

Effect of weight loss and ketosis on postprandial cholecystokinin and free fatty acid concentrations¹⁻³

Supornpim Chearskul, Elizabeth Delbridge, Arthur Shulkes, Joseph Proietto, and Adamandia Kriketos

ABSTRACT

Background: Weight regain after weight loss may not be due primarily to voluntary return to social habits but may be explained by changes in peripheral hormonal signals activating hunger and encouraging feeding behavior.

Objective: The objective of this study was to investigate physiologic adaptations to weight loss that may encourage weight regain.

Design: The study had a within-subject repeated-measure design [12 healthy, obese men, 33-64 y, body mass index (in kg/m²) 30-46] and was a clinical intervention investigation of circulating metabolites and hunger-satiety responses before and after weight loss. Measures included anthropometry (bioelectrical impedance, body weight, and waist circumference), concentrations of circulating hormones and metabolites [ketone bodies, free fatty acids (FFAs), insulin, leptin, glucose, and cholecystokinin (CCK)], and measures of hunger and satiety at baseline, 8 wk after weight loss with a very-low-energy diet, and 1 wk after weight maintenance.

Results: Weight loss led to a reduction in postprandial CCK secretion ($P = 0.016$). However, when subjects were ketotic (elevated circulating β -hydroxybutyrate concentrations), CCK secretion was sustained at concentrations before weight loss. After weight loss, there were reduced postprandial FFA concentrations ($P = 0.0005$). The presence of ketosis sustained FFA to concentrations before weight loss ($P = 0.60$).

Conclusion: Rapid weight loss of $\approx 10\%$ of initial body weight results in a reduction in postprandial CCK and FFA concentrations. *Am J Clin Nutr* 2008;87:1238-46.

INTRODUCTION

Feelings of hunger and satiety are important determinants of energy intake and hence of body weight regulation. Although humans can modulate food intake by voluntary control in the short term, the almost invariable weight regain that occurs in obese persons after weight loss suggests that in the long term, biologically determined feelings of hunger and satiety may be more important than voluntary control of food intake. A complex central mechanism is emerging with powerful influence on hunger and satiety. This central regulator is modulated by circulating peripheral signals that are responsive to the nutritional state [for review see Hellstrom et al (1)]. The known circulating modulators of hunger and satiety include nutrients such as glucose, ketone bodies, and free fatty acids (FFAs), and hormones such as ghrelin, leptin, insulin, cholecystokinin (CCK), glucagon-like peptide 1, and peptide YY, with others possibly yet to be described (2-7).

Leptin circulates throughout the body and is transported into the brain by an active saturable mechanism (8). Its role is to inhibit food intake and increase energy expenditure and does so by altering the expression of hypothalamic neurotransmitters (9). One possible reason for weight regain is that leptin concentrations profoundly decrease after weight loss (10-13) disproportionately to changes in adiposity (10, 13-17). After this drop, the dieting person then experiences the effects of leptin deficiency, namely hunger and lethargy (16).

Considerable research has confirmed CCK as a hormone that mediates meal termination (satiety) and possibly early-phase satiety. After a meal, CCK is released into the blood from endocrine I cells of the duodenum and jejunum, leading to inhibition of food intake (18). Exogenous administration of CCK shortens the duration of a meal, and CCK receptor antagonism increases food intake, reverses the inhibitory effects of exogenous CCK, and was shown to reverse the inhibitory effects of intestinal fat infusion on food intake and feeding behavior (19). Data on the relation between CCK and appetite in human studies are still limited.

The ketones β -hydroxybutyrate and acetoacetate are nonhormonal circulating metabolites of fatty acids that anecdotally are said to suppress hunger. This is the basis of the reported low-carbohydrate satiety-associated, ketotic diets that are so popular in the lay press. There is however little scientific evidence for an appetite-suppressing effect of ketones in humans. The aim of this study was to characterize some of the mechanisms associated with hunger after rapid weight loss. In particular, assessment of postprandial circulating concentrations of CCK and FFA and their association with hunger and satiety ratings and to confirm the effects of ketones on feelings of hunger. This was accomplished through a cross-sectional and longitudinal examination

¹ From the Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (SC), and the Departments of Medicine (ED, JP, and AK) and Surgery (AS), Austin Health/Northern Health, University of Melbourne, VIC, Australia.

² Supported by a University of Melbourne International Collaborative Research Grant (SC and AK), Austin Hospital Medical Foundation Research Grant (AK), and a National Health and Medical Research Council of Australia Career Development Award (AK). AS is a National Health and Medical Research Council (NHMRC) Senior Principal Research Fellow.

³ Address reprint requests to A Kriketos, Department of Medicine (AH/NH), 300 Waterdale Road, Heidelberg Heights, VIC 3181, Australia. E-mail: a.kriketos@unimelb.edu.au.

Received July 20, 2007.

Accepted for publication January 4, 2008.

of 12 obese male subjects who underwent a rapid weight loss and who were then instructed to maintain the reduced body weight for a further 1 wk after the reintroduction of food.

SUBJECTS AND METHODS

Study population

Twelve healthy, obese sedentary, nonsmoking men aged between 33 and 64 y (body mass index, in kg/m²: 30–46) without a history of diabetes, hypertension, and cardiovascular or endocrine disease were recruited from advertisements in newspapers and on campus. Subjects who were obese in early childhood were also excluded to avoid persons with monogenic or syndromic forms of obesity. Each subject's weight was stable (<2 kg of change) for the previous 3 mo. No significant difference was observed in the level of routine physical activity (exercise or home duties) undertaken by the subjects (based on frequency questionnaire).

Study protocol

Procedures were undertaken after a 10-h overnight fast, and all were conducted within the Clinical Research Unit (CRU) of the Department of Medicine at the Heidelberg Repatriation Hospital. Subjects were asked to refrain from consuming alcohol the night before and from participating in strenuous exercise for 24 h before testing. Three long visits at weeks 0, 8, and 9 and shorter fortnightly visits between week 1 and the end of week 8 (during the weight-loss phase) were required from each subject. At the completion of the study, the subjects were advised to continue following a low-fat, high-protein diet that was assigned to help them maintain the new body weight.

Baseline (week 1)

All testing occurred after the subject had fasted from 2200 the night before. Soon after arrival at the CRU at 0800, measures of anthropometry were taken, and later an intravenous line was inserted for blood sampling. Blood was taken to measure concentrations of glucose, insulin, FFA, leptin, CCK, and β -hydroxybutyrate. The subjects were then given a breakfast meal designed by the dietitian to provide similar calories between subjects on the basis of each person's lean body mass as determined from bioelectrical impedance analysis (BIA; Tanita TBF-300; WW Wedderburn Pty Ltd, Sydney, Australia). Hunger, satiety, desire to eat, and blood nutrients and hormones were then measured at 30, 60, 90, 120, 180, and 240 min (until \approx 1300) when the subjects were offered a self-chosen meal. An assortment of foods was offered that varied in amounts of protein, carbohydrate, and fat. The quantity of the meal voluntarily ingested for lunch was measured, as was the macronutrient composition of the meal.

Weight-loss phase (weeks 0–7)

During weeks 0–7 subjects underwent the weight-loss phase of the study. The dietitian instructed the subjects to follow a weight-loss program with the use of a very-low-energy diet (VLED) (Optifast; Novartis Nutrition, Mulgrave, Australia) and maintained regular contact with the subjects during the 8-wk weight-loss phase. During this weight-loss phase, the 3 meals of the day were replaced with the VLED, and \leq 2 cups vegetables/d

were allowed. Subjects were required to lose at least 10% of their body weight. This diet has been used extensively in our CRU.

After weight-loss (week 8)

At the beginning of week 8, subjects returned to the CRU for a repeat of the baseline testing. At this time, subjects were considered to be ketotic because of the lack of carbohydrate content in their diet during the preceding weeks. The same breakfast meal provided at baseline was provided to each subject (and the caloric content adjusted for the new lean body mass). Blood sampling and measures of hunger and satiety occurred as described above.

After weight loss (week 9)

During week 8 and before returning to the CRU at the beginning of week 9, subjects were instructed to begin replacing the VLED supplements with light meals, being careful not to regain any weight. Subjects were advised to follow dietary instructions that met their energy requirements for weight maintenance. The diet recommended was low in saturated fat, featured carbohydrates with low glycemic index values, and had a moderately higher protein intake relative to the typical protein intake for Australian men. Subjects were encouraged to be more physically active throughout the entire weight-loss and maintenance periods. At the beginning of week 9, subjects were no longer considered to be ketotic. They returned to the CRU for a repeat of the baseline testing. The same breakfast meal provided at baseline was provided to each subject (and the caloric content adjusted for the new lean body mass). Blood sampling and measures of hunger and satiety occurred as described above.

Anthropometry

Body weight, body fat mass, and waist and hip circumferences were measured at the beginning of each testing session and fortnightly throughout the study period of weeks 0–7. Anthropometry measures were made with the subject wearing light clothing and barefoot. Weight was recorded to the nearest 0.1 kg from a digital scale, and height was recorded to the nearest 0.5 cm with the use of a stadiometer. Waist circumference was determined at the level of the umbilicus and hip circumference at the level of the greater trochanters to the nearest 0.5 cm with the use of a spring-load tape measure. Body mass index (in kg/m²) was calculated, and waist-to-hip ratio was calculated by dividing waist circumference by hip circumference (in cm). Body fat distribution was estimated by BIA with the use of the standard adult mode of measurement. The BIA apparatus also estimated fat-free mass as the sum of muscle, bone, tissue, water, and all other fat-free mass in the body.

Visual analogue scales

Measures of hunger, satiety, and desire to eat were assessed with the use of 100-mm visual analogue scales (VASs) preceded by questions, such as "Do you feel hungry?" The VAS was extensively used in dietary studies (20–23) and is anchored with "not at all" and "extremely" at the left and right ends, respectively. The distance from the extreme left to the subject's vertical dash represents the rating score, expressed in millimeters. The measures of hunger, satiety, and desire to eat with the use of a VAS were determined every hour throughout the postprandial testing sessions.



The three-factor eating questionnaire was used before each breakfast testing session. It is a 51-item self-assessment instrument of eating behavior that measures cognitive restraint, disinhibition, and hunger (24). Its purpose is to assess 3 factors related to cognitions and behaviors associated with eating. These factors are cognitive dietary restraint, disinhibition, and susceptibility to hunger. More precisely, cognitive dietary restraint is the intent to restrict food intake to control body weight (21 items; score ranging from 0 to 21). Disinhibition is an overconsumption of food in response to a variety of stimuli, such as emotional stress, associated with a loss of control on food intake (16 items, score ranging from 0 to 16). Finally, susceptibility to hunger refers to food intake in response to feelings and perceptions of hunger (14 items; score ranging from 0 to 14) (24). The questionnaire was widely used in both overweight and normal-weight subjects (25, 26).

Blood sampling

Blood was collected in evacuated tubes with and without various anticlotting agents or left to clot for 20–30 min and then spun in a refrigerated centrifuge at 1000 rpm for 20 min. Serum or plasma were divided into aliquots and stored for analyses of metabolites. Evacuated tubes used were no clotting agents (for serum to measure glucose, leptin, and FFA), lithium heparin (for plasma to measure insulin), and K3 EDTA (for plasma to measure β -hydroxybutyrate and CCK). Serum and plasma for FFA, β -hydroxybutyrate, and CCK were stored at -80°C and thawed only once for analyses; all other aliquots were stored at -20°C .

Breakfast meal

The breakfast meal consisted of a boiled egg, 1 slice of toast with margarine, orange juice, 2 cereal biscuits (Weet-Bix; Sanitarium, Berkeley Vale, Australia) with low-fat milk, and a banana. This meal was $\approx 53\%$ carbohydrate, 25% fat, and 22% protein. Meals were prepared on the morning of testing, consumed within 20 min, and were well tolerated by the subjects. Adjustments of the caloric content of the meal were made on a per kilogram fat-free mass basis by modifying the quantities of ingredients; on average the meal provided 2000 kJ. This meal represented a typical breakfast eaten by an Australian adult man. No other food or beverage (with the exception of water) was offered throughout the testing period. Subjects remained seated quietly for the duration of the testing, ie, for 30 min before the meal and for 4 h subsequent to the consumption of the meal. The lunch meal was served 4 h after completion of the breakfast meal. Subjects were encouraged to serve themselves lunch from a buffet-style setting.

Assessment of energy intake (lunch meal)

For lunch an assortment of foods, varying in amounts of protein, carbohydrate, and fat, were offered (including pasta salad, sandwiches, ice cream, fruit, chocolate cake). The subjects selected their own lunch meal and, unknown to them, the quantity of the meal voluntarily ingested was measured, and the macronutrient composition of the meal was calculated (FOOD WORKS; Xyris Software, Brisbane, Australia).

Biochemical assays

Circulating concentrations of plasma and serum glucose, insulin, FFAs, β -hydroxybutyrate, and leptin were measured with

the use of appropriate diagnostic kits. Plasma and serum samples were batched for analyses to maximize capacity of the diagnostic kits and to minimize interassay error. Plasma CCK was measured in ethanol-extracted plasma with the use of antiserum 92128 (generous donation of Prof Jens Rehfeld, University Hospital, Copenhagen, Denmark) and ^{125}I -Bolton-Hunter-CCK8 label (GE Healthcare, Rydalmere NSW, Australia). The antiserum is specific for CCK-amide with negligible cross-reactivity to gastrin-amide or gly-extended forms of gastrin and CCK. The inhibitory dose 50% is 0.61 ± 0.06 pmol/L and the intra- and interassay CVs are 7% and $<14\%$, respectively. Insulin and leptin concentrations were assayed by commercial radioimmunoassay (Linco, St Charles, MO), and FFAs were assayed by enzymatic colorimetry (Wako, Osaka, Japan). The glucose oxidase method was used to measure plasma glucose (by the GM7 Analox glucose analyser; Helena Laboratories Australia Pty Ltd, Melbourne, Australia), and β -hydroxybutyrate concentrations were measured with the use of a 3-hydroxybutyrate II reagent kit (Helena Laboratories Australia Pty Ltd). Within our laboratory, inter- and intraassay CVs were $<10\%$ for these assays.

Statistical analysis

Data are expressed as mean \pm SEM. One-factor analysis of variance was performed to determine an overall *P* value for each measured marker. Comparisons between groups were performed with the use of Students' *t* tests. The Bonferroni correction was used to assess significance. Relations between continuous variables were assessed by simple regression analyses as appropriate. Data were analyzed with the use of STATVIEW 5 (SAS Institute Inc, Cary, NC).

We certify that all applicable institutional and governmental regulations about the ethical use of human volunteers were followed during this research. The Austin Health Human Research Ethics Committee approved the study, and all subjects provided written informed consent.

RESULTS

Effect of rapid weight loss on anthropometric measures

Eight weeks of caloric restriction (≈ 600 kJ/meal, ≈ 1800 kJ daily intake) in 12 obese men resulted in a significant average weight loss of 15% (116.7 ± 5.8 kg compared with 98.8 ± 5.3 kg; $P < 0.0001$), which was maintained for an additional week (99.3 ± 5.2 kg) once normal food was reintroduced into the daily diet (Table 1). Body mass index dropped from 37.2 ± 1.8 to 31.5 ± 1.6 after weight loss ($P < 0.0001$). The anthropometric changes that resulted from the weight loss in these 12 obese men are shown in Table 1. Significant reductions were observed in fat mass (average: 14.6 kg) and waist circumference (average: 20.9 cm). In addition to weight loss, blood pressure was significantly reduced by 25 mm Hg as was the resting pulse rate (Table 1).

Effect of rapid weight loss on fasting circulating markers

There were nonsignificant improvements in circulating concentrations of glucose and insulin after the rapid weight loss in these subjects (Table 2) that resulted in a nonsignificant reduction in insulin resistance, as estimated by homeostasis model assessment of insulin resistance (6.1 arbitrary units compared with 3.0 arbitrary units; $P = 0.059$; Table 2). These improvements were sustained after a week of weight maintenance. As

TABLE 1
Subject characteristics ($n = 12$)¹

	Week 0	Week 8	Week 9	Overall P^2
Body weight (kg)	116.7 ± 5.8 ^a	98.8 ± 5.3 ^b	99.3 ± 5.2 ^b	0.043
BMI (kg/m ²)	37.2 ± 1.8 ^a	31.5 ± 1.6 ^b	31.6 ± 1.6 ^b	0.032
Fat mass (kg)	45.1 ± 5.1 ^a	30.5 ± 4.0 ^b	29.4 ± 3.9 ^b	0.028
Fat-free mass (kg)	70.4 ± 2.1	67.1 ± 1.7	68.8 ± 2.2	0.523
Waist circumference (cm)	122.2 ± 5.1 ^a	101.3 ± 4.2 ^b	102.2 ± 4.1 ^b	0.003
Hip circumference (cm)	117.1 ± 3.7 ^a	107.1 ± 3.0 ^b	106.8 ± 3.1 ^b	0.005
Pulse (beats/min)	75.8 ± 2.2 ^a	66.3 ± 1.5 ^b	62.8 ± 1.6 ^b	0.001
Diastolic BP (mm Hg)	83.3 ± 2.9 ^a	71.5 ± 2.1 ^b	70.3 ± 2.0 ^b	0.001
Systolic BP (mm Hg)	137.4 ± 4.6 ^a	112.5 ± 3.4 ^b	111.6 ± 3.1 ^b	0.001

¹ All values are $\bar{x} \pm \text{SEM}$. BP, blood pressure. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni analysis).

² Calculated with one-factor ANOVA.

expected, plasma leptin concentrations also decreased significantly after the rapid weight loss (20.9 ± 4.2 ng/mL to 8.2 ± 2.0 ng/mL; $P = 0.0005$) and did not increase with the reintroduction of food but remained at this lower value at the end of week 9 (9.0 ± 1.9 ng/mL) (Table 2). The VLED diet during 8 wk resulted in a significant rise in circulating ketone bodies (β -hydroxybutyrate) from 0.28 ± 0.05 mmol/L to 1.02 ± 0.18 mmol/L ($P = 0.0007$). After reintroduction of food during 1 wk of weight maintenance, β -hydroxybutyrate concentrations returned to baseline (0.26 ± 0.05 mmol/L). Circulating concentrations of FFA were not different from baseline at week 8 while the subjects were ketotic (Table 2), but, by the end of week 9, FFA concentrations had decreased to a value significantly below that seen at baseline ($P = 0.0006$). After an overnight fast, fasting CCK concentrations were unchanged after weight loss at week 8 (1.62 ± 0.50 pmol/L compared with 1.44 ± 0.37 pmol/L) and after the reintroduction of food (1.05 ± 0.26 pmol/L).

Effect of weight loss on postprandial circulating markers

Compared with baseline and across a 4-h postprandial period, the plasma glucose and insulin responses [absolute area under the curve (AUC)] were reduced after weight loss (AUC glucose: 51.4 ± 2.6 mmol · L⁻¹ · 4 h⁻¹ compared with 48.2 ± 2.0 mmol · L⁻¹ · 4 h⁻¹; $P = 0.059$ and AUC insulin: 518.5 ± 96.9 mmol · L⁻¹ · 4 h⁻¹ compared with 315.3 ± 47.2 mmol · L⁻¹ · 4 h⁻¹; $P = 0.031$) and remained significantly lower at the end of the weight-maintenance period (AUC glucose: 46.4 ± 1.8 mmol · L⁻¹ · 4 h⁻¹; $P = 0.045$ and AUC insulin: 317.2 ± 36.8

mmol · L⁻¹ · 4 h⁻¹; $P = 0.035$). This finding implies an expected improvement in postprandial insulin sensitivity with weight loss.

As mentioned earlier, fasting concentrations of β -hydroxybutyrate were significantly increased after 8 wk of the VLED because subjects were in a ketotic phase. The postprandial concentrations of β -hydroxybutyrate were measured during the first postprandial hour (and shown in Figure 1A as absolute AUC). The postprandial β -hydroxybutyrate concentrations were not different before weight loss (week 0) and after weight maintenance (week 9) (Figure 1A). However, while subjects were in a ketotic phase (week 8), the postprandial concentrations of β -hydroxybutyrate (AUC) were significantly elevated compared with weeks 0 and 9 (Figure 1A).

FFA concentrations decreased in a similar pattern during the postprandial period across each of the 3 phases of the study (Figure 1B). A greater variation was observed in postprandial FFA concentrations (absolute AUC) at week 8 across the 12 subjects studied (presumably because of ketosis and increased β -oxidation of FFA), so there was no significant difference in the postprandial FFA response to the meal after weight loss at week 8 compared with baseline ($P = 0.60$). While still maintaining the lower body weight but after the reintroduction of some food during 1 wk (and therefore in the absence of ketosis), there was a significantly reduced postprandial AUC for FFA at week 9 than at week 0 ($P = 0.0005$) and as well at week 8 ($P = 0.001$).

CCK concentrations after an overnight fast were not significantly lower after weight loss or 1 wk later than at baseline (Table 2). However, at 30 min after the breakfast meal, there was a

TABLE 2
Fasting plasma and serum markers ($n = 12$)¹

	Week 0	Week 8	Week 9	Overall P^2
Plasma glucose (mmol/L)	6.08 ± 0.32 ³	5.36 ± 0.23	5.57 ± 0.22	0.14
Plasma insulin (mU/L)	22.11 ± 5.23	12.25 ± 1.49	13.30 ± 1.71	0.081
HOMA-R	6.07 ± 1.43	3.04 ± 0.52	3.43 ± 0.58	0.059
HOMA- β	8.99 ± 2.03	7.38 ± 1.05	6.46 ± 0.58	0.421
Leptin (ng/mL)	20.9 ± 4.2 ^a	8.2 ± 2.0 ^b	9.0 ± 1.9 ^b	0.006
β -Hydroxybutyrate (mmol/L)	0.28 ± 0.05 ^a	1.02 ± 0.18 ^b	0.26 ± 0.05 ^a	0.001
Free fatty acids (μ mol/L)	353 ± 15 ^a	441 ± 89 ^b	256 ± 14 ^a	0.059
CCK (pmol/L)	1.62 ± 0.50	1.44 ± 0.37	1.05 ± 0.26	0.575

¹ All values are $\bar{x} \pm \text{SEM}$. HOMA-R, homeostasis model assessment of insulin resistance; HOMA- β , homeostasis model assessment of β cell function; CCK, cholecystokinin. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni analysis).

² Calculated with one-factor ANOVA.

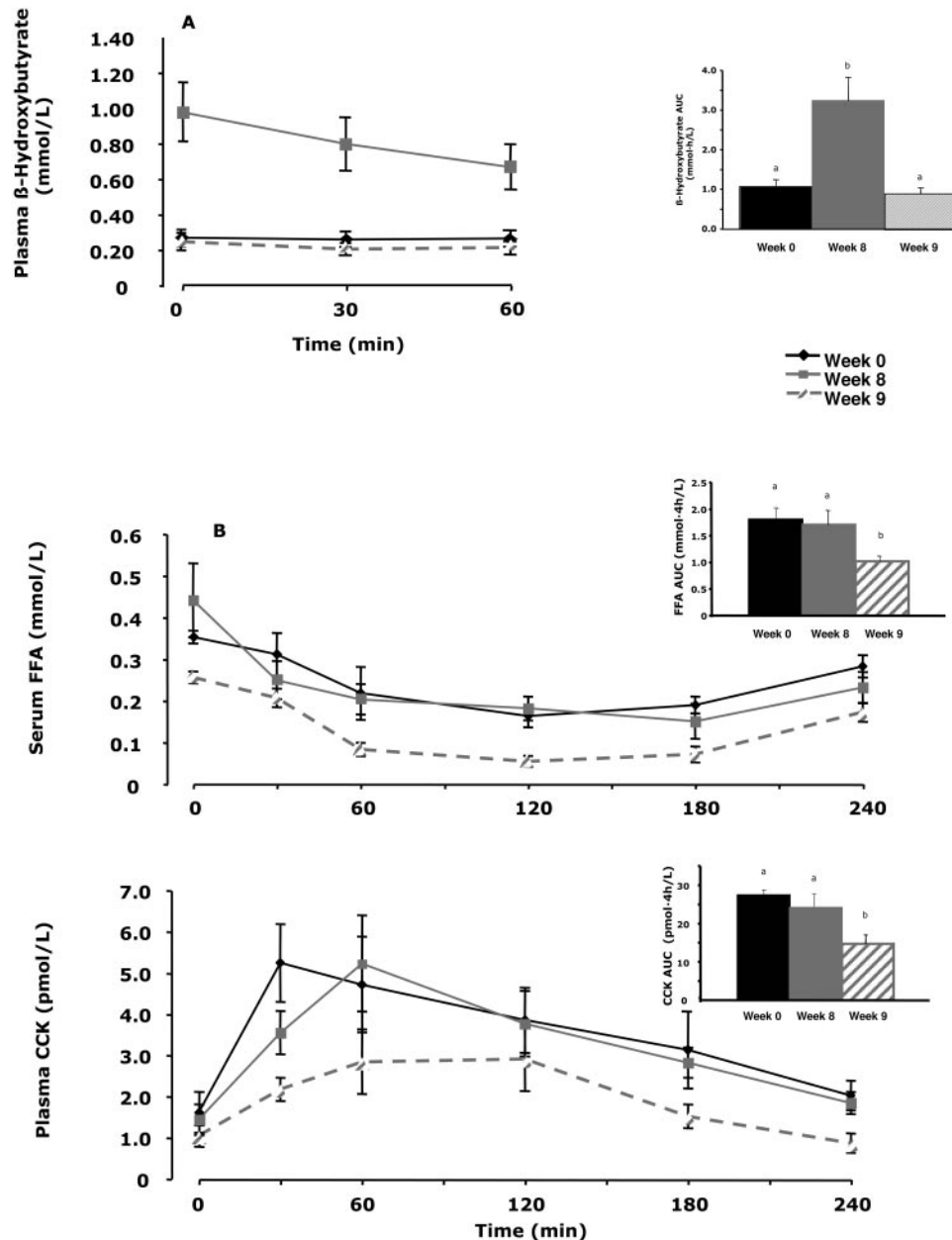


FIGURE 1. Postprandial concentrations of β -hydroxybutyrate (A) and free fatty acids (FFA), and cholecystokinin (CCK) (B) are shown before and after weight loss (error bars indicate SEM; $n = 12$). The bar graphs depict the cumulative postprandial responses (area under the curve) of the metabolites. Overall P value was calculated with the use of one-factor ANOVA. Means with different superscript letters are significantly different at $P < 0.05$, determined by Bonferroni analysis. The breakfast meals were consumed by all subjects within 10 min of receiving their meals. The postprandial times referred to in the figure begin from the end of the consumption of the breakfast meal.

nonsignificant trend for the concentration after weight loss (week 8) to be reduced compared with the concentration before weight loss (5.25 ± 0.94 pmol/L compared with 3.56 ± 0.52 pmol/L; $P = 0.08$), whereas at 60 min the postprandial responses were not different before and after weight loss (4.73 ± 1.16 pmol/L compared with 5.23 ± 1.16 pmol/L; $P = 0.57$). At week 9, the 30-min CCK concentrations were significantly lower than the concentrations at baseline ($P = 0.008$) and week 8 ($P = 0.037$). At week 9 the postprandial CCK response (absolute AUC) was significantly reduced compared with before weight loss ($P = 0.016$) and reduced compared with week 8 ($P = 0.006$). These data support the hypothesis that CCK appears to be changing in a

direction that may encourage eating behavior after weight loss because there was a delayed CCK response to a meal (Figure 1B).

Effect of rapid weight loss on ratings of hunger

Subjects were asked to rate their hunger with the use of a 3-factor eating questionnaire. In the fasting state, the perception of hunger showed a drop in the rating of hunger after weight loss (5.4 ± 1.1 arbitrary units at week 8 compared with 8.1 ± 1.1 arbitrary units at baseline; $P = 0.025$) and maintained a reduced rating of hunger after the reintroduction of food (6.0 ± 1.3 arbitrary units at week 9). The perception of cognitive restraint in

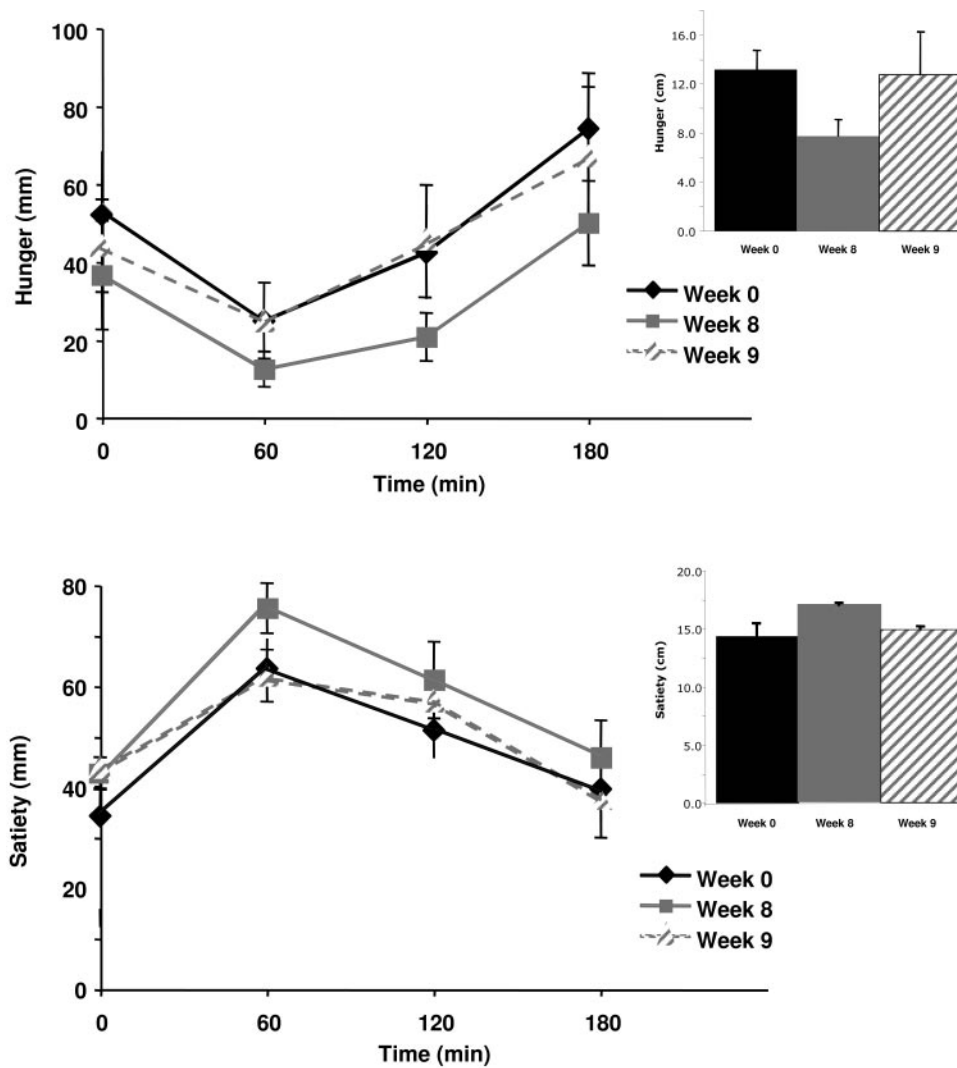


FIGURE 2. Perceptions of hunger and satiety measured with the use of visual analogue scales are shown during a 3-h postprandial period after a breakfast meal (error bars indicate SEM; $n = 12$). The bar graphs depict the cumulative responses (area under the curve) of the perceptions of hunger and satiety. Overall P value was calculated with the use of one-factor ANOVA. Means with different superscript letters are significantly different at $P < 0.05$, determined by Bonferroni analysis.

eating significantly increased after 8 wk of caloric restriction (11.4 ± 1.3 arbitrary units) compared with baseline (5.5 ± 0.7 arbitrary units; $P = 0.002$) and remained elevated after a week of reintroduction of food when the aim was to maintain the weight loss (11.8 ± 1.3 arbitrary units; $P = 0.002$). Further, the rating of disinhibition of eating was significantly reduced at week 8 compared with baseline (10.5 ± 1.0 arbitrary units compared with 8.0 ± 1.2 arbitrary units; $P = 0.02$) and remained reduced at week 9 (8.3 ± 1.3 arbitrary units).

In addition to hunger and satiety ratings being recorded in the fasting state with the use of VAS, postprandial hunger and satiety ratings were recorded at 60, 120, and 180 min after a breakfast meal (Figure 2). After 8 wk of the VLED, subjects' perceptions of hunger were blunted during the postprandial period but were not different at weeks 0 and 9. Conversely, satiety was perceived to be greatest after 8 wk of the VLED compared with weeks 0 and 9. Because of the variation in responses to hunger and satiety perceived by each subject and the limited number of subjects in the study, the postprandial responses in terms of absolute AUC

for hunger and satiety were not significant between testing periods, although there appeared to a slight, nonsignificant reduction in hunger ($P = 0.058$) and nonsignificant increase in satiety ($P = 0.067$) at week 8 when the subjects were ketotic (Figure 2).

Energy intake of self-selected lunch meal

The nutrient data (in terms of absolute gram quantities) of the self-selected lunch meals that were offered to each subject ≈ 4 h after breakfast before and after weight loss are shown in Table 3. Overall, no significant change was observed in carbohydrate, fat, or protein intake of the self-selected meal between study periods. Analyzed as the percentage of calories consumed, no significant changes were observed also in nutrient intake of the self-selected meal.

The energy content of the self-selected lunch meal consumed at week 0 was on average 2810 ± 256 kJ, and this did not change after weight loss before the reintroduction of normal food (week 8) (2859 ± 217 kJ). However, at the end of week 9, when food had been reintroduced into the subjects' diets for a week with

TABLE 3

Energy content of self-selected lunch meal ($n = 12$)¹

	Week 0	Week 8	Week 9	Overall P^2
Carbohydrate (g)	70.1 ± 6.1	62.4 ± 5.8	73.8 ± 7.7	0.469
Fat (g)	31.4 ± 4.1	33.9 ± 2.7	40.7 ± 3.2	0.148
Protein (g)	25.7 ± 2.4	32.1 ± 3.0	34.6 ± 2.9	0.083
Energy (kJ)	2810 ± 256	2859 ± 217	3345 ± 274	0.262

¹ All values are $\bar{x} \pm$ SEM. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni analysis).

² Calculated with one-factor ANOVA.

dietary instruction to maintain weight loss by increasing their protein intake, the energy content of the self-selected lunch meal tended to increase to 3345 ± 274 kJ ($P = 0.05$). This finding coincides with the significant reduction in CCK and FFA concentrations during and at the end of the postprandial testing period at week 9 compared with week 0 and week 8 (Figure 1B), and therefore reduced satiety after the breakfast meal.

DISCUSSION

The perplexing fact that weight loss is difficult to maintain, even by those who are desperate to be leaner, deserves investigation. Many factors that participate in meal initiation are not hard-wired into the physiology of energy homeostasis. Learned behaviors, sensory cues, emotional inputs, palatability of available food, and social or societal variables all influence the decision to eat, and none of these are tied directly to neuroendocrine circuits involved in energy homeostasis (27–29). Indeed, it was argued that meal initiation is a process driven more by learned and other “nonbiological” markers than by defined hormonal stimuli and that long-term regulation of energy intake occurs predominantly by the control of meal termination (which is also sensitive to input from insulin and leptin) rather than by meal onset (30–33).

Our data, in which 12 obese men underwent a rapid weight loss during 8 wk, would support the notion that physiologic adaptations to the meal termination mechanism may play a role in weight regain after weight loss. In particular, postprandial CCK concentrations changed in a direction that would promote increased eating behavior after weight loss and during a time when subjects were in energy balance at the reduced body weight. At week 9 there was a significant reduction of the postprandial CCK response to a meal, implying a suppression of satiety as a result of reduced body weight. This was followed by a significantly increased intake of energy in a meal offered to subjects, further supporting suppression of satiety by reduced CCK concentrations.

In humans and rodents, peripheral administration of CCK lead to decreased meal size and duration (34–37). CCK concentrations increase over 10–30 min after meal initiation (38). Dietary fat and protein in the small intestine are the main stimulants of CCK release, with carbohydrates only providing a weak stimulus (38). A recent report suggests that CCK may work synergistically with either leptin or insulin in the regulation of appetite and food intake (39, 40). Intravenous administration of CCK1 receptor antagonists in humans reduces satiety and increases hunger and meal size (41, 42). Oral CCK agonist GI181771X delays gastric emptying in healthy volunteers with minimal adverse effects (43). CCK agonists may have an important role in the treatment of weight maintenance after successful weight loss.

In the current study, although not statistically significant, there was a peak CCK response at 60 min after weight loss (week 8) compared with a peak at 30 min at baseline (week 0). This leads to the possibility that pancreatic or gastric and intestinal functions might have changed to cause this shift. It is known that obese persons have attenuated pancreatic output (amylase, trypsin, bicarbonate) when fasting compared with normal-weight subjects. The loss of a peak CCK response in week 9 might also be reflected by a decreased CCK response to dietary fat or protein response because of altered digestive function. Therefore, other intestinal factors (such as CCK-independent vagal afferent stimulation) might be involved in the altered food intake response.

In a recent elegant set of experiments Peters et al (31) show that the hormone leptin and the gut hormone CCK synergistically interact to enhance the process of satiation. Their results support the hypothesis that vagal afferent sensitivity to CCK and leptin is concentrated in neurons that innervate the stomach and duodenum. These specific visceral afferent populations are likely to comprise a substrate through which acute leptin or CCK interactions enhance satiation. Peripheral injection of CCK enhances the process of satiation through activation of abdominal vagal afferent neurons (44, 45) and can also affect food intake through abdominal vagal independent pathways (46). The findings by Peters et al (31) are consistent with the hypothesis that leptin and CCK act locally within the upper gastrointestinal tract to activate vagal afferent neurons and contribute to the control of food intake. Thus, the lower CCK response after weight loss coupled with a profoundly reduced leptin concentration may result in reduced satiety after meals.

Interestingly, no difference was observed in the postprandial response of FFA before (week 0) and after weight loss (week 8), but a significant reduction in postprandial FFA response at the end of the weight maintenance period (week 9). Elevated circulating FFA concentrations may contribute to enhanced FFA entry into the brain, leading to enhanced insulin sensitivity and reduced food intake, as shown by Morgan et al (47).

Only during the ketotic phase of investigation (week 8) was there a significant difference in the concentrations of postprandial β -hydroxybutyrate, with a progressive decline in β -hydroxybutyrate concentrations during the first hour of the postprandial study period (Figure 1A). Our study shows that while at reduced body weight but still in ketosis (elevated circulating β -hydroxybutyrate concentrations at week 8), postprandial circulating CCK and FFA concentrations were sustained at the concentrations before weight loss (therefore reversing the effect of weight loss at week 9). Both of these adaptations (sustained CCK secretion and circulating FFA concentrations) are mitigated by being in ketosis. Therefore, our results support the anecdotal notion (48) that ketones enhance satiety and raise the

possibility of an interaction between ketone bodies and CCK secretion (34–43, 47). It is not well established in humans that an increase in ketones is directly linked to a decrease in food intake or satiety, and more long-term studies are needed to address this important issue.

Clearly, the change in CCK release and the lower FFA concentrations after weight loss showed here are only 2 components of a multifaceted response to weight loss. In addition to the reduction in the CCK response and the lowered FFA concentrations, the >10% loss of body weight in these subjects resulted in the expected reduction of leptin, glucose, and insulin concentrations. Because all 5 factors (CCK, leptin, glucose, FFA, and insulin) known to be involved in the satiety signal are lower after weight loss (30–33), together with the previously shown increase in ghrelin concentrations (49), and other possible adaptations such as changes in sympathetic tone (50), it is possible that a tendency to have larger meals will result, encouraging weight regain. Indeed, as shown in the present study, at week 9 after weight loss, the subjects selected a meal that tended to be more energy dense and provided more fat (Table 3).

A limitation of this study is the low number of subjects investigated. Because of the limited number of subjects and the effect of variability in human responses to VAS, our measures of hunger and satiety did not produce statistically significant results. However, there were indications that in general subjects experienced increased satiety and decreased hunger perceptions at the end of the weight-loss phase, while still in ketosis.

Future work should improve our understanding of the tight mechanisms that are provided by peripheral and central mechanisms to regulate body weight, food intake, and appetite. It is possible that ketones enhance CCK release, and studies involving infusion of β -hydroxybutyrate and examination of CCK secretion are required to improve our understanding in this area.

We thank Mrs Elizabeth Maclean for her assistance in dietary aspects of this study.

The author's responsibilities were as follows—SC, ED, and AK: conducted the study; AS: performed the CCK assays; JP and AK: were responsible for the design of the study, interpretation of the results, and completion of the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES

- Hellstrom PM, Geliebter A, Naslund E, et al. Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr* 2004;92(suppl 1):S47–57.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372(6505):425–32.
- Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. *Nature* 2001;409(6817):194–8.
- Murphy KG, Bloom SR. Gut hormones in the control of appetite. *Exp Physiol* 2004;89(5):507–16.
- Burton-Freeman B, Davis PA, Schneeman BO. Plasma cholecystokinin is associated with subjective measures of satiety in women. *Am J Clin Nutr* 2002;76(3):659–67.
- Romon M, Lebel P, Velly C, Marecaux N, Fruchart JC, Dallongeville J. Leptin response to carbohydrate or fat meal and association with subsequent satiety and energy intake. *Am J Physiol* 1999;277(5 Pt 1):E855–61.
- Little TJ, Horowitz M, Feinle-Bisset C. Role of cholecystokinin in appetite control and body weight regulation. *Obes Rev* 2005;6(4):297–306.
- Wong ML, Licinio J, Yildiz BO, et al. Simultaneous and continuous 24-hour plasma and cerebrospinal fluid leptin measurements: dissociation of concentrations in central and peripheral compartments. *J Clin Endocrinol Metab* 2004;89(1):258–65.
- Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 1996;98(5):1101–6.
- Geldszus R, Mayr B, Horn R, Geisthovel F, von zur Muhlen A, Brabant G. Serum leptin and weight reduction in female obesity. *Eur J Endocrinol* 1996;135(6):659–62.
- Keim NL, Stern JS, Havel PJ. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *Am J Clin Nutr* 1998;68(4):794–801.
- Wisse BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R, Gougeon R. Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women. *Am J Clin Nutr* 1999;70(3):321–30.
- Jenkins AB, Markovic TP, Fleury A, Campbell LV. Carbohydrate intake and short-term regulation of leptin in humans. *Diabetologia* 1997;40(3):348–51.
- Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. *J Clin Endocrinol Metab* 1996;81(12):4406–13.
- Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab* 1997;82(2):561–5.
- Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 1996;81(9):3419–23.
- Dubuc GR, Phinney SD, Stern JS, Havel PJ. Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. *Metabolism* 1998;47(4):429–34.
- Huda MS, Wilding JP, Pinkney JH. Gut peptides and the regulation of appetite. *Obes Rev* 2006;7(2):163–82.
- Geary N. Endocrine controls of eating: CCK, leptin, and ghrelin. *Physiol Behav* 2004;81(5):719–33.
- Sepple CP, Read NW. Gastrointestinal correlates of the development of hunger in man. *Appetite* 1989;13(3):183–91.
- Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord* 2000;24(1):38–48.
- Parker BA, Sturm K, MacIntosh CG, Feinle C, Horowitz M, Chapman IM. Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr* 2004;58(2):212–8.
- Cornier MA, Grunwald GK, Johnson SL, Bessesen DH. Effects of short-term overfeeding on hunger, satiety, and energy intake in thin and reduced-obese individuals. *Appetite* 2004;43(3):253–9.
- Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* 1985;29(1):71–83.
- Lindroos AK, Lissner L, Mathiassen ME, et al. Dietary intake in relation to restrained eating, disinhibition, and hunger in obese and nonobese Swedish women. *Obes Res* 1997;5(3):175–82.
- Laessle RG, Tuschl RJ, Kothaus BC, Pirke KM. A comparison of the validity of three scales for the assessment of dietary restraint. *J Abnorm Psychol* 1989;98(4):504–7.
- de Castro JM. Genes, the environment and the control of food intake. *Br J Nutr* 2004;92(suppl 1):S59–62.
- Berthoud HR. Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. *Obesity (Silver Spring)* 2006;14(suppl 5):197S–200S.
- Berthoud HR. Mind versus metabolism in the control of food intake and energy balance. *Physiol Behav* 2004;81(5):781–93.
- Bray GA. Afferent signals regulating food intake. *Proc Nutr Soc* 2000;59(3):373–84.
- Peters JH, Ritter RC, Simasko SM. Leptin and CCK selectively activate vagal afferent neurons innervating the stomach and duodenum. *Am J Physiol Regul Integr Comp Physiol* 2006;290(6):R1544–9.
- Peters JH, Simasko SM, Ritter RC. Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. *Physiol Behav* 2006;89(4):477–85.
- Peters JH, Ritter RC, Simasko SM. Leptin and CCK modulate complementary background conductances to depolarize cultured nodose neurons. *Am J Physiol Cell Physiol* 2006;290(2):C427–32.



34. Gibbs J, Young RC, Smith GP. Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* 1973;245(5424):323–5.
35. Moran TH, Schwartz GJ. Neurobiology of cholecystokinin. *Crit Rev Neurobiol* 1994;9(1):1–28.
36. Muurahainen N, Kissileff HR, Derogatis AJ, Pi-Sunyer FX. Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. *Physiol Behav* 1988;44(4–5):645–9.
37. Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in man. *Am J Clin Nutr* 1981;34(2):154–60.
38. Liddle RA, Goldfine ID, Rosen MS, Taplitz RA, Williams JA. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *J Clin Invest* 1985;75(4):1144–52.
39. Riedy CA, Chavez M, Figlewicz DP, Woods SC. Central insulin enhances sensitivity to cholecystokinin. *Physiol Behav* 1995;58(4):755–60.
40. Matson CA, Wiater MF, Kuijper JL, Weigle DS. Synergy between leptin and cholecystokinin (CCK) to control daily caloric intake. *Peptides* 1997;18(8):1275–8.
41. Matzinger D, Gutzwiller JP, Drewe J, et al. Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans. *Am J Physiol* 1999;277(6 Pt 2):R1718–24.
42. Beglinger C, Degen L, Matzinger D, D'Amato M, Drewe J. Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. *Am J Physiol Regul Integr Comp Physiol* 2001;280(4):R1149–54.
43. Castillo EJ, Delgado-Aros S, Camilleri M, et al. Effect of oral CCK-1 agonist GI181771X on fasting and postprandial gastric functions in healthy volunteers. *Am J Physiol Gastrointest Liver Physiol* 2004;287(2):G363–9.
44. Smith GP, Gibbs J. Cholecystokinin: a putative satiety signal. *Pharmacol Biochem Behav* 1975;3(1 suppl):135–8.
45. Smith GP, Jerome C, Norgren R. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. *Am J Physiol* 1985;249(5 Pt 2):R638–41.
46. Reidelberger RD. Abdominal vagal mediation of the satiety effects of exogenous and endogenous cholecystokinin in rats. *Am J Physiol* 1992;263(6 Pt 2):R1354–8.
47. Morgan K, Obici S, Rossetti L. Hypothalamic responses to long-chain fatty acids are nutritionally regulated. *J Biol Chem* 2004;279(30):31139–48.
48. Adam-Perrot A, Clifton P, Brouns F. Low-carbohydrate diets: nutritional and physiological aspects. *Obes Rev* 2006;7(1):49–58.
49. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 2002;346(21):1623–30.
50. Levin BE. Why some of us get fat and what we can do about it. *J Physiol* 2007;583(Pt 2):425–30.

