Effects of acute and longer-term dietary restriction on upper gut motility, hormone, appetite, and energy-intake responses to duodenal lipid in lean and obese men1–3

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ABSTRACT

Background: A 4-d 70% energy restriction enhances gastrointestinal sensitivity to nutrients associated with enhanced energy-intake suppression by lipid. To our knowledge, it is unknown whether these changes occur with 30% energy restriction and are sustained in the longer term.

Objectives: We hypothesized that 1) a 4-d 30% energy restriction would enhance effects of intraduodenal lipid on gastrointestinal motility, gut hormones, appetite, and energy intake in lean and obese men and 2) a 12-wk energy restriction associated with weight loss would diminish effects of acute energy restriction on responses to lipid in in obese men.

Design: Twelve obese males were studied before (day 0) and after 4 d (day 5), 4 wk (week 4), and 12 wk (week 12), and 12 lean males were studied before and after 4 d of consumption of a 30% energy-restricted diet. On each study day, antropyloroduodenal pressures, gut hormones, and appetite during a 120-min (2.86-kcal/min) intraduodenal lipid infusion and energy intake at a buffet lunch were measured.

Results: On day 5, fasting cholecystokinin was less, and ghrelin was higher, in lean (P < 0.05) but not obese men, and lipid-stimulated cholecystokinin and peptide YY and the desire to eat were greater in both groups (P < 0.05), with no differences in energy intakes compared with on day 0. In obese men, a 12-wk energy restriction led to weight loss (9.7 ± 0.7 kg). Lipid-induced basal pyloric pressures (BPPs), peptide YY, and the desire to eat were greater (P < 0.05), whereas the amount eaten was less (P < 0.05), at weeks 4 and 12 compared with day 0.

Conclusions: A 4-d 30% energy restriction modestly affects responses to intraduodenal lipid in health and obesity but not energy intake, whereas a 12-wk energy restriction, associated with weight-loss, enhances lipid-induced BPP and peptide YY and reduces food intake, suggesting that energy restriction increases gastrointestinal sensitivity to lipid. This trial was registered at the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au) as 12609000943246.

INTRODUCTION

The gastrointestinal tract plays a critical role in signaling information about the presence of nutrients in the gastrointestinal lumen to the brain, and we have recently determined that specific gastrointestinal functions, particularly the magnitude of pyloric stimulation and release of gastrointestinal hormones, including cholecystokinin, in response to nutrients are major determinants of the suppression of subsequent energy intake (1). There is increasing evidence that these gastrointestinal responses to nutrients are compromised in obesity. For example, both fasting and postprandial peptide YY and glucagon-like peptide-1 concentrations are reduced in obesity (2, 3). In this context, the effects of lipid are of particular interest because obese individuals appear to be less sensitive to intraduodenal administration (which bypasses the stomach and allows the standardized delivery of nutrients to nutrient receptors in the small intestine) of fatty acid than lean subjects (4). In particular, the effects of intraduodenal oleic acid to stimulate pyloric pressures and gut hormones, including cholecystokinin and peptide YY, are markedly diminished in obese individuals compared with healthy, lean control subjects (4). Thus, intraduodenal lipid infusion appears to represent an experimental model that allows for the evaluation of gastrointestinal sensitivity to lipid.

The concept of a role of diet in the changes in gastrointestinal sensitivity to nutrients observed in obesity is supported by observations that previous patterns of energy intake, in excess or restriction, have the capacity to modify gastrointestinal responses to nutrients (5–8). For example, the consumption of a high-fat diet for 14 d accelerated gastric emptying and mouth-to-cecum...
transit (5) and reduced the pyloric response to intraduodenal lipid (5–9). Conversely, a 4-d fast slows gastric emptying of glucose in both lean and obese individuals (6) and a 4-d very-low-calorie diet (VLCD)4 (70% energy restriction; ~1000 kcal/d) in obese subjects enhanced the effects of an intraduodenal lipid infusion to stimulate pyloric pressures and plasma peptide YY and suppressed both ghrelin and energy intake (10). Thus, acute energy excess appears to attenuate, and acute severe energy restriction appears to enhance, the gastrointestinal sensitivity to intraduodenal nutrients, particularly lipid.

Dietary management remains the most common approach to the management of obesity; however, despite continued adherence to weight-loss diets, body weight often stabilizes over time or even increases. This effect may, at least in part, reflect adaptive responses to reduced energy availability. For example, dietary restriction is associated with a fall in the basal metabolic rate (11, 12) so that energy requirement is less and hunger increases (13, 14). It is also possible that, over time and in contrast to acute dietary restriction, adaptive changes in gastrointestinal mechanisms occur that favor an increase in energy intake and weight regain. Indeed, Cummings et al showed that a 6-mo weight-loss program by using a VLCD substantially increased both fasting and postprandial ghrelin concentrations in obese men and women (15). In a recent study in obese men and postmenopausal women, fasting and postprandial peptide YY and cholecystokinin were reduced and ghrelin increased in response to a 10-wk VLCD (~500–550 kcal/d), and this difference remained significant 1 y after the initial weight loss (16). Such changes may potentially reflect the modulation of gastric emptying or small intestinal transit (ie, reduced nutrient delivery to and along the small intestine) may lead to reduced cholecystokinin and peptide YY release and the suppression of ghrelin. The observed effects, particularly on ghrelin, may increase hunger and, thus, compromise compliance with a diet. To our knowledge, it is not known whether a more moderate (~30%) dietary restriction has significant effects on gastrointestinal function and energy intake, and there is not any information about potential temporal changes in gastrointestinal function during a period of dietary restriction and, in case of any changes, their relation to energy intake and weight loss in obesity.

Therefore, the aim of this study was to evaluate the hypothesis that a 4-d 30% energy restriction would enhance the effects of intraduodenal lipid on gastrointestinal function and appetite in lean and obese men, whereas following a 12-wk energy restriction in obese men, the effects on gastrointestinal function and appetite would be diminished and compromise the potential for sustained weight loss.

**SUBJECTS AND METHODS**

**Subjects**

Of 31 eligible men (see Supplemental Figure 1 under “Supplemental data” in the online issue), 12 healthy, lean [median age (range): 44 y (35–60 y); median BMI (in kg/m²) (range): 23 (20.9–25)] and 12 obese [median age (range): 49 y (24–59 y); median BMI (range): 32.6 (30.9–37.6)] but otherwise healthy men completed the study. The remaining 7 men (all lean) withdrew from the study during the first visit because they did not tolerate the nasoduodenal tube or intraduodenal infusion (ie, they reported having nausea). The 7 men were unwilling to return to repeat the tests, and thus, there were no data that could be included in the statistical analysis. On the basis of our previous data (10), we calculated that a mean difference in energy intake between visits of ~740 kJ (within-subject SD: 700 kJ) would be detectable with a sample size of 12 subjects at 80% power and a significance level of 5% (adjusted as appropriate for the number of post hoc comparisons in the statistical analysis).

Subjects were recruited through advertisements in local newspapers and from a pool of volunteers available in the University of Adelaide Discipline of Medicine department and studied between January 2010 and May 2011. Only men were included because they may be more sensitive to dietary manipulation than women are (17) and to avoid the potential influences of the menstrual cycle on gastrointestinal function and energy intake (18). All subjects were questioned before their inclusion to exclude significant gastrointestinal symptoms, disease or surgery, current use of medications that may affect gastrointestinal motor function or appetite, diabetes (glycated hemoglobin >6.5%), epilepsy, cardiovascular or respiratory disease, any other significant illness, allergy to local anesthetic, intake of >20 g alcohol/d, smoking, food intolerances or allergies, vegetarians, and any individuals who had recently commenced a dietary restriction or attempted to lose weight. All subjects were required to be weight stable for 3 mo (ie, <5% fluctuation) before study entry and asked to maintain their normal activity levels during the entire study. In addition, the degree of eating restraint was assessed (19). Lean men were only included if they were unrestrained eaters as determined by a score ≥12 on the eating-restraint component of the eating questionnaire. This variable was not used as an exclusion criterion in the obese because many obese subjects have some degree of eating restraint. The median (range) score in lean subjects [4.5 (3–9)] did not differ from that in the obese [10 (1–14)]. The study was approved by the Royal Adelaide Hospital Ethics Committee and registered as a clinical trial [Australian New Zealand clinical trials registry (www.actr.org.au); registration no. 12609000943246]. All subjects provided informed, written consent before participation. Subjects were informed that the purpose of the study was to evaluate effects of changes in the diet on the function of the stomach and small intestine.

**Study outline**

The study evaluated the effects of a 4-d acute (in lean and obese men) and a 12-wk prolonged (in obese men only) 30% energy restriction on antropyloroduodenal motility, gut hormone, appetite, and energy-intake responses to a 120-min intraduodenal infusion (2.86 kcal/min) of a long-chain triglyceride emulsion (10% Intralipid, 300 mOsmol/kg, 1.1 kcal/mL; Fresenius Medical Care Australia Pty Ltd) and on body weight.

**Determination of energy requirements and diets**

To assess the habitual diet and determine energy requirements, subjects completed a weighed food diary over 5 consecutive days (3 weekdays and 2 weekend days) before study commencement.
For this purpose, subjects received standardized instructions as to how to weigh and record all foods and beverages consumed over the 5 d from an accredited dietitian (PT) and were provided with digital kitchen scales. Subsequently, 3 (2 weekdays and 1 weekend day) of the 5 d were selected at random for analysis by the dietitian (20). The energy intake (kJ) and amount eaten (g) were calculated by using specialized software (Foodworks Professional Edition, version 5, 1998–2007; Xyris Software Inc). On the basis of each subject’s food diary, a meal plan was formulated to achieve a 30% reduction in the subject’s habitual energy intake by using a macronutrient-balanced diet that consisted of ~50% carbohydrate, ~30% fat, and ~20% protein in the subsequent dietary intervention.

Lean men underwent a 4-d period of dietary restriction (to avoid significant weight loss), whereas obese men underwent a 12-wk period of dietary restriction that would be predicted to be associated with weight loss. To optimize compliance with the dietary intervention, subjects were provided with all foods and snacks by using ready-to-eat meals (Lite n’ Easy). Lite n’ Easy provided 3 dietary templates of 1200-, 1500-, and 1800-kcal meal plans, which included 375 mL skim milk to ensure adequate calcium intake. To match dietary requirements of individual subjects beyond the energy provided by the Lite n’ Easy meal plan, additional food was provided (eg, fruit and muesli bars) as necessary. Subjects were also asked to record, in a dietary checklist, any food that was consumed in addition to that provided. Obese men attended fortnightly, individual counseling sessions with the dietitian at consulting rooms at CSIRO Animal, Food and Health Science on days 13, 27, 41, 55, and 69 to review their diets and record their body weights. Lean men attended the laboratory on the following 4 occasions: before starting the diet, during phase I of the migrating motor complex, a baseline blood sample was collected, and a VAS was completed, every 15 min (ie, during phase I of the migrating motor complex), a baseline blood sample was taken, and the subject completed a visual analog scale (VAS) questionnaire for the assessment of appetite perceptions (23). At t = 0 min, an intraduodenal infusion of lipid commenced and continued for 120 min. During the infusion, 10-mL blood samples were collected, and a VAS was completed, every 15 min. At t = 120 min, the infusion ceased, and the subject was extubated and offered a cold, buffet-style meal (t = 120–150 min) from which energy intake was assessed (24). After this time, the subject was allowed to leave the laboratory.

Study protocol

An outline of the study protocol is shown in Figure 1. During the dietary intervention period, obese men attended the laboratory on 4 occasions [ie, at baseline before starting the diet (day 0), day 5 (day 5), day 29 (week 4), and day 85 (week 12)], whereas lean men attended the laboratory on 2 occasions (ie, days 0 and 5) to evaluate the effects of an acute (day 5) and prolonged (weeks 4 and 12; obese men only) dietary restriction on gastrointestinal function and energy intake in response to a 120-min intraduodenal lipid infusion. An intraduodenal infusion was used to bypass any orosensory (taste) influences and exclude potential confounding effects of interindividual variations in gastric emptying. The infusion rate of 2.86 kcal/min was within the physiologic range of gastric emptying of nutrients (21).

Subjects were provided with a standardized meal [beef lasagna (energy content: 2170 kJ); McCain Foods] to consume at 1900 the evening before each study. Thereafter, subjects were asked to refrain from consuming all solids and liquids, except water, and any strenuous physical activity before attending the laboratory in the Discipline of Medicine at 0830 the following morning. Studies were performed by RVS. A silicone-rubber manometry catheter that incorporated 16 channels (Dentsleeve International Ltd) was inserted through an anesthetized nostril into the stomach and allowed to pass into the duodenum by peristalsis (22). Six side holes (channels 1–6) were positioned in the antrum, a 4.5-cm pyloric sleeve sensor (channel 7), with 2 channels present on the back of the sleeve (channels 8 and 9), was positioned across the pylorus, and 7 side holes (channels 10–16) were positioned in the duodenum. An additional channel, 11.75 cm distal to the pylorus, was used for the intraduodenal infusion. Both the most-distal antral (channel 6; approximately ~40 mV) and most-proximal duodenal (channel 10; ~0 mV) channels were perfused with degassed 0.9% saline so that the correct positioning of the catheter was maintained continuously by measurement of the transmucosal potential difference (22). For this measurement, an intravenous cannula was placed subcutaneously in the left forearm and filled with sterile saline as a reference electrode (22). All other channels were perfused with degassed, distilled water at 0.15 mL/min. After correct positioning of the catheter, fasting motility was monitored until the occurrence of phase III of the migrating motor complex. An intravenous cannula was inserted into a forearm vein for regular blood sampling (10 mL). At t = −15 min (ie, during phase I of the migrating motor complex), a baseline blood sample was taken, and the subject completed a visual analog scale (VAS) questionnaire for the assessment of appetite perceptions (23).

Measurements

Antropyloroduodenal pressures

Antropyloroduodenal pressures were digitized and recorded on a computer-based system and analyzed for 1) the number and amplitude of antral pressure waves (PWs), 2) the number and amplitude of isolated pyloric pressure waves (IPPWs), 3) basal pyloric pressure (BPP), and 4) the number and amplitude of duodenal PWs, as previously described (21).

Gastrointestinal hormone concentrations

Blood samples were collected in ice-chilled EDTA-treated tubes that contained 400 kIU aprotinin/mL blood (Trayslol;
Bayer Australia Ltd) and centrifuged at 3200 rpm for 15 min at 4°C within 30 min of collection. Plasma was stored at −70°C until assayed.

Plasma cholecystokinin concentrations (pmol/L) were measured by radioimmunoassay by using an adaptation of a previously described method (25). Samples were extracted in 66% ethanol, and extracts were dried down and resuspended in assay buffer (50 mmol phosphate/L, 10 mmol EDTA/L, and 2 g gelatin/L; pH 7.4). Standards were prepared by using synthetic sulfated cholecystokinin-8 (Sigma), and antibody (C2S81, lot 105H4852; Sigma) was added at a working dilution of 1:17,500, and sulfated cholecystokinin-8 [125I]-labeled with Bolton and Hunter reagent (Perkin Elmer) was used as tracer. Samples were incubated for 7 d at 4°C. The antibody-bound fraction was separated by adding dextran-coated charcoal-containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal in 30 mL assay buffer,) and the radioactivity was determined in the supernatant fluid after centrifugation. The intraassay CV was 7.1%, the interassay CV was 17.8%, and the detection limit was 1 pmol/L.

Plasma peptide-YY concentrations (pmol/L) were analyzed by radioimmunoassay by using an adaptation of a previously described method (26). An antiserum raised in rabbits against human peptide YY (1–36) (Sigma) was used, and thus, the assay measured both peptide YY (1–36) and peptide YY (3–36). The antiserum showed <0.001% cross-reactivity with human pancreatic polypeptide and sulfated cholecystokinin-8 and 0.0025% cross-reactivity with human neuropeptide Y. The intraassay CV was 6.5%, the interassay CV was 4.2%, and the detection limit was 1.5 pmol/L.

Plasma ghrelin concentrations (pg/mL) were measured by radioimmunoassay with some modifications to a published method (27). The radiolabel (NEX388) was purchased from Perkin Elmer. The standard and samples were incubated with the antibody for 3–4 d before incubation with the radiolabel for an additional 24 h at 4°C. The intraassay CV was 8.5%, the interassay CV was 15%, and the detection limit was 40 pg/mL.

**Habitual dietary intake, appetite, and energy intake**

The habitual dietary intake was quantified by calculating, from the weighed-food diaries, the mean energy intake (kJ/3 d) with commercially available software (Foodworks, version 3.01; Xyris Software) (24). Appetite perceptions, including the desire to eat and fullness, were assessed by using a validated VAS questionnaire (23). Nausea and bloating were also quantified. Each VAS consisted of a 100-mm horizontal line, whereby 0 mm represented “sensation not felt at all,” and 100 mm represented “sensation felt the greatest.” Subjects were asked to place a vertical mark along the line to indicate the strength of each sensation. Energy intake in response to intraduodenal lipid was quantified from the amount eaten at the buffet meal, the ingredients of which were weighed before and after consumption to quantify the amount (g) of food and beverages consumed (24). Energy intake (kJ) was calculated with software (Foodworks, version 3.01).

**Statistical analysis**

All data were analyzed with SPSS software (version 17; SPSS Inc) under the supervision of a professional biostatistician (Kylie Lange). Primary outcomes included energy intake, gut hormones, gut motility, and body weight, and secondary outcomes included appetite and gastrointestinal symptoms. Baseline values for the VAS and hormone concentrations were calculated as the means of values at $t = -15$ and 0 min. Baseline values for numbers and amplitudes of antral and duodenal PWs, BPP, and IPPWs were obtained from means of values between $t = -15$ and 0 min. During the 120-min infusion period, antral and duodenal PWs were expressed as a motility index (MI) calculated from total numbers and mean amplitudes as described elsewhere (28), whereas the number of IPPWs was expressed as the total number, and amplitudes of IPPWs and BPP were expressed as means, over 15-min intervals. In addition, AUCs, which were calculated by using the trapezoidal rule, were determined for numbers and amplitudes of IPPWs, BPP, plasma hormones, and VAS scores.

Repeated-measures ANOVA, with visit and group as factors (part 1) and visit as a factor (part 2) was used to evaluate the MI for antral and duodenal PWs, the total number and mean amplitude of IPPWs, the mean BPP, AUCs for hormones and VAS scores, body weight, waist circumference, energy intake (kJ), and the amount eaten (g) from the buffet meal. Post hoc comparisons, adjusted for multiple comparisons by using Bonferroni correction, were performed when ANOVAs revealed significant effects. Habitual energy intakes (kJ) were compared between lean and obese subjects by using an independent-sample $t$ test.

Relations between antropyloroduodenal motility (antral and duodenal MI, the AUC of numbers and amplitudes of IPPWs and BPP) and gut hormones (AUC of plasma cholecystokinin, peptide YY, and ghrelin) with energy intake, the amount eaten, and body weight were evaluated by using an ANCOVA model across all visits to obtain within-subject correlations ($r$).

All data are means ± SEMs. Statistical significance was accepted at $P < 0.05$.

**RESULTS**

Compliance with the 30% dietary restriction was very good, with an achieved restriction that amounted to 28 ± 2% in both lean and obese men. Two lean men achieved only 19% and 20% restriction, and 3 obese men achieved 16%, 18%, and 20% restriction. All but 2 subjects tolerated the study conditions on study days well; one obese man vomited at week 12, 75 min after the commencement of the lipid infusion, and another obese man experienced diarrhea at week 4, 60 min after the commencement of the lipid infusion, at which times the infusions were discontinued. For the purpose of the statistical analysis, in these cases, the last measured data value was carried forward up to $t = 120$ min.

**Habitual energy intake**

There were no differences in reported habitual energy intakes between lean and obese men (Table 1).

**Part 1: effects of acute (4-d) 30% dietary restriction in lean and obese men**

**Body weight, BMI, and waist circumference**

There were no changes in body weight (lean:76 ± 2 kg on day 0 and 75 ± 2 kg on day 5; obese: 106 ± 4 kg on day 0 and 104 ± 3 kg on day 5), BMI (lean: 23.3 ± 0.6 on day 0 and 23 ± 0.5 on day 5; obese: 33.1 ± 0.6 on day 0 and 32.6 ± 0.6 on day 5), or...
Habitual energy intake and amount eaten

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kJ)</td>
<td>10,456 ± 636</td>
<td>10,705 ± 460</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>2581 ± 228</td>
<td>2955 ± 185</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. n = 12 lean and 12 obese men. The independent-sample t test was used to determine statistical differences. There were no significant effects on energy intake or amount eaten.

Antral pressures

There were no differences in the fasting MI of antral PWs between visits or groups because antral motility during phase I was quiescent (ie, MI: 0 mm Hg). The MI of antral PWs increased in response to intraduodenal lipid; however, there was no effect of visit or group (Table 2).

Antropyloroduodenal pressures

During phase I was quiescent (ie, MI: 0 mm Hg). The MI of antral PWs between visits or groups because antral motility was not significant (Table 2).

TABLE 2
Antropyloroduodenal motility responses during a 120-min intraduodenal infusion of a long-chain triglyceride emulsion (10% Intralipid, 2.86 kcal/min; Fresenius Medical Care Australia Pty Ltd) on days 0 and 5 (in lean and obese men) and weeks 4 and 12 (in obese men only) of a 30% energy-restricted diet

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 5</th>
<th>Week 4</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antral MI (mm Hg)</td>
<td>6.6 ± 0.5</td>
<td>7 ± 0.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total IPPWs (n)</td>
<td>125 ± 29</td>
<td>179 ± 44</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amplitude of IPPWs (mm Hg)</td>
<td>34 ± 8</td>
<td>47 ± 11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BPP (mm Hg)</td>
<td>2.2 ± 1</td>
<td>4.1 ± 1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Duodenal MI (mm Hg)</td>
<td>8.0 ± 0.4</td>
<td>8.4 ± 0.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Obese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antral MI (mm Hg)</td>
<td>5.7 ± 0.8</td>
<td>7 ± 0.3</td>
<td>6.6 ± 0.4</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>Total IPPWs (n)</td>
<td>167 ± 32</td>
<td>149 ± 25</td>
<td>191 ± 26</td>
<td>168 ± 22</td>
</tr>
<tr>
<td>Amplitude of IPPWs (mm Hg)</td>
<td>34 ± 6</td>
<td>46 ± 7</td>
<td>49 ± 9</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>BPP (mm Hg)</td>
<td>1 ± 0.9²</td>
<td>1.2 ± 1²</td>
<td>2 ± 1.2</td>
<td>3.1 ± 0.3³</td>
</tr>
<tr>
<td>Duodenal MI (mm Hg)</td>
<td>7.5 ± 0.4</td>
<td>8.6 ± 0.3</td>
<td>8.1 ± 0.2</td>
<td>8.2 ± 0.2</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. n = 12 lean and 12 obese men. Repeated-measures ANOVA with visit and subject group as factors was used to determine statistical differences. There was no effect of visit or group on antral or duodenal MI or the number or amplitude of IPPWs. BPP, basal pyloric pressure; MI, motility index; IPPW, isolated pyloric pressure wave.

²Significantly different from lean men, P-group effect < 0.05.

³Significantly different from day 0 and week 4, P-visit effect < 0.001.

Gastrointestinal hormones

Plasma cholecystokinin. There was a small but significant difference in fasting cholecystokinin between visits but not groups (P < 0.05) (lean: 2.3 ± 0.3 pmol/L on day 0 and 1.9 ± 0.2 pmol/L on day 5; obese: 2.9 ± 0.7 pmol/L on day 0 and 5: 2.6 ± 0.8 pmol/L on day 5). During all visits, plasma cholecystokinin increased in response to intraduodenal lipid, peaking at ~15–30 min, after which time concentrations plateaued. There was an effect of visit but not group on plasma cholecystokinin (P < 0.01) (Figure 2A, Table 3) whereby plasma cholecystokinin was less on day 5 than day 0 (P < 0.05).

Plasma peptide YY. There was no difference in fasting peptide YY between visits but groups (lean: 34.8 ± 3.8 pmol/L on day 0 and 36.3 ± 4 pmol/L on day 5; obese: 33.9 ± 3.4 pmol/L on day 0 and 33.5 ± 3.4 pmol/L on day 5). Peptide YY rose steadily throughout the lipid infusion. There was an effect of visit but not group on plasma peptide YY (P < 0.01) (Figure 2B, Table 3) whereby peptide YY was greater on day 5 than day 0 (P < 0.05).

Plasma ghrelin. There were significant differences in fasting ghrelin between visits and groups (lean: 2063 ± 221 pg/mL on day 0 and 2317 ± 241 pg/mL on day 5; obese: 1321 ± 242 pg/mL on day 0 and 1254 ± 224 pg/mL on day 5), whereby fasting ghrelin was greater in lean men on day 5 than day 0 (P < 0.05) and greater in lean men on both days 0 and 5 than in obese men (P < 0.05). The magnitude of ghrelin suppression [ie, Δghrelin (t = 0 min − t = 120 min) in response to lipid was greater on days 0 (P < 0.05) and 5 (P < 0.001) in lean compared with obese men (Figure 2C, Table 3). There was a visit × group interaction for ghrelin (P < 0.05) whereby ghrelin was greater in lean men on both days 0 and 5 than in obese men (P < 0.05) and greater in lean men on day 5 than day 0 (P < 0.05).
Appetite perceptions and gastrointestinal symptoms

There was a difference in fasting desire-to-eat scores between visits in both lean (day 0: 45 ± 8; day 5: 64 ± 8; *P*, 0.05) and obese (day 0: 38 ± 6; day 5: 51 ± 8; *P*, 0.05) men, with no difference between groups. There was a significant effect of visit but not group on the desire-to-eat (*P*, 0.05) (Figure 3A) whereby the desire to eat was greater on day 5 than day 0 (*P*, 0.05). Scores for prospective consumption and hunger were similar to those for the desire to eat (data not shown).

There were no effects of visit or group on fullness, nausea, or bloating. Fasting scores were very low and only increased slightly in response to lipid, with no apparent differences between groups or visits (data not shown).

Energy intake

There was no effect of visit or group on energy intake (kJ) or the amount of food consumed (g) from the buffet meal (Table 4).

Part 2: effects of prolonged (12-wk) 30% dietary restriction in obese men

Body weight, BMI, and waist circumference

There was a significant effect of visit on body weight, BMI, and waist circumference (*P* < 0.001 for all), whereby all of these variables were less at weeks 4 (100 ± 3 kg, 31.5 ± 0.5, and 109 ± 2 cm, respectively) and 12 (96 ± 3 kg, 30.2 ± 0.5, and 103 ± 2 cm, respectively) than on day 0 (106 ± 4 kg, 33.1 ± 0.6, 114 ± 2 cm, respectively) (*P*, 0.01 for all) and less at week 12 than week 4 (*P*, 0.01).

Antropyloroduodenal pressures

Antral pressures. There were no differences in the fasting MI of antral PWs between visits (ie, MI: 0 mm Hg). The MI of antral PWs increased in response to intraduodenal lipid; however, there was no effect of visit (Table 2).

 IPPWs. There were no differences in the fasting number (day 0: 0 ± 0; week 4: 0 ± 0; week 12: 0 ± 0) or amplitude (day 0:...
Obese men (n = 12 lean and 12 obese men). Repeated-measures ANOVA with visit and subject group as factors was used to determine statistical differences.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Week 4</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystokinin (pmol · L⁻¹ · min)</td>
<td>716 ± 65</td>
<td>585 ± 60²</td>
<td>——</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Peptide YY (pmol · L⁻¹ · min)</td>
<td>12,835 ± 1332</td>
<td>9076 ± 1027²</td>
<td>——</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Ghrelin (pg · mL⁻¹ · min)</td>
<td>205,563 ± 20,617⁴</td>
<td>223,251 ± 25,028¹⁴</td>
<td>——</td>
<td>——</td>
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<tr>
<td>Obese</td>
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<td></td>
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<tr>
<td>Cholecystokinin (pmol · L⁻¹ · min)</td>
<td>726 ± 89</td>
<td>653 ± 89²</td>
<td>718 ± 80</td>
<td>680 ± 80</td>
<td></td>
</tr>
<tr>
<td>Peptide YY (pmol · L⁻¹ · min)</td>
<td>7097 ± 1127</td>
<td>7674 ± 760²</td>
<td>8355 ± 905</td>
<td>8652 ± 1202</td>
<td></td>
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<tr>
<td>Ghrelin (pg · mL⁻¹ · min)</td>
<td>102,895 ± 16,758</td>
<td>104,245 ± 16,980</td>
<td>112,917 ± 18,202</td>
<td>117,858 ± 17,986</td>
<td></td>
</tr>
</tbody>
</table>

¹All values are means ± SEMs. n = 12 lean and 12 obese men. Repeated-measures ANOVA with visit and subject group as factors was used to determine statistical differences.

²Significantly different from day 0, P-visit effect < 0.01.

³Significantly different from obese men, P-visit × group interaction < 0.05.

⁴Significantly different from day 0, P-visit × group interaction < 0.05.

0 ± 0 mm Hg; week 4: 0 ± 0 mm Hg; week 12: 0 ± 0 mm Hg) of IPPWs between visits. The number and amplitude of IPPWs increased in response to intraduodenal lipid; however, there was no effect of visit on these variables (Table 2).

**BPPs.** There were no differences in fasting BPP between visits (day 0: 1 ± 3 mm Hg; week 4: 0.3 ± 2.2 mm Hg; week 12: 0 ± 0.3 mm Hg). BPP increased in response to intraduodenal lipid, and there was a significant effect of visit on mean BPP (P < 0.001), whereby at week 12, mean BPP was greater than at day 0 and week 4 (P < 0.01) (Table 2).

**Duodenal pressures.** There were no differences in the fasting MI of duodenal PWs between visits (ie, MI: 0 mm Hg). The MI of duodenal PWs increased in response to intraduodenal lipid; however, there was no effect of visit (Table 2).

**Gastrointestinal hormones**

**Plasma cholecystokinin.** There were no differences in fasting cholecystokinin between visits (day 0: 2.9 ± 0.7 pmol/L; week 4: 2.6 ± 0.6 pmol/L; week 12: 2.6 ± 0.6 pmol/L). There was no effect of visit on plasma cholecystokinin (Figure 2D, Table 3). Plasma cholecystokinin increased in response to lipid on all visits and peaked at ~30 min, after which time concentrations plateaued.

**Plasma peptide YY.** There were no differences in fasting peptide YY between visits (day 0: 33.9 ± 3.4 pmol/L; week 4: 31.3 ± 2.6 pmol/L; week 12: 33.1 ± 4 pmol/L). There was an effect of visit on plasma peptide YY (P < 0.01) (Figure 2E, Table 3), so that plasma peptide YY was greater at weeks 4 and 12 compared with day 0.

**Plasma ghrelin.** There was an effect ofvisit on fasting ghrelin (P < 0.05), although post-hoc comparisons revealed no difference between visits (day 0: 1321 ± 242 pg/mL; week 4: 1513 ± 265 pg/mL; week 12: 1502 ± 246 pg/mL). There was no effect of visit on plasma ghrelin (Figure 2F, Table 3), which moderately decreased in response to lipid during all visits (P < 0.05).

**Appetite perceptions and gastrointestinal symptoms**

There were differences in fasting scores between visits (P < 0.001). The fasting desire to eat was greater at weeks 4 (60 ± 8; P < 0.01) and 12 (62 ± 9; P < 0.05) than day 0 (38 ± 6), with no differences between weeks 4 and 12. There was an effect of visit on scores for the desire to eat (P < 0.05) (Figure 3B). Desire-to-eat scores did not differ significantly between week 4 and day 0 (P = 0.054) but were greater at week 12 than day 0 (P < 0.05), with no difference between weeks 4 and 12. Scores for prospective consumption and hunger were similar to those of the desire to eat (data not shown). There were no effects
of visit or group on fullness, nausea, or bloating (data not shown).

Energy intake

There was no significant effect of visit on energy intake ($P = 0.06$) but a significant effect of visit on the amount of food consumed ($g$) ($P < 0.01$) (Table 4), whereby the amount of food consumed was less at weeks 4 and 12 (both $P < 0.05$ than day 0, with no differences between weeks 4 and 12.

Relations between energy intake and body weight with antropyloroduodenal motility and hormones

There were inverse relations between the amount eaten with the AUC of plasma peptide YY ($r = -0.29, P < 0.05$) and between energy intake ($r = -0.36, P < 0.01$) and body weight ($r = -0.29, P < 0.05$) with the AUC of BPP (see Supplemental Figure 2 under “Supplemental data” in the online issue).

Relations between lipid-stimulated gastrointestinal hormone concentrations

There were no relations between AUCs of plasma cholecystokinin and peptide YY, cholecystokinin and ghrelin, or peptide YY and ghrelin concentrations.

DISCUSSION

This study evaluated the effects of acute (in lean and obese men) and prolonged (in obese men) 30% energy restriction on antropyloroduodenal motility, gastrointestinal hormone, appetite, and energy-intake responses to an intraduodenal lipid infusion. After a 4-d restriction, ghrelin, both fasting and in response to lipid, was greater in lean but not obese men, and cholecystokinin was less, whereas peptide YY and the desire to eat were greater in both groups, although all observed differences were small, and there were no differences in energy intakes compared with on day 0. Accordingly, an acute 30% dietary restriction was associated with only modest changes in gastrointestinal function in response to lipid and no change in energy intake. In contrast, a 12-wk energy restriction in obese men with significant weight loss was associated with increased BPP and plasma peptide YY responses to intraduodenal lipid compared with on day 0 and a reduction in the amount eaten, despite an increased fasting desire to eat. Moreover, there were inverse relations between the amount eaten and plasma peptide YY and between energy intake and body weight with BPP. This suggests that the 12-wk dietary restriction enhanced gastrointestinal sensitivity to lipid in obese subjects, leading to a reduction in the amount eaten and facilitating weight loss.

We elected to evaluate gastrointestinal responses to an acute 30% dietary restriction in both lean and obese men because we have shown, in a comparable sample size, that a 70% dietary restriction for 4 d markedly enhanced the stimulation of tonic and phasic pyloric pressures, suppression of antral and duodenal pressures, and stimulation of peptide YY in response to intraduodenal lipid (at the same caloric load of 2.86 kcal/min as in the current study), with no differences in cholecystokinin compared with in the prediet condition. A 30% energy restriction is also concordant with many weight-loss diets. Although fasting ghrelin and hunger ratings were elevated after the dietary restriction, the magnitude of their suppression by intraduodenal lipid was enhanced, and energy intake was markedly reduced (10). In the current study, we observed increased peptide YY and reduced cholecystokinin in response to intraduodenal lipid and an increase in the desire to eat on day 5; however, there were no differences in BPP, IPPWs, antral and duodenal pressures, ghrelin, or energy intakes. The small changes that were evident after the dietary restriction, including a reduction in plasma cholecystokinin and greater stimulation of peptide YY by intraduodenal lipid in both lean and obese men, indicated that even modest and acute changes in the diet have the capacity to modulate gastrointestinal functions, but the magnitude of these effects was either insufficient (ie, a threshold was not reached) or counteractive mechanisms, potentially including increased ghrelin concentrations, prevented any reduction in energy intake.

A prolonged caloric restriction has been reported to result in marked increases in ghrelin (15) and rapid reductions in circulating leptin concentrations (29) and energy expenditure (12), and an increase in appetite (30), which may explain why body weight often stabilizes, despite continued attempts by individuals to adhere to prescribed weight-loss diets (31). Thus, we had hypothesized that such adaptations would also affect gastrointestinal function. To our surprise, we showed that the stimulation of both BPP and peptide YY by intraduodenal lipid increased over the 12-wk dietary period. Moreover, although the fasting desire to eat was elevated, the magnitude of its decline in response to lipid was greater, whereby, immediately before the meal, there were no differences in scores between study days. This result may suggest

### TABLE 4

<table>
<thead>
<tr>
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<th>Day 0</th>
<th>Day 5</th>
<th>Week 4</th>
<th>Week 12</th>
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<tbody>
<tr>
<td>Lean</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Energy intake (kJ)</td>
<td>4370±376</td>
<td>4199±471</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Amount eaten (g)</td>
<td>1073±88</td>
<td>974±124</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Obese</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>4579±436</td>
<td>4306±459</td>
<td>3854±502</td>
<td>4146±505</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>1037±109</td>
<td>966±113</td>
<td>798±107</td>
<td>839±110</td>
</tr>
</tbody>
</table>

1 All values are means ± SEMs. n = 12 lean and 12 obese men. Repeated-measures ANOVA with visit and subject group as factors was used to determine statistical differences.

2 Significantly different from day 0, $P$-visit effect < 0.05.
that, although higher fasting concentrations reflected increased hunger and a negative energy balance, subjects were also more sensitive to the effects of intraduodenal lipid, which led to a greater suppression of intake, whereby the amount of food eaten was less, and there was a trend for a reduced energy intake, at weeks 4 and 12 than day 0. The ghrelin response was similar and in line with our previous work (10, 32). Thus, the reduction in intake (which led to a mean weight loss of >9%) may have reflected a greater sensitivity to the gastrointestinal effects of intraduodenal lipid.

Lipid-stimulated plasma cholecystokinin was reduced after the 4-d dietary restriction. These changes were consistent with a study in rats in which a 3-d food deprivation led to a rapid decrease in plasma cholecystokinin associated with reduced duodenal concentrations of cholecystokinin messenger RNA (33) as well as a study in lean humans which reported that overfeeding for a period of 2 wk increased plasma-cholecystokinin responses to a mixed meal (34). In analogy to the interpretation by the authors (34), a reduction in cholecystokinin concentrations may be indicative of a corresponding upregulation in and enhanced sensitivity of cholecystokinin receptors that are responsible for the feedback inhibition of cholecystokinin release.

Because cholecystokinin has the capacity to suppress ghrelin release (35, 36), the reduction in cholecystokinin may, at least in part, have contributed to the lack of ghrelin suppression and the increase in the desire to eat. However, cholecystokinin also stimulates peptide YY (35, 36), but peptide YY was actually increased after the 4-d dietary restriction, which suggests that, despite a reduction in cholecystokinin release, an increase in receptor sensitivity to cholecystokinin may have allowed the ongoing stimulation of peptide YY. The interrelations between the actions of these hormones remain complex and require additional investigation.

A number of studies have evaluated changes in gut hormone release after a longer-term energy restriction by using VLCDs (15, 16). For example, a 6-mo VLCD, which was associated with ~17% weight loss, increased plasma ghrelin by 24% (15), and both fasting and postprandial ghrelin and peptide YY were reported to be increased, and fasting and postprandial plasma cholecystokinin was reported to be reduced, after a 10-wk VLCD, with these effects still evident 1 y after the initial weight loss (16). Thus, although VLCDs, in both the short term [as in our previous study (10)] and the longer term (15, 16), may have effects on gastrointestinal hormones, that favor a reduction in energy intake, the data from the current study demonstrate that a more moderate dietary restriction that may be associated with better patient compliance in the longer term may lead to comparable weight-loss outcomes.

The observed changes in BPP warrant discussion. Pyloric pressure is stimulated by the arrival of nutrients in the small intestinal lumen (21) and a major determinant of the slowing of gastric emptying of a meal (37). As discussed, the stimulation of pyloric pressures by intraduodenal oleic acid is diminished in obese compared with lean men (4), and the magnitude of stimulation of pyloric pressures by intraduodenal lipid or carbohydrate is an independent determinant of subsequent energy intake (1). In the light of these findings, the current results are of particular interest. BPP did not increase significantly in lean men after the 4-d dietary restriction, probably because it is not possible to substantially increase BPP in this group. In contrast, in obese men, in whom baseline BPP was significantly less than that of lean controls, the dietary restriction enhanced BPP in response to intraduodenal lipid and was associated with a reduction in food intake and body weight, which established that 1) even modest longer-term dietary restriction can, at least in part, restore gastrointestinal sensitivity to the actions of intraluminal lipid and lead to a reduction in food intake (extending our previous findings), and 2) the development of these changes appears to take considerable time. Thus, to our knowledge, the data provide initial evidence for a potential role of intact gastrointestinal sensing of intraduodenal lipid to initiate gastrointestinal responses that are fundamental to the acute regulation of food intake.

Although it could be argued that the intraduodenal infusion of lipid may not represent a physiologic stimulus, outcomes from the current and our previous studies suggested that the intraduodenal infusion of lipid is an excellent model to evaluate gastrointestinal responses as a marker for gastrointestinal sensitivity to lipid in healthy subjects and different population and patients groups as well as any changes in the response to different diets (4, 7, 9, 10, 26, 38). Because gastrointestinal motor and hormone functions are independent determinants of acute energy intake (1), but these functions are diminished in obese subjects (4), strategies that enhance the sensitivity of the upper gastrointestinal tract to nutrients, particularly lipid, will, on the basis of the current study, at least in part restore the gastrointestinal feedback loops that activate those gastrointestinal functions, thereby allowing subjects to eat less and achieve the same degree of satiety. However, much more research is needed to investigate interventions that will be required to reliably achieve these gastrointestinal changes in free-living, obese individuals associated with an energy intake reduction and maintenance of body weight loss in the longer-term to establish the broad relevance of this approach to clinical obesity.

The limitations of our study should be recognized. We administered the lipid emulsion directly into the duodenum because our primary focus was to identify changes in small intestinal sensitivity to lipid. As a result, potential gustatory and gastric influences were bypassed, and we could not comment on any effects on gastric emptying. Moreover, the effects of carbohydrate or protein were not assessed. The observed outcomes may have been stronger if the 5 subjects in whom compliance was suboptimal had complied fully with the dietary restriction. We monitored dietary compliance during the study, and although this monitoring is difficult in intervention studies, the substantial reduction in weight that occurred as a result of the dietary restriction strongly suggested that the majority of subjects complied with the prescribed diet. Self-reported dietary records may be prone to bias and underreporting but are, nevertheless, an accepted method of dietary assessment (39). We could not exclude a potential order effect because baseline and dietary periods were not randomized. However, we have previously shown an excellent reproducibility of gastric emptying, gut hormone, and energy intake in response to the same nutrient preload (24) and are, therefore, confident that the observed differences were not a result of changes in measured variables over time.

In conclusion, in both lean and obese men, gastrointestinal responses to intraduodenal lipid were only affected modestly by an acute 30% dietary restriction and not associated with a reduction in energy intake. In contrast, in obese men, the 12-wk
dietary restriction, which was associated with weight loss, enhanced the gastrointestinal responses to intraduodenal lipid, particularly BPP and peptide YY release, and led to a reduction in food intake despite a greater fasting desire to eat, which suggests that longer-term, moderate dietary restriction has the capacity to enhance gastrointestinal sensitivity to lipid. Although the somewhat unexpected direction of outcomes from our study (ie, marked weight loss and no evidence of gastrointestinal adaptive responses) does not allow us to directly accept or refute our hypothesis, the data provide additional evidence of the importance of gastrointestinal mechanisms for the control of energy intake and, in the longer term, body weight regulation. Thus, identifying novel dietary interventions that potently modulate those gastrointestinal mechanisms that are important for energy intake regulation has important implications for the development of effective dietary strategies for sustained weight loss.

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The authors’ responsibilities were as follows—RVS: designed and conducted the research, analyzed data, performed the statistical analysis, and wrote the manuscript; TJL: designed the research and wrote the manuscript; PT and MN: designed and conducted the research; SS: performed gut-hormone assays; MH and PMC: designed the research, analyzed data, and wrote the manuscript; and CF-B: designed the research, analyzed and interpreted data, wrote the manuscript, and had primary responsibility for the final content of the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES