

Original Research

Effects of Leptin Resistance on Acute Fuel Metabolism after a High Carbohydrate Load in Lean and Overweight Young Men

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Objective: Six lean ($BMI = 20.8 \pm 0.7$) and seven overweight ($BMI = 30.8 \pm 1.7$) young men (18–27 years old) were studied to investigate the acute effect of a high-carbohydrate meal on leptin levels and its relation to energy expenditure as well as to protein, carbohydrate and fat oxidation.

Methods: Study participants were given a high-carbohydrate meal (17% as protein, 80% as carbohydrates and 3% as lipids) covering 40% of their estimated daily energy requirements. Serum leptin, insulin, glucose, free fatty acids and triglycerides levels were measured before meal intake and during the four postprandial hours. Furthermore, energy expenditure (EE), protein, carbohydrate and lipid oxidation were measured in fasted and fed conditions.

Results: Fasting leptin was found to be positively correlated with circulating insulin concentrations ($r = .748; p = 0.011$) and body fat in kg ($r = .827; p = 0.001$). During the measured postprandial period no statistically significant changes were found in leptin levels as compared with pre-meal values in either lean or overweight men, nor differences in leptin changes between both groups. After load intake, carbohydrate oxidation was lower in overweight individuals ($p < 0.05$), while no significant differences were observed in protein oxidation. Cumulative lipid oxidation was found to be negatively associated with post-meal leptin values, being significantly lower in the overweight as compared with lean men ($p < 0.05$). This study demonstrates that the acute postprandial fuel substrate utilization is altered in overweight men with a lower carbohydrate oxidation and a strong inhibition of lipid oxidation, which could be attributed to some leptin resistance.

Conclusion: These data also suggest that short-term meal-related metabolic responses may explain the long-term body adiposity if they are sustained over long intervals.

INTRODUCTION

The maintenance of body weight stability requires that average fuel oxidation matches the macronutrient intake in order to maintain body weight homeostasis [1]. Furthermore, energy intake and energy expenditure appear to be regulated by short-term meal-related factors such as hormonal information, and metabolic signals that influence tissue fuel utilization [2] and adipose tissue tend to remain constant over time by long-term adiposity-related signals such as insulin and leptin [3]. Therefore, body adiposity may be affected if changes of these short-term factors are sustained over long intervals.

Leptin, the product of the *ob* gene [4], is presently assigned

to have a critical function in the regulation of body weight, playing a key role in the regulation of body fat mass [5–7]. This hormone, produced mainly by the adipose tissue, conveys information to the brain about the size of energy stores and activates hypothalamic centers that regulate energy intake and energy expenditure [8]. Serum leptin levels are elevated in obesity and usually correlate with body fat content and insulin both in lean and obese individuals [9,10].

In normal weight men, recent studies have demonstrated the acute effects of food intake on postprandial leptin. While some authors [11] found an increase in leptin levels five hours after overfeeding, other investigators [12] reported a rise in plasma leptin levels four hours after a normal mixed meal, claiming

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that food intake stimulates leptin secretion in humans. The mechanisms of this short-term effect have not been totally elucidated. Nevertheless, insulin which stimulates leptin secretion in a clamp intervention [13] may play a physiological role in this process [14]. Consistent with this hypothesis, a single carbohydrate meal induced higher postprandial leptin levels than an isoenergetic fat meal in lean subjects, while high-carbohydrate meals also increased the 24-hour circulating leptin levels as compared with high-fat meals [16].

Obesity is commonly accompanied by a pattern of fasting hyperinsulinemia and higher plasma insulin values in response to a mixed meal or glucose load [17,18]. If, as suggested, insulin mediates the effects of energy-yielding nutrients on leptin [13], then impaired insulin-induced leptin production in obese and insulin-resistant subjects could contribute to the development or worsening of obesity. On the other hand, it has been suggested as physiologically significant for leptin resistance in obese subjects in the regulation of energy expenditure and fuel metabolism [19]. Nevertheless, an area of uncertainty is whether a possible resistance to leptin may play a role in substrate metabolism, particularly in obese individuals. Therefore, the current trial aimed to compare leptin response and its relation to nutrient metabolism after a high-carbohydrate load between normal weight and overweight men.

MATERIAL AND METHODS

Subjects

Thirteen young male, six lean ($BMI < 24 \text{ kg/m}^2$) and seven overweight ($BMI > 27 \text{ kg/m}^2$) were studied. All study participants were healthy, non-diabetic, non-smokers, taking no oral prescription medications and with a stable body weight during the previous three months. The intervention was approved by the Clinical Investigation Ethical Committee of Navarra, and informed consent was obtained from all participant subjects, according to the Helsinki II declaration. The characteristics of the 13 participants are shown in Table 1.

Study Protocol

The protocol consisted of a three-day baseline period followed by one experimental day. During the three-day baseline period participants consumed a eucaloric diet designed to maintain body weight, which provided 55% of energy as carbohydrates, 15% as protein and 30% as fat. On the experimental day, participants entered the metabolic unit between 07:30 and 07:45 a.m. (after a 10-hour overnight fast) and remained until 8.00 p.m. After the assessment of body weight and body composition, participants lay on a bed during 30 minutes until resting energy expenditure by indirect calorimetry was determined. A venous catheter was inserted into an antecubital vein for blood sampling. The fasting period was prolonged during

Table 1. Characteristics of the 13 Study Participants in Basal Conditions

	Lean	Overweight
Subjects (n)	6	7
Age (years)	22.1 ± 1.0	20.5 ± 0.6
Height (cm)	174 ± 10	176 ± 10
Body weight (kg)	63.5 ± 2.8	96.1 ± 6.2^2
Body mass index (kg/m^2)	20.8 ± 0.7	30.8 ± 1.7^2
Body fat (% weight)	15.4 ± 1.6	26.7 ± 1.2^2
Body fat (kg)	9.9 ± 1.3	26.1 ± 3.0^2
Glucose (mmol/L)	4.72 ± 0.04	5.06 ± 0.12^1
FFA (mmol/L)	1.19 ± 0.07	1.03 ± 0.14
Triglycerides (mmol/L)	0.53 ± 0.07	0.83 ± 0.1
Insulin ($\mu\text{U}/\text{mL}$)	3.66 ± 0.27	7.60 ± 0.96^1
Leptin (ng/mL)	0.27 ± 0.07	2.02 ± 0.4^2

FFA = Free fatty acids

Results are the mean \pm SEM.

¹ $p < 0.05$; ² $p < 0.01$; statistical significance between lean and overweight men.

7.5 hours in order to obtain a depletion of glycogen stores and match all individuals for glycogen reserves [20].

Diet Formula

Subjects received a test formula after a total of 17.5 hours of fasting and the postprandial period was studied during the following four hours. The high-carbohydrate test meal provided 40% of each subject's daily energy requirements, which was assumed to be $1.2 \times$ measured sedentary energy expenditure estimated by indirect calorimetry in the morning of the experiment day. The test meal was a liquid formula (Meritene Polvo, Novartis Nutrition), to which sugar and corn oil were added and provided 80% of energy as carbohydrates (53% as sucrose and 27% as lactose), 17% as proteins and 3% as lipids (Table 2).

Body Measurements

Body composition determinations included height, which was measured with a stadiometer to the nearest 1.0 mm, and

Table 2. Nutritional Content of the Formula Test (Meritene, Novartis Nutrition) and Energy and Nutrient Intake of the Test Meal

	g/100g of Product	% Total energy
Energy (kJ)	87.7	
Moisture	11.8	
Carbohydrates (lactose)	53	60
Proteins (casein)	33.3	37
Lipids (corn oil)	0.9	3
Normal Weight (n = 6)		Overweight (n = 7)
Energy (kJ)	3316 ± 86	4140 ± 217
Protein (g)	33.6 ± 0.8	41.9 ± 2.2
Carbohydrate (g)	158.9 ± 4.0	198.0 ± 10.3
Sucrose (g)	104.4 ± 2.7	131.0 ± 6.9
Lactose (g)	53.5 ± 1.3	66.9 ± 3.4
Fat (g)	2.6 ± 0.1	3.3 ± 0.1

Results are the mean \pm SEM.

weight assessed to the nearest 100g with a Seca[®] scale. Moreover, skinfold thickness was measured at three sites (biceps, triceps and suprailiac) using a Holtain caliper and the equation of Durnin and Womersley was used to estimate percent body fat [21].

Energy Expenditure and Substrate Oxidation

The measurements of fasted and fed energy expenditure and the rates of substrate utilization were carried out by indirect calorimetry, which was performed with a continuous open-circuit ventilated-hood system (Deltatrac Monitor MBM-200, Datex-Engstrom Division, Instrumentarium Corp., Helsinki, Finland). A calibration of oxygen, carbon dioxide and air flow was carried out with reference gas before the beginning of each test. Energy expenditure (EE), non-protein respiratory quotient (NPRQ) and substrate oxidation rates were calculated from oxygen consumption, carbon dioxide production (recorded by Deltatrac once per minute and averaged over 20 minutes) and urinary nitrogen excretion values, according to equations previously described [1,22]. Three urine collections were taken: the first during the night prior to the day of the experiment (10 hours fasting), the second during the 7.5-hour fasted period and the third during the postprandial period (four hours). The nitrogen content of the three samples was analyzed by the Kjeldahl method.

Post-meal measurements were assessed at 30 minute intervals over 240 minutes following meal intake. Cumulative EE as well as protein, carbohydrate and lipid oxidation, expressed as mg/kJ · 4 hours over the fed period, was calculated as the area under the curve as compared to pre-meal absolute values and computed with a trapezoidal rule [23].

Biochemical Measurements

Blood samples were collected before and after the high CHO load intake, frozen and centrifuged immediately and then stored at -40°C before assay. Enzymatic determinations were performed to measure serum glucose (Glucosa PAP, Roche, Spain), free fatty acids (NEFA C, Wako Chemicals GmbH, Germany) and triacylglycerol concentrations (Unimate 5 Trig, Roche, Spain). Insulin was analyzed by duplicate radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products Corporation, USA). Serum leptin levels were assessed with a enzyme-linked immunosorbent (ELISA) kit (Active Human Leptin) supplied by Diagnostic System Laboratories, USA. In this assay, detection limit was 0.05 ng/mL with a intra-and inter-assay variability of 4.4% and 4.9%, respectively.

Statistical Analysis

All results are expressed as mean ± SEM. Based on the results of the Kolmogorov-Smirnov and Shapiro-Wilks normality tests; differences between groups were analyzed with either Student's *t* test or Mann-Whitney U test as appropriate.

Associations between variables were evaluated with Pearson correlation coefficients. A stepwise multiple regression analysis was used to identify postprandial substrate oxidation predictors. The postprandial incremental area under the curve (AUC) was calculated for glucose, insulin, triglycerides and free fatty acids, using the trapezoidal method, taking into account the pre-meal values [16]. Statistical analysis was performed with the SPSS 9.0 statistical software package (Microsoft, Redmond, WA) and the power test calculation with the Granmo 4.0 for Windows.

RESULTS

Pre-meal fasting values (measured after 17.5 hours of fasting) were significantly higher in overweight men for serum leptin ($p < 0.01$), glucose ($p < 0.05$) and insulin ($p < 0.05$) as shown in Table 1. Postabsorptive leptin levels were found to be positively correlated with body fat in kg ($r = 0.827, p < 0.001$), serum insulin ($r = 0.748, p = 0.011$) and triglycerides ($r = 0.887, p = 0.001$). After the high-CHO intake, both glucose and insulin increased in the two groups ($p < 0.05$), although no significant differences were found in the postprandial AUC changes (Table 3) between lean and overweight men in glucose, insulin, FFA or triglycerides concentrations (power test between 40% and 50%). Leptin concentrations were positively associated with serum insulin ($r = 0.642; p = 0.018$) one hour after meal intake as well as with body fat mass ($p < 0.01$), triglyceride levels ($p < 0.01$) and NPRQ ($p < 0.01$) along the rest of the measured period (data not shown). However, pre- and post-meal leptin changes (% pre-meal values) were not significantly different in either group, and no significant differences were found in postprandial leptin changes between lean and overweight men (Fig. 1).

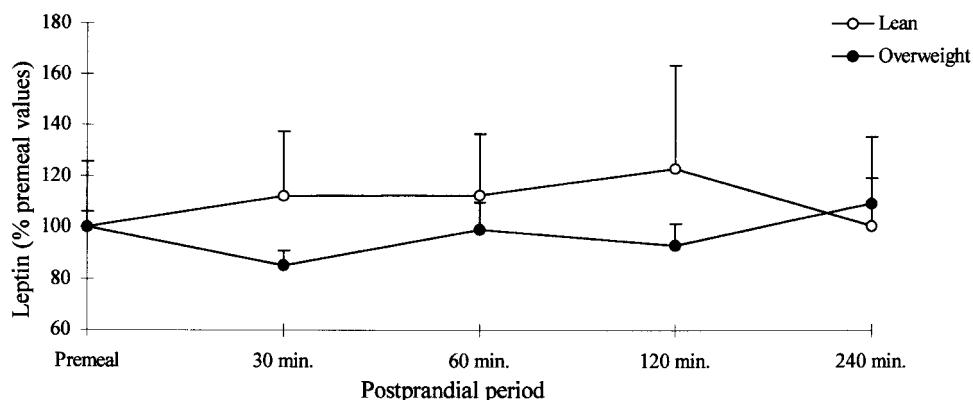
Over the measured post-meal period, overweight men presented a significantly higher EE ($p < 0.05$) as well as a significantly lower ($p < 0.05$) carbohydrate and lipid oxidation as compared with lean men (Table 4). Including or excluding two outliers (one lean and one overweight), the postprandial lipid oxidation was negatively associated ($p = 0.01$) with the

Table 3. Postprandial Changes Measured over the Four hours after Meal Intake as Calculated by the Incremental Area under the Curve Taking into Account the Pre- and Post-Meal Values

	Lean n = 6	Overweight n = 7
Glucose (mmol/L · 4 hours)	7.93 ± 1.26	5.21 ± 0.96
FFA (mmol/L · 4 hours)	-3.71 ± 0.27	-2.66 ± 0.56
Triglycerides (mmol/L · 4 hours)	0.17 ± 0.08	0.38 ± 0.14
Insulin (μU/mL · 4 hours)	132.1 ± 24.8	222.8 ± 42.6

FFA = Free fatty acids

Results are the mean ± SEM.

**Fig. 1.** Postprandial leptin changes as compared with premeal fasting levels.**Table 4.** Cumulative Energy Expenditure (EE) and Nutrient Oxidation over the Four Hours after Meal Intake¹

	Lean n = 6	Overweight n = 7
EE (MJ · 4 h)	1.33 ± 0.05	2.08 ± 0.12 ²
Protein Oxidation (mg/kJ · 4 hours)	13.60 ± 1.85	16.22 ± 2.3
CHO Oxidation (mg/kJ · 4 hours)	34.04 ± 2.03	27.37 ± 1.91 ²
Lipid Oxidation (mg/kJ · 4 hours)	6.75 ± 2.20	0.91 ± 0.98 ²

Results are the mean ± SEM.

¹ Cumulative values calculated as the area under the curve taking into account pre and post meal values.

² p < 0.05—statistical significance between lean and overweight men.

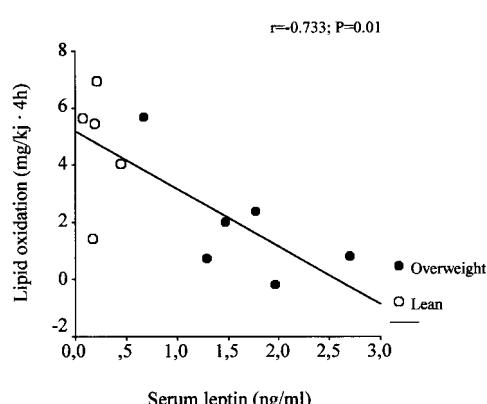
leptin concentrations (Fig. 2), and a stepwise multiple regression analysis including postprandial metabolic variables showed that final leptin concentration was the major predictor of lipid oxidation ($p = 0.042$). Protein and carbohydrate oxidations were also negatively associated ($p < 0.05$).

DISCUSSION

Several studies have suggested that short-term energy balance has an impact on circulating leptin levels [11,15,24], but

the role of specific macronutrients with respect to leptin and its relation with fuel substrate utilization is still a matter of debate. In this trial, no statistically significant changes in leptin levels during the four hours following CHO intake were observed, either in lean or overweight men; this is in agreement with a previous report in which leptin response to carbohydrate intake is delayed for four to five hours after meal intake [15]. Thus, the apparent lack of response observed in our volunteers may be due to the short-time measurement period, since food ingestion may have a delayed effect on circulating leptin levels [12,15,25].

It also has been suggested that carbohydrate intake may have an important role in the regulation of leptin levels, possibly due to insulin mediated-changes in adipose tissue glucose disposal [15,16]. Pre-meal and 60-minute post-meal insulin levels correlated positively with leptin concentrations, in agreement with data from others studies [25,26]. However, the postprandial insulin response was not accompanied by changes of the same magnitude in leptin concentrations, suggesting that acute short-term hyperinsulinemia did not immediately affect leptin levels in lean or overweight men. The rise in plasma leptin observed during weight gain and obesity may be a consequence of resistance to leptin action [11,19,27], which could consequently promote a greater fat accumulation and other metabolic changes. Indeed, during the measured postprandial period, overweight men showed a significantly lower carbohydrate and lipid oxidation rate as compared with lean men. The data concerning macronutrient metabolism indicates that, after carbohydrate intake, insulin-inhibited lipid utilization was more pronounced in overweight individuals and was accompanied by higher circulating leptin levels. Furthermore, leptin has been implicated in controlling insulin release by inhibiting insulin expression and its anabolic effects on adipose tissue [28,29] and also in promoting lipid oxidation in other tissues [30,31]. Accordingly, resistance to leptin action or a reduced leptin sensitivity in these overweight subjects could subsequently be responsible for a lower lipid oxidation in the presence of carbohydrates. This is also supported by the

**Fig. 2.** Association between serum leptin levels at four hours after meal intake with cumulative postprandial lipid oxidation by Pearson's Correlation Test (two outliers values were removed).

autocrine/paracrine actions on leptin-induced lipolysis in some *in vitro* adipocyte experiments [32].

In our study, a negative association between protein and carbohydrate oxidation was found. Obesity is characterized by some muscle insulin resistance, which could be responsible for a lower insulin induced muscle glucose disposal and a subsequently higher protein utilization as fuel substrate showed by overweight men, although the later measurement was not statistically significant.

As other authors [33], we observed no relationship between EE (absolute values or corrected for fat free mass) and leptin values both during fasting and after load intake. These results may be due to the small number of subjects or to the fact that lean and overweight men have a distinct sensitivity to leptin [7] which could explain the lack of association between leptin and EE.

In a previous study [19], it was reported that postabsorptive serum leptin was inversely correlated with NPRQ in obese individuals, with leptin concentrations higher in those subjects with low NPRQ. Interestingly, we found a positive trend ($p = 0.09$) between fasting leptin and NPRQ values and a strong positive relation ($p < 0.01$) between leptin concentrations and NPRQ values in all post-meal measurements. These findings indicate that higher leptin values were associated with higher proportion of oxidized carbohydrate in relation to fat, confirming the hypothesis that some degree of peripheral leptin resistance in overweight subjects may reduce lipid oxidation, at least after carbohydrate intake.

If one accepts that leptin values are an index of the degree of obesity [7] and that a low rate of fat oxidation is considered a metabolic predictor of weight gain [34,35], it may be speculated with these data that circulating levels of leptin may also indicate fuel substrate utilization in obesity. Although further investigation of the impact of circulating leptin on fuel metabolism after food and macronutrient distribution intake is needed, these data suggest that the reduced fat oxidation commonly observed in the obese state may be, in part, due to some peripheral resistance to the lipolytic actions of leptin.

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