responsibilities were as follows – DM, KG, CW: contributed to concept development and designed study; SF, MT recruited participants, performed the tests; SF, AE performed metabolic regulator analysis; DM, SF analysed other data, performed the statistical analysis; SF, DM drafted the manuscript; SF, DM, KG and CW revised manuscript. None of the authors had a personal or financial conflict of interest to disclose.

Acknowledgement

This study was conducted as a part of the PhD of Sadia Fatima, funded by Khyber Medical University (Pakistan). Authors are thankful to all the participants for their time and dedication.

References

Almiron-Roig, E., Palla, L., Guest, K., Ricchiuti, C., Vint, N., Jebb, S. A., et al. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews, 71*(7), 458–473.

Cawood, A. L., Elia, M., & Stratton, R. J. (2012). Systematic review and meta-analysis of the effects of high protein oral nutritional supplements. *Ageing Research Reviews, 11*(2), 278–296.

Chaudhri, O. B., Field, B. C. T., & Bloom, S. R. (2008). Gastrointestinal satiety signals. *International Journal of Obesity, 32*, S28–S31.

Clements, J. A., Heading, R. C., Nimmo, W. S., & Prescott, L. F. (1978). Kinetics of acetaminophen absorption and gastric emptying in man. *Clinical Pharmacology and Therapeutics, 24*(4), 420–431.

Crovetti, R., Porrini, M., Santangelo, A., & Testolin, G. (1998). The influence of thermic effect of food on satiety. *European Journal of Clinical Nutrition, 52*(7), 482.

De Graaf, C., Blom, W. A. M., Smeets, P. A. M., Stafleu, A., & Hendriks, H. F. J. (2004). Biomarkers of satiation and satiety. *The American Journal of Clinical Nutrition, 79*(6), 946–961.

Doucet, É., Laviolette, M., Imbeault, P., Strychar, I., Rabasa-Lhoret, R.., & Prud'homme, D. (2008). Total peptide YY is a correlate of postprandial energy expenditure but not of appetite or energy intake in healthy women. *Metabolism, 57*(10), 1458–1464.

Flint, A., Gregersen, N. T., Gluud, L. L., Moller, B. K., Raben, A., Tetens, I., et al. (2007). Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. *British Journal of Nutrition, 98*(1), 17–25.

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 24*(1), 38–48.

Frankenfield, D. C., Muth, E. R., & Rowe, W. A. (1998). The Harris-Benedict studies of human basal metabolism: history and limitations. *Journal of the American Dietetic Association, 98*(4), 439–445.

Frayn, K. N., & Macdonald, I. A. (1997). Assessment of substrate and energy metabolism in vivo. In B. Draznin, & R. Rizza (Eds.), *Clinical research in diabetes and obesity, Part I: Methods, assessments and metabolic regulation*. Totowa, NJ: Humana Press.

Gielkens, H. A. J., Verkijk, M., Lam, W. F., Lamers, C. B. H. W., & Masclee, Ad A. M. (1998). Effects of hyperglycemia and hyperinsulinemia on satiety in humans. *Metabolism, 47*(3), 321–324.

Goris, A. H. C., Vermeeren, M. A. P., Wouters, E. F. M., Schols, A. M. W. J., & Westerterp, K. R. (2003). Energy balance in depleted ambulatory patients with chronic obstructive pulmonary disease: the effect of physical activity and oral nutritional supplementation. *British Journal of Nutrition, 89*(05), 725–729.

Hetherington, M. M., Anderson, A. S., Norton, G. N. M., & Newson, L. (2006). Situational effects on meal intake: a comparison of eating alone and eating with others. *Physiology & Behavior, 88*(4), 498–505.

Karl, J. P., Young, A. J., Rood, J. C., & Montain, S. J. (2013). Independent and combined effects of eating rate and energy density on energy intake, appetite, and gut hormones. *Obesity, 21*(3), E244–E252.

Lemmens, S. G., Martens, E. A., Kester, A. D., & Westerterp-Plantenga, M. S. (2011). Changes in gut hormone and glucose concentrations in relation to hunger and fullness. *The American Journal of Clinical Nutrition, 94*(3), 717–725.

Mansour, M. S., Ni, Yu-M., Roberts, A. L., Kelleman, M., RoyChoudhury, A., & St-Onge, M.-P. (2012). Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: a pilot study. *Metabolism, 61*(10), 1347–1352.

Milne, A. C., Avenell, A., & Potter, J. (2005). Oral protein and energy supplementation in older people: a systematic review of randomized trials. *Nestle Nutr Workshop Ser Clin Perform Programme, 10*, 103–120. http://dx.doi.org/10.1159/000083301. discussion 120-105.

Miodownik, S., Melendez, J., Carlon, V. A., & Burda, B. (1998). Quantitative methanol-burning lung model for validating gas-exchange measurements over wide ranges of. *Journal of Applied Physiology, 84*(6), 2177–2182.

Näslund, E. (2000). Gastric emptying: comparison of scintigraphic, polyethylene glycol dilution, and paracetamol tracer assessment techniques. *Scandinavian Journal of Gastroenterology, 35*(4), 375–379.

Poustie, V. J., Russell, J. E., Watling, R. M., Ashby, D., & Smyth, R. L. (2006). Oral protein energy supplements for children with cystic fibrosis: CALICO multicentre randomised controlled trial. *British Medical Journal, 332*(7542), 632–636. http://dx.doi.org/10.1136/bmj.38737.600880.AE.

Ravn, A.-M., Gregersen, N. T., Christensen, R., Rasmussen, L. G., Hels, O., Belza, A., et al. (2013). Thermic effect of a meal and appetite in adults: an individual participant data meta-analysis of meal-test trials. *Food & Nutrition Research, 57*.

Stratton, R. J., & Elia, M. (1999). The effects of enteral tube feeding and parenteral nutrition on appetite sensations and food intake in health and disease. *Clinical Nutrition, 18*(2), 63–70.

Stratton, R. J., & Elia, M. (2010). Encouraging appropriate, evidence-based use of oral nutritional supplements. *Proceedings of the Nutrition Society, 69*(04), 477–487.

Stratton, R. J., Hébuterne, X., & Elia, M. (2013). A systematic review and meta-analysis of the impact of oral nutritional supplements on hospital readmissions. *Ageing Research Reviews, 12*(4), 884–897.

Stratton, R. J., Stubbs, R. J., & Elia, M. (2008). Bolus tube feeding suppresses food intake and circulating ghrelin concentrations in healthy subjects in a short-term placebo-controlled trial. *The American Journal of Clinical Nutrition, 88*(1), 77–83.

Suzuki, K., Simpson, K. A., Minnion, J. S., Shillito, J. C., & Bloom, S. R. (2010). The role of gut hormones and the hypothalamus in appetite regulation. *Endocrine Journal, 57*(5), 359–372.

Tappy, L. (1996). Thermic effect of food and sympathetic nervous system activity in humans. *Reproduction Nutrition Development, 36*(4), 391–397.

Veldhorst, M., Smeets, A. J. P. G., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., et al. (2008). Protein-induced satiety: effects and mechanisms of different proteins. *Physiology & Behavior, 94*(2), 300–307.

Wang, G.-J., Tomasi, D., Backus, W., Wang, R., Telang, F., Geliebter, A., et al. (2008). Gastric distention activates satiety circuitry in the human brain. *Neuroimage, 39*(4), 1824–1831.

Westerterp-Plantenga, M. S., Wijckmans-Duijsens, N. E. G., Verboeket-Van De Venne, W. P. H. G., De Graaf, K., Weststrate, J. A., & Van Het Hof, K. H. (1997). Diet-induced thermogenesis and satiety in humans after full-fat and reduced-fat meals. *Physiology & Behavior, 61*(2), 343–349.