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Effects of short-term carbohydrate or fat overfeeding on energy expenditure and plasma leptin concentrations in healthy female subjects

M Dirlewanger[1], V di Vetta[1], E Guenat[1], P Battilana[1], G Seematter[1], P Schneiter[1], E Jéquier[1] and L Tappy[1]*

[1]*Institute of Physiology, University of Lausanne, 7 rue du Bugnon, 1005 Lausanne Switzerland*

OBJECTIVE: To determine the effects of excess carbohydrate or fat intake on plasma leptin concentrations and energy expenditure.
DESIGN: Ten healthy lean females were studied: (a) during a 3 day isoenergetic diet (ISO); (b) during 3 day carbohydrate overfeeding (CHO OF); and (c) during 3 day fat overfeeding (FAT OF). During each test, basal metabolic rate, the energy expended during mild physical activity and recovery, and 24 h energy expenditure (24 h EE) were measured with indirect calorimetry. The concentrations of glucose and lactate were monitored in subcutaneous interstitial fluid over a 24 h period using microdialysis. Plasma hormone and substrate concentrations were measured in a blood sample collected in the morning of the fourth day.
RESULTS: CHO OF increased plasma leptin concentrations by 28%, and 24 h EE by 7%. Basal metabolic rate and the energy expended during physical activity were not affected. FAT OF did not significantly change plasma leptin concentrations or energy expenditure. There was no relationship between changes in leptin concentrations and changes in energy expenditure, suggesting that leptin is not involved in the stimulation of energy metabolism during overfeeding. Interstitial subcutaneous glucose and lactate concentrations were not altered by CHO OF and FAT OF.
CONCLUSIONS: CHO OF, but not FAT OF, increases energy expenditure and leptin concentration.
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Introduction

Maintenance of a constant body weight depends on the balance between energy intake and energy expenditure. Such a balance is known to occur not on a day-to-day basis but on average over extended periods of time. Chronic ingestion of energy in excess of energy expenditure will invariably result in energy storage, essentially as fat tissue with only a modest stimulation of energy expenditure.[1] The magnitude of the weight gain shows considerable interindividual variations, possibly due in part to genetic factors.[2–4] This observation of interindividual variations in net energy deposition during a fixed energy overload suggests that overfeeding triggers a variable increase in energy expenditure. In support of this hypothesis, it has recently been observed that overfeeding stimulates 24 h energy expenditure (24 h EE), an increase negatively correlated with weight gain.[5] The mechanisms by which energy expenditure increases during overfeeding remains unknown.

Overfeeding has been shown to increase leptin gene expression in adipose tissue and to increase plasma leptin concentrations.[6] Increased plasma leptin levels may contribute to limiting weight gain by exerting a negative feedback on energy intake and by stimulating energy expenditure.[7–9] This latter effect of leptin remains, however, poorly documented. In rats, an increase in energy expenditure in response to exogenous leptin administration was observed only after several days.[10] In contrast with this slow activation of energy expenditure, leptin administration effectively prevented the decrease in energy expenditure normally observed rapidly in food-restricted animals.[11] It also prevented the drop in brown adipose tissue activity when administered to fasted rats.[12] No data are available regarding the effects of leptin on energy expenditure in humans. In non-human primates, however, leptin administration was shown to activate the sympathetic nervous system,[13] which may in turn stimulate energy expenditure.

There is considerable evidence that a high fat content of the diet is a factor favouring passive overconsumption and the development of obesity in both animals and man.[14] It has recently been observed that plasma leptin concentration decreases in humans switched from a normal to a high-fat diet.[15] This decrease in plasma leptin concentration may favour excessive food intake and lower energy expenditure.

*Correspondence: L Tappy, Institut de physiologie, 7 rue du Bugnon, 1005 Lausanne, Switzerland.
E-mail: Luc.Tappy@iphysiol.unil.ch
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The metabolic factors which relate energy and macronutrient intake to leptin secretion by adipose tissue remain incompletely understood. Insulin concentrations may be a major factor linking carbohydrate intake to leptin secretion.[16–18] One mechanism by which insulin may increase leptin production is via its action to increase glucose utilization by adipose tissue.[19] More specifically, stimulation of the oxidative, but not non-oxidative glucose pathway appears to be associated with leptin production.[19] Catecholamines[20] and cortisol[21] have also been shown to modulate leptin secretion, and changes in the concentrations of these hormones during dietary manipulations may participate in the regulation of plasma leptin concentrations.

In order to further assess the specific effects of nutrients during overfeeding on plasma leptin concentration and on energy expenditure, we studied a group of healthy lean women during isoenergetic conditions and during a 3 day period with an excess of 40% energy above requirements administered either as fat or as carbohydrate. Energy expenditure was assessed under each condition, and was related to changes in plasma leptin concentration. In addition, the concentration of glucose and lactate in subcutaneous adipose tissue was monitored over 24 h periods by means of *in vivo* microdialysis.

Subjects and methods

Subjects

Ten healthy lean women were selected to take part in this study. Their percentage fat was determined from skinfold thickness measurements.[22] Their anthropometric characteristics are shown in Table 1. All were in good physical condition, were not presently taking any medication, and had no family history of diabetes mellitus or metabolic disorders. The experimental protocol was approved by the Ethical Committee of Lausanne Medical School and all participants provided informed, written consent.

Dietary conditions

At inclusion, resting energy expenditure was measured by indirect calorimetry during a 45–60 min period, using a ventilated hood. Twenty-four-hour energy requirement was estimated to amount to resting energy expenditure (in MJ/day) multiplied by 1.3. The subject was placed on a controlled diet during three periods of 3 days. On one occasion (isoenergetic diet, ISO) they received a diet containing 50% of total energy as carbohydrate, 35% as lipid and 15% as protein (which consisted mainly of a liquid formula (Fresubin energy, Fresenius, Stans, CH), supplemented with orange juice, yoghurt and cream), to be consumed at specified times. They were instructed not to consume any other food or drink. Subjects were carefully instructed to strictly adhere to the dietary recommendations but no assessment of compliance could be performed during these 2 days spent as outpatients. Food was administered under direct control on the third day which the subjects spent in the respiratory chamber (see below). On a second occasion, they received an hyperenergetic diet providing 40% excess energy as carbohydrate (carbohydrate overfeeding: CHO OF). For this purpose, the isoenergetic diet was supplemented with bread, rice, biscuits and sugar. On a third occasion, they received the same 40% excess of energy as fat (fat overfeeding: FAT OF (isocaloric diet supplemented with cheese, potato chips and chocolate)). The average energy intake and macronutrient composition corresponding to these three conditions are shown in Table 2. The order of administration of each diet was randomized and each condition was separated by at least a 7 day interval. All studies were performed during the follicular phase of the menstrual cycle.

Experimental protocol

In the morning of the third day of each dietary condition, the subjects came to the Institute of Physiology after an overnight fast. A microdialysis catheter (CMA 100, CMA, Stockholm, Sweden) was inserted into the periumbilical subcutaneous adipose tissue and was perfused with 0.3 µl/min phosphate-buffered saline by means of a portable infusion pump (CMA 106). Thereafter, the subjects moved to a respiratory chamber and their energy expenditure was monitored by indirect calorimetry from 8:00 am to 7:00 am the next day.[23] During this 23 h period, they received a breakfast at 8:30 am, lunch at 12:00 mid-day and dinner at 6:00 pm, and they exercised on a treadmill at 4 km/h with a slope of 3% between 10:00 am and 10:30 am. They were required to lay in bed with the light off between 11:00 pm and 7:00 am the next day. Dialysate was collected as 1 h fractions between 8:00 am and 11:00 pm, and as 4 h fractions between 11:00 pm and 7:00 am the next day. A urine collection was performed for analysis of urinary nitrogen excretion. The next day, basal energy expen-

Table 1 Characteristics of the subjects

Age (y)	*Weight (kg)*	*Height (m)*	*Body mass index (kg/m²)*	*Fat mass (%)*
22.4 (20–26)	60.9 ± 2.4 (54–78)	1.67 ± 0.03 (1.52–1.79)	21.9 ± 2.2 (19.3–25.3)	27.2 ± 1.4 (21.5–35.4)

Values are expressed as mean ± s.d. (range).

Table 2 Diet compositions

	Energy intake (MJ/day)	*Carbohydrate (%)*	*Fat (%)*	*Protein (%)*
ISO	7.5 ± 0.6	50	35	15
CHO OF	10.3 ± 0.4	64	25	11
FAT OF	10.5 ± 0.5	35	55	11

diture was measured by indirect calorimetry during 60 min (ventilated hood) with the subject fasted overnight and lying quietly, and two blood samples were obtained 10 min apart for determination of basal hormone and substrate concentrations.

Analytical procedure

Plasma glucose concentrations were measured with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA); plasma beta-hydroxybutyrate concentrations were measured enzymatically using a kit from Boehringer Mannheim, Mannheim, Germany. Plasma leptin (kit from Linco, St Charles, MO), insulin (kit from Biochem Immunosystems GmbH, Freiburg, Germany), glucagon (kit from Linco), and cortisol (kit from DPC, Los Angeles, CA) were determined by radioimmunoassay. Plasma epinephrine and norepinephrine concentrations were determined with HPLC using electrochemical detection.[24]

Calculation

Twenty-four-hours EE and basal energy expenditure were calculated from $\dot{V}O_2$, $\dot{V}CO_2$ and urinary nitrogen using the equations of Livesey and Elia.[25] Suprabasal energy expenditure was calculated as (24 h EE) − (basal energy expenditure).

The effect of exercise on energy expenditure (energy for physical activities) was assessed by calculating energy expenditure between 10:00 and 11:00 am, ie during the 30 min exercise plus the 30 min recovery period.

Statistical analysis

Plasma hormone and substrate concentrations and energy expenditure data were compared with ANOVA and paired *t*-tests with Bonferroni adjustment. Correlations between changes in plasma leptin concentration and 24 h EE were assessed using linear regression analysis.

Results

Compared to an isoenergetic diet, carbohydrate overfeeding led the next day in the postabsorptive state to a 28% increase in plasma leptin concentrations, a 24% decrease in plasma non-esterified fatty acid concentrations and a 59% decrease in plasma β-hydroxybutyrate concentration, but did not change the plasma concentrations of major glucoregulatory hormones, catecholamines and glucose. Fat overfeeding did not significantly affect any of these parameters (Table 3). Figure 1 shows the glucose and lactate concentrations in subcutaneous tissue interstitial fluid over 24 h periods. Both glucose and lactate showed three major peaks at 9:00 – 10:00 am, 1:00 pm and 7:00 pm, corresponding to postprandial periods. No significant difference was observed between the isoenergetic and the carbohydrate or fat overfeeding conditions.

Table 3 Basal plasma substrate and hormone concentrations

	Isocaloric diet	*Carbohydrate overfeeding*	*Fat overfeeding*
Glucose (mmol/1)	4.12 ± 0.07	4.40 ± 0.11	4.30 ± 0.04
Non esterified fatty acids (mmol/1)	0.495 ± 0.031	0.374 ± 0.035*	0.458 ± 0.030
β-hydroxybutyrate (mmol/1)	0.143 ± 0.023	0.077 ± 0.015*	0.148 ± 0.030
Insulin (mU/1)	9.8 ± 1.2	10.7 ± 1.4	10.0 ± 1.2
Glucagon (ng/1)	49 ± 3	48 ± 4	47 ± 4
Cortisol (nmol/1)	509 ± 78	459 ± 77	519 ± 86
Leptin (μg/1)	9.8 ± 1.7	12.5 ± 1.6*	10.9 ± 1.4
Epinephrine (pmol/1)	26 ± 4	26 ± 3	26 ± 3
Norepinephrine (pmol/1)	205 ± 22	207 ± 17	199 ± 13

*$P < 0.05$ *vs* isocaloric diet.

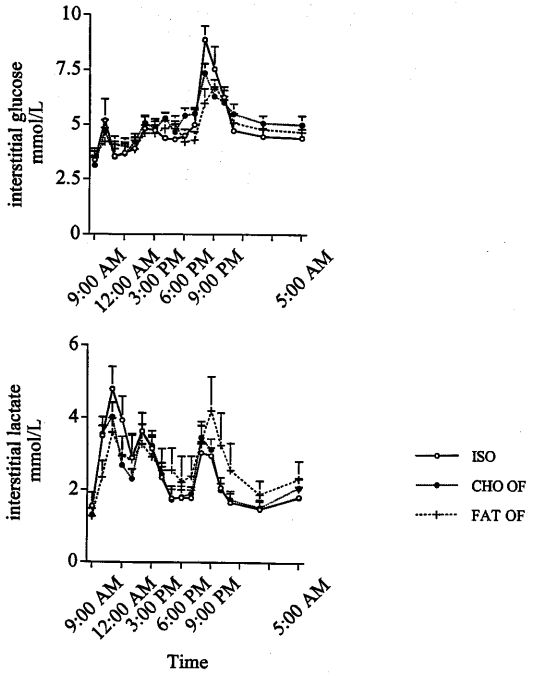

Figure 1 Interstitial glucose and lactate concentrations in subcutaneous adipose tissue in isonergetic conditions (ISO) or during carbohydrate (CHO OF) or fat (FAT OF) overfeeding.

Twenty-four-hour EE was increased by 7% after carbohydrate overfeeding compared to isoenergetic conditions ($P < 0.05$). Basal energy expenditure and the energy expended during physical activity were not affected by carbohydrate overfeeding. Suprabasal 24 h EE was increased by 31%, but the difference did not reach statistical significance ($P = 0.07$). Fat overfeeding did not significantly affect any component of EE (Figure 2). There was no correlation between changes in plasma leptin concentrations and changes in energy expenditure either expressed as absolute values (carbohydrate overfeeding $r^2 = 0.038$, fat overfeeding $r^2 = 0.040$), or relative values ($r^2 = 0.074$ and 0.051).

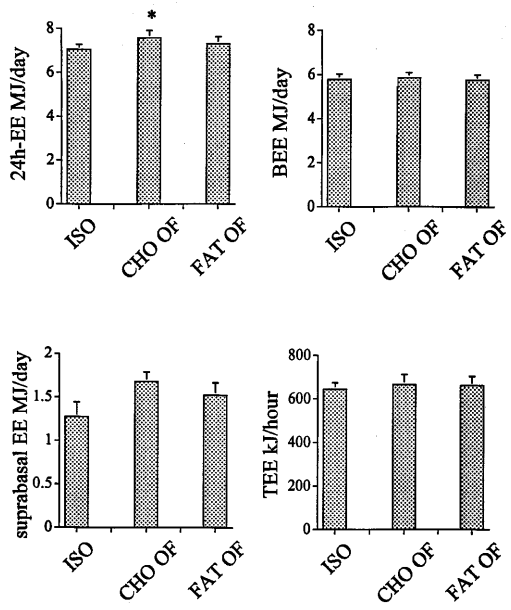

Figure 2 Twenty-four-hour energy expenditure (24 h EE), basal energy expenditure (BEE), suprabasal energy expenditure (suprabasal EE), and thermic effect of exercise (TEE) in isoenergetic conditions (ISO) and during carbohydrate (CHO OF) or fat (FAT OF) overfeeding. TEE was measured over 1 h, corresponding to 30 min exercise + 30 min recovery.

There was also no relationship between changes in leptin concentrations and suprabasal energy expenditure ($r^2 = 0.186$ during carbohydrate overfeeding and 0.249 during fat overfeeding).

Discussion

Several observations performed in humans have demonstrated that leptin secretion and leptin gene expression in adipocytes are regulated by nutrient balances. In a study performed in healthy humans, it was observed that massive overfeeding (120 kcal/kg body weight) increased plasma leptin concentrations within a 12 h period; prolonged overfeeding leading to a 10% weight gain further increased leptin concentrations up to three-fold. This increase was positively correlated with body fat gain, suggesting that it was primarily attributable to changes in body composition.[6] However, short-term fasting led to substantial reductions of plasma leptin concentrations within 24 h.[26] In both cases, plasma leptin concentrations rapidly returned to normal levels after the intervention periods.

The mechanisms responsible for changes in plasma leptin concentration during over- or underfeeding remain incompletely elucidated. The drop in plasma leptin concentration during fasting is concomitant with the rise in ketone body concentrations, but is not elicited by infusion of exogenous ketone bodies.[26] The fasting-induced drop in plasma leptin levels coincides with decreases in both plasma glucose and insulin concentrations and is prevented by glucose infusion.[27] However, overfeeding increases both plasma insulin and glucose concentrations and it is likely that the high plasma insulin levels stimulate leptin gene expression and secretion of leptin by the adipocytes. Insulin-stimulated glucose oxidation in adipocytes has been shown to regulate leptin secretion and therefore is also a candidate for stimulating leptin secretion during carbohydrate overfeeding.[19] In this study, we measured interstitial glucose and lactate concentrations in subcutaneous adipose tissue throughout a 24 h period. Fat or carbohydrate overfeeding did not alter these two parameters. However, it has to be kept in mind that interstitial glucose concentration does not provide information on the flux of glucose into adipocytes, which can be expected to have been increased during carbohydrate overfeeding. It is therefore likely that the absence of an increased interstitial lactate concentration during carbohydrate overfeeding was due to stimulation of glucose utilization and oxidation in the adipose tissue, with concomitant reduction of relative non-oxidative glucose disposal. In a recent study, it was observed that leptin concentration decreased in women who switched from a high-carbohydrate to a high-fat isoenergetic diet, and that this effect may have been related to decreased plasma glucose and insulin concentrations.[15] In the present study we observe that a short period (3 days) of carbohydrate overfeeding leads to a moderate 25% increase in plasma leptin concentration. This effect of carbohydrate overfeeding occurred without changes in interstitial adipose tissue concentrations of glucose or lactate. Postabsorptive plasma insulin, glucagon, cortisol and catecholamine concentrations, measured the morning after the 3 day tests, were not significantly altered by the three nutritional conditions. For practical reasons, it was not possible to obtain blood samples during the experimental days within the respiration chamber. Since carbohydrate overfeeding is known to stimulate insulin secretion, it is likely that hyperinsulinaemia was responsible for the increase in plasma leptin concentration.

Interestingly, fat overfeeding failed to stimulate leptin secretion; this condition does not stimulate insulin secretion, which probably accounts for the absence of leptin response. These results are consistent with the hypothesis of Havel *et al*, which suggests that high-fat feeding leads to an excessive spontaneous intake of energy due to a failure to elicit leptin-mediated suppression of food intake.[15]

Carbohydrate, but not fat overfeeding, led to a modest 7% (about 580 kJ/day) increase in 24 h EE. Of this 580 kJ/day increase in energy expenditure, about 150 kJ (5% of the energy content of excess carbohydrate) can be attributed to the obligatory thermic effect of the extra amount of carbohydrate intake. The rest remains unaccounted for. Basal energy expenditure and the energy expended during an imposed bout of exercise and its recovery period were not altered, indicating no change in the energetic

efficiency of exercise. This modest increase in 24 h EE may be attributed to the stimulation of the facultative components of dietary thermogenesis, or to an increase in the amount of energy expended in non-exercise physical activity. A recent report indicates that 8-weeks of overfeeding increases total energy expenditure while leaving basal energy expenditure, the thermic effect of food and the energy cost and physical activity unchanged.[5] In this study, this increase in energy expenditure was attributed to non-exercise physical activity, that is to voluntary movements not associated with exercise, but occurring throughout the day. It was also observed that this component of 24 h EE was inversely related to weight gain. It is therefore possible that carbohydrate overfeeding stimulates non-exercise physical activity through mechanisms which remain to be specified.

In contrast with the effects of carbohydrate overfeeding, fat overfeeding failed to increase significantly any component of energy expenditure. This may be partially explained by the fact that the obligatory energy cost of storing and processing fat is low compared to carbohydrate.[28] This difference may also be due to the lack of activation of sympathetic nervous system activity or to a lower stimulation of thyroid hormone secretion.

Several observations suggest that leptin administration increases energy expenditure when administered in fasted and leptin-deficient animals,[12,29] as well as in normal animals[10] (although prolonged periods of leptin administration are required in the latter case). In humans, a relationship between plasma leptin concentrations and energy expenditure has been observed.[30] Our present observation indicates that carbohydrate overfeeding increases fasting leptin concentrations, but not basal energy expenditure. There was also no correlation between changes in fasting leptin concentration and in 24 h EE. Since we did not measure plasma leptin concentrations throughout the day, we cannot exclude a relationship post-prandial leptin concentration and suprabasal energy expenditure. However, our results do not support the hypothesis of a leptin-driven stimulation of energy expenditure during carbohydrate overfeeding in humans. These conclusions are consistent with the recent report that a 9-y-old leptin-deficient girl had normal basal energy expenditure in regard of her body composition which did not increase after 12 months of leptin replacement therapy.[31] We cannot, however, discard the possibility that the important fat mass loss would have resulted in a decrease in energy expenditure which was prevented by leptin treatment. In a recent study,[5] Levine and collaborators observed that 24 hour energy expenditure was stimulated in healthy lean volunteers submitted to a period of overfeeding. This stimulation corresponded essentially to an increase in suprabasal energy expenditure, while the resting energy expenditure, the thermic effect of food and the energetic efficiency of skeletal muscle work were all unchanged, and was attributed by these authors to 'non-volitional physical activity'. Furthermore, stimulation of this component of energy expenditure showed considerable interindividual variability and was inversely correlated with weight gain. Further studies will be required to assess whether increased leptin production contributes to stimulate this component of energy expenditure during carbohydrate overfeeding.

In conclusion, our present observations show that carbohydrate, but not fat overfeeding, leads to modest increases in both plasma leptin concentrations and 24 h EE but no correlation was found between these two responses. Stimulation of leptin secretion occurred without alterations of interstitial glucose concentrations in adipose tissue. Hyperinsulinaemia or increased insulin-mediated glucose utilization in adipose tissue is likely to be involved. Stimulation of 24 h EE by carbohydrate overfeeding was not explained by alterations of basal energy expenditure or the energetic efficiency of exercise; it can be attributed to either stimulation of facultative dietary thermogenesis or to stimulation of the amount of energy expended in non-exercise physical activity. The present data do not support the hypothesis that stimulation of energy expenditure after carbohydrate overfeeding is mediated by leptin secretion in humans.

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