Leptin responses to physical inactivity induced by simulated weightlessness

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Received 28 September 1999; accepted in final form 31 March 2000

Space flight induces endocrine and metabolic changes. This altered metabolism affects the body composition of astronauts and the energy requirements necessary to maintain their health. Total body water (TBW), lean body mass (LBM), and fat mass are lost while the subjects are in negative energy balance due to a reduction in energy intake and no reduction in energy expenditure (12, 30). Moreover, the living conditions in space, i.e., isolation and confinement, appear to produce stress, which in turn affects fuel homeostasis. Recent in-flight data have shown that endocrine perturbations are involved in the body composition changes (31). This involved thyroid hormones, prostaglandins, and catecholamines, whereas the role of cortisol, growth hormone (GH), and insulin appeared not certain, at least in part, in the muscle loss. These responses are dependent on the transient metabolic stress observed earlier in flight and the severity of the negative energy balance (29, 30). During head-down bed rest (HDBR), the most commonly used ground-based simulation model of weightlessness, our group (2) has observed that body composition was altered although the subjects were in energy balance due to a concomitant reduction in energy intake and expenditure related to physical activity. LBM dropped, and fat mass increased (despite an overall stability of fat mass) in the non-load-bearing segments of the body (legs and trunk). The hormonal responses, cortisol, GH, and catecholamines, suggested as in-flight that a transient metabolic stress participates in these modifications. Overall, although a growing body of data raises evidence for an endocrine participation in the deleterious adaptations to actual or simulated microgravity, these hormones alone cannot explain the underlying mechanisms, thus suggesting the implication of other factors.

The leptin hormone may be one of these factors. It is principally produced by fat mass and has numerous biological effects, such as cardiovascular and neuroendocrine regulation or cell differentiation. However, the principal function is the regulation of energy stores and body composition through centrally and peripherally mediated effects on ingestive behavior and metabolism (reviewed in Ref. 17). Several factors, gender dependent, are involved in the regulation of leptin levels. Among these, insulin, corticosteroids, nonsterified fatty acids (NEFA), catecholamines, and food intake have been implicated, but little is known about the role of physical activity (17). Effectively, no meaningful acute or chronic effects of exercise independent of the amount of body fat were induced (4, 7, 9, 11, 21, 22).

The leptin responses to simulated and actual microgravity are unknown. Therefore, the question of the
leptin implication in the microgravity-induced body composition and fuel homeostasis changes remains to be answered. This study was carried out to determine the leptin and counterregulatory hormone responses to 7 days of HDBR and to dissociate putative gender differences. Because HDBR is a model of physical inactivity, such an experiment has relevant clinical implications. Effectively, sedentary lifestyle has been implicated in the onset of some pathology such as insulin resistance, obesity, and glucose intolerance (reviewed in Ref. 15). However, the basic mechanisms due to inactivity have been poorly investigated.

MATERIALS AND METHODS

Subjects

A group of 16 healthy, normally active Caucasian subjects (8 males and 8 females) volunteered for this study. Their anthropometric data are summarized in Table 1. The subjects were free of clinical or biochemical disease and did not take any medications 3 mo before the study. Within the same period, women were required to stop any oral contraception. Subjects were thoroughly briefed about the experimental procedures and signed a consent form approved by the Comité Consultatif de Protection de Personnes dans les Recherches Biomédicales Midi-Pyrénées I (France). No medication, smoking, alcohol, or caffeinated drinks were allowed during the study. The experimental conditions were well tolerated by all volunteers.

Protocol

Two HDBRs were realized at 1-yr intervals, one in males and the other in females. The studies were divided into three periods. In the first period, all subjects resided at the bed-rest facility at Clinique de l’Espace (Hôpital de Rangueil, Toulouse, France) for 4 days of ambulatory control period (control day −5 to day −1). The subjects then underwent 7 days of (−6°) HDBR. Finally, the subjects remained in the hospital for 4 days of ambulatory recovery period.

Volunteers were fed with weighed conventional foods ad libitum. Dietary records were kept for calculation of nutrient intakes using the Regal software (Geni, Micro 6, Nancy, France) and the French Food Tables published by the Centre Informatique sur la Qualité des Aliments (6). A senior dietitian ensured the accuracy of the diet records. Dietary sodium was limited to 3 g/day and water intake to 2.5 l/day.

Daily Hormone Measurements

Plasma levels of leptin, insulin, epinephrine (Epi), norepinephrine (NE), GH, cortisol, glucose, and NEFA were measured in the fasting state at 0700 and in the supine position. Blood was sampled daily in males, but for ethical reasons, blood withdrawal was reduced in females and was performed every 2 days.

Leptin measurements were performed using a Human-Leptin-RIA-sensitive kit (Mediagnost, Tübingen, Germany), which has a sensitivity of 0.01 ng/ml. The intra-assay variation was lower than 5%, and the interassay variations did not exceed 7.6%. GH assay was performed with a GH IRMA kit (Immunotech, Marseille, France) having a sensitivity below 0.05 μg/l and an intra- and interassay of 1.15 and 13.5%, respectively. Plasma corticosterone measurement was performed by HPLC with ultraviolet detection using a classical method (20) modified in the laboratory. Under these conditions, the intra- and interassay variation coefficients were 1.97 and 6.98%, respectively, and the sensitivity was below 1 ng/ml. The other hormonal and metabolite assays were performed by classical, previously described, methods: Epi and NE by HPLC with electrochemical detection (25), insulin by RIA (1), and glucose and NEFA by enzymatic methods (1).

Body Composition

Body composition was determined before (control day −4) and during HDBR (HDBR day +6) using the isotope dilution method. After blood samples required for hormone and metabolite measurements were taken, the subjects provided baseline saliva samples and then drank 0.9 g/kg H218O 2% on control day −4 and 0.7 g/kg H218O 10% (Isotec, St Quentin en Yvelines, France) on HDBR day +6. Two different enrichments were used to avoid differences in background enrichment due to the first probe. Then salivary samples were collected 3, 4, 5, and 6 h after ingestion of labeled water to optimize the equilibration plateau determination of isotope with body fluids. Samples were stored at −20°C in cryogenically stable tubes until analysis by isotope ratio mass spectrometry at the Centre de Recherche en Nutrition Humaine de Lyon, as previously described (2). The TBW (liters) was

<table>
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<tr>
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<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td></td>
<td>Control day −4</td>
<td>HDBR day 6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>32.4 ± 1.9</td>
<td>27.9 ± 0.9</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.77 ± 0.99</td>
<td>1.64 ± 0.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.94 ± 0.69</td>
<td>23.90 ± 0.9</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>74.81 ± 2.76</td>
<td>75.08 ± 2.69</td>
</tr>
<tr>
<td>Total water</td>
<td>liters</td>
<td>%</td>
</tr>
<tr>
<td>%</td>
<td>60.21 ± 0.87</td>
<td>59.48 ± 1.00</td>
</tr>
<tr>
<td>Lean mass</td>
<td>kg</td>
<td>%</td>
</tr>
<tr>
<td>%</td>
<td>61.40 ± 1.96</td>
<td>60.82 ± 1.79</td>
</tr>
<tr>
<td>Fat mass</td>
<td>kg</td>
<td>%</td>
</tr>
<tr>
<td>%</td>
<td>13.42 ± 1.23</td>
<td>14.26 ± 1.38</td>
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Values are means ± SE; n = 8 men and 8 women. HDBR, head-down bed rest; BMI, body mass index; %, percentage of body mass. No significant differences were observed by repeated-measure analysis of variance (RM-ANOVA).
Table 2. Dietary intake

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Men</th>
<th>Women</th>
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<tr>
<td></td>
<td>Control</td>
<td>HDBR</td>
</tr>
<tr>
<td>Energy intake, MJ/day</td>
<td>10.5 ± 0.2</td>
<td>10.3 ± 0.3</td>
</tr>
<tr>
<td>Water, g/day</td>
<td>3,088 ± 91</td>
<td>2,861 ± 66</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>51 ± 1</td>
<td>51 ± 1</td>
</tr>
<tr>
<td>Fat %</td>
<td>84 ± 2</td>
<td>86 ± 2</td>
</tr>
<tr>
<td>Protein g/day</td>
<td>114 ± 2</td>
<td>109 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 men and 8 women. %, percentage of daily energy intake.

determined from the dilution space of 18 oxygen after adjusting it by a factor of 1.01 to account for isotope exchange. Because our group previously observed that the hydration coefficient of the LBM was maintained during HDBR near the expected value of 73.2% (2), LBM and fat mass were calculated from TBW and body mass.

Statistical Analysis

Each subject acted as his own control, with the pre-HDBR ambulatory period taken as the control period. The effects of HDBR were evaluated by a repeated-measure analysis of variance with time as the variable. Fisher's protected least-significant differences test was used for post hoc comparisons. All analyses were performed with StatView (Abacus Concepts, Berkeley, CA, 1992), and reported values are means ± SE, with P < 0.05 considered to be statistically significant.

RESULTS

Body Composition

Bed rest did not induce any significant modifications in body mass (Table 1). TBW, LBM, and fat mass also were similar before and after the bed rest, expressed either in kilograms or in percentage of body mass.

Energy and Dietary Intake

Energy and nutrient intakes, as the macronutrient composition of the diet, were unchanged during the bed rest (Table 2).

Hormones and Metabolites

The hormonal and metabolite data were averaged within each period to clarify the results. Moreover, the statistical level of significance (P < 0.05) in males was unchanged when data were normalized to the number of samples realized in females. We will not detail the well-known and described basal gender differences in hormones and metabolites that are not the purpose of this study.

Leptin. The individual plasma leptin evolution is represented by period (control, HDBR, and recovery) in Fig. 1. The average pattern of leptin response to HDBR is represented in Fig. 2. In men, the plasma levels of leptin increased significantly by 40% during HDBR (P = 0.003). In the recovery period, the hormone levels were also 34% higher than in the control period (P = 0.021), and therefore no normalization was observed. In women, HDBR induced a 20% increase in the leptin levels (P = 0.050). Conversely to men, the levels were not significant during the recovery period (P = 0.204).

Insulin and insulin-to-glucose ratio. Insulin was increased during HDBR in both sexes (men 34%, P = 0.018; women 26%, P = 0.022), as was the insulin-to-glucose ratio (men 30%, P = 0.049; women 25%, P = 0.031) (Fig. 2). The insulin levels were not normalized during recovery in men and stayed 33% higher than in the control period (P = 0.040).

Glucose and NEFA. HDBR did not induce changes in the glucose levels in men (P = 0.29) but induced a hypoglycemia in women (6%, P = 0.001) that was not normalized during the recovery period (6%, P = 0.001) (Fig. 3). Men's and women's NEFA concentrations were lower during both HDBR (men 37%, P = 0.004; women 25%, P = 0.033) and the recovery period (men 38%, P = 0.004; women 30%, P = 0.005).

Catecholamines, cortisol, and GH. In men, HDBR did not result in changes in plasma concentrations of Epi (P = 0.170), NE (P = 0.096), and cortisol (P = 0.621) (Table 3). The results were similar in women, i.e., no changes in Epi (P = 0.063), NE (P = 0.404), and cortisol (P = 0.104) were noted. No changes in the GH plasma concentrations were observed during HDBR in men (P = 0.051) and women (P = 0.897).

Correlation Analysis

Analysis of correlation was performed in both sexes during HDBR. However, to assess nontruncated gender comparison, correlation in men was performed in data adjusted for number of samples performed in women. We should mention that such an operation did not modify the results (data not shown). In these conditions, significant correlation was observed in men between leptin and insulin (r = 0.657, P = 0.011), insulin-to-glucose ratio (r = 0.681, P = 0.007), energy intake (r = 0.791, P = 0.001), and GH (r = 0.438, P = 0.032). Nonsignificance was obtained with NEFA, glucose, NE, Epi, and cortisol. Conversely, none of the above variables correlated with leptin in women: insu-
Leptin and Head-Down Bed Rest

**DISCUSSION**

HDBR reproduces the hypokinesia, hypodynamia, and thoracocephalic fluid shift observed in space and therefore induces LBM loss, body fat mass gain, and cardiovascular alterations (3). Recent in-flight data suggest that anabolic and catabolic endocrine relationships may have a role in the body composition changes (31). Leptin, the product of the ob gene, has numerous biological effects, among which regulation of energy stores is the most important. This hormone is regulated by complex loops involving intake behavior, catecholamines, cortisol, GH, and insulin (17). To our knowledge, no studies have examined the extent to which systemic leptin levels may be altered in the context of the chronic perturbations in energy expenditure associated with physical inactivity, and HDBR experiments offer a unique opportunity to study such adaptation.

**Leptin Circadian Rhythms and Blood Sampling Time**

Leptin exhibits a circadian rhythm with a peak observed in the early morning hours and the nadir in the afternoon (28). Therefore, it has been suggested that measurement of a 24-h profile of leptin secretion is a better indicator than a single daily sample. Bed-rest studies are extremely heavy experiments, and 24-h measurements are impossible (amount of blood and time required). However, to limit such variations, special attention has been taken to respect the blood withdrawing hour (0700). Moreover, a recent study has shown that fasting plasma leptin levels measured over 1 mo between 0700 and 0800 are reproducible in healthy, free-living lean and obese persons who maintain a stable body weight (13). Therefore, changes in leptin levels triggered during this study were not due to circadian variations.

**Body Composition and Energy Intake**

We did not observe changes in body composition after 7 days of HDBR; conversely, longer bed rest induced loss of lean mass and redistribution of fat mass in the non-load-bearing parts of the body (2). The $H_{2}^{18}O$ dilution method did not allow us to determine such regional changes. However, the main conclusion is that fat mass and LBM overall are not altered after 7 days of bed rest. In the same way, the energy intake expressed either in kilojoules or in percentage of daily intake was unchanged, suggesting that 7 days of bed rest did not induce changes in the macronutrient composition of the diet. This is of special importance in studying the effects of physical inactivity in the absence of concomitant changes in adipose tissue mass and energy intake.

**Responses of Leptin and Counterregulatory Hormones to HDBR**

In this study, we report that plasma leptin concentrations increased significantly during HDBR in both sexes and that no normalization was observed during the recovery period in men. In men, leptin levels were strongly correlated with insulin concentrations and insulin-to-glucose ratio (index of insulin resistance). Interestingly, in women, both insulin and insulin-to-glucose ratio increased, but no correlation was observed with leptin. Resistance to the insulin effects was previously reported during bed rest (5). It was due to physical inactivity, because a single bout of daily exercise restored the sensitivity of insulin to control values. Insulin seems to influence leptin levels in the long term, independently of age (21) or glucose tolerance status (14) by either stimulating leptin mRNA expression and protein release or exercising a trophic effect on the adipose tissue (23). As a result, there is a significant correlation between plasma leptin and fast-
ing insulin in cross-sectional studies on normal subjects, even after adjustment for overall adiposity (8). However, whether hyperinsulinemia accompanying insulin resistance is associated with higher leptin levels in humans is unclear and still remains under debate, but our results demonstrate that hyperleptinemia is strongly linked to a decrease in insulin sensitivity, at least in part, during physical inactivity and simulated weightlessness. In support of this, it has been reported that leptin is associated with insulin resistance only in men (8). In the same way but in another context, Segal et al. (27) observed that hyperinsulinemia was associated with higher plasma leptin levels in severe insulin-resistant patients with other insulin-resistant states such as non-insulin-dependent diabetes mellitus. The development of insulin resistance during bed rest could be the onset signal for the increase in leptin concentrations observed during our study. Because a stronger correlation was observed between leptin and insulin-to-glucose ratio rather than with insulin, we could hypothesize that prolonged inactivity-induced insulin resistance could lead to the leptin resistance, as hypothesized in obesity. This issue is only speculative and warrants further studies. Metabolites, glucose, and NEFA decreased during both HDBR and recovery. These changes must be linked with the higher insulin levels. However, glucose in men was not modified. These results suggest that HDBR both in women and in men induce insulin resistance but that peripheral uptake of glucose and NEFA is less altered in women than in men.

**Fig. 2.** Time course of leptin (A), insulin (B), and the insulin-to-glucose ratio (C) during the control, HDBR, and recovery periods. The values are expressed as means ± SE with n = 8 males and 8 females. Repeated-measure (RM) ANOVA protected least-significant difference (PLSD) Fisher’s test results: *P < 0.05 vs. control; **P < 0.01 vs. control.
Plasma cortisol, a known stimulator of leptin secretion (17), was unchanged throughout the experiment. For longer bed rest, urinary excretion of cortisol has been shown to increase after 1 wk of bed rest (2), whereas during space flight, corticoid increased earlier in flight. Vernikos et al. (32) have shown that, during a 7-day HDBR, urinary excretion of cortisol increased in men but not in women. This point is not observed in this study, but the use of punctual plasma measurements instead of daily 24-h urine samples limits the comparison of the two studies. However, cortisol's implication in the body composition changes is still a matter of debate (31). Plasma catecholamines also were not modified, conversely to the urinary excretion that is well known to decrease during bed rest (2, 3). GH decreased nonsignificantly in males during the study (despite being close to the level of significance). The increase in ob protein could be partly due to a reduced inhibition of GH on leptin secretion, as previously described (17), and this is supported by the correlation analysis. No changes were noted in females, but the interindividual variations strongly limit the interpretation of the results. This hormone was found unchanged during space flight (31) and increased during bed rest (2).

**Leptin and Exercise Training**

It is well known that three major factors modulate body weight: 1) metabolic factors, 2) diet, and 3) physical activity, and each is influenced by genetic traits. Our results, as diverse trends of decreasing energy intake and increasing body weight, suggest that reduced physical activity may be the most important factor for body composition regulation. Some authors have postulated that energy expenditure due to physical activity may also be a regulatory signal for leptin production through several potential mechanisms, such as changes in insulin sensitivity, fatty acid concentrations, or alterations in sympathoadrenal activ-

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**Table 3. Daily hormone levels**

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<th>Men</th>
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<th>Women</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HDBR</td>
<td>Recovery</td>
<td>Control</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>63 ± 9</td>
<td>53 ± 7</td>
<td>52 ± 6</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>266 ± 24</td>
<td>236 ± 24</td>
<td>244 ± 31</td>
<td>282 ± 31</td>
</tr>
<tr>
<td>Cortisol, mmol/l</td>
<td>193 ± 27</td>
<td>197 ± 32</td>
<td>179 ± 17</td>
<td>184 ± 9</td>
</tr>
<tr>
<td>Growth hormone, µg/l</td>
<td>0.28 ± 0.06</td>
<td>0.16 ± 0.05</td>
<td>0.16 ± 0.06</td>
<td>0.30 ± 0.93</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 men and 8 women. No significant differences were observed by RM-ANOVA.
Prolonged and strenuous exercise, such as marathon or ultramarathon training, may decrease leptin concentration (11). However, daily physical activity is not associated dependently with circulating leptin in normal males (16), and physiological studies have failed to reveal changes of leptin levels in response to moderate-intensity aerobic capacity in males (4, 9, 21, 22). In contrast, this type of exercise may dependently affect leptin levels in females (7). In addition, leptin may be independently associated with resting and total energy expenditure in females and possibly children but not in males (6, 10, 19, 26). Thus the relationship between exercise and leptin is extremely complex but appears to be modified by gender and, according to our data, by physical inactivity.

In conclusion, 50 years ago, Mayer et al. (18) hypothesized that the mechanisms controlling energy balance are accurate in persons with higher levels of physical activity, but in sedentary persons there is a threshold of physical activity below which these mechanisms become imprecise and result in overweight. During this 7-day HDBR, we observed that physical inactivity in men and women induced an increase in leptin levels independently of changes in fat mass, in dietary intake, or of a stress response. This increase was linked with the development of insulin resistance in men. These results are based on small changes in leptin levels and have to be considered as preliminary, but they are the first report of ob protein response to inactivity.

Perspectives

Understanding how physical inactivity alters body composition and energy requirements is of paramount importance, because it is implied in the onset of several pathologies related to an increased sedentary lifestyle. Therefore, HDBR experiments have relevance not limited only to space medicine. An interesting finding in our study is the correlation between leptin levels and energy intake in men. Energy intake is known to decrease during longer bed rest (2, 30), a phenomenon resulting from a spontaneous behavior. This phenomenon observed in flight, associated with space motion sickness, is partly attributed to the development of a negative energy balance (29, 30). Leptin is known to reduce food intake (17), and the increased leptin levels observed in this study, if continued, may be involved in the reduction of the energy intake observed for longer bed-rest periods and in flight. Further studies are required to delineate how the inactivity-induced increase in leptin levels may respond during longer bed rest and how it may be related to the well known 1) decrease in energy intake and 2) body composition changes.

The authors thank Dr. John Carew for English editorial assistance. S. Blanc is a fellow of the Centre National d’Etudes Spatiales.

This work was supported by grants from the Centre National d’Etudes Spatiales.

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