Regulation of average 24 h human plasma leptin level; the influence of exercise and physiological changes in energy balance

DPC van Aggel-Leijssen1*, MA van Baak1, R Tenenbaum2, LA Campfield2 and WHM Saris1

1Nutrition Research Centre (NUTRIM), Department of Human Biology, Maastricht University, Maastricht, The Netherlands and 2Hoffmann-La Roche, Department of Metabolic Diseases, Nutley, New Jersey 07110, USA

OBJECTIVE: The effects of short-term moderate physiological changes in energy flux and energy balance, by exercise and over- or underfeeding, on a 24 h plasma leptin profile, were investigated.

DESIGN: Subjects were studied over 24 h in four randomized conditions: no exercise/energy balance (energy intake (EI) = energy expenditure (EE) = 11.8 ± 0.8 MJ); exercise/energy balance (EI = EE = 15.1 ± 0.6 MJ); exercise/negative energy balance (EI = 11.8 ± 0.8 MJ, EE = 15.1 ± 0.8 MJ); exercise/positive energy balance (EI = 18.6 ± 0.7 MJ, EE = 15.1 ± 0.6 MJ).

SUBJECTS: Eight healthy, lean men (age: 23.5 ± 7.0 y, body fat 14.1 ± 5.4%, body mass index (BMI): 21.4 ± 2.3 kg/m²).

MEASUREMENTS: Blood was sampled every hour during the daytime (09.00 - 23.00 h) and every two hours during the night (01.00 - 09.00 h) for analysis of plasma leptin, insulin, glucose, FFA and catecholamines.

RESULTS: Plasma leptin levels were highest around 01.00 h (mean ± s.e.m. 4.9 ± 2.0 ng/ml) and lowest around 11.00 h (2.3 ± 0.7 ng/ml). An increased 24 h EE, induced by exercise under conditions of energy balance, significantly decreased the peak and average 24 h plasma leptin concentration. A positive energy balance, by overfeeding, resulted in a significantly higher amplitude of the 24 h plasma leptin curve, compared to a condition of energy balance.

CONCLUSION: Exercise decreases peak and average 24 h plasma leptin concentration and a moderately positive energy balance increases the amplitude of the 24 h plasma leptin profile. These effects are not acute, but are manifest within 24 h. The variations of average 24 h FFA and average 24 h glucose concentrations almost fully explained the variation in average 24 h leptin concentration across trials.

Keywords: energy expenditure; short-term; insulin; energy restriction; overfeeding

Introduction

Leptin, the product of the ob gene which has been cloned in mice1 and from human adipose tissue,2,3 is a hormone secreted by adipocytes. Leptin interacts with specific receptors in the brain, thereby altering food intake and energy expenditure (EE).4-7 Hypothalamic neuropeptide Y,8 melanocortin-4 receptor and melanocyte-stimulating hormone9,10 appear to be involved in the transduction of the leptin action in the brain. In rodents and humans, a strong positive correlation between serum leptin concentrations and percentage body fat has been found.11-14

Based on these and other findings, leptin is proposed to be an important factor in the regulation of energy balance.15 It provides the signal from the adipose tissue triglyceride stores to the regulatory centre in the brain that is involved in the regulation of food intake and EE. However, it is likely that leptin secretion is regulated by factors other than adipose tissue stores alone.15-17

Like many other endocrine hormones, leptin concentrations in humans show a clear diurnal pattern, with the highest plasma concentrations between midnight and early morning and the lowest around noon to midafternoon.18-20 In addition, ultradian oscillations in leptin secretion have been observed in humans.21 Plasma leptin levels exhibit a pattern indicative of pulsatile release with 32.0 pulses per 24 h and a pulse duration of 32.8 min.22 Schoeller et al20 indicated that acute sleep deprivation did not alter the diurnal variation of plasma leptin concentrations and that day/night reversal produced a rapid shift in the diurnal variation, which led, after three days, to the occurrence of the plasma leptin nadir 11 h earlier than during the control day. This indicates that the diurnal variation of leptin is unlikely to be controlled by the circadian clock.20 The study of Schoeller et al20 also indicated that the diurnal rhythm of plasma leptin is entrained to meal timing. Leptin levels continued their overnight decrease when breakfast was delayed.20

The effects of fasting (36 h) and massive overfeeding (12 h) on leptin have been studied in humans.16,23,24 Fasting resulted in a decrease in plasma leptin, overfeeding in increased leptin concentrations, indicating.

*Correspondence: Dorien P.C. van Aggel-Leijssen, Nutrition Research Centre (NUTRIM), Department of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands.
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that plasma leptin levels are regulated by short-term disturbances in energy balance (EB). However, no acute changes in leptin concentration have been found in relation to feeding. According to Considine, leptin concentrations can only be regulated by the extremes of fasting or massive overfeeding. Apart from changing energy balance, fasting and overfeeding also affect energy flux by affecting diet-induced thermogenesis. Therefore, it is possible that the effects of changes in energy balance on plasma leptin are mediated by changes in energy flux. Increases in energy flux can also be attained by exercise. However, no acute effects of exercise on plasma leptin concentrations have been found, but longer term effects have not been excluded. Therefore, we studied the effects of exercise in combination with moderate physiological changes in 24 h energy balance on 24 h leptin profile.

Methods

Subjects
Eight healthy, lean, male volunteers participated in this study. The subjects had a body mass index (BMI) of 21.4 ± 2.3 kg/m², a fat percentage of 14.1 ± 5.4% and a mean age of 23.5 ± 7.0 y. They were untrained (< 1 h/week sports activities). The study protocol was approved by the Ethics Committee of the University of Maastricht.

Study design
Prior to the actual study, basal metabolic rate (BMR) and maximal oxygen consumption (VO2 max) on a cycle ergometer were determined in each subject. EE on a day without exercise was estimated to be 1.5 × BMR in each subject and was 11.8 ± 0.8 MJ on average. During the exercise trials, each subject exercised at 50% of his VO2 max (corresponding work load 103 ± 7 W) for 2 h (4 times 30 min). In each subject, the extra energy cost of this exercise was estimated from the oxygen consumption and CO2 production at this work load minus BMR. The estimated 24 h energy expenditure on a day with exercise was 15.1 ± 0.6 MJ.

Four different experimental conditions, in which exercise (E) and/or energy balance (EB) were varied, were studied in each subject in randomized order with one week separating the trials (Table 1): no exercise/energy balance (E−/EB0), exercise/energy balance (E+/EB0), exercise/negative energy balance (E+/EB−) and exercise/positive energy balance (E+/EB+).

Experimental protocol
After an overnight fast, subjects reported to the laboratory at 08.30 h. An intravenous catheter was placed in an ante-cubital vein in the arm for blood sampling. At 09.00 h a fasting blood sample was drawn, after which, blood was sampled every hour (up to 23.00 h). Blood sampling was performed just before feeding or exercise bouts. Therefore, the next blood sample was taken one hour after the start of the meal and 30 min after the end of the exercise bout. Subjects went to bed at 23.00 h. During the night, blood was sampled every two hours without waking the subject. Subjects rose at 07.00 h the next morning. The last blood sample was obtained at 09.00 h.

The subjects received breakfast at 09.00 h, lunch at 13.00 h, dinner at 18.00 h and snacks at 11.00 h, 16.00 h and 21.00 h. In all trials, subjects received food with a macronutrient composition of 50% carbohydrates, 35% fat and 15% protein in each meal or snack. This macronutrient composition is according to the Dutch guidelines for healthy food intake, which resembles habitual Dutch food intake. When subjects were in EB, the energy intake (EI) was divided over the day as follows: breakfast 20%, lunch 30% dinner 30% and snacks 20%. The extra EI during the E+/EB0 and E+/EB+ trials was supplemented in the form of snacks and during the main meals. The snacks had the same macronutrient composition as described above. During the daytime, subjects were told to be as little physically active as possible, except for the cycling exercise, which was part of the protocol. Cycling was performed at 10.00 h 12.00 h, 15.00 h and 17.00 h.

The subjects went home 24 h after the start of the trial. They received food for that day (1.5 × BMR), which had the same composition as the food in the E−/EB0 trial and were instructed not to be physically active. They reported to the laboratory the next morning at 08.30 h, which was 48 h after the start of the trial. An intravenous catheter was again inserted and a blood sample was drawn at 09.00 h.

Experimental procedures

Body composition. Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volulograph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the formula from Siri.

BMR. BMR was measured by an open-circuit ventilated hood system (Oxycon β, Mijnhardt) while the
subjects were at rest after an overnight fast. EE was calculated by the abbreviated formula from Weir.31

Maximum aerobic capacity. One week before the experiment started each subject performed an incremental exercise test on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands) to determine the maximal O₂ uptake (VO₂ max). After a 5 min warming up at 100 W, workload was increased by 50 W every 3 min until heart rate was > 160 beats/min. Thereafter the workload was increased by 25 W every 3 min until exhaustion of the subject. During the test, oxygen consumption was measured continuously (Oxycon β, Mijnhardt). Heart rate was continuously recorded by a heart rate monitor (Polar, Almere, The Netherlands).

Analytical procedures. After blood sampling, the blood was immediately put into either an EDTA containing tube (for analysis of glucose, FFA and insulin) or a heparin containing tube with or without 100 μl glutathione solution (45 g/l saline) (for analysis of leptin or catecholamine concentrations, respectively). Plasma was separated, frozen and stored at −70°C until analysis. Leptin concentrations were measured in plasma using a double-antibody 'sandwich' ELISA assay using a monoclonal antibody specific for human leptin. This assay measures total (free and bound) leptin. The lower level of detection is 0.25 ng/ml and the upper limit is 50 ng/ml. The intra- and interassay variation are 9% and 12%, respectively. Plasma insulin concentrations were measured using a specific radioimmunoassay (RIA) using human insulin standards and radiolabelled porcine insulin.32 Plasma glucose levels were measured with a COBAS BIO centrifugal spectrophotometer using a glucose hexokinase kit (Hoffmann-La Roche, Basel, Switzerland), whereas plasma FFA levels were measured on a COBAS FARA centrifugal spectrophotometer using a non-esterified fatty acid colorimetric kit (Wako Chemicals, Neuss, Germany). The plasma catecholamine samples were pooled per 24 h trial in each subject. Plasma catecholamine concentrations were analysed by HPLC with electrochemical detection.35

Calculations. Weighted average 24 h plasma concentrations of leptin, insulin, FFA and glucose were calculated. The diurnal rhythm of the leptin concentration was quantitatively described with cosinor analysis, as described previously.34 Cosinor analysis was implemented using multiple regression to estimate the parameters of a cosine function. The function M + A cosine (0.262t + p), with a fixed period of 24 h (the duration of one complete rhythmic cycle), was fitted through all data to obtain individual parameter estimates (t= clock time). The following parameters were obtained: acrophase (p; time between reference time (0 h) and time of peak value), amplitude (A; half of the total predictable change in a rhythm) and mesor (M; average value of a cosine curve fitted to the data; the mesor and the average 24 h leptin concentration are equivalent).

Statistical analysis. Data are presented as means ± s.e.m. Differences between the E−/EB0 and E+/EB0 trials were analysed by the Wilcoxon signed ranks test. Differences among E+ trials were analysed with a nonparametric repeated measurements analysis of variance (Friedman test). Post-hoc pairwise comparisons between these trials were performed by the Wilcoxon signed rank tests; P-values were corrected for multiple comparisons according to Bonferroni’s inequalities. P < 0.05 was regarded as statistically significant.

Results

The plasma leptin profile showed a diurnal pattern in all trials (Figure 1, a and b). This pattern is the same for all trials and all subjects. There is a zenith around 01.00 h and a nadir around 11.00 h. The plasma leptin concentrations measured at zenith were significantly higher in all trials than those measured at nadir (P < 0.05). The plasma leptin concentrations at baseline (09.00 h) were not significantly different among trials.

The weighted average 24 h plasma leptin concentrations, as well as the mesor of the plasma leptin concentration estimated by cosinor analysis of the 24 h plasma leptin profile, were significantly lower in the E/EB trial (E+/EB0) than in the non E/EB trial (E−/EB0) (P < 0.05) (Table 2 and Table 3). The peak leptin concentration of the exercise trial (E+/EB0) (3.4 ± 3.9) was significantly lower than the peak of the non exercise trial (E−/EB0) (5.0 ± 5.6) (P < 0.05) (Figure 1a). Although the amplitude of the leptin concentration curve was 2-fold lower in the E+/EB0 trial than in the E−/EB0 trial, this difference failed to reach statistical significance (Table 2).

The absolute amplitude of the plasma leptin concentration curve in the positive energy balance trial (E+/EB+) was significantly increased compared to the energy balance (E+/EB0) and the negative energy balance (E+ /EB−) trial (2- respectively 3-fold) (P < 0.05) (Table 2). Although the weighted average 24 h leptin concentration and the mesor of the cosinor analysis were higher in the E+/EB+ trial than in the E+/EB0 and E+/EB− trials, these differences failed to reach statistical significance (Table 2 and Table 3).

No statistically significant difference among the four trials was observed with respect to the acrophase (Table 2), suggesting that manipulation of energy flux
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and/or EB did not cause a phase shift in the 24 h leptin concentration curve.

Figure 2, Figure 3 and Figure 4 show the plasma concentrations of glucose, insulin and FFA during the trials. The pattern of plasma glucose changes is not very consistent, but exercise and meals both appear to increase plasma glucose concentration (Figure 2, a and b). The plasma insulin concentration changes in a more reproducible manner, increasing after meals (Figure 3).

Exercise does not influence this pattern and a positive energy balance reduces the fluctuations during the day. The 24 h FFA profile (Figure 4) shows an increase in FFA levels after an exercise bout and higher FFA levels in the negative EB trial (E+/EB−).

Figure 1 Plasma leptin levels (ng/ml)±s.e.m. during 24 h and after 48 h for the (a) E−/EB0 (no exercise and energy balance) trial and the E+/EB0 (exercise and energy balance) trial and (b) for trials E+/EB− (exercise and negative energy balance) and E+/EB+ (exercise and positive energy balance) (n=8). *Significantly different from E−/EB0 (P<0.05).

Table 2 Mesor (ng/ml), amplitude (ng/ml) and acrophase (h)±s.e.m. of leptin for the different trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Mesor (ng/ml)</th>
<th>Amplitude (ng/ml)</th>
<th>Acrophase (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E−/EB0</td>
<td>3.21±1.09*</td>
<td>1.23±0.61</td>
<td>0.11±0.88</td>
</tr>
<tr>
<td>E+/EB0</td>
<td>2.56±1.15</td>
<td>0.61±0.17**</td>
<td>−0.82±0.62</td>
</tr>
<tr>
<td>E+/EB−</td>
<td>2.55±0.88</td>
<td>0.41±0.09**</td>
<td>−0.38±1.09</td>
</tr>
<tr>
<td>E+/EB+</td>
<td>3.04±1.00</td>
<td>1.29±0.42</td>
<td>−1.17±0.36</td>
</tr>
</tbody>
</table>

Significantly different from *trial E+/EB0 (P<0.05) and **trial E+/EB+ (P<0.05).

Table 3 Weighted average 24 h concentrations of leptin (ng/ml), insulin (ng/ml), FFA (mmol/L), glucose (mmol/L), noradrenaline (ng/l) and adrenaline (ng/l)±s.e.m. for the different trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Leptin (ng/ml)</th>
<th>Insulin (ng/ml)</th>
<th>FFA (mmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>Noradrenaline (ng/l)</th>
<th>Adrenaline (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E−/EB0</td>
<td>3.18±1.10</td>
<td>1.24±0.21</td>
<td>309.7±22.7</td>
<td>4.82±0.08</td>
<td>349.2±28.5</td>
<td>30.4±2.8</td>
</tr>
<tr>
<td>E+/EB0</td>
<td>2.53±1.14*</td>
<td>1.29±0.17</td>
<td>358.8±33.3</td>
<td>4.88±0.08</td>
<td>364.6±36.6</td>
<td>30.1±2.1</td>
</tr>
<tr>
<td>E+/EB−</td>
<td>2.51±0.88</td>
<td>1.21±0.18</td>
<td>358.8±23.7</td>
<td>4.85±0.07</td>
<td>377.9±24.9</td>
<td>40.6±3.4</td>
</tr>
<tr>
<td>E+/EB+</td>
<td>3.03±1.00</td>
<td>1.52±0.19****</td>
<td>270.5±18.0</td>
<td>4.99±0.12</td>
<td>324.9±19.9</td>
<td>30.0±1.4***</td>
</tr>
</tbody>
</table>

Significantly different *from E−/EB0 (P<0.05), **from E+/EB0 (P<0.05) and ***from E+/EB− (P<0.05).
Table 3 shows that the average plasma insulin concentrations in the EB (E+/EBO) and negative EB trial (E+/EB-) were significantly lower than in the positive EB trial (E+/EB+) \((P < 0.05)\), whereas the 24 h plasma FFA concentration in the EB trial (E+/EBO) was significantly higher than in the positive EB trial (E+/EB+) \((P < 0.05)\). The average 24 h plasma adrenaline concentration was significantly higher during the E+/EB- than the E+/EB+ trial \((P < 0.05)\). Simple regression analysis showed that most of the variation in average 24 h plasma concentration of leptin across trials was explained by the average 24 h FFA concentration \((r= -0.85)\). Correlations between leptin and glucose, insulin, noradrenaline and adrenaline were 0.14, 0.39, -0.79 and -0.59, respectively. Stepwise regression analysis showed that the combination of plasma FFA and glucose explained 98% of the variation in leptin across trials.

**Discussion**

Serum leptin concentration is proportional to the amount of adipose tissue.\(^{10-14}\) However, serum leptin concentration has been shown to be regulated by other factors as well, independent of fat mass. Considine\(^{12}\) concluded recently that leptin can be regulated by EI, but only by the extremes of fasting or massive overfeeding, since leptin levels do not change acutely after consumption of a normal meal. However, since it appears that changes in leptin levels are mediated by changes in ob gene expression in adipose tissue,\(^{11-13}\) it may take more time for changes in plasma leptin concentration to take place. Plasma leptin concentrations may not only be related to EI, but also to changes in energy flux. Therefore we studied the effect of moderate changes in EB, modulated by EI and expenditure, on 24 h plasma leptin concentrations.

As in other studies,\(^{11,19,25}\) we did not find evidence for an acute effect of meals on leptin concentration, although our study was not specifically designed to study this issue. This would have required another protocol with more frequent blood sampling around the meals. This study, as well as others,\(^{18,20,21,35}\) clearly demonstrated a diurnal variation in plasma leptin concentration with the lowest levels during the day and the highest during the night.

The main finding of the present study is that an approximately 28% increased 24 h EE, induced by
exercise under conditions of EB, significantly decreased mesor and average 24 h plasma leptin levels by 20%. There is a slight difference between the mesor and the weighted average 24 h plasma leptin concentration, since the mesor is measured from the cosine fit of the plasma leptin curve and the average indicates an average of measured leptin concentrations. Previous studies have reported the absence of an acute effect of exercise on plasma leptin concentration.14,26,27 In addition, Pérusse et al26 reported no effects of exercise training on plasma leptin, independent of changes in fat mass. The results of our study indicate that an increased EE decreases plasma leptin secretion, but that this effect is delayed. The effect can be detected over a 24 h period, but not immediately after the exercise bout.

A disturbance in EB, induced by overfeeding on a day of relatively high physical activity, increased the absolute amplitude of the cosine fit of the 24 h plasma leptin curve significantly, whereas the mesor and average 24 h leptin concentration showed a tendency to increase. In a condition of underfeeding on a day with a relatively high physical activity, no significant effect on average plasma leptin levels or amplitude was detected. However, the amplitude of the 24 h plasma leptin curve showed a tendency to decrease and, because of the small number of subjects, a type II error can not completely be excluded. However, the calculated power of the statistical test was ± 80%. The question of whether a negative energy balance affects plasma leptin profile on a rest day cannot be answered, because this condition was not studied.

In contrast to the statement of Considine17 (see above), the results of this study show that leptin can be regulated by moderate increases in EB and exercise. The effect is detectable over a 24 h period, rather than acutely or by fasting leptin levels only. This implies that short term changes in EB and energy flux during the day, are associated with a change in leptin profile over 24 h. The most prominent effects on plasma leptin concentration are seen during the night. These changes may contribute to the achievement and maintenance of energy homeostasis over days under these conditions.

Changes in energy balance are associated with changes in energy flux: EE will be higher under positive EB conditions than under negative EB conditions. The results of this study show that an increase in EE induced by exercise is associated with a reduction of peak, mesor and average 24 h plasma leptin concentration. It is therefore unlikely that the increased leptin concentration during a positive EB is due to the increased energy flux associated with this condition.

Several factors, such as beta-adrenergic nervous system stimulation, insulin and glucocorticoids, adipocyte lipolysis and glucose metabolism have been shown to affect plasma leptin concentration in humans. Two hours of beta-adrenoceptor stimulation by infusion of isoprenaline reduces plasma leptin concentration.38 A strong positive correlation between fasting plasma leptin and fasting plasma insulin concentration37,38 and between 24 h plasma leptin and insulin concentration35 has been reported.

Prolonged exposure to high insulin concentrations in a hyperinsulinemic euglycaemic clamp has been found to increase plasma leptin concentration24,38,39 It has been concluded that this effect is not mediated by changes in plasma FFA levels,24 because infusion of Intralipid did not (acutely) affect leptin concentration. Short-term insulin administration (2–3 h) does not lead to acute changes in plasma leptin.38–40 However, the effect on 24 h plasma leptin concentration has not been studied. The role of cortisol is not fully clear. Cortisol has been found to be inversely related to leptin concentrations25 and rhythms in humans.30,35 On the other hand, the diurnal rhythm of plasma leptin demonstrated a very rapid phase shift, relative to plasma cortisol during simulated jet lag.20 Glucocorticoid treatment did not affect fasting leptin concentration,41 but increased 24 h leptin secretion.42 The reduction in leptin concentration during fasting was shown to be correlated with parameters that reflect decreased glucose availability and increased lipolysis.43 Our data support these findings: average 24 h leptin concentration across trials showed the highest correlation with average 24 h plasma FFA concentration. The combination of FFA and glucose concentration explained almost fully the variation in plasma leptin across trials.

Conclusion

The present study shows that exercise decreases the peak and average 24 h plasma leptin concentration and that a positive EB in combination with relatively high physical activity mainly increases the amplitude of the plasma leptin profile. These effects are not acute, but are manifest within 24 h. The variations of average 24 h FFA and average 24 h glucose concentrations almost fully explained the variation in average 24 h leptin concentration across trials.

References


