Original Article

Acute effects of oral preloads with increasing energy density on gastric emptying, gut hormone release, thermogenesis and energy intake, in overweight and obese men

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This study investigated the effect of high- and low-energy density preloads on gastrointestinal and metabolic factors, which act to regulate acute energy intake. Sixteen overweight and obese men (BMI range: 27.2-36.5 kg/m^2) each received 3 oral preloads in randomised order: i) high-energy-density, high-fat (1.5 kcal/g), ii) low-energy-density, high-fat (1.1 kcal/g), and iii) low-energy-density, high-protein (1.1 kcal/g). Over 180 min, gastric emptying, plasma glucagon-like peptide-1 concentrations, and diet-induced thermogenesis were assessed, and subsequent energy intake was determined. Total energy intake did not differ between preloads (high-energy-density, high-fat, 2059±72 kilocalories (kcal); low-energy-density, high-fat, 1876±91 kcal; and low-energy-density, high-protein, 1867±63 kcal). Gastric emptying was slower following the high-energy-density, high-fat preload (158±8 min) compared with the low-energy-density, high-protein preload (130±9 min) (p=0.05), but did not differ between the high-energy-density, high-fat and low-energy-density, high-fat (147±8 min) preloads. Plasma glucagon-like peptide-1 did not differ substantially between preloads. Diet-induced thermogenesis was lower following high-energy-density, high-fat (10.4±0.7 %) than low-energy-density, high-fat (14.9±1.2 %) and low-energy-density, high-protein (18.1±1.1 %) preloads (p<0.01 for both). We conclude that an increased energy density slows gastric emptying and reduces thermogenesis, but that a high fat content overrides the effect of energy density on gastric emptying. The counter-regulatory modulation of these gastric and metabolic factors may explain, at least in part, the lack of differences in subsequent energy intake in response to oral preloads with increasing energy density.

Key Words: gut function, body weight regulation, high-fat diet, high-protein diet, humans

INTRODUCTION

It is widely regarded that the consumption of energy-dense food contributes to obesity, even when the increase in energy density is subtle.[1] Nevertheless, the largest long-term prospective studies failed to observe any association between the consumption of foods with a high-energy-density and overweight/obesity.[2,3] Furthermore, results from randomised clinical trials are mixed - no effect of lowering dietary energy density was found on body weight at 1-4 years,[4,5] while others have reported greater weight loss at 6 months when the energy density of individuals' habitual diet was reduced by only 0.56±0.3 kcal/g compared with those who maintained their habitual diet.[6] There are potentially a number of reasons for these inconsistencies including substantial intra-individual variation in the physiological responses to mixed-meals that, in turn, promote or limit further food intake.

Persuasive evidence indicates that inter-related gastrointestinal factors, particularly pyloric motor activity, and cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) release, provide inhibitory feedback that slows gastric emptying (GE) and suppresses energy intake in response to varying combinations of intraduodenal fat and/or carbohydrate.[7-10] Moreover, there is emerging, but still relatively little, evidence that the modulation of some

gastrointestinal factors in response to specific nutrients, differs in lean and obese individuals. For example, Rolls *et al* reported that lean, healthy males reduced their energy intake after 500-kcal yogurt preloads that were high in either fat or carbohydrate when compared with a no-preload condition,[11] and they also found accurate compensation when energy was delivered intragastrically, but not when administered intravenously.[12] Collectively, these studies demonstrate the existence of mechanisms in the gastrointestinal tract for the rapid detection of nutrients in lean, healthy subjects. In contrast, our group has recently observed that increased BMI was related inversely to gastrointestinal sensitivity to fat, and with greater intakes of energy and fat.[13,14] It has also been established that body weight is related inversely to both GE,[15,16] and GLP-1 release.[17,18] In addition, GLP-1, itself, is related inversely to GE.[19-21] In lean men and women, it has also been established that an isocaloric meal with an increased, compared to reduced, fat-to-protein ratio, reduces the GLP-1 response and blunts diet-induced thermogenesis (DIT). Since an increased DIT, itself, has been associated directly with increased fullness and reduced hunger,[22-24] it is conceivable that an increased fat-to-protein ratio, independent of energy density (that is, a preload with a low-energy-density and increased fat content compared with one of equal energy density, but with a reduced fat and increased protein content), reduces feelings of satiety and hence promotes excess energy consumption. While the implications of the above associations remain unclear, it is conceivable that 'insufficient' modulation of these potentially important acute gastrointestinal and metabolic regulatory factors, in response to preloads of high- and low- energy density, may promote excess energy intake, particularly in overweight and obese individuals.

Accordingly, the aim of this study was to evaluate the effects of high- compared to low-energy-density preloads, and an increase in the fat-to-protein ratio of two preloads matched in energy density, on gastrointestinal and metabolic factors, including GE, GLP-1 and DIT, and on appetite and energy intake, in overweight and obese men.

METHODS
Subjects
Sixteen overweight and obese men (mean±SD [range]; aged 36±13 [18-57] years, BMI 32±2.7 [27.2-36.5] kg/m^2; waist circumference 110±6 [103-124] cm; eating restraint score 7±4 [2-12]) were recruited through advertisements placed in the local paper, and on notice boards around the local universities and hospitals within the metropolitan areas of Adelaide, South Australia. Men only were studied to avoid the established effect of the menstrual cycle on energy intake.[25] Sample size was based on statistical power calculations using within-subject contrasts with p=0.05 and a power of (β) ≥0.8 to detect a minimal difference of 150 kcal between treatments in the primary outcome of energy intake (using an average within-subject standard deviation for energy intake of 120 kcal[26]) and to determine correlations of r ≥0.6 between energy intake with GE, GLP-1, and DIT. Subjects were included if they were healthy and had maintained a stable body weight for at least 3 months prior to the study (within 5 % of the screening weight), and were unrestrained eaters

(score ≤12 on the eating restraint component of the Three Factor Eating questionnaire).[27] Exclusion criteria included a history of gastrointestinal disease, taking any medication known to affect gastrointestinal function, energy metabolism, body weight or appetite, smoking, consumption of >20 g per day of alcohol, and an intolerance of lactose (or a disliking of yoghurt). The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, all subjects provided written, informed consent, prior to their inclusion, and the study was carried out in accordance with the Declaration of Helsinki. The study was registered as a clinical trial with the Australia and New Zealand Clinical Trial Registry (www.anzctr.org.au; trial number ACTRN12611000941965). At the screening visit, which was ~7-10 days prior to the first test day, subjects were informed that the study aim was to compare the acute effects of preloads with increasing energy density on the function of the stomach, including gastric emptying, the release of gut hormones into the blood, and on the amount of energy burnt/expended, but were unaware that energy intake was measured.

Study design
Each subject was assessed on three occasions, each separated by 3-7 days. On each occasion, they received one of three semi-solid yoghurt-based test preloads: i) high-energy-density, high-fat (HEDHF); ii) low-energy-density, high-fat (LEDHF); and iii) low-energy-density, high-protein (LEDHP), in a randomised, single-blind, cross-over fashion. Randomisation was performed by an investigator not involved in the assessments using the free software "Research Randomiser",[28] and the investigator performing the assessments was blinded to the randomisation and preload conditions. Gastric emptying, concentrations of blood glucose, serum insulin and plasma GLP-1, postprandial DIT, appetite perceptions, and subsequent energy intake, in response to the preloads, were measured.

Preloads
The ingredients, energy density and macronutrient composition of the HEDHF, LEDHF and LEDHP semi-solid yoghurt-based test preloads are depicted in Table 1; all preloads were matched for weight (g) and carbohydrate content (g), palatability, smell, texture and appearance and the caloric content of the HEDHF preload was 222 kcal greater than both LED preloads. To increase the energy density of the HEDHF preload, the fat content of the LEDHF and LEDHP preloads was increased by 24 g and 40 g, respectively. The protein content of the HEDHF and LEDHF preloads was matched and was 35 g less than with LEDHP. The water content of HEDHF was 52 and 54 g less than with the LEDHF and LEDHP preloads, respectively. The differential macronutrient composition of both LED preloads was achieved by manipulating the fat and protein content such that LEDHF contained 16 g more fat and 35 g less protein than LEDHP. For all preloads, the fat and protein contents were manipulated using full-fat thickened cream (~68 % saturated fat) and whey protein isolate, respectively. For the purpose of measuring GE, using the [^{14}C]-octanoic acid breath test,[29] 5 ml of egg yolk labelled with 75 KBq of [$^{1-14}$C]-sodium octanoic acid (Biomedicals Australasia, Sydney, New South Wales,

Australia) was added to each preload.

Protocol

Subjects were provided with a standard dinner that consisted of a beef lasagne (McCain Foods, Wendouree, Victoria, Australia), a slice of wholemeal bread, 250 mL orange juice (Daily Juice Co, Docklands, Victoria, Australia), and a chocolate chip muesli bar (Nestle Cereal Partners Australia Ltd, Rhodes, NSW, Australia) which they consumed the evening prior to each study day. From 8 pm, they fasted from all solids and liquids, except water. In addition, subjects refrained from strenuous exercise and alcohol for 24 hours prior to the study. Subjects arrived at the Discipline of Medicine at 8.30 am. Upon arrival, subjects rested in the supine position for 40 min, during which time fasting resting energy expenditure (REE) was measured by indirect calorimetry. A cannula was then inserted into a forearm vein, and a baseline blood sample (14 mL) collected for subsequent determination of blood glucose, serum insulin and plasma GLP-1 concentrations. A baseline breath sample for the assessment of GE, and ratings for the assessment of hunger and fullness using a visual analogue scale (VAS) questionnaire, were collected. Subjects then sat upright to consume one of the yoghurt test preloads within 10 min, after which time they returned to the supine position and rested quietly under the ventilated hood for a further 3 hours to measure postprandial DIT. Immediately after the preload (t=0 min) and at t=15, 30, 45, 60, 75, 90, 120, 150 and 180 min, blood and breath samples, and appetite ratings were collected;

additional breath samples were collected at t=105, 135 and 165 min. At t=180 min, subjects were presented with a standardised cold buffet-style meal to assess energy intake. A 3-hour interval between the test preload and buffet meal was selected since we anticipated (based on our knowledge of gastric emptying rates) that the stomach would still contain approximately 30 % of the preload content, and hence, upper gut and metabolic satiety-related signals would potentially still be exerting an influence on energy intake.[6,30] At t=210 min, final blood and breath samples, and appetite ratings, were collected following which the cannula was removed and subjects permitted to leave the laboratory.

Measurements

Energy and macronutrient intake

The cold buffet-style meal comprised 4 slices (125 g) wholemeal bread, 4 slices (125 g) white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 20 g mayonnaise, 20 g margarine, 170 g apple, 190 g banana, 200 g strawberry yogurt, 150 g chocolate custard, 140 g fruit salad, 375 mL iced coffee, 300 mL orange juice and 600 mL water. The amount of food offered was in excess of what the subject was expected to eat, and each subject was allowed up to 30 min to freely consume from the buffet meal until comfortably full. Food consumption was assessed by weighing each food item of the buffet-style meal, before and after presentation and energy intake and macronutrient composition were calculated using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, QLD, Australia).[31]

Appetite and gastrointestinal symptom ratings: VAS questionnaires were used to assess perceptions of appetite (hunger and fullness) and gastrointestinal symptoms (bloating and nausea).[32] Immediately following the consumption of each test preload its palatability was assessed. The strength of each sensation was represented by a vertical mark being placed along a 100-mm horizontal line, where 0 represented "sensation is not felt at all' and 100 represented that "sensation is felt the greatest". Scores were expressed as changes from baseline (ie, t=-15 min).

Gastric emptying

Gastric emptying was determined by the [^{14}C]-octanoic acid breath test, which is a validated, indirect test to quantify GE from $^{14}CO_2$ excreted in the breath.[29,33] End-expiratory breath samples were collected in glass scintillation vials containing a proprietary non-toxic metallic hydroxide, CO_2 trapping solution (RAH Nuclear Medicine, Adelaide, South Australia) that effectively collected 0.5 mmol of CO_2. Samples were then solubilised in 10 ml Starscint liquid scintillation counting solution (Packard Instruments, Meriden, Connecticut, USA) and counted in a Packard 2100TR Tri-Carb liquid scintillation counter (Packard Instruments) to a 1 % coefficient of variation. Resultant disintegrations per minute (DPM) of $^{14}CO_2$ were expressed as a percentage of the original dose and plotted against time and non-linear regression modelling of the data was used to determine the 50 % emptying time ($T_{50,}$ min), the highest concentration of $^{14}CO_2$ is re-

Table 1. Composition and ingredients of the test preloads

	HEDHF	LEDHF	LEDHP
Composition[†]			
Energy content (kcal)	999	777	777
Weight (g)	673	673	673
Energy density (kcal/g)	1.48	1.15	1.15
Protein (g)	30	29	64
Fat (g)	62	38	22
Carbohydrate (g)	83	83	81
Ingredients (g)			
Natural yoghurt[‡]	385	375	375
Frozen raspberries[§]	35	60	68
Cream[¶]	170	100	55
Sugar[††]	40	40	40
Cornflour[‡‡]	5	5	5
Gelatine[‡‡]	3	4	4
Whey protein isolate[§§]	0	0	40
Egg yolk[††]	5	5	5
Artificial sweetener[¶¶]	0	0	0.2
Water	33	87	85

[†]Energy intake and macronutrient composition were calculated using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, QLD, Australia)[31]; [‡]Paul's Natural Yoghurt, Clarence Gardens, South Australia, Australia; [§]McCAIN Season Choice Raspberries, Ballart, Victoria, Australia; [¶]Bulla Full Fat Cream, Colac, Victoria, Australia; [††]Woolworths, Bella Vista, New South Wales, Australia; [‡‡]WhiteWings Food, Macquarie Park, New South Wales, Australia; [§§]Pure Nutrition Whey Protein Isolate, Nexus Pty Ltd, Ermington, New South Wales, Australia; [¶¶]Merisant Australia Pty Ltd, Crows Nest, New South Wales, Australia. HEDHF, high-energy-density, high-fat; LEDHF, low-energy-density,

leased (T_{max}, min), the time the at which emptying begins (T_{lag}), and the GE coefficient (GEC), as previously described.[29]

Glucose, insulin, and GLP-1 concentrations

Blood glucose concentrations (mmol/L) were determined immediately by a portable glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA).[34] Venous blood samples were collected in ice-chilled EDTA-treated tubes containing 400 kIU aprotinin (Trasylol, Bayer Australia, Pymple, Australia) per mL blood. Serum and plasma were obtained by centrifugation of blood samples at 3200 rpm for 15 min at 4℃. The serum and plasma samples were frozen at -70℃ for later analysis. Serum insulin concentrations (mU/L) were measured by solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000 Insulin, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Minimum detectable concentration was 2 mU/L, the intra-assay coefficient of variation (CV) was 3.9 % and the inter-assay CV was 5.0 %. Plasma GLP-1$_{7-36}$ concentrations (pmol/L) were determined after ethanol extraction of plasma samples by a radioimmunoassay (GLPIT-36HK, Linco Research, St. Charles, Missouri) as described previously.[9] Minimum detectable concentration was 3 pmol/L, the intra-assay CV was 5.0 % and the inter-assay CV was 9.2 %.

Energy expenditure and DIT

Fasting REE was indirectly calculated from measurements of ventilatory oxygen consumption (VO_2, mL/min) and carbon-dioxide production (VCO_2, mL/min), which were recorded continuously over a minimum of 40 min using a TrueOne 2400 Metabolic Measurement System (ParvoMedics, Inc Sandy, Utah, USA). The first 15 min of data were discarded to ensure that all subjects were completely rested and that the values of VO_2 and VCO_2 were relatively stable (ie, fluctuating by $\leq 1\%$). From the remaining data, the most stable consecutive values over 20 min were averaged and represented the value for fasting REE.[35] Postprandial REE was measured continuously for 180 min after preload ingestion. DIT was determined from the average postprandial REE and expressed as a percentage of the energy consumed in the preload.[35] The intra-individual day-to-day variation for REE is 1.7±0.41 % (range 0.1-4.5%) and for DIT 7.8±1.5% (range 0.1-14.7%). The intra-class correlation coefficient, index of reliability, is 0.97 for fasting REE and 0.74 for DIT.

Statistical analysis

Baseline values (ie, t= -15 min) of individual parameters were compared between study days using one-way ANOVA; since there were no differences, all statistical analyses were performed using raw data. Repeated-measures ANOVA was used to evaluate appetite ratings, glucose, insulin and GLP-1 concentrations, with time and treatment as factors. One-way ANOVA was used to analyse energy intake (kcal), amount eaten (g) and macronutrient distribution (%), all GE parameters (ie, T_{50}, T_{max}, T_{lag} and GEC), and DIT. Post-hoc, paired comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed when ANOVA revealed significant effects. Data from all study days were combined to evaluate relationships between GE and the energy and macronutrient content of the preloads, areas under the response curves (AUC; calculated using the trapezoidal rule) and t=180 min values for glucose, insulin, GLP-1, hunger and fullness, and postprandial DIT, using a linear correlation (r) for each subject. Relationships between GE with GLP-1 and glucose concentrations, and between energy intake with T_{50}, AUCs and t=180 min values for glucose, insulin, GLP-1, hunger and fullness, and postprandial DIT, were also evaluated. An r value ≥ 0.6, and which was statistically significant ($p \leq 0.05$), was considered physiologically relevant for incorporation into a multivariate linear regression analysis to establish key determinants of energy intake. All data are presented as means ±SE, and statistical significance was accepted at $p < 0.05$. Statistical analysis was performed by SPSS (v.17.0 for windows, SPSS Inc, Chicago, USA).

RESULTS

All subjects tolerated the study protocol well and completed all study days. The palatability of the test preloads, as rated by the subjects immediately after consumption, did not differ significantly (mm; HEDHF 51±8; LEDHF 56±7; LEDHP 67±8).

Energy intake

Energy intake from the buffet meal did not differ between preloads, nor did total energy intake (ie, buffet + test preload) (Table 2). Overall food intake (g) from the buffet, and the intake (g and % total energy) derived from fat, protein and carbohydrate, did not differ between preloads.

Appetite and gastrointestinal symptom ratings

Hunger and fullness responses are depicted in Figures 1A and 1B, respectively. Baseline ratings for hunger and fullness did not differ between study days. There was no

Table 2. Energy and macronutrient intakes at the buffet-style meal 180 minutes after ingestion of the test preloads[†-‡]

	HEDHF	LEDHF	LEDHP
Energy intake at buffet			
(kcal)	1111±71	1135±90	1125±67
Total energy intake[§]			
(kcal)	2059±72	1876±91	1867±63
Weight			
(g)	1094±69	1118±76	1093±81
Protein			
(g)	62±5	62±6	61±4
(% energy)	22±1	22±1	21±1
Fat			
(g)	42±4	44±4	44±4
(% energy)	33±1	33±1	34±1
Carbohydrate			
(g)	119±7	121±8	119±6
(% energy)	44±2	45±2	44±2

[†]Data are mean±SE, n=16. [‡]Data were analysed by one-way ANOVA and post-hoc paired comparisons were performed when a significant preload effect was found (ie, $p < 0.05$). [§]Buffet + preload. HEDHF, high-energy-density, high-fat; LEDHF, low-energy-density, high-fat; LEDHP, low-energy-density, high-protein.
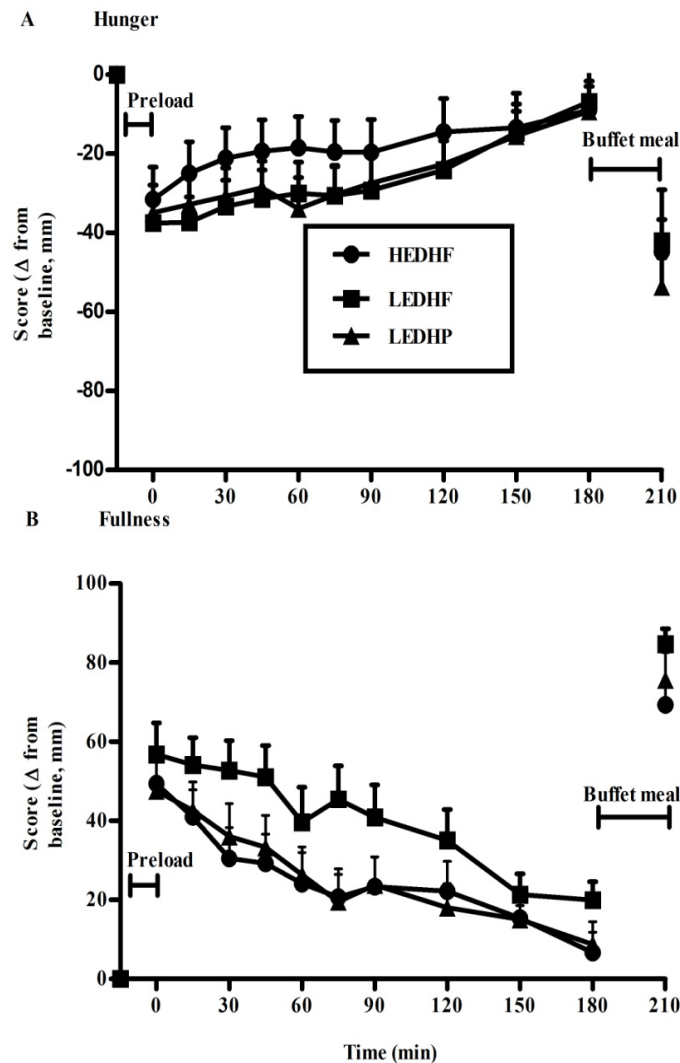There were no significant differences between any of the preloads for any of the reported outcomes.

Figure 1. Scores for hunger (A) and fullness (B) before the test preload (t= -15 min), during the postprandial period (t= 0-180 min) and after the buffet (t=210 min). Data are presented as mean±SE (n=16). Data were analysed using repeated-measures ANOVA with time and treatment as factors. Post-hoc, paired comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed when ANOVA revealed significant effects of preload (ie, *p*<0.05). There was no significant difference in hunger or fullness between preloads. HEDHF, high-energy-density, high-fat, LEDHF, low-energy-density, high-fat, LEDHP, low-energy-density, high-protein**.**

effect of preload on the ratings of hunger from t=0-180 min; ratings were reduced immediately following ingestion of each preload before progressively returning towards baseline over the 180 min period (effect of time, $F_{(2, 135)}$=13.3, *p*<0.001). Similarly, fullness scores from t= 0-180 min were not different between preloads. In response to all preloads, fullness increased immediately and then progressively returned towards baseline over the 180 min (effect of time, $F_{(2, 135)}$=13.3, *p*<0.001).

Baseline ratings for nausea and bloating did not differ between study days (data not shown). There was no effect of preload, or time, on the ratings of nausea. While there was an effect of both preload ($F_{(2, 30)}$=4.3, *p*<0.05) and time ($F_{(9, 135)}$=4.7, *p*=0.05) on ratings of bloating, post-hoc analysis revealed no significant differences between any of the preloads.

Gastric emptying

T_{50}, T_{max}, T_{lag} (min) and GEC values for each preload are summarized in table 3. In one subject, values for T_{50} and T_{max} in response to the LEDHP were uninterpretable and, hence, excluded from analysis. There was an effect of

preload on T_{50} ($F_{(2, 46)}$=3.0, *p*=0.05); T_{50} was slower following HEDHF than LEDHP (*p*=0.05), but did not differ between HEDHF and LEDHF, or the two LED, preloads. T_{max}, T_{lag} and GEC did not differ between preloads.

Glucose, insulin and GLP-1 concentrations

Baseline concentrations for blood glucose, serum insulin and plasma GLP-1 did not differ between study days (Figures 2A – C). There was no effect of preload on glucose concentrations from t= 0-180 min. Following each preload, mean blood glucose concentrations peaked within the first 15 min before progressively returning to baseline by approximately 60 min (effect of time, $F_{(9, 135)}$=16, *p*<0.001). There was an effect of preload on serum insulin concentrations ($F_{(2, 28)}$= 8.9, *p*<0.01); they were greater following LEDHP than both HEDHF (*p*<0.01) and LEDHF (*p*<0.05). There was an effect of preload on plasma GLP-1 concentrations ($F_{(2, 28)}$=3.9, *p*<0.05), but post-hoc analyses revealed that the concentrations were not substantially greater following HEDHF compared with either LEDHP or LEDHF (*p*=0.07 and *p*=0.1, respectively).
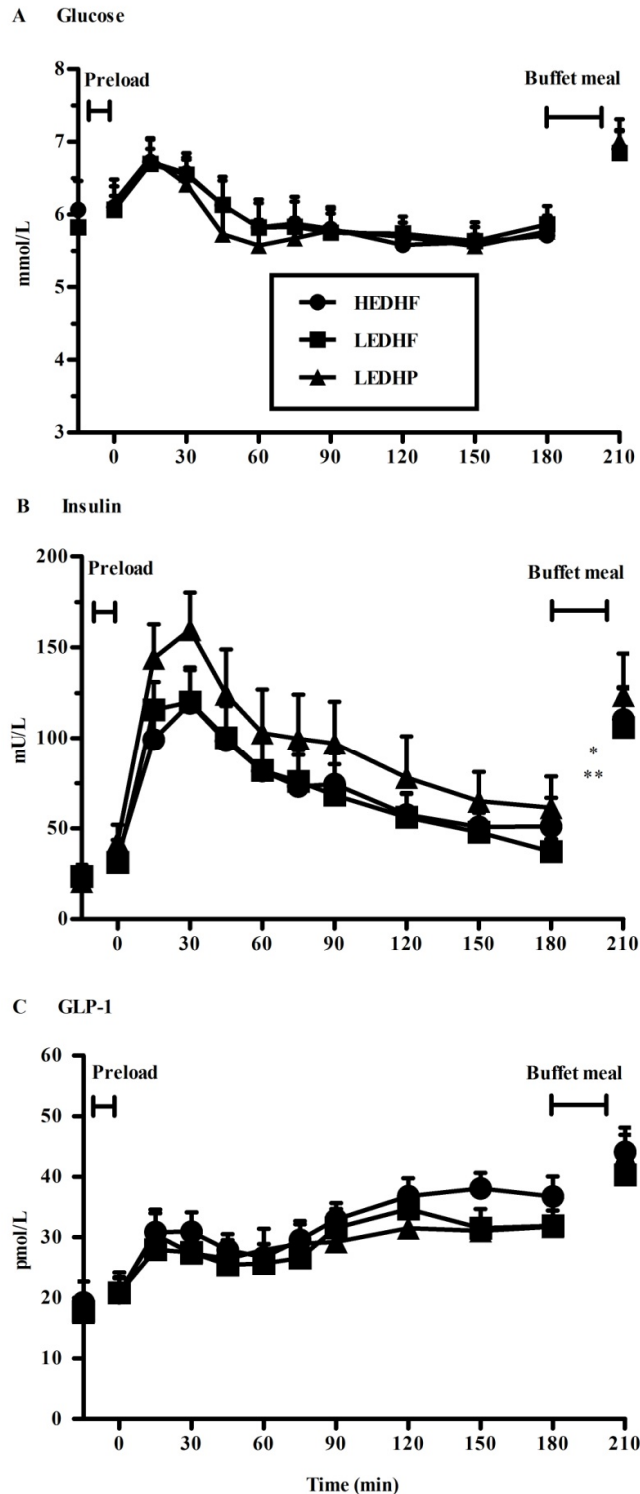
A   Glucose

B   Insulin

C   GLP-1

Figure 2. Blood glucose (A), serum insulin (B) and plasma glucagon-like peptide-1 (GLP-1) (C) before the preload (t= -15 min), during the postprandial period (t= 0-180 min) and after the buffet (t=210 min). Data are presented as mean±SE (n=16). Data were analysed using repeated-measures ANOVA with time and treatment as factors. Post-hoc, paired comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed when ANOVA revealed significant effects of preload (ie, $p<0.05$). Figure B: [*]Insulin concentrations from t= 0-180 min were greater after LEDHP than after HEDHF ($p<0.01$); [**]Insulin concentrations from t= 0-180 min were greater after LEDHP than after LEDHF ($p<0.05$). Post hoc analysis revealed that there was no significant difference in glucose or GLP-1 concentrations, between preload. HEDHF, high-energy-density, high-fat, LEDHF, low-energy-density, high-fat, LEDHP, low-energy-density, high-protein.

Energy expenditure and DIT
Fasting REE did not differ between study days. There was an effect of preload on DIT ($F_{(2, 45)}=13.8$, $p<0.001$); DIT was greater following both LED preloads than following HEDHF (both $p<0.01$) (Table 3).

Relationships between GE with energy content of the preloads and with glucose concentrations and energy intake from the buffet
T_{50} (min) was related directly, albeit weakly, to the energy content of the preload (r=0.337, $p<0.05$). T_{50} was also related inversely to plasma glucose AUC in response to the preloads (r= -0.3, $p=0.05$). Energy intake at the buf-

Table 3. Gastric emptying and energy metabolism parameters after ingestion of the test preloads[†,‡]

	HEDHF	LEDHF	LEDHP
Gastric emptying parameters			
T_{50} (min)	158±7	147±8	130±9[*]
T_{max} (min)	106±14	79±10	71±12
T_{lag} (min)	90±8	77±8	68±6
GEC	1.88±0.09	1.96±0.09	2.04±0.09
Energy metabolism parameters			
Fasting REE (kcal/d)	2038±50	2080±49	2007±52
DIT (%)	10.4±0.7	14.9±1.2[**]	18.1±1.1[**]

[†]Data are mean±SE, n=16 (except LEDHP T_{50} and GEC, where n=15). [‡]Data were analysed by one-way ANOVA and post-hoc paired comparisons were performed when a significant preload effect was found (ie, $p<0.05$). [*]vs. HEDHF, $p=0.05$. [**]vs. HEDHF, $p<0.01$. HEDHF, high-energy-density, high-fat; LEDHF, low-energy-density, high-fat; LEDHP, low-energy-density, high-protein

fet-meal was related directly to GE (r=0.277, $p=0.05$). No associations met the criteria (ie, r ≥0.6) for inclusion into a multivariate linear regression model, and hence, regression analysis was not performed.

DISCUSSION
The main finding from this acute feeding study was that a small increase in the energy density of yoghurt preloads, by increasing the fat-to-protein ratio, slowed GE and reduced DIT, in overweight and obese individuals. While the changes in GE in response to the HEDHF preload were in the direction that would be expected to limit further energy intake, the counter-regulatory reduction in DIT may explain, at least in part, why energy intake at the next meal was not actually different between the three test preloads. In addition, increasing the fat-to-protein ratio, while maintaining energy density, did not substantially affect these factors.

The small difference in energy density between the HEDHF and two LED preloads used in this study was 0.33 kcal/g (or 222 kcal) and was based on randomised, controlled trials which had compared diets with similar differences in energy density and which had found variable effects on body weight loss over 6 months to 4 years – i.e., some reported no effect of reducing the energy density of diets on weight loss, whereas another showed greater weight loss following a 0.56 kcal/g reduction in energy density.[4-6] Our data indicate that a small increase in energy density did slow GE, but the effect was not substantial enough to reduce subsequent energy intake in the HEDHF when compared to either LED condition. Given that we have confirmed that the HEDHF meal elicited a substantially reduced DIT compared with the two LED preloads (reduced by 4-8 %), it is conceivable that the effect of slowed GE on energy intake was neutralised by the counter-regulatory effect of DIT, but this is speculative since we did not find any relationship between DIT and energy intake. Alternatively, individuals may have a threshold for 'energy ingested' before physiological mechanisms act to limit further intake.

While the modulation of the physiological factors examined herein did not differentially suppress energy intake, it is possible that other biological and external factors such as time of day, the sensory quality and palatability, as well as 'liking', of the foods presented as the preloads and also at the buffet meal, and situational events, may have diluted these satiety-related signals.[36] Although

this study minimised many external cues, it is unlikely they were removed completely. The overweight and obese participants rated the palatability of the yoghurt preloads as not different, and indicated that they 'liked' and were familiar with all of the items presented at the buffet meal; as such, palatability and liking should not have confounded the findings. There was no difference in the macronutrient composition of the food eaten at the buffet, and hence, the variety of foods offered should not have impacted our results. In contrast, the subjects' habitual dietary intakes were not assessed, and it is possible that habitual diet may have affected our results since young healthy men have been reported to have an impaired ability to change their habitual level of substrate oxidation when switched from a high-fat to high-carbohydrate diet, or vice-versa.[37]

An additional observation from this study that is worthy of mentioning was that after pooling all GE data from the three preloads, GE was related directly, albeit weakly, to energy intake from the buffet, as well as to postprandial plasma glucose concentrations. While the data do not establish cause and effect, they do confirm that GE is most probably an important mediator of both energy intake[30,38] and glycemic control.[19,39] Given the latter relationship, it is likely that a slowing of GE preceded the small rise in glucose that occurred within the first 30 min after the preloads; particularly, since all preloads were matched for carbohydrate, and fasting glucose concentrations did not differ between test days. As such, the slowing of GE due to a very small increase in energy density (provided saturated fat contributes minimally to the total grams of fat) may have some relevance for the management of obesity-related conditions, including type 2 diabetes.

In an attempt to separate the acute effect of increasing energy density from that of fat content, *per se,* we evaluated two preloads that were matched for energy density, carbohydrate content and palatability, but which replaced the energy derived from fat with energy from protein. When energy density and palatability were controlled, we postulated that reducing the fat content of LEDHF by 16 g in exchange for 35 g of protein (ie, the LEDHP preload) would reduce energy intake at the buffet as a result of increased GLP-1 (and possibly insulin) release, increased DIT, and reduced hunger and increased fullness.[40-42] We observed no substantial difference in the magnitude of the effects elicited by the two LED conditions on any of these

parameters, but acknowledge that the assessment of DIT over 3 hours has a CV of 7.8±1.5 %, and therefore, the study may have been underpowered to detect small differences in DIT. In hindsight, a more appropriate study design to distinguish the independent influences of energy density and fat content, would have been to compare two HED preloads (one high and one low in fat) with a LED preload that has the same fat content as the HEDLF preload.

Caution in the interpretation of our findings has been applied due to several limitations in the design of this study. For example, a difference in energy intake between the HEDHF and two LED preloads was probably not detected, because there was no consistent trend in the direction of the difference across subjects, despite the fact that the average within-subject standard deviation for paired comparisons was comparable to that reported previously in an obese population where differences of 150-200 kcal were found.[26] It is also possible that subtle sensory differences in the preloads and buffet food,[36] as well as habitual diet, influenced some individuals' responses,[37] and altering the diet composition of an individual's diet may require days to weeks to impact on appetite and energy intake.[41,43] The study included only male volunteers and the results may, therefore, not reflect responses in females, although this is unlikely.[12,44] Finally, inclusion of a lean control group would have enabled us to determine whether gastrointestinal and metabolic factors involved in the regulation of energy intake are less responsive in overweight and obese, than lean, men.

In conclusion, an increase in the energy density of yoghurt preloads slowed GE, and reduced DIT, in overweight and obese individuals. The counter-regulatory modulation of these factors may explain, at least in part, why energy intake was not different, despite the consumption of an additional 222 kcal from the HEDHF preload. In addition, increasing only the fat-to-protein ratio, while maintaining energy density, had no substantial affect on any of the assessed outcomes. Taken together, our findings indicate that an increase in the energy density of a single meal, by virtue of an increased fat-to-protein content, does not necessarily promote energy intake acutely, in overweight and obese individuals.

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Original Article

Acute effects of oral preloads with increasing energy density on gastric emptying, gut hormone release, thermogenesis and energy intake, in overweight and obese men

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增加能量密度的口服配方對於過重及肥胖男性之胃排空、腸道荷爾蒙釋放、攝食產熱效應及能量攝取的急性影響

此研究為偵測高能量密度及低能量密度配方，對於調節急性能量攝取的腸胃道及代謝因子的影響。計 16 位過重及肥胖的男性(身體質量指數：27.2-36.5 kg/m²)，每位受試者隨機接受三種口服配方：i)高能量密度高脂配方(1.5 kcal/g)，ii)低能量密度高脂配方(1.1 kcal/g)，以及 iii)低能量密度高蛋白配方(1.1 kcal/g)。服用配方 3 小內，評估胃排空狀況、血漿類升糖激素胜肽-1(GLP-1)濃度、飲食誘導的產熱效應，並偵測後續的能量攝取。合計後續進食與高能量密度高脂配方、低能量密度高脂配方或低能量高蛋白配方的平均總能量攝取分別為 2059±72 kcal、1876±91 kcal 及 1867±63 kcal，三者無顯著差異。攝入高能量密度高脂配方，所需胃排空時間較低能量密度高蛋白配方長，但與低能量密度高脂配方無顯著差異。不同配方對於受試者血漿 GLP-1 濃度無顯著差異。高能量密度高脂配方，所誘導的攝食產熱效應為 10.4±0.7%，顯著低於低能量密度高脂的 14.9±1.2%及低能量密度高蛋白的 18.1±1.1%。從上述結果，推論增加能量密度會減緩胃排空及減少攝食產熱效應，但若同時含有高量脂肪，則會覆蓋能量密度對於胃排空的影響。胃與代謝因子兩者相反的調控模式，或許可以用於解釋，為何增加能量密度配方的攝入後對於後續總能量的攝取並無影響。

關鍵字：腸道功能、體重調節，高脂飲食、高蛋白飲食、人類