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

Sadia Fatimab, Konstantinos Gerasimidisa, Charlotte Wrighta, Melina Tsiountsiouraa, Eirini-Iro Arvanitidoa, Dalia Malkovaa,

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

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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/m² consumed HENDS (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 HENDS trial but the net cumulative effect on energy intake was 1.07 ± 0.34 MJ higher in the HENDS compared to PLACEBO. Plasma concentration of CCK and PYY and insulin were significantly (P < 0.05) higher in the HENDS trial while appetite measures were not significantly different between HENDS 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.

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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 micro-nutrients, providing between 6 3 kJ/ml to 10 1 kJ/ml per typical serving of 125–220 ml (Stratton & Elia, 2010). Although HENDS 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 HENDS 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

References:

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).

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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 (Wandish et al., 2008) and with higher diet induced thermogenesis (Crogett, 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/m²), 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), supplementing 2.5 MJ, or a low calorie drink made up with skimmed milk, cocoa and artificial sweeteners (PLACEBO trial), supplementing 0.4 MJ (Table 1). Participants were blinded of the preload drink and they were not told which drink they would consume and 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).

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 white meal breakfast 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.

Table 1

<table>
<thead>
<tr>
<th>Energy (MJ)</th>
<th>HENSD</th>
<th>PLACEBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO (g)</td>
<td>68.8 g</td>
<td>11.3 g</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>30.4 g</td>
<td>1.3 g</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>11.9 g</td>
<td>9.4 g</td>
</tr>
<tr>
<td>Energy from CHO (%)</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Energy from Fat (%)</td>
<td>46</td>
<td>13</td>
</tr>
<tr>
<td>Energy from Protein (%)</td>
<td>8</td>
<td>39</td>
</tr>
</tbody>
</table>

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

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Induced thermogenesis for the pre-lunch period (60 min) of the metabolic rate measured for 60 min after supplement consumption (Frayn, 2000) by using indirect calorimetry equations described elsewhere (Frayn, 1997). Diet induced thermogenesis for the pre-lunch period was assessed by using indirect calorimetry equations described elsewhere (Frayn, 1997). These values were then used to calculate rate of energy expenditure (VO₂ and VCO₂) and rate of carbon dioxide production (VCO₂) were recorded every minute for the duration of 20 min. These values were then used to calculate rate of energy expenditure (VO₂ and VCO₂) and rate of carbon dioxide production (VCO₂) were recorded every minute for the duration of 20 min. These values were then used to calculate rate of energy expenditure (VO₂ and VCO₂) and rate of carbon dioxide production (VCO₂). Energy, carbohydrate (CHO), protein and fat intake in HENSD and PLACEBO trials. Values are presented as Mean ± SE, (n = 23).

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 (VO₂) and rate of carbon dioxide production (VCO₂) were recorded every minute for the duration of 20 min. These values were then used to calculate rate of energy expenditure (VO₂ and VCO₂) and rate of carbon dioxide production (VCO₂). Energy, carbohydrate (CHO), protein and fat intake in HENSD and PLACEBO trials. Values are presented as Mean ± SE, (n = 23).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>HENSD</th>
<th>PLACEBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>2.49</td>
<td>0.38</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>1.92 ± 0.17</td>
<td>2.89 ± 0.23**</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>80 ± 8</td>
<td>113 ± 1.1*</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>11 ± 1</td>
<td>16 ± 1.4</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>12 ± 1</td>
<td>21.2 ± 2**</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>4.41 ± 0.17</td>
<td>3.27 ± 0.23**</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>149 ± 7.65</td>
<td>125 ± 9.70*</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>42 ± 2</td>
<td>23 ± 2**</td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>2.16 ± 0.16</td>
<td>2.27 ± 0.20</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>69 ± 5</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>22 ± 2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>18 ± 1.6</td>
<td>18 ± 1.9</td>
</tr>
<tr>
<td>Breakfast + Lunch</td>
<td>4.09 ± 0.26</td>
<td>5.16 ± 0.32**</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>150 ± 11</td>
<td>186 ± 13**</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>33 ± 3</td>
<td>40 ± 3**</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>30 ± 2</td>
<td>40 ± 3**</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>21 ± 11</td>
<td>197 ± 13</td>
</tr>
<tr>
<td>Breakfast + Lunch + Supplement</td>
<td>6.58 ± 0.26</td>
<td>5.54 ± 0.32**</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>45 ± 3</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>60 ± 2</td>
<td>41 ± 3**</td>
</tr>
</tbody>
</table>

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.

3.3. 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 IU activity per ml, Sigma-Aldrich, UK) and then centrifuged at 1400 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,
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/m² (range: 17–20 kg/m²) 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 PLA-
CEBO 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 differ-
ence 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 con-
sumption of the supplement, breakfast and lunch achieving
102 ± 8% and 83 ± 6% of daily energy requirement in HENSD and
PLACEBO trials, respectively.

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 trials.

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 be-
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 post-
prandial 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 PLA-
CEBO 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 per-
centage of energy provided by the supplement) was not signifi-
cantly 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 of 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 hor-
monal appetite regulators, calculated as time-averaged AUC for
pre-lunch and pre-breakfast periods, are presented in Table 4.
During the pre-breakfast period, time-averaged AUC for plasma glucose was significantly (P = 0.0001) higher in HENSD than PLACERBO trial, whilst during the pre-lunch period there was no significant difference (P = 0.06) between trials for this measure. 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 PLACERBO trial. Time-averaged AUC for CCK (P = 0.001) and PYY (P = 0.006) were also significantly higher in the HENSD than PLACERBO 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; PLACERBO, 26 ± 2 μl/ml) was significantly (P = 0.01) lower in HENSD trial than in PLACERBO trial, whilst during the pre-lunch period (HENSD, 68 ± 4 μl/ml; PLACERBO, 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 R² 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 PLACERBO trials. Values are presented as Mean ± SE, (n = 21).
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HENSD</td>
</tr>
<tr>
<td>Pre-breakfast</td>
<td>27.5 ± 5.2</td>
</tr>
<tr>
<td>Pre-lunch</td>
<td>143.0 ± 19.1</td>
</tr>
<tr>
<td>Over 240 min</td>
<td>296.6 ± 37.8</td>
</tr>
</tbody>
</table>

Significantly different (*P < 0.05, **P < 0.001) from HENSD trial.
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: $R^2 = 0.5 \pm 0.09, P < 0.001$), 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.

### 6. Discussion

The aim of this study was to provide insight into the appetite, metabolic, hormonal and gastric emptying responses to HENSD nutritional supplements. Appetite (2015), http://dx.doi.org/10.1016/j.appet.2015.06.010

#### Table 5

<table>
<thead>
<tr>
<th>Slope</th>
<th>95% CI</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satiety vs PYY</td>
<td>$0.5 \pm 0.1^*$</td>
<td>$0.3 \pm 0.08$</td>
</tr>
<tr>
<td>Fullness vs PYY</td>
<td>$0.5 \pm 0.1^*$</td>
<td>$0.3 \pm 0.07$</td>
</tr>
<tr>
<td>Hunger vs PYY</td>
<td>$-0.3 \pm 0.1^*$</td>
<td>$-0.5 \pm 0.1$</td>
</tr>
<tr>
<td>Desire to Eat vs PYY</td>
<td>$-0.4 \pm 0.3^*$</td>
<td>$-0.7 \pm 0.2$</td>
</tr>
<tr>
<td>Satiety vs CCK</td>
<td>$3.3 \pm 6.3$</td>
<td>$0.1 \pm 0.03$</td>
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<tr>
<td>Fullness vs CCK</td>
<td>$2.8 \pm 7.8$</td>
<td>$0.1 \pm 0.05$</td>
</tr>
<tr>
<td>Hunger vs CCK</td>
<td>$-0.5 \pm 1.3$</td>
<td>$-1.26 \pm 12.4$</td>
</tr>
<tr>
<td>Desire to Eat vs CCK</td>
<td>$-4.0 \pm 6.9$</td>
<td>$-20.5 \pm 12.4$</td>
</tr>
<tr>
<td>Satiety vs Insulin</td>
<td>$0.9 \pm 0.2^*$</td>
<td>$0.5 \pm 0.12$</td>
</tr>
<tr>
<td>Fullness vs Insulin</td>
<td>$0.9 \pm 0.2^*$</td>
<td>$0.5 \pm 0.13$</td>
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<tr>
<td>Hunger vs Insulin</td>
<td>$-0.8 \pm 0.2^*$</td>
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<td>Desire to Eat vs Insulin</td>
<td>$-0.9 \pm 0.2^*$</td>
<td>$-1.3 \pm 0.5$</td>
</tr>
<tr>
<td>Satiety vs Glucose</td>
<td>$7.0 \pm 4.0^*$</td>
<td>$22.16 \pm 11.1$</td>
</tr>
<tr>
<td>Fullness vs Glucose</td>
<td>$7.7 \pm 3.0^*$</td>
<td>$0.8 \pm 14.6$</td>
</tr>
<tr>
<td>Hunger vs Glucose</td>
<td>$8.7 \pm 2.1$</td>
<td>$-13.8 \pm 3.7$</td>
</tr>
<tr>
<td>Desire to Eat vs Glucose</td>
<td>$-8.0 \pm 2.4^*$</td>
<td>$-14.5 \pm 3.6$</td>
</tr>
</tbody>
</table>

**PYY, Peptide YY; CCK, cholecystokinin.** Mean of the regression slopes significantly different from zero, $P < 0.05$ (Student’s one sample t-test).
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 HENSD 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 other words, 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 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 PY and CCK, which are known to elicit anorexigenic effects (Chaudhari, Field, & Bloom, 2008; Suzuki, Simpson, Minnon, 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 (Nalund, 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, & Ella, 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 PY, 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, Smets, Staafleu, & Hendriks, 2004; Doucet et al., 2008; Gielkens, Verkijk, Lam, Lamers, & Maslee, 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 R² 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 PY 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 (Ravns 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...
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.

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References


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