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ORIGINAL ARTICLE

Effect of high-fat meals and fatty acid saturation on postprandial levels of the hormones ghrelin and leptin in healthy men

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Objective: Ghrelin and leptin play a role in control of food intake and adiposity but mechanisms regulating these hormones in man are poorly defined and evidence that dietary fats may have adverse effects is inconclusive. We investigated whether high-fat meals, which differed in saturated fatty acid (SFA) content acutely modified these hormones.
Design: Randomised, double-blind, crossover trial. A high-fat (HF) test meal (59 ± 4 g fat; 71% of energy as fat) was given for breakfast on two occasions. Meals comprised either high ($\sim$70:30) or low ($\sim$55:45) saturated:unsaturated fatty acid (SFA:USFA) ratio. Fasting and postprandial measurements of serum total ghrelin (RIA), leptin (enzyme-linked immunosorbent assay (ELISA)) and insulin (RIA) were made over 6 h. Postprandial measurements were also made at 10 and 24 h following a fat-exclusion lunch, snack and dinner.
Subjects: A total of 18 lean, healthy men.
Results: There was no significant effect of the fatty meal (time, $P>0.05$), nor a differential effect of SFA:USFA ratio (treatment*time, $P>0.05$) on ghrelin over 6 h. Leptin decreased in response to both HF treatments (time, $P<0.001$) but increased SFA content did not further inhibit hormone secretion (treatment*time, $P>0.05$). There was no significant correlation between ghrelin or leptin and circulating insulin ($P>0.05$).
Conclusion: We conclude that HF diets may adversely effect serum leptin, although the circadian decrease may account in part for this response. Increasing dietary SFAs had no deleterious effects on leptin or total ghrelin.
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Keywords: postprandial; high-fat; saturated fatty acids; leptin; ghrelin; lean men

Introduction

The hormones ghrelin and leptin both appear to play a role in the regulation of food intake and adiposity. Ghrelin stimulates hunger and promotes food ingestion, whereas leptin may increase satiety and reduce food consumption, at least in animal models, yet the mechanisms which regulate these hormones in man remain poorly defined.

Ghrelin, produced primarily by the stomach, stimulates food intake by increasing hunger (Cummings *et al*., 2001; Wren *et al*., 2001). Fasting increases endogenous levels (Ariyasu *et al*., 2001; Cummings *et al*., 2001), as does diet-induced weight loss (Cummings *et al*., 2002) and circulating levels are lower in the obese (Shiiya *et al*., 2002). There is little response of ghrelin to changes in gastric distension

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which suggests energy load and nutrient composition rather than volume of food may be the important signalling mechanisms (Shiiya *et al*., 2002; Callahan *et al*., 2004). However, evidence that nutrient intake acutely decreases ghrelin (Tschop *et al*., 2001; Shiiya *et al*., 2002) and reduces hunger has recently been questioned since the postprandial decline induced by high-carbohydrate (CHO) feeding (Tschop *et al*., 2001; Shiiya *et al*., 2002; Monteleone *et al*., 2003; Greenman *et al*., 2004) may not be replicated when intake is high in protein (Erdmann *et al*., 2003; Knerr *et al*., 2003; Greenman *et al*., 2004) or lipid (Monteleone *et al*., 2003; Erdmann *et al*., 2004). A recent study has also shown a potentially adverse increase in endogenous ghrelin circulation following a fatty meal (Erdmann *et al*., 2004).

Leptin is secreted predominantly by adipose tissue (Friedman and Halaas, 1998), is proportional to body fat content (Havel *et al*., 1996) and may have an antagonistic role to ghrelin (Knerr *et al*., 2003). Weight gain increases (Kolaczynski *et al*., 1996a; Levine *et al*., 1999) and weight loss decreases (Havel *et al*., 1996; Weigle *et al*., 1997; Levine *et al*., 1999) plasma leptin, but variability between individuals with comparable adipose mass suggests factors other than adipocyte size and lipid content influence leptin production. Hypotheses that rate of change and not total content of adipocyte lipid were critical (Coleman and Herrmann, 1999) led to trials acutely modulating fat balance but the results were disappointing (Murgatroyd *et al*., 2003). Fasting (Boden *et al*., 1996; Kolaczynski *et al*., 1996a; Weigle *et al*., 1997) and exercise (Hilton, 2000) cause an acute drop, and overfeeding a rise (Kolaczynski *et al*., 1996a) in leptin. Whether high-fat (HF) diets associated with obesity may modulate leptin is less clear, failing to alter fasting leptin during energy balance (Schrauwen *et al*., 1997) and weight stability (Weigle *et al*., 1997), or during overfeeding (Dirlewanger *et al*., 2000). The postprandial response to HF feeding set against a diurnal change in circulating leptin is even more variable and circulating levels may increase (Dallongeville *et al*., 1998; Imbeault *et al*., 2001), decrease (Havel *et al*., 1999; Romon *et al*., 1999; Evans *et al*., 2001; Imbeault *et al*., 2001; Koutsari *et al*., 2003), or not change (Weigle *et al*., 1997; Guerci *et al*., 2000; Monteleone *et al*., 2003; Tentolouris *et al*., 2003) following a fatty meal. Changes in both hormones in response to dietary fat are commonly less pronounced than for CHO (Havel *et al*., 1999; Romon *et al*., 1999; Monteleone *et al*., 2003; Greenman *et al*., 2004) and it has been suggested that insulin may play a critical role in regulation (Evans *et al*., 2001; Mohlig *et al*., 2002; Saad *et al*., 2002; Flanagan *et al*., 2003; Koutsari *et al*., 2003; Fogteloo *et al*., 2004).

In this study, we wanted to investigate whether a HF meal induced postprandial changes in these peptides and also, since meals rich in saturated fats are known to reduce postprandial insulin sensitivity (Robertson *et al*., 2002), whether the composition of dietary fat further modulated the hormonal response to eating.

Methods

Subjects

18 lean healthy young men were recruited into this trial. All subjects provided written informed consent to participate and ethics approval for this study was obtained from the Auckland Ethics Committees, Auckland, New Zealand.

Protocol

This was a double blind, randomised intervention trial where subjects spent 24 h on two separate occasions within the Human Nutrition Unit metabolic facility at the University of Auckland, New Zealand. Participants were given a HF test meal, containing 71% of energy as fat (71 en%), upon arrival and blood samples were collected over 24 h. The test meals contained butter fat with a high or low saturated:unsaturated fatty acid (SFA:USFA) ratio (Table 1). The major alterations in the test lipids were a decrease in palmitic acid (16:0) and an increase in oleic (18:1) and linoleic (18:2) acid in the $low_{SFA:USFA}$ treatment group. Details of industrial preparation of this modified dairy fat can be found in a previous publication in which other aspects of this trial were previously reported (Poppitt *et al*., 2004).

Participants were randomised such that half of the subjects were allocated $high_{SFA:USFA}$ treatment and half $low_{SFA:USFA}$ treatment during their first 24 h intervention period. All subjects then crossed over on to the other treatment arm for the second period. Treatments were separated by a minimum 3 day washout period during which the subjects returned home and were requested to maintain their usual dietary and exercise habits. During intervention periods the men were confined to the nutrition facility at the University of Auckland and were provided with all meals and snacks. Subjects were maintained in energy balance over 24 h based upon an estimate of basal metabolic rate (BMR) and minimal physical activity during a sedentary day, calculated as

Table 1 Composition of dietary lipids in the test meals showing the major fatty acid constituents

	$High_{SFA:USFA}$	$Low_{SFA:USFA}$
Total SFA (% fat)	70.5	54.4
Lauric C12:0	3.8	2.7
Myristic C14:0	12.0	8.3
Palmitic C16:0	31.5	18.8
Stearic C18:0	10.1	13.4
Total MUFA (% fat)	22.1	32.0
$C18:1_{total}$	18.6	30.0
$C18:1_{trans}$	4.3	4.7
Total PUFA (% fat)	3.0	10.5
Linoleic C18:2	1.2	7.2
α-Linolenic C18:3	0.8	2.3
Cholesterol mg/100 g butter	222	191

SFA, saturated fatty acid; USFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; minor peaks not shown; % fat, percentage of total fat.

1.4 × BMR. They arrived fasted at the nutrition unit at 0730 h, an indwelling venous cannula was inserted and a baseline blood sample collected. At 0800 the HF breakfast was served. Subjects were required to consume the breakfast within 15 min. Blood samples were collected at 1, 3, 6 and 10 h. A fat-exclusion lunch was given following the 6 h blood sample, a fat-exclusion afternoon snack at 8 h and a fat-exclusion dinner following the 10 h blood sample. Subjects remained within the facility overnight and the final fasted blood sample was collected at 24 h.

Test meals

The two dairy lipids were cooked into blueberry muffins and given with a milk- and sugar-free decaffeinated hot beverage and/or glass of cold water as the HF breakfast test meal. Lipid content of the breakfast was scaled to body size based upon total daily energy intake of each subject. Each muffin contained 5.3 g butter fat per MJ of daily intake. The average total daily intake for the 18 men was 11.2 ± 0.2 (s.d.) MJ/day, the average energy content of the HF breakfast was 3.13 ± 1.9 (s.d.) MJ and the average butter fat in the test meal was 59 ± 4 (s.d.) g, equivalent to approximately 73 g of dairy butter. Macronutrient composition of the HF breakfast was 70.8 en% fat, 23.2 en% CHO and 4.9 en% protein. The fat-exclusion lunch comprised vegetarian pasta, bread roll, orange juice (3.1 g fat, 3.4en% fat, 78.8 en% CHO, 14.2 en% protein), the fat-exclusion snack comprised fruit cake and apple juice (0.7 g fat, 1.8 en% fat, 90.6 en% CHO, 7.2 en% protein), the fat-exclusion dinner comprised vegetarian risotto, raspberry dessert, carbonated beverage (2.4 g fat, 2.8 en% fat, 85.1 en% CHO, 9.9 en% protein). These meals and snacks were designed to be identical on both treatment arms.

Analytical methods

Blood samples were centrifuged and serum stored at −80°C until later batch analyses of ghrelin, leptin and insulin. Total ghrelin concentrations were measured by radioimmunoassay (RIA) using a Linco Research commercial kit (Saint Charles, MI, USA), using ^{125}I-labelled ghrelin as the tracer. Primary antibody was rabbit antighrelin, secondary antibody was goat anti-rabbit IgG and precipitation was achieved using polyethylene glycol in a phosphate and Triton-X100 buffer. In all, 100 μl of sample and standards were incubated with primary antibody at 4°C overnight. After 24 h 100 μl of tracer was added and incubated overnight at 4°C. After 48 h 1.0 ml of precipitating reagent was added, tubes incubated at room temperature for 20 min then centrifuged at 1700 g for 20 min at 4°C. The pellets were counted on a Wallac 1480 Wizard gamma counter (Wallac Finland Oy, Turku, Finland). Linco Research reagents were prepared and stored according to the manufacturer's instructions. Curve fitting and sample concentration were computed using the MultiCalc software supplied with the counter. Coefficient of variation for this assay across the full standards range was 3.3%. The lowest level of detection was 0.311 ng/ml. Serum leptin was measured by enzyme-linked immunosorbent assay (ELISA) using a Duoset commercial kit (R & D System, Minneapolis, USA). 100 μl of sample was applied to 96-well microtitre plates and incubated with the individual coating antibodies for 2 h at room temperature. After washing with PBS, the detection antibodies were applied for another 2 h at room temperature. The bound immune-complexes were detected at 450 nm. The standard curve was generated for every set of samples assayed using the standards provided in the kit. Serum insulin concentrations were measured by RIA using a Peninsula Laboratories commercial kit (Belmont, USA). Polyclonal antibodies from guniea-pig antiserum raised against bovine insulin were incubated with the human insulin standard Actrapid (Novo Nordisk A/S, Bagsværd, Denmark) and unknowns. Iodinated ^{125}I tracer was added, samples centrifuged and the bound fraction counted on a Wallac 1480 Wizard 3 gamma counter (Wallac Finland Oy, Turku, Finland). Peninsula Laboratories reagents were prepared and stored according to the manufacturers instructions. The lowest level of detection was 10 pmol/l.

Statistical analyses

Metabolic outcomes were analysed as absolute values using linear mixed model ANOVA (SAS: PROC MIXED, SAS version 8.0, SAS Institute Inc, Cary, NC, USA, 2001). Repeated measure ANOVA tested within-diet and between-diet interactions over time periods of 0–1, 0–6 and 0–24 h for each individual parameter. Treatment group, ID, time of sample, run order and block effects were included in the analysis. In all measures, when there was no differential effect of fat quality the two treatments were combined and the quantitative effects of the dietary lipid analysed over time. Baseline comparisons were carried out using paired Student's *t*-tests. Correlations between leptin, ghrelin and insulin were analysed using Pearson's correlation. Statistical significance was based on 95% limits ($P<0.05$).

Results

18 men were recruited for this trial. There were no exclusions or drop-outs on either treatment regime. All subjects completed both arms of the intervention. The subjects were young, lean, healthy men. Mean age was 23 ± 4.2 (s.d.) years, body mass index was 22.9 ± 2.0 (s.d.) kg/m^2 and waist circumference was 79.5 ± 6.1 (s.d.) cm. All subjects had normal glucose control when defined by fasting plasma glucose <5.5 mmol/l, (mean 4.7, 0.3 s.d. mol/l), and were normotensive (mean SBP: 123, 10.5 s.d. mmHg; mean DBP: 78, 9.0 s.d. mmHg) with no evidence of hyperlipidaemia based on fasting total-cholesterol (mean 4.3, 0.8 s.d. mmol/l), LDL-cholesterol (mean 2.5, 0.7 s.d. mmol/l), HDL-cholesterol (mean 1.4, 0.4 s.d. mmol/l) and triacylglycerol

(TAG, mean 0.8, 0.3 s.d. mmol/l). Mean total ghrelin at baseline was 1.065 (0.26, s.d.) ng/ml and mean leptin at baseline was 1.434 (0.41, s.d.) ng/ml. There was no significant difference between treatments at baseline for any parameter.

Changes in ghrelin relative to fasting baseline are shown in Figure 1. Over the initial 1 h there was a decrease in circulating ghrelin of up to 12% of baseline on the high$_{SFA:USFA}$ ($\delta = -0.105$ ng/ml) and low$_{SFA:USFA}$ ($\delta = -0.106$ ng/ml) treatments, but neither decrease reached significance in this trial of 18 men possibly due to high between-subject variability ($P > 0.05$). When analysed independent of dietary treatment over the 6 h postprandial period there was no significant effect of the fatty meal *per se* (time, $P > 0.05$), nor were there significant differential effects of the high$_{SFA:USFA}$ or low$_{SFA:USFA}$ treatments (treatment*time, $P > 0.05$, Table 2) on total ghrelin, although low$_{SFA:USFA}$ treatment kept ghrelin close to 10% below baseline over 6 h, while high$_{SFA:USFA}$ returned to baseline. Subjects were given an identical high-CHO lunch (L) and afternoon snack (S) on both treatments at 6.5 and 8 h postbreakfast, and ghrelin fell to 12–15% below baseline in both diet groups at 10 h as expected following a high-CHO meal. The overnight fast returned circulating ghrelin to baseline levels on both treatment arms.

Changes in leptin relative to fasting baseline are shown in Figure 2. The pattern of change in circulating leptin was similar on both high$_{SFA:USFA}$ and low$_{SFA:USFA}$ treatments. When analysed independent of dietary treatment there was a significant decrease between baseline and 6 h (time, $P < 0.001$) over which time leptin decreased by 8% on the high$_{SFA:USFA}$ and 10% on the low$_{SFA:USFA}$ treatment. Circulating leptin was consistently higher on the low$_{SFA:USFA}$ arm at all points between baseline and 6 h (treatment $P < 0.05$, see Table 2) but there was no differential change over time in response to treatment group hence the interaction term was not significant at 6 h (treatment*time, $P > 0.05$). Following the high-CHO lunch (L) and snack (S) at 6.5 and 8 h,

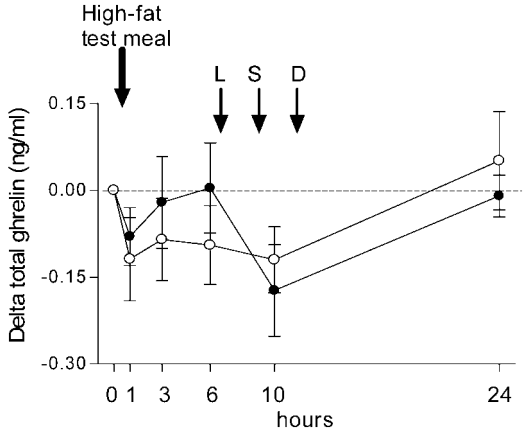

Figure 1 Postprandial changes in circulating total ghrelin following a high-fat breakfast containing a high- (71:29, ●) or low- (55:45, ○) saturated:unsaturated fatty acid (SFA:USFA) profile. No significant decrease (time, $P > 0.05$; ANOVA) nor differential effect of treatment (treatment*time, $P > 0.05$; ANOVA) over 6 h. Mean ± s.e.m. for 18 lean men. L, lunch; S, snack; D, dinner.

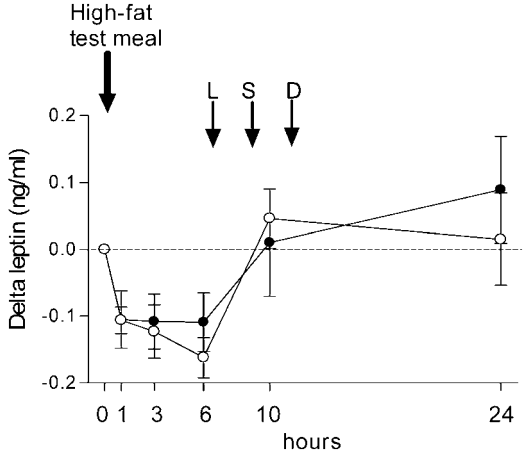

Figure 2 Postprandial changes in circulating leptin following a high-fat breakfast containing a high- (71:29, ●) or low- (55:45, ○) saturated:unsaturated fatty acid (SFA:USFA) profile. Significant decrease (time, $P < 0.001$; ANOVA) but no significant differential effect of treatment (treatment*time, $P > 0.05$; ANOVA) over 6 h. Mean ± s.e.m. for 18 lean men. L, lunch; S, snack; D, dinner.

Table 2 Postprandial effects of the two high-fat breakfasts measured over 6 and 24 h

	0 h (baseline)	*1 h*[1]	*3 h*	*6 h*[2]	*10 h*	*24 h*[3]
High$_{SFA:USFA}$ ratio breakfast						
Insulin (pmol/l)	127 ± 15	619 ± 62***	217 ± 68	110 ± 13***	982 ± 98	183 ± 27***
Ghrelin (ng/ml)	1.097 ± 0.06	1.017 ± 0.06 ns	1.075 ± 0.07	1.101 ± 0.08 ns	0.923 ± 0.08	1.086 ± 0.06*
Leptin (ng/ml)	1.344 ± 0.15	1.239 ± 0.16***$^{\#}$	1.235 ± 0.15	1.234 ± 0.14***$^{\#}$	1.354 ± 0.16	1.433 ± 0.13***$^{\#\#\#}$ $
Low$_{SFA:USFA}$ ratio breakfast						
Insulin (pmol/l)	124 ± 19	539 ± 75***	251 ± 80	141 ± 26***	1034 ± 72	151 ± 12***
Ghrelin (ng/ml)	1.033 ± 0.07	0.914 ± 0.05 ns	0.948 ± 0.07	0.937 ± 0.08 ns	0.912 ± 0.06	1.083 ± 0.07*
Leptin (ng/ml)	1.525 ± 0.16	1.419 ± 0.15***$^{\#}$	1.403 ± 0.16	1.364 ± 0.15***$^{\#}$	1.573 ± 0.14	1.540 ± 0.15***$^{\#\#\#}$ $

Fat exclusion lunch, snacks and dinner were given after the 6 h blood sample.
SFA, saturated fatty acid; USFA, unsaturated fatty acid; h, hours. Mean ± s.e.m. Repeated measures ANOVA, analysed as [1]0-1 h, [2]0-6 h, [3]0-24h: time *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; treatment $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$; treatment*time $^{\$}P < 0.05$, ns, no significant effects.

respectively, circulating leptin returned to baseline levels by the 10 h blood sample on both treatments.

There was a significant change in insulin over 6 h in response to the HF breakfast (time, $P<0.001$) with a rapid peak at 1 h on both treatments (time, $P<0.001$), but no differential effects of $high_{SFA:USFA}$ and $low_{SFA:USFA}$ treatment over 6 h (treatment*time, $P>0.05$, Table 2). There was no evidence of a significant negative correlation between change in insulin and change in either leptin or ghrelin when calculated both at individual time points 1, 3 and 6 h, and as the area under the curve over 6 h using the premeal blood sample as baseline ($P>0.05$).

Discussion

While there is evidence that ghrelin and to a lesser extent leptin respond to acute changes in energy intake (Kolaczynski *et al*., 1996a; Weigle *et al*., 1997; Shiiya *et al*., 2002; Monteleone *et al*., 2003; Greenman *et al*., 2004), the effect of nutrient composition on their postprandial response is less well understood. Our study of lean men showed a decrease in leptin but not ghrelin over 6 h following a HF meal, and an increase in saturation of the fatty meal did not further alter response of either hormone. Since meals rich in SFA reduce postprandial insulin sensitivity (Robertson *et al*., 2002), and insulin may be an important modulator of leptin (Havel *et al*., 1999; Evans *et al*., 2001; Koutsari *et al*., 2003; Romon *et al*., 2003; Fogteloo *et al*., 2004) and ghrelin (Mohlig *et al*., 2002; Saad *et al*., 2002; Flanagan *et al*., 2003), we wanted to determine whether increasing dietary SFA may differentially alter these hormones. Interpretation of the postprandial response of leptin is complicated by diurnal changes in hormone release, driven in part by meal time and/or composition (Schoeller *et al*., 1997; Fogteloo *et al*., 2004), with a decline postbreakfast followed by a midnight peak (Sinha *et al*., 1996; Coleman and Herrmann, 1999; Havel *et al*., 1999). While a high-CHO breakfast may reverse the morning decline (Romon *et al*., 1999; Evans *et al*., 2001; Herrmann *et al*., 2001; Romon *et al*., 2003) irrespective of meal time (Schoeller *et al*., 1997), we had no evidence from our trial of a similar effect following fatty meals.

Although there is growing consensus that insulin is an important regulator of leptin, our review of the literature has shown the data to not yet be conclusive. There is little or no correlation between the two hormones in several postprandial studies (Dagogo-Jack *et al*., 1996; Sinha *et al*., 1996; Pratley *et al*., 1997; Guerci *et al*., 2000; Herrmann *et al*., 2001; Poretsky *et al*., 2001) and this has lead to a revived interest in the influence of other dietary nutrients, including lipids, which have a blunted effect on insulin compared to CHO. Other trials which have shown a decrease in leptin after a fatty meal include lean (Romon *et al*., 1999) and obese subjects (Imbeault *et al*., 2001), and where HF test meals have followed high- and low-CHO acclimation (Koutsari *et al*., 2003). In a study of lean women given three HF (60 en%) or three high-CHO meals over 24 h, leptin continued to rise from an 0800 h nadir on both treatments but the postprandial response was less pronounced following the HF meals (Havel *et al*., 1999). The authors suggested this was due to decreased insulin-mediated glucose metabolism in adipose tissue, and that decreased postprandial exposure of leptin resulting from a fat bolus could be adipogenic (Havel *et al*., 1999), citing the hyperphagia and gross obesity resultant in humans from genetic impairment of leptin production (Montague *et al*., 1997). Whether a decrease in circulating leptin over 24 h may represent an adverse risk for obesity remains controversial and recombinant leptin weight loss therapy in hyperleptinaemic obese subjects has been met with limited success (Hukshorn *et al*., 2002; Fogteloo *et al*., 2003; Lejeune *et al*., 2003; Wong *et al*., 2004). It has been suggested that the role of leptin is not prevention of obesity, rather a response to low-energy availability (Havel, 2004) such that during starvation leptin falls triggering an increase in hunger (Hukshorn *et al*., 2003). Administration of recombinant leptin has resulted in decreased appetite (Hukshorn *et al*., 2003), in line with animal models (Pelleymounter *et al*., 1995) and also with leptin deficiency and nonfunctional leptin receptor defects which occur rarely in man (Montague *et al*., 1997). Not all HF trials however show a decrease in serum leptin. Hormone levels were unchanged in lean and obese subjects given HF meals both morning and evening (Guerci *et al*., 2000), in lean (Monteleone *et al*., 2003) and obese subjects (Weigle *et al*., 1997) who responded to neither HF or high-CHO, and those who responded to high-CHO but not to HF (Tentolouris *et al*., 2003). Numerous studies investigating long-term effects of HF feeding have failed to show a response of fasting leptin to diet (Havel *et al*., 1996; Schrauwen *et al*., 1997; Weigle *et al*., 1997) but the collection of only a morning fasted sample for hormone analysis may make the interpretation of these studies difficult.

The mechanism by which leptin is controlled remains poorly defined. Studies where obese subjects failed to respond to HF or high-CHO led to conclusions that total adipose mass rather than diet may be driving circulating leptin (Weigle *et al*., 1997). Gradually the consensus has moved towards insulin playing a pivotal role but the relationship with leptin in the postprandial state is not clear-cut. Cross-sectional studies show an association between fasting leptin and insulin (Rosenbaum *et al*., 1997), as do long-term (Kolaczynski *et al*., 1996b; Boden *et al*., 1997) but not short-term (Dagogo-Jack *et al*., 1996) insulin infusion and some postprandial studies (Romon *et al*., 1999; Koutsari *et al*., 2003). Our data were in line with a number of studies which fail to show significant correlation between leptin and insulin after consumption of HF, mixed and even high-CHO meals (Sinha *et al*., 1996; Pratley *et al*., 1997; Guerci *et al*., 2000; Herrmann *et al*., 2001; Poretsky *et al*., 2001). If insulin is a driving force in the control of leptin release (Havel *et al*., 1999; Romon *et al*., 1999, 2003; Evans *et al*., 2001; Koutsari

et al., 2003; Fogteloo *et al*., 2004), it is possible that this mechanism is important only when a major portion of the diet comprises CHO.

Total circulating ghrelin is believed to be an important initiator of ingestion in humans. It tends to rise during fasting (Ariyasu *et al*., 2001; Cummings *et al*., 2001), increasing hunger, and fall following a meal (Caixas *et al*., 2002; Monteleone *et al*., 2003; Erdmann *et al*., 2004; Greenman *et al*., 2004), decreasing hunger. The consensus that ghrelin decreases postprandially is based mainly on high-CHO trials, however, and trials of obese subjects (English *et al*., 2002) and also of HF feeding (Mohlig *et al*., 2002; Erdmann *et al*., 2004) are less conclusive. Table 3 shows details of four acute HF interventions highlighting the variable outcome of these trials. Ghrelin was unchanged (Mohlig *et al*., 2002), decreased (Monteleone *et al*., 2003; Greenman *et al*., 2004) and increased (Erdmann *et al*., 2004) over 1–3 h following a lipid challenge, possible a consequence of the other macronutrient components of the load. Certainly evidence is growing that fatty meals induce a muted response compared with high-CHO meals (Monteleone *et al*., 2003; Greenman *et al*., 2004), and the relative lack of response of ghrelin to both HF regimes in our trial lends support to this idea. Conversely, we should note that although not reaching significance the dietary lipid load did induce a 12% decrease in circulating ghrelin. It is possible that factors such as between-subject variability in glucose control or even previous dietary intake may have reduced the power of this trial to detect this change as statistically significant. Acute changes to the fat loads may also not have been detected using our protocol of blood collection at 1 and 3 h, although data from lean subjects given an 85 en% fat meal (Erdmann *et al*., 2004) has shown plasma ghrelin to peak at 45–60 min after eating.

Although high-CHO foods appear to elicit the greatest suppression of ghrelin secretion, the role of glucose and/or insulin as initiators of hormone change is not well defined. A number of studies have shown ghrelin to decrease in response to oral (Caixas *et al*., 2002; Nakagawa *et al*., 2002; Shiiya *et al*., 2002) or i.v. glucose (Nakagawa *et al*., 2002; Shiiya *et al*., 2002; Briatore *et al*., 2003), but others do not (Schaller *et al*., 2003). An inhibitory role of insulin on ghrelin has also been shown in some (Mohlig *et al*., 2002; Saad *et al*., 2002; Flanagan *et al*., 2003) but not all (Caixas *et al*., 2002; Schaller *et al*., 2003) studies. Physiological hyperinsulinaemia has been shown to decrease ghrelin (Saad *et al*., 2002), while increasing insulin and glucose through infusion has not (Caixas *et al*., 2002) until reaching supraphysiological insulin levels (Schaller *et al*., 2003). Two trials in type 1 diabetics lacking endogenous insulin have also been contradictory. When exogenous insulin was withheld and the patients given a mixed high-CHO meal, plasma ghrelin was unaffected (Murdolo *et al*., 2003), yet meals with high- and low-glycaemic indices decreased total ghrelin suggesting that insulin was not an essential signal controlling hormone release (Spranger *et al*., 2003). Diet-induced changes in

Table 3 The effect of high-fat meals on postprandial ghrelin response

Reference	*Subjects*	*Energy content (kJ)*	*Fat*	*CHO*	*Protein*	*Effect on circulating insulin*	*Effect on circulating ghrelin*
Mohlig *et al*. (2002)	$n=5$ healthy males	N/A	20% lipid influsion	0 g	0 g	No significant change	No significant change over 2 h
Monteleone *et al*. (2003)	$n=14$ healthy females	840 kJ	17 g 75%	8 g 15%	5 g 10%	Increased by 650% at 1 h	Significant decrease over 3 h ($P<0.05$)
Erdmann *et al*. (2004)	$n=8$ healthy males; $n=6$ healthy females	13.5 kJ/g (total not reported)	85.5%	0%	14.5%	No significant change	Significant increase over 45 min ($P<0.05$)
Greenman *et al*. (2004)	$n=13$ nondiabetic lean and obese males/females	1506 kJ	40 g 100%	0 g	0 g	No significant change	Significant decrease ($P<0.035$)
Poppitt *et al*. (current trial)	$n=18$ healthy males	3100 kJ	59 g 71% (42 g SFA)	45 g 23%	9 g 5%	Increased by 480% at 1 h	Nonsignificant decrease over 1 h and 6 h
		3100 kJ	59 g 71% (32 g SFA)	45 g 23%	9 g 5%	Increased by 430% at 1 h	Nonsignificant decrease over 1 h and 6 h

insulin also appear unable to explain the differential effects of HF meals on circulating ghrelin (see Table 3). Lipid plus CHO caused a high insulin peak but a decrease (Monteleone *et al*., 2003) or no significant change (our data) in ghrelin. Lipid alone had no effect on insulin but no change (Mohlig *et al*., 2002) or a decrease (Greenman *et al*., 2004) in ghrelin. Lipid plus protein had no effect on insulin but an increase in ghrelin (Erdmann *et al*., 2004).

In conclusion, this study of lean men has shown that in contrast to high-CHO meals consumption of a fatty meal acutely decreased circulating leptin with no evidence of reversal of the morning nadir, which in turn raises the question of possible adipogenic effects of this decreased postprandial exposure as previously suggested by Havel *et al*. (1999). Increasing the saturated fat content of the diet did not further modify circulating leptin. HF meals did not significantly decrease ghrelin below fasting baseline levels, nor was there evidence of a differential effect of fatty acid composition on total ghrelin and no correlation between either of these hormones and circulating insulin. While insulin may be an important regulator of serum leptin and/or ghrelin when a high-CHO diet is eaten, it may play a lesser role following consumption of fatty meals.

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