Ketosis and appetite-mediating nutrients and hormones after weight loss

Priya Sumithran M.B., B.S., Ph.D, Luke A. Prendergast Ph.D., Elizabeth Delbridge Ph.D.,
Katrina Purcell B.Sc. (Hons), Arthur Shulkes Sc.D., Adamandia Kriketos Ph.D., Joseph
Proietto M.B., B.S., Ph.D.

From the University of Melbourne, Department of Medicine (P.S., L.A.P., E.D., K.P., A.K.,
J.P.), and Department of Surgery (A.S.) (Austin Health), and La Trobe University,
Department of Mathematics and Statistics (L.A.P.), Victoria, Australia

Running title: Ketosis and appetite after weight loss

Author for correspondence and reprint requests:

Professor Joseph Proietto
Department of Medicine, Level 2, Boronia Building
Heidelberg Repatriation Hospital
300 Waterdale Rd
Heidelberg, Vic. 3081
Australia
phone: +61 3 9496 2250
fax: +61 3 9497 4554
email: j.proietto@unimelb.edu.au

ClinicalTrials.gov Identifier: NCT00870259.
ABSTRACT

Background: 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.

Objective: To examine the effect of ketosis on a number of circulating factors involved in appetite regulation, following diet-induced weight loss.

Subjects and 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 ± SEM) 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, NEFA, and cholecystokinin 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 increases in circulating ghrelin and subjective appetite which accompany dietary weight reduction were mitigated when weight-reduced participants were ketotic.

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. While 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 (GLP-1), peptide YY (PYY), and pancreatic polypeptide (PP) (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 pre-weight 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 post-menopausal women (mean ± SD age 54.4 ± 10.9 years) undertook a very low energy diet (VLED) for 8 weeks, during which all 3 daily meals were replaced with a VLED formulation (Optifast® VLCD, Nestlé Nutrition, Sydney, Australia) and 2 cups of low-starch vegetables, according to the manufacturer’s guidelines, which provided 2.1 to 2.3 MJ (500 to 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, Australia) 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 standardised breakfast was provided, which consisted of a boiled egg, toast, margarine, orange juice, cereal biscuits.
(Weet-Bix; Sanitarium, Berkeley Vale, Australia) and whole milk. This meal contained 2.3 MJ (550 kcal), of which approximately 51% energy was from carbohydrate, 33% fat and 16% protein. Blood samples and VAS ratings of appetite were collected 30, 60, 120, 180 and 240 minutes 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 post-prandial plasma acylated ghrelin, active GLP-1, total GIP, PP, amylin and PYY concentrations were measured using the human gut hormone panel Lincoplex kit (Millipore), 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, Sydney, Australia). CCK was measured in ethanol-extracted plasma using antiserum 92128 (generous donation of Prof Jens Rehfeld, University Hospital, Copenhagen, Denmark) and 125I-Bolton-Hunter-CCK8 label (Perkin Elmer, Melbourne, 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 analyser, Helena Laboratories, Melbourne, Australia). BHB was measured using a colorimetric assay (Unicel DxC 800 Synchron Clinical System analyser, Beckman Coulter, Sydney, 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 (21).

**Statistical analysis**

Analyses included the 39 of 50 subjects who completed all 3 data collection visits. At baseline, data were missing from one subject for the gut hormone multiplex, two subjects for CCK and 3 subjects for NEFA, due to difficulty obtaining sufficient blood for analysis. Of the remaining data, less than 2% was missing at random, and was replaced using linear interpolation. Analyses were carried out using R version 2.13.1 (22). Repeated measures ANOVA to compare measurements between study visits was done by fitting a generalized least squares (gls) model with an unstructured error covariance. Comparison between weeks was carried out using Wald tests applied to the gls fitted model. For data which was not normally distributed, the log or square-root transformation was used, although means and SEMs 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 (23) 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-hour 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 were calculated using the bootstrap approach. Insulin resistance was estimated by the homeostasis model of assessment (HOMA-IR), using the formula HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (mU/L)]/22.5 (24). Values are given as means ± SEM 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 $0.07 \pm 0.00$ to $0.48 \pm 0.07$ mmol/L after 8 weeks of VLED ($p<0.001$), and fell to $0.19 \pm 0.03$ 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-hour post-prandial values for glucose, NEFA, ghrelin and amylin at weeks 0, 8 and 10 are depicted in Figure 1. Mean fasting and 4-hour area under the curve (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 HOMA-IR, from week 0 to 8. There were no significant changes in these measurements between weeks 8 and 10.

4-hour post-prandial area under the curve (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 ($\rho=0.40$; 95% CI [0.06, 0.67]), and between changes in BHB and AUC glucose from week 8 to 10 ($\rho=0.49$; 95% CI [0.20, 0.71]).

NEFA

4-hour post-prandial 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 ($\rho=0.49$; 95% CI [0.18, 0.69]), and between changes in BHB and AUC NEFA from week 8 to 10 ($\rho=0.43$; 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 ($\rho = -0.44; 95\% \text{ CI } [-0.68, -0.09]$), and between changes in leptin and BHB from weeks 8 to 10 ($\rho = -0.33; 95\% \text{ CI } [-0.61, -0.01]$).

188

Gastrointestinal peptides

At week 8, weight-reduced subjects had significantly lower fasting ghrelin, PYY, amylin and PP, compared with week 10 values. Fasting GIP, GLP-1 and CCK were not different in weight-reduced subjects between weeks 8 and 10 (Table 2).

190

4-hour 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.

194

There were significant inverse correlations between BHB and AUC ghrelin at week 8 ($\rho = -0.34; 95\% \text{ CI } [-0.62, -0.04]$), and between changes in BHB and AUCs for ghrelin ($\rho = -0.48, 95\% \text{ CI } [-0.70, -0.22]$) and amylin ($\rho = -0.43, 95\% \text{ CI } [-0.68, -0.12]$) 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 ($\rho = 0.38; 95\% \text{ CI } [0.11, 0.61]$).

200

AUCs for PYY, GLP-1 and PP were not significantly different between weeks 8 and 10.

202

Appetite during ketosis and after refeeding in weight-reduced participants

204
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 to 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 pre-weight 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, 25). It has been shown that post-prandial release of CCK is maintained at the pre-weight 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
The present findings are in keeping with a recent report of a 12-week carbohydrate-restricted diet, during which 28 overweight subjects lost approximately 6.5% of their starting weight without a significant change in fasting plasma ghrelin (26). In our study, post-prandial ghrelin concentrations were also measured, and found to remain unchanged following weight loss as long as subjects were ketotic. After refeeding, fasting and post-prandial 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 (27). It has been hypothesised 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 post-prandial blood glucose concentrations is interesting. Since post-prandial reductions in blood glucose may increase hunger, (7), (the “glucostatic theory” of food intake regulation, first proposed by Mayer more than 60 years ago (28)), the effect of ketosis on post-prandial glucose may contribute to appetite reduction. Reports of ketogenic low carbohydrate diets having beneficial effects on insulin sensitivity in humans (29, 30) have used the homeostasis model assessment of insulin resistance (HOMA-IR) or quantitative insulin sensitivity check index (QUICKI), which take into account only fasting glucose and insulin measurements. Conversely, using hyperinsulinaemic euglycaemic clamps, it was demonstrated that a
ketogenic diet reduces the ability of insulin to suppress endogenous glucose production, and
impairs insulin-stimulated glucose oxidation (31). Elevated NEFA may also contribute to
insulin resistance (32).

In rats, intracerebroventricular infusion of β-hydroxybutyrate reduces food intake and body
weight (33), and there is recent in vitro evidence that BHB may directly reduce central
orexigenic signalling (34). Peripheral injection of BHB also reduces food intake, and this
effect is eliminated by transection of the common hepatic branch of the vagus nerve (35). 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 (36).
Ghrelin also conveys its orexigenic signal to the brain via the vagus nerve (37). The
preservation of pre-weight-loss profiles of ghrelin and CCK release may thereby contribute
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 (38), 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 (38). Amylin is co-
secreted with insulin by pancreatic β-cells, and reduces food intake directly in the area
postrema (39), and also via mediation of the anorexic effects of other hormones including
CCK (40). GIP, an incretin hormone, appears to promote energy storage (41). 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 randomised 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 (42). Only a minority of people have detectable urinary ketones after 3-6 months on low-carbohydrate diets (5, 42).

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 minimise 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 (43). 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 β-hydroxybutyrate 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 (44). 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.

Supplementary information is available at EJCN's website

ACKNOWLEDGEMENTS

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

JP was chairman of the Optifast medical advisory board at the time the study was conducted.

The other authors have no conflicts of interest.
REFERENCES


36. 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**: R120-126.


<table>
<thead>
<tr>
<th>Measure</th>
<th>Week 0</th>
<th>Δ week 0-8 (ketotic)</th>
<th>Δ week 0-10 (non-ketotic)</th>
<th>Δ week 8-10</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>96.2 (13.6)</td>
<td>-12.5 ± 0.5‡</td>
<td>-13.0 ± 0.5‡</td>
<td>-0.5 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.7 (3.5)</td>
<td>-4.5 ± 0.1‡</td>
<td>-4.7 ± 0.1‡</td>
<td>-0.2 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circ. (cm)</td>
<td>103.3 (10.6)</td>
<td>-9.9 ± 0.5‡</td>
<td>-10.6 ± 0.5‡</td>
<td>-0.7 ± 0.4</td>
<td>0.07</td>
</tr>
<tr>
<td>Hip circ. (cm)</td>
<td>120.3 (8.0)</td>
<td>-8.1 ± 0.4‡</td>
<td>-8.9 ± 0.4‡</td>
<td>-0.8 ± 0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>49.5 (11.2)</td>
<td>-13.4 ± 0.7‡</td>
<td>-14.6 ± 0.8‡</td>
<td>-1.2 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>136.0 (19.8)</td>
<td>-17.6 ± 2.4‡</td>
<td>-13.9 ± 2.4‡</td>
<td>3.7 ± 1.7</td>
<td>0.03#</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82.7 (11.1)</td>
<td>-9.2 ± 1.9‡</td>
<td>-10.0 ± 1.6‡</td>
<td>-0.8 ± 1.8</td>
<td>0.68</td>
</tr>
</tbody>
</table>

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

Symbols denote significant differences from week 0 (‡p≤0.001). Repeated measures ANOVA reported highly significant changes over weeks for all measures (all p-value < 0.001). # indicates pairwise comparisons which did not remain significant after adjustment for multiple comparisons.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Week 0</th>
<th>Δ week 0-8 (ketotic)</th>
<th>Δ week 0-10 (non-ketotic)</th>
<th>Δ week 8-10</th>
<th>p-value wk 8 vs. 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose§</td>
<td>5.8 (0.9)</td>
<td>-0.6 ± 0.1‡</td>
<td>-0.4 ± 0.1†</td>
<td>0.2 ± 0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Insulin§</td>
<td>18.1 (9.8)</td>
<td>-9.0 ± 1.2‡</td>
<td>-8.3 ± 1.2‡</td>
<td>0.7 ± 0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>HOMA-IR§</td>
<td>4.7 (2.8)</td>
<td>-2.6 ± 0.4‡</td>
<td>-2.4 ± 0.4‡</td>
<td>0.3 ± 0.1</td>
<td>0.08</td>
</tr>
<tr>
<td>BHB</td>
<td>0.07 (0.0)</td>
<td>0.43 ± 0.08‡</td>
<td>0.12 ± 0.03‡</td>
<td>-0.3 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NEFA§</td>
<td>0.5 (0.3)</td>
<td>0.3 ± 0.1‡</td>
<td>0.1 ± 0.05</td>
<td>-0.2 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin§</td>
<td>33.2 (18.3)</td>
<td>-23.4 ± 2.2‡</td>
<td>-21.1 ± 2.2‡</td>
<td>2.3 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin/Fat mass§</td>
<td>0.66 (0.3)</td>
<td>-0.41 ± 0.04‡</td>
<td>-0.34 ± 0.04‡</td>
<td>0.07 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ghrelin§</td>
<td>122.0 (89.2)</td>
<td>4.4 ± 9.1</td>
<td>52.8 ± 9.0‡</td>
<td>49.2 ± 9.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PYY§</td>
<td>68.3 (33.0)</td>
<td>-19.7 ± 4.4†</td>
<td>-13.6 ± 4.2</td>
<td>6.4 ± 3.2</td>
<td>0.02#</td>
</tr>
<tr>
<td>GIP</td>
<td>18.3 (10.5)</td>
<td>-4.1 ± 2.3</td>
<td>-1.3 ± 2.5</td>
<td>2.8 ± 1.8</td>
<td>0.12</td>
</tr>
<tr>
<td>GLP-1§</td>
<td>40.2 (17.2)</td>
<td>-6.2 ± 2.1†</td>
<td>-5.3 ± 2.5†#</td>
<td>-0.1 ± 2.1</td>
<td>0.88</td>
</tr>
<tr>
<td>PP§</td>
<td>66.4 (67.5)</td>
<td>-19.2 ± 10.2</td>
<td>5.7 ± 10.5</td>
<td>24.9 ± 8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amylin§</td>
<td>83.1 (52.1)</td>
<td>-50.5 ± 7.9‡</td>
<td>-34.8 ± 8.2‡</td>
<td>15.5 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCK§</td>
<td>1.7 (1.0)</td>
<td>-0.6 ± 0.2‡</td>
<td>-0.5 ± 0.2‡</td>
<td>0.1 ± 0.1</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>4-hour AUC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1487 (262)</td>
<td>25.8 ± 42</td>
<td>-66 ± 32*#</td>
<td>-90.6 ± 30</td>
<td>0.003</td>
</tr>
<tr>
<td>Insulin§</td>
<td>12086 (7728)</td>
<td>-4722 ± 974‡</td>
<td>-4574 ± 899‡</td>
<td>147 ± 422</td>
<td>0.36</td>
</tr>
<tr>
<td>NEFA§</td>
<td>71.9 (29.1)</td>
<td>40.1 ± 6.8‡</td>
<td>9.4 ± 4.4</td>
<td>-30.7 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ghrelin§</td>
<td>23034 (16703)</td>
<td>1136 ± 2023</td>
<td>9696 ± 1937‡</td>
<td>8877 ± 1743</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PYY§</td>
<td>18190 (6384)</td>
<td>-2580 ± 665‡</td>
<td>-1771 ± 571†</td>
<td>754 ± 602</td>
<td>0.21</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Mean (SD)</td>
<td>Change from Baseline (Mean ± SEM)</td>
<td>P Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>----------------------------------</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIP§</td>
<td>18384 (8471)</td>
<td>9011 ± 2004‡ 6755 ± 2057† -2537 ± 1220</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP1</td>
<td>11471 (4087)</td>
<td>-460 ± 608 -353 ± 560 -130 ± 431</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>40991 (23757)</td>
<td>7358 ± 3404 3775*#</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylin§</td>
<td>35900 (3340)</td>
<td>3049‡ 3170‡</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK</td>
<td>730 (317)</td>
<td>-64 ± 44 -100 ± 47†# -29 ± 31</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Fasting

| Hunger§ | 31.9 (26.3) | 2.6 ± 4.7 9.6 ± 4.8*# 7.0 ± 3.8 | 0.03#   |
| 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§ | 42.8 (18.8) | -0.2 ± 3.1 6.0 ± 2.9*# 6.2 ± 2.6 | 0.02#   |
| Urge§   | 32.7 (23.3) | 2.5 ± 3.8 11.1 ± 4.2*# 8.5 ± 3.1 | 0.006#  |
| Preoccupied | 36.2 (23.3) | -4.9 ± 4.0 -5.0 ± 4.0 0.0 ± 3.0 | 1.00    |

### 4-hour AUC

| Hunger | 5181 (2538) | 847 ± 499 1463 ± 542*# 616 ± 397 | 0.10    |
| Full   | 12806 (5326) | 491 ± 848 783 ± 853 292 ± 534 | 0.59    |
| Desire§ | 5694 (2902) | 796 ± 542 1456 ± 562*# 660 ± 334 | 0.05    |
| Prospective | 7125 (2883) | 715 ± 525 992 ± 534 277 ± 257 | 0.40    |
| Urge   | 5457 (2922) | 831 ± 455 1473 ± 533*# 642 ± 346 | 0.07    |
| Preoccupied | 5144 (3279) | 597 ± 524 880 ± 537 283 ± 293 | 0.42    |

Table 2: Fasting and 4-hour area under curve (AUC) of nutrient, hormone and VAS values at week 0 (mean (SD)), and changes from baseline at weeks 8 and 10 (mean ± SEM). 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 to 100mm for fasting values. Repeated measures ANOVA reported.
significant changes over weeks for measures denoted by §. Symbols denote significant differences from week 0 (‡ p≤0.001, † p≤0.01, * p<0.05). # indicates pairwise comparisons which did not remain significant after adjustment for multiple comparisons.
Figure 1: Mean fasting and post-prandial glucose, NEFA, ghrelin and amylin at weeks 0, 8 and 10.

Symbols indicate significant differences in AUCs compared with week 0 (*p<0.05; ‡p<0.001).
Author/s: Sumithran, P; Prendergast, LA; Delbridge, E; Purcell, K; Shulkes, A; Kriketos, A; Proietto, J

Title: Ketosis and appetite-mediating nutrients and hormones after weight loss

Date: 2013-07-01

Citation: Sumithran, P; Prendergast, LA; Delbridge, E; Purcell, K; Shulkes, A; Kriketos, A; Proietto, J, Ketosis and appetite-mediating nutrients and hormones after weight loss, EUROPEAN JOURNAL OF CLINICAL NUTRITION, 2013, 67 (7), pp. 759 - 764

Persistent Link: http://hdl.handle.net/11343/123873

File Description: Accepted version