European Journal of Clinical Nutrition (2013) **67**, 759–764

www.nature.com/ejcn

ORIGINAL ARTICLE

Ketosis and appetite-mediating nutrients and hormones after weight loss

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BACKGROUND/OBJECTIVES: Diet-induced weight loss is accompanied by compensatory changes, which increase appetite and encourage weight regain. There is some evidence that ketogenic diets suppress appetite. The objective is to examine the effect of ketosis on a number of circulating factors involved in appetite regulation, following diet-induced weight loss.
SUBJECTS/METHODS: Of 50 non-diabetic overweight or obese subjects who began the study, 39 completed an 8-week ketogenic very-low-energy diet (VLED), followed by 2 weeks of reintroduction of foods. Following weight loss, circulating concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), leptin, gastrointestinal hormones and subjective ratings of appetite were compared when subjects were ketotic, and after refeeding.
RESULTS: During the ketogenic VLED, subjects lost 13% of initial weight and fasting BHB increased from (mean ± s.e.m.) 0.07 ± 0.00 to 0.48 ± 0.07 mmol/l ($P<0.001$). BHB fell to 0.19 ± 0.03 mmol/l after 2 weeks of refeeding ($P<0.001$ compared with week 8). When participants were ketotic, the weight loss induced increase in ghrelin was suppressed. Glucose and NEFA were higher, and amylin, leptin and subjective ratings of appetite were lower at week 8 than after refeeding.
CONCLUSIONS: The circulating concentrations of several hormones and nutrients which influence appetite were altered after weight loss induced by a ketogenic diet, compared with after refeeding. The increase in circulating ghrelin and subjective appetite which accompany dietary weight reduction were mitigated when weight-reduced participants were ketotic.

European Journal of Clinical Nutrition (2013) **67,** 759–764; doi:10.1038/ejcn.2013.90; published online 1 May 2013

Keywords: appetite; ketosis; very-low-energy diet; weight loss

INTRODUCTION

The increasing prevalence of overweight and obesity has been widely reported. Ketogenic low-carbohydrate diets are a popular means of weight loss, and in the short-term, often result in greater weight loss than low-fat diets.[1] During fasting or restriction of dietary carbohydrate intake, fatty acid oxidation in the liver results in the production of ketones. Although the mechanism of the efficacy of ketogenic diets has not been definitively established, it is commonly proposed that ketones suppress appetite,[2,3] and it has been observed that study participants on *ad libitum* ketogenic diets spontaneously restrict their energy intake.[4,5]

In the hypothalamus, signals from several circulating hormones and nutrients are integrated to regulate appetite and energy expenditure.[6] The peripheral modulators of appetite include glucose,[7] free-fatty acids[8] and hormones from the gastrointestinal tract, pancreas and adipose tissue, such as leptin, insulin, ghrelin, cholecystokinin (CCK), glucagon-like peptide 1, peptide YY and pancreatic polypeptide.[9–15]

Following diet-induced weight loss, a number of compensatory changes occur, which encourage weight regain and restoration of energy balance. These include reductions in energy expenditure[16] and circulating leptin,[17] and an increase in the orexigenic hormone ghrelin.[18] It was recently reported that postprandial release of CCK, a hormone which increases satiety, was significantly reduced after diet-induced weight loss.[19] However, when weight-reduced subjects were ketotic due to restriction of dietary carbohydrate, CCK release was maintained at preweight loss concentrations, raising the possibility of an interaction between circulating ketones and hormonal mediators of appetite.

The aim of the present study was to examine whether a number of circulating hormones involved in appetite regulation are altered in the presence of ketosis following diet-induced weight loss.

SUBJECTS AND METHODS

The study was approved by the Austin Health Human Research Ethics Committee, and all subjects provided written informed consent. A detailed description of methods has previously been published.[20] In brief, 50 overweight or obese non-diabetic men and postmenopausal women (mean (± s.d.) age 54.4 ± 10.9 years) undertook a very-low-energy diet (VLED) for 8 weeks, during which all three daily meals were replaced with a VLED formulation (Optifast VLCD, Nestlé Nutrition, Sydney, New South Wales, Australia) and two cups of low-starch vegetables, according to the manufacturer's guidelines, which provided 2.1–2.3 MJ (500–550 kcal) per day. During the subsequent 2 weeks, subjects who lost ⩾10% of their starting weight ($n=39$) were instructed to gradually substitute the VLED meal replacements with regular foods, with dietary recommendations adjusted for individual energy requirements for weight maintenance.

Data collection

Data was collected at baseline (week 0), at week 8 and after the 2 week transition to regular foods (week 10). After an overnight fast, measures of anthropometry were taken with subjects wearing light clothing and barefoot. Bioelectrical impedance was used to measure body weight and composition (Tanita TBF-300, Tanita, Perth, Western Australia, Australia)

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Received 1 October 2012; revised 27 March 2013; accepted 3 April 2013; published online 1 May 2013

using the standard adult mode of measurement. A baseline blood sample was collected, and subjects were asked to rate their appetite using a validated visual analogue scale.[21] A standardized breakfast was provided, which consisted of a boiled egg, toast, margarine, orange juice, cereal biscuits (Weet-Bix; Sanitarium, Berkeley Vale, New South Wales, Australia) and whole milk. This meal contained 2.3 MJ (550 kcal), of which ~51% energy was from carbohydrate, 33% from fat and 16% from protein. Blood samples and VAS ratings of appetite were collected 30, 60, 120, 180 and 240 min thereafter.

Biochemical assays

Blood was collected into prepared tubes, spun in a refrigerated centrifuge, and frozen for later analysis. Plasma for β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and CCK were stored at −80 °C. All other aliquots were stored at −20 °C. Fasting and postprandial plasma acylated ghrelin, active glucagon-like peptide 1, total glucose-dependent insulinotropic polypeptide (GIP), pancreatic polypeptide, amylin and peptide YY concentrations were measured using the human gut hormone panel Lincoplex kit (Millipore, Sydney, New South Wales, Australia), a multiplex assay kit, which uses antibody-immobilised beads to simultaneously quantify peptide hormones. The sensitivity of the assay is 1.8, 5.2, 0.2, 2.4, 3.2 and 8.4 pg/ml respectively, for the hormones as listed above. Intra-assay and inter-assay variation are <11% and <19% respectively. Plasma insulin and leptin were measured by commercial radioimmunoassay (Millipore). CCK was measured in ethanol-extracted plasma using antiserum 92128 (generous donation of Prof Jens Rehfeld, University Hospital, Copenhagen, Denmark) and ^{125}I-Bolton-Hunter-CCK8 label (Perkin Elmer, Melbourne, Victoria, Australia). The antiserum is specific for CCK-amide with negligible cross-reactivity to gastrin-amide or gly-extended forms of gastrin and CCK. NEFA was measured by enzymatic colorimetry (Wako, Osaka, Japan). Glucose was measured by the glucose oxidase method (GM7 Analox glucose analyzer, Helena Laboratories, Melbourne, Victoria, Australia). BHB was measured using a colorimetric assay (Unicel DxC 800 Synchron Clinical System analyzer, Beckman Coulter, Sydney, New South Wales, Australia). Circulating levels of other ketones (acetoacetate and acetone) were not measured, as the increase in ketones during food restriction is predominantly due to BHB.[22]

Statistical analysis

Analyses included the 39 of 50 subjects who completed all three data collection visits. At baseline, data were missing from one subject for the gut hormone multiplex, two subjects for CCK and three subjects for NEFA, due to difficulty obtaining sufficient blood for analysis. Of the remaining data, <2% was missing at random, and was replaced using linear interpolation. Analyses were carried out using R version 2.13.1.[23] Repeated measures ANOVA to compare measurements between study visits was done by fitting a generalized least squares model with an unstructured error covariance. Comparison between weeks was carried out using Wald tests applied to the generalized least squares-fitted model. For data which was not normally distributed, the log or square-root transformation was used, although means and s.e.m. are reported on the original scale. The pairwise comparisons between weeks in Tables 1 and 2 were also adjusted by the Benjamini and Yekutieli method[24] to account for multiple comparisons, and *P*-values which no longer remain significant after adjustment are indicated on the tables. A table of changes in anthropometry, fasting and 4-h area under curve (AUC) of nutrients, hormones and VAS scores between weeks 0– 8, 0–10 and 8–10 showing *P*-values for comparisons between weeks before and after adjustment for multiple comparisons is provided in the Supplementary Data section. Correlations reported are Spearman rank correlations (ρ), and 95% confidence intervals (CI) were calculated using the bootstrap approach. Insulin resistance was estimated by the homoeostasis model of assessment of insulin resistance, using the formula homoeostasis model of assessment of insulin resistance (HOMA-IR) = (fasting glucose (mmol/l) × fasting insulin (mU/l))/22.5.[25] Values are given as means ± s.e.m. unless otherwise specified.

RESULTS

Effect of diet on anthropometric measurements

Measures of anthropometry and blood pressure at baseline, and changes following 8 weeks of VLED and after 2 weeks of reintroduction of food are shown in Table 1.

Eight weeks on a VLED resulted in a mean loss of 13% initial body weight, with significant reductions in adiposity, waist and hip circumferences and blood pressure. There were minor, but statistically significant, changes in anthropometric parameters between weeks 8 and 10.

Ketosis

Fasting BHB increased from 0.07 ± 0.00 to 0.48 ± 0.07 mmol/l after 8 weeks of VLED ($P<0.001$), and fell to 0.19 ± 0.03 mmol/l after 2 weeks of food reintroduction ($P<0.001$, compared with weeks 0 and 8).

Nutrients and hormones during ketosis (week 8) and after refeeding (week 10) in weight-reduced participants

Mean fasting and 4-h postprandial values for glucose, NEFA, ghrelin and amylin at weeks 0, 8 and 10 are depicted in Figure 1. Mean fasting and 4-h AUC values at baseline, and changes from baseline at weeks 8 and 10 for nutrients and hormones are shown in Table 2.

Glucose, insulin. Weight loss led to significant reductions in fasting glucose and insulin, resulting in a significant improvement in insulin resistance, estimated by homoeostasis model of assessment of insulin resistance, from week 0 to 8. There were no significant changes in these measurements between weeks 8 and 10.

Four-h postprandial AUC for insulin fell significantly with weight loss, and was not significantly different between weeks 8 and 10. In contrast, AUC glucose did not change significantly with VLED-induced weight loss, but fell after refeeding (Table 2). There were significant correlations between BHB and AUC glucose at week 8

Table 1. Anthropometric and blood pressure measurements at baseline (mean s.d.), and changes after weight loss (mean ± s.e.m.) when subjects were ketotic (week 8) and after refeeding (non-ketotic, week 10)

Measure	*Week 0*	*Δ Week 0–8 (ketotic)*	*Δ Week 0–10 (non-ketotic)*	*Δ Week 8–10*	P-*value (week 8 versus 10)*
Weight (kg)	96.2 (13.6)	−12.5 ± 0.5‡	−13.0 ± 0.5‡	−0.5 ± 0.1	<0.001
BMI (kg/m^2)	34.7 (3.5)	−4.5 ± 0.1‡	−4.7 ± 0.1‡	−0.2 ± 0.1	<0.001
Waist circumferance (cm)	103.3 (10.6)	−9.9 ± 0.5‡	−10.6 ± 0.5‡	−0.7 ± 0.4	0.07
Hip circumferance (cm)	120.3 (8.0)	−8.1 ± 0.4‡	−8.9 ± 0.4‡	−0.8 ± 0.3	0.002
Fat mass (kg)	49.5 (11.2)	−13.4 ± 0.7‡	−14.6 ± 0.8‡	−1.2 ± 0.3	<0.001
Systolic BP (mmHg)	136.0 (19.8)	−17.6 ± 2.4‡	−13.9 ± 2.4‡	3.7 ± 1.7	0.03[a]
Diastolic BP (mmHg)	82.7 (11.1)	−9.2 ± 1.9‡	−10.0 ± 1.6‡	−0.8 ± 1.8	0.68

Symbols denote significant differences from week 0 (‡$P\leqslant0.001$). Repeated measures ANOVA reported highly significant changes over weeks for all measures (all *P*-value <0.001). [a]Indicates pairwise comparisons, which did not remain significant after adjustment for multiple comparisons.

Table 2. Fasting and 4-h AUC of nutrient, hormone and VAS values at week 0 (mean s.d.), and changes from baseline at weeks 8 and 10 (mean ± s.e.m.)

Measure	*Week 0*	*Δ Week 0–8 (ketotic)*	*Δ Week 0–10 (non-ketotic)*	*Δ Week 8–10*	P-*value (week 8 versus 10)*
Fasting					
Glucose[a]	5.8 (0.9)	−0.6 ± 0.1‡	−0.4 ± 0.1†	0.2 ± 0.1	0.07
Insulin[a]	18.1 (9.8)	−9.0 ± 1.2‡	−8.3 ± 1.2‡	0.7 ± 0.5	0.17
HOMA-IR[a]	4.7 (2.8)	−2.6 ± 0.4‡	−2.4 ± 0.4‡	0.3 ± 0.1	0.08
BHB	0.07 (0.0)	0.43 ± 0.08‡	0.12 ± 0.03‡	−0.3 ± 0.06	<0.001
NEFA[a]	0.5 (0.3)	0.3 ± 0.1‡	0.1 ± 0.05	−0.2 ± 0.05	<0.001
Leptin[a]	33.2 (18.3)	−23.4 ± 2.2‡	−21.1 ± 2.2‡	2.3 ± 0.6	<0.001
Leptin/fat mass[a]	0.66 (0.3)	−0.41 ± 0.04‡	−0.34 ± 0.04‡	0.07 ± 0.02	<0.001
Ghrelin[a]	122.0 (89.2)	4.4 ± 9.1	52.8 ± 9.0‡	49.2 ± 9.5	<0.001
PYY[a]	68.3 (33.0)	−19.7 ± 4.4†	−13.6 ± 4.2	6.4 ± 3.2	0.02[b]
GIP	18.3 (10.5)	−4.1 ± 2.3	−1.3 ± 2.5	2.8 ± 1.8	0.12
GLP-1[a]	40.2 (17.2)	−6.2 ± 2.1†	−5.3 ± 2.5†,[b]	−0.1 ± 2.1	0.88
PP[a]	66.4 (67.5)	−19.2 ± 10.2	5.7 ± 10.5	24.9 ± 8.0	<0.001
Amylin[a]	83.1 (52.1)	−50.5 ± 7.9‡	−34.8 ± 8.2‡	15.5 ± 2.9	<0.001
CCK[a]	1.7 (1.0)	−0.6 ± 0.2‡	−0.5 ± 0.2‡	0.1 ± 0.1	0.19
4-h AUC					
Glucose	1487 (262)	25.8 ± 42	−66 ± 32*,[b]	−90.6 ± 30	0.003
Insulin[a]	12 086 (7728)	−4722 ± 974‡	−4574 ± 899‡	147 ± 422	0.36
NEFA[a]	71.9 (29.1)	40.1 ± 6.8‡	9.4 ± 4.4	−30.7 ± 5.5	<0.001
Ghrelin[a]	23 034 (16703)	1136 ± 2023	9696 ± 1937‡	8877 ± 1743	<0.001
PYY[a]	18 190 (6384)	−2580 ± 665‡	−1771 ± 571†	754 ± 602	0.21
GIP[a]	18 384 (8471)	9011 ± 2004‡	6755 ± 2057†	−2537 ± 1220	0.06
GLP-1	11 471 (4087)	−460 ± 608	−353 ± 560	−130 ± 431	0.97
PP	40 991 (23757)	7358 ± 3404	9384 ± 3775*,[b]	834 ± 3160	0.68
Amylin[a]	35 900 (3340)	−16 880 ± 3049‡	−11 875 ± 3170‡	5027 ± 1113‡	<0.001
CCK	730 (317)	−64 ± 44	−100 ± 47†[b]	−29 ± 31	0.36
Fasting					
Hunger[a]	31.9 (26.3)	2.6 ± 4.7	9.6 ± 4.8*[b]	7.0 ± 3.8	0.03[b]
Full	44.2 (25.9)	−0.5 ± 4.3	−7.2 ± 4.1	−7.2 ± 3.6	0.05
Desire	40.8 (25.1)	−0.9 ± 4.5	4.6 ± 4.5	5.4 ± 3.2	0.09
Prospective[a]	42.8 (18.8)	−0.2 ± 3.1	6.0 ± 2.9*,[b]	6.2 ± 2.6	0.02[b]
Urge[a]	32.7 (23.3)	2.5 ± 3.8	11.1 ± 4.2*,[b]	8.5 ± 3.1	0.006[b]
Preoccupied	36.2 (23.3)	−4.9 ± 4.0	−5.0 ± 4.0	0.0 ± 3.0	1.00
4-h AUC					
Hunger	5181 (2538)	847 ± 499	1463 ± 542*,[b]	616 ± 397	0.10
Full	12 806 (5326)	491 ± 848	783 ± 853	292 ± 534	0.59
Desire[a]	5694 (2902)	796 ± 542	1456 ± 562*,[b]	660 ± 334	0.05
Prospective	7125 (2883)	715 ± 525	992 ± 534	277 ± 257	0.40
Urge	5457 (2922)	831 ± 455	1473 ± 533*,[b]	642 ± 346	0.07
Preoccupied	5144 (3279)	597 ± 524	880 ± 537	283 ± 293	0.42

Abbreviations: AUC, area under curve; BHB, β-hydroxybutyrate; CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic polypeptide; HOMA-IR, homeostasis model of assessment of insulin resistance; NEFA, non-esterified fatty acids; PP, pancreatic polypeptide; PYY, peptide YY. Units are as follows: insulin mU/l; HOMA-IR units; leptin ng/ml; PYY, GIP, GLP-1, PP pg/ml; CCK fmol/ml, VAS millimetres (mm), with possible range 0–100 mm for fasting values. Repeated measures ANOVA reported significant changes over weeks for measures denoted by [a]Symbols denote significant differences from week 0 (‡$P \leqslant 0.001$, †$P \leqslant 0.01$, * $P < 0.05$). [b]Indicates pairwise comparisons, which did not remain significant after adjustment for multiple comparisons.

($P = 0.40$; 95% CI (0.06, 0.67)), and between changes in BHB and AUC glucose from week 8 to 10 ($P = 0.49$; 95% CI (0.20, 0.71)).

NEFA. Four-h postprandial AUC for NEFA was elevated at week 8, but after 2 weeks of refeeding was not significantly different from baseline values (Table 2). There were significant correlations between BHB and AUC NEFA at week 8 ($P = 0.49$; 95% CI (0.18, 0.69)), and between changes in BHB and AUC NEFA from week 8 to 10 ($P = 0.43$; 95% CI (0.11, 0.69)).

Leptin. Fasting leptin fell significantly with weight loss, and increased slightly following reintroduction of food, even when adjusted for fat mass. There were inverse correlations between leptin and BHB at week 8 ($P = -0.44$; 95% CI (-0.68, -0.09)), and between changes in leptin and BHB from weeks 8 to 10 ($P = -0.33$; 95% CI (-0.61, -0.01)).

Gastrointestinal peptides. At week 8, weight-reduced subjects had significantly lower fasting ghrelin, peptide YY, amylin and pancreatic polypeptide, compared with week 10 values. Fasting GIP, glucagon-like peptide 1 and CCK were not different in weight-reduced subjects between weeks 8 and 10 (Table 2).

Four-h AUC values for ghrelin and amylin were significantly lower at week 8 than at week 10 ($P < 0.001$ for both, Figure 1). AUC ghrelin increased significantly between weeks 0 and 8 in participants who did not achieve ketosis (BHB > 0.3 mmol/l) at week 8, but the weight loss induced increase in ghrelin was completely suppressed in subjects who were ketotic. There were significant inverse correlations between BHB and AUC ghrelin at

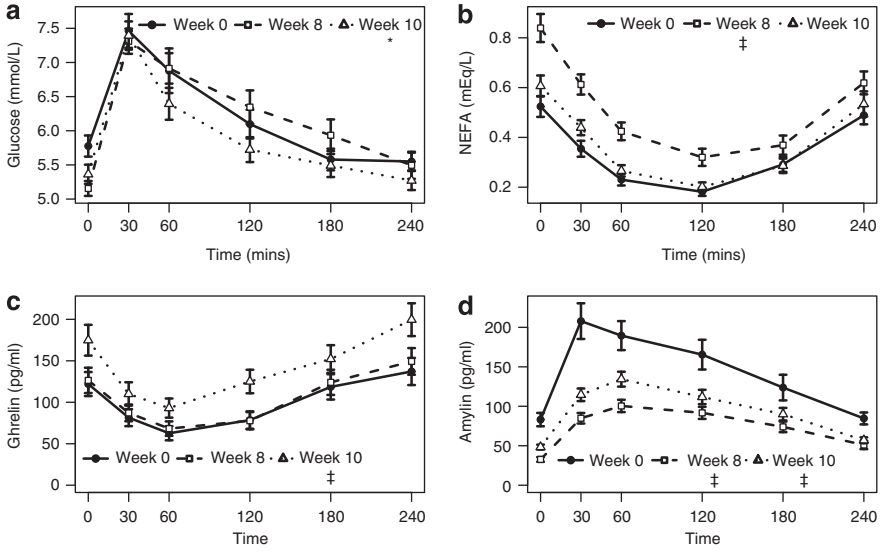

Figure 1. Mean fasting and postprandial glucose (**a**), NEFA (**b**), ghrelin (**c**) and amylin (**d**) at weeks 0, 8 and 10. Symbols indicate significant differences in AUCs compared with week 0 (*$P<0.05$; ‡$P\leqslant0.001$).

week 8 ($P=-0.34$; 95% CI (-0.62, -0.04)), and between changes in BHB and AUCs for ghrelin ($P=-0.48$, 95% CI (-0.70, -0.22)) and amylin ($P=-0.43$, 95% CI (-0.68, -0.12)) from week 8 to 10. AUC GIP tended to be higher at week 8 compared with week 10. AUC CCK was not significantly different from baseline at week 8, but was significantly lower than baseline values at week 10. At week 8, there was a significant correlation between BHB and AUC CCK ($P=0.38$; 95% CI (0.11, 0.61)).

AUCs for peptide YY, glucagon-like peptide 1 and pancreatic polypeptide were not significantly different between weeks 8 and 10.

Appetite during ketosis and after refeeding in weight-reduced participants

Appetite ratings at week 0, and changes from baseline at weeks 8 and 10 are presented in Table 2.

At week 8, fasting and AUC ratings of appetite were unchanged compared with baseline. However, after 2 weeks of refeeding (week 10), fasting scores for hunger, urge to eat and prospective consumption rose significantly, and fullness tended to decrease. At week 10, AUCs for hunger, urge and desire to eat were significantly higher than preweight loss levels ($P=0.02$, 0.02 and 0.04 respectively; all $P>0.05$ after adjustment for multiple comparisons).

DISCUSSION

Although an inhibitory effect of ketosis on appetite is widely assumed, there is little information regarding the effect of ketosis on circulating factors involved in mediating hunger and satiety.

It is well-established that diet-induced weight loss is accompanied by changes in energy expenditure and concentrations of appetite-regulating hormones, in a manner which encourages weight regain and restoration of energy balance.[16–20,26] It has been shown that postprandial release of CCK is maintained at the preweight loss level following an 8-week ketogenic VLED, but reduced when weight-reduced subjects are no longer ketotic.[19] The present study confirms this finding, and uncovers several other factors, which are altered in ketotic weight-reduced subjects. Subjective ratings of appetite were significantly lower when weight-reduced subjects were ketotic than following refeeding.

In mildly ketotic participants, the increase in the circulating concentration of ghrelin, a potent stimulator of appetite, which otherwise occurs as a result of diet-induced weight loss, was suppressed. The present findings are in keeping with a recent report of a 12-week carbohydrate-restricted diet, during which 28 overweight subjects lost ~6.5% of their starting weight without a significant change in fasting plasma ghrelin.[27] In our study, postprandial ghrelin concentrations were also measured, and found to remain unchanged following weight loss as long as subjects were ketotic. After refeeding, fasting and postprandial ghrelin concentrations rose significantly.

Our findings of elevated NEFA after 8 weeks on a low-carbohydrate VLED with return to baseline values after carbohydrate reintroduction are not surprising, as carbohydrate restriction stimulates adipocyte lipolysis and ketogenesis. In rodents, intracerebroventricular administration of a long-chain fatty acid markedly reduced food intake and hypothalamic expression of neuropeptide Y, a potent stimulator of appetite,[8] and peripheral infusion of lipids has been shown to reduce voluntary food intake in humans.[28] It has been hypothesized that fatty acids may provide a signal to the hypothalamus of nutrient abundance,[8] and this may contribute to the appetite-reducing effects of ketogenic low-carbohydrate diets.

The observation that ketosis did not affect fasting glucose, but was associated with elevated postprandial blood glucose concentrations is interesting. As postprandial reductions in blood glucose may increase hunger,[7] (the 'glucostatic theory' of food intake regulation, first proposed by Mayer more than 60 years ago[29]), the effect of ketosis on postprandial glucose may contribute to appetite reduction. Reports of ketogenic low-carbohydrate diets having beneficial effects on insulin sensitivity in humans[30,31] have used the homoeostasis model assessment of insulin resistance or quantitative insulin sensitivity check index (QUICKI), which take into account only fasting glucose and insulin measurements. Conversely, using hyperinsulinemic euglycemic clamps, it was demonstrated that a ketogenic diet reduces the ability of insulin to suppress endogenous glucose production, and impairs insulin-stimulated glucose oxidation.[32] Elevated NEFA may also contribute to insulin resistance.[33]

In rats, intracerebroventricular infusion of BHB reduces food intake and body weight,[34] and there is recent *in vitro* evidence

that BHB may directly reduce central orexigenic signalling.[35] Peripheral injection of BHB also reduces food intake, and this effect is eliminated by transection of the common hepatic branch of the vagus nerve.[36] Of note, this branch primarily contains afferent fibres originating in the proximal small intestine, stomach and pancreas, which have been shown to be sensitive to stimulation by CCK.[37] Ghrelin also conveys its orexigenic signal to the brain via the vagus nerve.[38] The preservation of preweight-loss profiles of ghrelin and CCK release may thereby contribute to the suppressive effect of ketosis on appetite.

It is interesting to note that not all hormones changed in a direction which would contribute to appetite suppression by ketosis. Leptin was lower after 8 weeks on a VLED than following 2 weeks of refeeding, even when adjusted for fat mass. This is consistent with previous reports of a lower plasma leptin during dynamic weight loss than after maintenance of the reduced weight,[39] and is in keeping with the hypothesis that the role of leptin is more as an emergency signal of energy depletion than an inhibitor of food intake.[39] Amylin is cosecreted with insulin by pancreatic β-cells, and reduces food intake directly in the area postrema,[40] and also via mediation of the anorexic effects of other hormones including CCK.[41] GIP, an incretin hormone, appears to promote energy storage.[42] The weight loss induced reduction in amylin and increase in GIP, which would be expected to encourage regain of lost body weight, were somewhat attenuated following refeeding. It is difficult to explain the seemingly contradictory effects of ketosis on different hormones and nutrients involved in appetite regulation. Nonetheless, subjective ratings of appetite were lower when participants were ketotic. It is possible that central sensitivity to the anorexic effect of hormones such as leptin and amylin may be altered by ketosis, or that interaction between various hormones is affected. The relative potency of the multitude of factors involved in appetite regulation is also unknown, and it may be that the increase in hunger following the reduction in ketosis reflects the strength of ghrelin as an orexigenic signal.

It should be noted that although the majority of randomized controlled trials comparing *ad libitum* ketogenic low-carbohydrate diets with low-fat diets have found greater weight loss over 6 months on the ketogenic diets, the difference is no longer observed at 12 months.[1] In one of these studies, urinary ketones were significantly higher in the low-carbohydrate group compared with the low-fat group over the first 12 weeks, but no relationship was found between urinary ketones and weight loss.[43] Only a minority of people have detectable urinary ketones after 3–6 months on low-carbohydrate diets.[5,43]

This study has limitations. Subjects were undergoing active weight loss after 8 weeks on the VLED, compared with relative weight stability after 2 weeks of food reintroduction, which could have influenced the concentration of measured hormones and nutrients. However, compensatory mechanisms aimed at restoring energy balance would be expected to be more pronounced during dynamic weight loss, so this is likely to minimize the predominantly anorexigenic changes detected while subjects were ketotic. There was a small but statistically significant reduction in body weight and adiposity between weeks 8 and 10. This difference, representing a reduction in initial weight of 13.6% (week 10) compared with 13.1% (week 8), is unlikely to be of sufficient magnitude to affect the hormone and appetite results. It has previously been shown that circulating ghrelin increases significantly following a diet-induced loss of 8.5% of initial body weight.[44] Perhaps due to fear of weight regain, it is likely that not all participants consumed the prescribed amount of carbohydrates during the period of food reintroduction, and although BHB concentrations decreased significantly between week 8 and 10, they did not return to baseline values by week 10. This is likely to underestimate the effect of ketosis on the appetite-regulating hormones and nutrients measured. Although we have demonstrated associations between ketones and appetite-regulating hormones, this does not indicate causality. However, it has been shown in mice that infusion of BHB increases circulating CCK and reduces food intake.[45] A similar study with measurement of other appetite-regulating hormones would be informative.

In conclusion, following diet-induced weight loss, the circulating concentrations of several hormones and nutrients which influence appetite were altered when participants were ketotic, compared with after refeeding. The increases in circulating ghrelin and subjective appetite which accompany dietary weight reduction were mitigated when weight-reduced participants were ketotic. Further research is needed to determine the precise mechanism of this effect.

CONFLICT OF INTEREST

JP was chairman of the Optifast medical advisory board at the time the study was conducted. The other authors have no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by NHMRC project grant (508920), Endocrine Society of Australia scholarship (PS), Royal Australasian College of Physicians Shields Research Entry scholarship (PS), and Sir Edward Dunlop Medical Research Foundation (JP). We thank Celestine Bouniu, John Cardinal, Sherrell Cardinal, Christian Rantzau, Rebecca Sgambellone, Sherley Visinoni and Mildred Yim for technical assistance.

REFERENCES

1 Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy Jr. WS, Brehm BJ *et al.* Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med* 2006; **166**: 285–293.
2 Astrup A, Meinert Larsen T, Harper A. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet* 2004; **364**: 897–899.
3 Erlanson-Albertsson C, Mei J. The effect of low carbohydrate on energy metabolism. *Int J Obes (Lond)* 2005; **29**(Suppl 2): S26–S30.
4 Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Ann Intern Med* 2005; **142**: 403–411.
5 Yancy Jr. WS, Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med* 2004; **140**: 769–777.
6 Schwartz MW, Woods SC, Porte Jr. D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661–671.
7 Campfield LA, Smith FJ, Rosenbaum M, Hirsch J. Human eating: evidence for a physiological basis using a modified paradigm. *Neurosci Biobehav Rev* 1996; **20**: 133–137.
8 Obici S, Feng Z, Morgan K, Stein D, Karkanias G, Rossetti L. Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* 2002; **51**: 271–275.
9 Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL *et al.* Gut hormone PYY[sub3-36] physiologically inhibits food intake. *Nature* 2002; **418**: 650–654.
10 Batterham RL, Le Roux CW, Cohen MA, Park AJ, Ellis SM, Patterson M *et al.* Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab* 2003; **88**: 3989–3992.
11 Gutzwiller JP, Goke B, Drewe J, Hildebrand P, Ketterer S, Handschin D *et al.* Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 1999; **44**: 81–86.
12 Moran TH, Kinzig KP. Gastrointestinal satiety signals II. Cholecystokinin. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G183–G188.
13 Rezek M. The role of insulin in the glucostatic control of food intake. *Can J Physiol Pharmacol* 1976; **54**: 650–665.
14 Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG *et al.* Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 5992–5995.
15 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**: 425–432.
16 Leibel RL, Hirsch J. Diminished energy requirements in reduced-obese patients. *Metabolism* 1984; **33**: 164–170.

17 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**: 659–662.
18 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**: 1623–1630.
19 Chearskul S, Delbridge E, Shulkes A, Proietto J, Kriketos A. Effect of weight loss and ketosis on postprandial cholecystokinin and free fatty acid concentrations. *Am J Clin Nutr* 2008; **87**: 1238–1246.
20 Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A *et al.* Long-term persistence of hormonal adaptations to weight loss. *New Engl J Med* 2011; **365**: 1597–1604.
21 Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M *et al.* The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 2000; **84**: 405–415.
22 Hall SE, Wastney ME, Bolton TM, Braaten JT, Berman M. Ketone body kinetics in humans: the effects of insulin-dependent diabetes, obesity and starvation. *J Lipid Res* 1984; **25**: 1184–1194.
23 R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, 2011.
24 Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Statist* 2001; **29**: 1165–1188.
25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homoeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
26 Pfluger PT, Kampe J, Castaneda TR, Vahl T, D'Alessio DA, Kruthaupt T *et al.* Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. *J Clin Endocrinol Metab* 2007; **92**: 583–588.
27 Ratliff J, Mutungi G, Puglisi MJ, Volek JS, Fernandez ML. Carbohydrate restriction (with or without additional dietary cholesterol provided by eggs) reduces insulin resistance and plasma leptin without modifying appetite hormones in adult men. *Nutr Res* 2009; **29**: 262–268.
28 Gil KM, Skeie B, Kvetan V, Askanazi J, Friedman MI. Parenteral nutrition and oral intake: effect of glucose and fat infusions. *J Parenter Enteral Nutr* 1991; **15**: 426–432.
29 Mayer J. Glucostatic mechanism of regulation of food intake. *N Engl J Med* 1953; **249**: 13–16.
30 Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J *et al.* A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003; **348**: 2074–2081.
31 Volek JS, Sharman MJ, Gomez AL, DiPasquale C, Roti M, Pumerantz A *et al.* Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *J Am Coll Nutr* 2004; **23**: 177–184.
32 Bisschop PH, de Metz J, Ackermans MT, Endert E, Pijl H, Kuipers F *et al.* Dietary fat content alters insulin-mediated glucose metabolism in healthy men. *Am J Clin Nutr* 2001; **73**: 554–559.
33 Liu J, Jahn LA, Fowler DE, Barrett EJ, Cao W, Liu Z. Free fatty acids induce insulin resistance in both cardiac and skeletal muscle microvasculature in humans. *J Clin Endocrinol Metab* 2011; **96**: 438–446.
34 Arase K, Fisler JS, Shargill NS, York DA, Bray GA. Intracerebroventricular infusions of 3-OHB and insulin in a rat model of dietary obesity. *Am J Physiol* 1988; **255**: R974–R981.
35 Laeger T, Pohland R, Metges CC, Kuhla B. The ketone body beta-hydroxybutyric acid influences agouti-related peptide expression via AMP-activated protein kinase in hypothalamic GT1-7 cells. *J Endocrinol* 2012; **213**: 193–203.
36 Langhans W, Egli G, Scharrer E. Selective hepatic vagotomy eliminates the hypophagic effect of different metabolites. *J Auton Nerv Syst* 1985; **13**: 255–262.
37 Horn CC, Friedman MI. Separation of hepatic and gastrointestinal signals from the common 'hepatic' branch of the vagus. *Am J Physiol Regul Integr Comp Physiol* 2004; **287**: R120–R126.
38 Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H *et al.* The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120–1128.
39 Rosenbaum M, Nicolson M, Hirsch J, Murphy E, Chu F, Leibel RL. Effects of weight change on plasma leptin concentrations and energy expenditure. *J Clin Endocrinol Metab* 1997; **82**: 3647–3654.
40 Riediger T, Schmid HA, Lutz T, Simon E. Amylin potently activates AP neurons possibly via formation of the excitatory second messenger cGMP. *Am J Physiol Regul Integr Comp Physiol* 2001; **281**: R1833–R1843.
41 Lutz TA, Tschudy S, Rushing PA, Scharrer E. Attenuation of the anorectic effects of cholecystokinin and bombesin by the specific amylin antagonist AC 253. *Physiol Behav* 2000; **70**: 533–536.
42 Hauner H, Glatting G, Kaminska D, Pfeiffer EF. Effects of gastric inhibitory polypeptide on glucose and lipid metabolism of isolated rat adipocytes. *Ann Nutr Metab* 1988; **32**: 282–288.
43 Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS *et al.* A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003; **348**: 2082–2090.
44 Garcia JM, Iyer D, Poston WSC, Marcelli M, Reeves R, Foreyt J *et al.* Rise of plasma ghrelin with weight loss is not sustained during weight maintenance. *Obesity (Silver Spring)* 2006; **14**: 1716–1723.
45 Visinoni S, Khalid NFI, Joannides CN, Shulkes A, Yim M, Whitehead J *et al.* The role of liver fructose-1,6-bisphosphatase in regulating appetite and adiposity. *Diabetes* 2012; **61**: 1122–1132.

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