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LEPTIN, GASTROINTESTINAL AND STRESS HORMONES IN RESPONSE TO EXERCISE IN FASTED OR FED SUBJECTS AND BEFORE OR AFTER BLOOD DONATION

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Leptin, an ob gene product of adipocytes, plays a key role in the control of food intake and energy expenditure but little is known about leptin response to strenuous exercise in fasted and fed subjects or before and after blood donation. This study was designed to determine the immediate effects of strenuous exercise in healthy volunteers under fasting or fed conditions and before and one day after blood donation (450 ml) on plasma levels of leptin and gut hormones [gastrin, cholecystokinin (CCK), pancreatic polypeptide (PP) and insulin], as well as on "stress" hormones (cortisol, catecholamines and growth hormone). Two groups (A and B) of healthy non-smoking male volunteers were studied. All subjects performed incremental exercise tests until exhaustion (up to maximal oxygen uptake — \( \text{VO}_{2\text{max}} \)), followed by 2 h of rest session. Group A performed the tests on a treadmill, while group B on a cycloergometer. In group A, one exercise was performed under fasting conditions and the second following ingestion of a standard liquid meal. In group B, one exercise test was performed as a control test and the second 24 h after blood donation (450 ml). Blood samples were withdrawn 5 min before the start of the test, at the \( \text{VO}_{2\text{max}} \), and 2 h after finishing the exercise. No significant change in plasma leptin were observed both immediately and 2 h after the exercise in fasted subjects, but after the meal the plasma leptin at \( \text{VO}_{2\text{max}} \) and 2 h after the test was significantly higher, while after blood donation was significantly reduced. The postprandial rise in plasma leptin was accompanied by a marked increment in gut hormones; gastrin, CCK and PP and stress hormones such as norepinephrine, cortisol and GH. These hormonal changes could contribute to the postprandial rise in plasma leptin concentrations, while the fall of leptin after blood donation could be attributed to the inadequate response of stress hormones and autonomic nervous system to exhausting exercise. We conclude that strenuous physical exercise: 1) fails to affect plasma leptin level but when performed after meal but not after blood withdrawal it results in an increase and fall in plasma leptin, and 2) the release of gut hormones (gastrin, CCK and PP) and stress hormones (norepinephrine, cortisol, GH) increase immediately after exercise independently of feeding or blood donation and 3) following blood donation the strenuous exercise resulted in a marked reduction in the plasma leptin, cortisol and GH concentrations, possibly due to the impairment in the autonomic nervous control of these hormones.

Key words: exercise, leptin, gastrin, pancreatic polypeptide, insulin, catecholamines, growth hormone, blood donation.
INTRODUCTION

It is well known that body mass is determined by the balance between food (caloric) intake and energy expenditure, both being regulated by neurohormonal mechanisms. Since 1994, when leptin, an ob gene encoded protein, was discovered by Zhang et al., (1) numerous studies attempted to determine the role of this hormone in the control of caloric intake, body mass and energy expenditure (2, 3). Fasting, which is known to reduce energy expenditure, was reported to decrease plasma concentrations of leptin (4, 5) and the short- or long-term decrease in body weight and body fat content, resulting from the limitation of caloric intake have been correlated with the reduction in plasma levels of this hormone (6). Little information is available, however, regarding the short-term effects of feeding on plasma leptin release in humans.

Unlike fasting, exercise, ranging from moderate to exhausting, failed to cause in immediate alterations in plasma leptin level (7—9) though when the hormone level was measured at least two hours after strenuous exercise, a significant decrease in this level was recorded in the postexercise recovery (10). No data so far are available concerning the influence of strenuous exercise, feeding or their combination and blood withdrawal on plasma leptin and gut hormones such as gastrin, CCK, pancreatic polypeptide (PP) and insulin and on stress hormones including catecholamine, cortisol and growth hormone (GH).

The aim of this study was to investigate whether plasma leptin level might be modified by strenuous exercise without or with feeding and without or with blood withdrawal and what is the relationship of leptin to other hormones of the gut as well as to stres hormones.

METHODS

Subjects

Two groups (A and B) of healthy non-smoking males volunteers were included into the study. The study was reviewed and approved by local ethical committee and a written consensus was obtained from the studied subjects. The physical characteristics (mean ± S.E.M.) of the subjects from group A and B are presented in Table 1 and 2.

Table 1. Physical characteristics of the studied subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Body height (cm)</th>
<th>Body weight (kg)</th>
<th>BMI (kg·m⁻²)</th>
<th>VO₂ max (ml·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=7)</td>
<td>21.00 ± 0.69</td>
<td>184.57 ± 2.83</td>
<td>76.57 ± 1.97</td>
<td>22.51 ± 0.65</td>
<td>42.00 ± 1.72</td>
</tr>
<tr>
<td>B (n=12)</td>
<td>22.67 ± 0.74</td>
<td>176.75 ± 2.58</td>
<td>73.50 ± 2.47</td>
<td>23.52 ± 0.62</td>
<td>39.16 ± 1.10</td>
</tr>
<tr>
<td>Group</td>
<td>Heart rate (bt·min⁻¹)</td>
<td>VO₂ (ml·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------</td>
<td>----------------</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Basal</td>
<td>VO₂max</td>
<td>Basal</td>
<td>VO₂max</td>
<td></td>
</tr>
<tr>
<td>A(n = 7)</td>
<td>82.4 ± 1.9</td>
<td>191.9 ± 3.3</td>
<td>279 ± 11</td>
<td>2904 ± 135</td>
<td></td>
</tr>
<tr>
<td>A*(n = 7)</td>
<td>82.9 ± 2.5</td>
<td>195.7 ± 2.1</td>
<td>293 ± 14</td>
<td>3163 ± 145</td>
<td></td>
</tr>
<tr>
<td>B(n = 12)</td>
<td>90.6 ± 5.5</td>
<td>189.3 ± 2.7</td>
<td>307 ± 9</td>
<td>2864 ± 91</td>
<td></td>
</tr>
<tr>
<td>B*(n = 12)</td>
<td>93.9 ± 3.4</td>
<td>190.1 ± 2.5</td>
<td>311 ± 12</td>
<td>2698 ± 130*</td>
<td></td>
</tr>
</tbody>
</table>

* — significantly different (p<0.05) from control of group B subjects.

**Exercise protocol applied in group A**

The subjects included into group A (n = 7) performed the incremental exercise tests according to Bruce (11) protocol, using the treadmill (2000 Treadmill, SensorMedics, USA). Before each test 6 min resting period was allowed to determined the resting stage of the cardio-respiratory parameters, as well as to withdraw the blood samples. The first bout of exercise was performed at the running velocity of 2.7 km·h⁻¹ and the grade of 10%, followed by a gradual increase of both the running velocity and the running velocity and the grade every 3 min for details see 11). The test was interrupted when the subject could no longer maintain the applied running velocity at a given grade or developed fatigue. The test exercise was performed twice, once, under fasting conditions and another following a standard meal. The interval between both tests was about 7—10 days. The standard meal (Abbott, UK) containing ~10 g of proteins, ~33.8 g of carbohydrates, ~8.1 g of fat, 250 kcal, in volume of 250 ml, with osmolarity of 300 mOsm/L, swallowed within 2—3 min, was ingested about 5 min prior to the onset of exercise. During both tests gas exchange variables were registered using Vmax 29c (SensorMedics, USA). Heart rate was determined continuously from the ECG curve registered by Corina Marquette Hellige GmbH, Germany). The duration of the exercise test was about 15—20 min.

**Exercise protocol applied in group B**

The subjects included into group B (n = 12) performed incremental exercise tests until exhaustion using the cycloergometer Ergoline 800s, the Netherlands. Before the test 6 min resting period was allowed to determine the resting stage of the cardio-respiratory parameters, as well as to withdraw the blood samples. The exercise test started at power output of 20 W, followed by gradual increase amounting to 20 W every 3 min and it was continued until exhaustion. The pedalling rate was 70 rev·min⁻¹. This test was performed twice. The first test was considered as a control and the second as the main test. The main test was performed 24 h after withdrawal of 450 ml of blood (honorary blood donation). The tests were separated by a period of about 7—10 days. Both tests in this group of subjects, were performed after a standard breakfast (containing ~30 g of protein, ~120 g of carbohydrates, 30 g of fat all ~870 kcal), ingested 2—3 hour prior to the test. Gas exchange variables in this test were measured continuously by breath-to-breath using Oxycon Champion Jaeger, Germany as previously described (12) starting 6 min prior to the exercise — until the test was stopped. Heart rate was determined continuously from the ECG curve registered by the Hellige SMS 181 unit, Germany. The duration of the exercise test in this styd was about 30—40 min.
Blood sampling

Abbott Int-Catheter, Ireland (18G/1.2 x 45) was inserted into the antecubital vein about 15 min prior to the onset of exercise. Venous blood samples, 10 ml each, were withdrawn via catheter at 5 min before the onset of exercise at the VO_2max (the end of exercise protocol) and 2 h after the test. Before taking each blood sample, 1 ml of blood volume was taken to eliminate the blood from the catheter as described before (13). For hormone assay the samples of blood were taken into the chilled tubes containing EDTA. Subsequently, the samples were centrifuged, and the plasma was separated and frozen at -20°C. The hematocrit was measured before and 24 h after blood donation (5 minutes prior to the main exercise test and it showed ~7% decrease that is from 43.86 ± 1.60 to 40.75 ± 2.09 in these subjects.

Hormone assay

Plasma leptin was measured using the radioimmunoassay-kit for human leptin purchased from Linco Research Inc. (St. Louis, MO, USA) with rabbit anti-human leptin serum and purified recombinant human leptin as a standard. The antisera was highly specific and did no recognise any other known gut hormones. The detection limit of assay was about 0.1 pM and the intra- and inter-assay variations were 7% and 10% respectively, as described before (14).

Plasma gastrin was determined using antisera (rabbit antigastrin serum no 4562 kindly donated by Professor J. F. Rehfield of Copenhagen, Denmark) in a final dilution of 1: 280,000, as described previously (15, 16). Human gastrin (G-17-I) purchased from Bachem Biosciences, Bubendorf, Switzerland) was labelled by an iodogen method and the tracer was purified on SEP PAK cartridge C18. Human G-17-I was used as a standard in the above RIA. The detection limits was about 1.5 pM and the intra- and inter-assay variations were, respectively, 12%, and 9%.

Plasma CCK was determined using antisera (rabbit antisera no NY 112 kindly provided by Professor N. Yanaihara, Shizuoka, Japan) that recognised the sulphated residue of CCK-8 and CCK-33 equally but had only negligible cross-reactivity with sulphated G-17 (17) and did not cross-react with unrelated gastrointestinal hormones. Plasma samples were extracted with ethanol/acetic acid mixture, dried in vacuum and restituted with diluent just before the assay. Synthetic sulphated human CCK-8 was used as a standard and was labelled with ^125I using Bolton and Hunter reagent (Amersham, Poole, UK) as a tracer. The assay system was sufficiently sensitive to detect about 0.5 pM of CCK. Intra- and inter-assay variations were 8% and 12%, respectively.

Plasma PP concentration was measured using antisera against hPP kindly provided by Dr R E Chance (Lilly Lab, Indianapolis, IA, USA). Antiserum was used in a final dilution of 1: 400,000. Highly purified hPP and labelled with ^125I was used as a standard (purchased from Life Science Products, Boston, USA). The detection limit was 2 pM and the intra- and inter-assay variations were 5% and 11%, respectively, as described before (16).

Plasma insulin was measured using kits (Polatom, Swierk-Otwood, Poland) in accordance with manufacturer's instructions. The detection limit was 2.5 μU/ml and the intra- and inter-assay variations were 4% and 4.8%, respectively. GH was determined using human GH lit (Polatom, Otwood-Swierk, Poland) in accordance with the producer's instruction. The detection limit of the assay was 0.5 μU/ml and intra- and inter-assay variations were, respectively, 3.4% and 5.1%.

Plasma cortisol levels were determined by solid phase RIA (Coat-a-count Cortisol kit, Diagnostic Products Corporation, Los Angeles, CA, USA). The detection limit was 0.5 μg/l and the intra- and inter-assay variations were, respectively, 3% and 5%. The plasma levels of epinephrine and norepinephrine were determined by HPLC system using 7125 Rheodyne injection syringe and LC-4B (BAS) electrochemical detector. The chromatographic data were analysed using 1.27 BAS program. The detection limit for epinephrine and norepinephrine was about 10 ng/ml, as described before (18).
Statistics

Results are expressed as means ± standard error mean (S.E.M.). Statistical comparison was performed with StatView II software (Abacus Concept). Wilcoxon's test was used in the calculations between paired items. P < 0.05 was considered to indicate significance.

RESULTS

The determined concentrations of plasma leptin, gut and stress hormones are presented for the following measured points; Basal = resting pre-exercise stage, VO₂max = the exercise intensity at which the maximal oxygen uptake was reached (end of the exercise test) and 2 h = two hours after the end of exercise test.

Effect of exercise on plasma leptin

Effects of exercise on plasma leptin in fasted and fed subjects and in subjects before and after blood donation are shown on Figs. 1 and 2. In fasted subjects of group A, plasma leptin was low (~120 pM) and was not significantly affected at VO₂max or 2 h after the exercise. In fed subjects at the VO₂max, the plasma leptin showed significant elevation by about 90% above basal and remained significantly elevated at 2 h after the exercise (Fig. 1). In subjects who exercised before the blood donation, plasma leptin was unaltered both at VO₂max and after 2 h of resting session (Fig. 2). Day after blood donation (450 ml), the initial plasma leptin was significantly reduced (by about 30%) as compared to the level measured before the withdrawal of the blood. Exercise failed again to affect significantly this reduced plasma levels of leptin both immediately and after 2 h following exercise.

Effect of exercise on gut hormones

Plasma gastrin was significantly increased by exercise at the VO₂max level in fasted subjects and those before blood donation. After 2 h following exercise the hormone returned to the preexercise level (Figs. 3 and 4). In fed subjects, plasma gastrin during exercise at the VO₂max reached significantly higher level as compared to basal value and it was significantly higher than that observed at VO₂max in fasted subjects. Plasma CCK levels was also significantly increased by exercise in fasting patients (group A) and showed significant elevation after meal both at the VO₂max and 2 h after exercise. After a standard breakfast meal in group B subjects, plasma CCK was significantly higher than in fasted subjects of group A and showed further significant increment, at the VO₂max and 2 h after exercise, both before and after blood donation (Figs. 3 and 4).
Fig. 1. Plasma levels of leptin in group A subjects under basal conditions and immediately following exercise (at VO$_2$ max) and after 2 h of rest session in tests without and with standard meal. Mean ± SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant change as compared to the value recorded in tests without standard meal.

Fig. 2. Plasma levels of leptin in group B subjects under basal conditions and immediately following exercise (at VO$_2$ max) and after 2 h of rest session in tests before and 24 h after blood donation. Mean ± SEM of 12 tests on 12 subjects. Cross indicates significant change as compared to the value recorded in tests before blood donation.
Fig. 3. Plasma levels of gastrin and CCK in group A subjects under basal conditions and immediately following exercise (at VO$_{2\text{max}}$) and after 2 h of rest session in tests without and with standard meal. Mean ± SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant reduction as compared to the value recorded at VO$_{2\text{max}}$.

Fig. 4. Plasma levels of gastrin and CCK in group B subjects under basal conditions and immediately following exercise (at VO$_{2\text{max}}$) and after 2 h of rest session in tests before and 24 h after blood donation. Mean ± SEM of 12 tests on 12 subjects. Asterisk indicates significant change as compared to the value recorded at VO$_{2\text{max}}$. Cross indicates significant reduction as compared to the value recorded at VO$_{2\text{max}}$. 
Plasma PP concentration in fasted subjects showed a marked (by about 40%) and significant rise in response to exercise at the VO$_{2\text{max}}$ and this returned to the preexercise level after 2 h of resting session. In fed subjects exercise almost doubled plasma PP level, which significantly declined 2 h after the exercise but still remained significantly elevated above basal level (Fig. 5). Also in subjects before the blood donation, the plasma PP level showed significant increase at the VO$_{2\text{max}}$. Plasma PP was significant increased after blood donation and tended to showed further though not significant increment in response to exercise to fell below the initial concentration 2 h after exercise (Fig. 6).

Exercise both in fasted and fed subjects caused a significant decrease of plasma insulin ad this fall was observed at the VO$_{2\text{max}}$ also in fed subjects and in those who donated the blood. In both groups of subjects the insulin concentration returned to preexercise level after 2 h of resting period (Fig. 6).

Effects of exercise on stress hormones

Plasma cortisol concentration raised significantly (by about 30%) at the VO$_{2\text{max}}$ both in fasted and fed subjects and this rise returned to preexercise level after 2 h of resting session (Fig. 7). A marked increment in plasma cortisol was also observed in subjects who performed exercise before blood donation. However, after blood donation, plasma cortisol, that showed initially higher level, failed to exhibit any significant increment at the VO$_{2\text{max}}$. After 2 h of resting session plasma cortisol fell significantly below the basal level (Fig. 8).

Plasma epinephrine in both groups of subjects remained at the similar levels and exercise failed to significantly increase the level of this catecholamine in fasted and fed subjects or before and after blood donation (Figs. 9 and 10). In contrast, plasma norepinephrine showed about 10 fold increase that appeared at the VO$_{2\text{max}}$ but after 2 h of resting session the level of this cathecholamine returned to the preexercise level both in fasted and fed subjects as well as before and after blood donation (Figs. 9 and 10).

Basal growth hormone (GH) level, which averaged ~ 0.2 nM, showed a dramatic increment at the VO$_{2\text{max}}$ both in fasted and fed subjects and this increment was observed before and after blood donation though this rise after blood withdrawal was significantly smaller than that before blood loss (Figs. 11 and 12). Following blood loss, the rise in plasma GH at the VO$_{2\text{max}}$ reached only about 30% of the increment observed before blood donation and this rise returned to basal level 2 h after exercise.
Fig. 5. Plasma levels of PP and insulin in group A subjects under basal conditions and immediately following exercise (at VO$_2$$_{max}$) and after 2 h of rest session in tests without and with standard meal. Mean ± SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant change as compared to the value recorded at VO$_2$$_{max}$. Double cross indicates significant increase above the value recorded in tests without standard meal.

Fig. 6. Plasma levels of PP and insulin in group B subjects under basal conditions and immediately following exercise (at VO$_2$$_{max}$) and after 2 h of rest session in tests before and 24 h after blood donation. Mean ± SEM of 12 tests on 12 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant reduction as compared to the value recorded at VO$_2$$_{max}$. Double crosses indicate significant increase above the value recorded in tests before blood donation.
Fig. 7. Plasma levels of cortisol in group A subjects under basal conditions and immediately following exercise (at $\text{VO}_2\text{max}$) and after 2 h of rest session in tests without and with standard meal. Mean $\pm$ SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at $\text{VO}_2\text{max}$.

Fig. 8. Plasma levels of cortisol in group B subjects under basal conditions and immediately following exercise (at $\text{VO}_2\text{max}$) and after 2 h of rest session in tests without and with standard meal. Mean $\pm$ SEM of 12 tests on 12 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at $\text{VO}_2\text{max}$.
Fig. 9. Plasma levels of epinephrine and norepinephrine in group A subjects under basal conditions and immediately following exercise (at VO$_{2\text{max}}$) and after 2 h of rest session in tests without and with standard meal. Mean ± SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at VO$_{2\text{max}}$.

Fig. 10. Plasma levels of epinephrine and norepinephrine in group B subjects under basal conditions and immediately following exercise (at VO$_{2\text{max}}$) and after 2 h of rest session in tests before and 24 h after blood donation. Mean ± SEM of 12 tests on 12 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at VO$_{2\text{max}}$. 
**Fig. 11.** Plasma levels of growth hormone (GH) in group A subjects under basal conditions and immediately following exercise (at VO\(_{2\text{max}}\)) and after 2 h of rest session in tests without and with standard meal. Mean ± SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at VO\(_{2\text{max}}\). Double crosses indicate significant change as compared to the value recorded in tests without standard meal.

**Fig. 12.** Plasma levels of growth hormone (GH) in group B subjects under basal conditions and immediately following exercise (at VO\(_{2\text{max}}\)) and after 2 h of rest session in tests before and 24 h after blood donation. Mean ± SEM of 12 tests on 12 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at VO\(_{2\text{max}}\). Double crosses indicate significant change as compared to the value recorded in tests before blood donation.
**Fig. 13.** Plasma levels of glucose and fatty acids (FFA) in A group subjects under basal conditions and immediately following exercise (at VO$_2$$_{max}$) and after 2 h of rest session in tests without and with standard meal. Mean ± SEM of 7 tests on 7 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at VO$_2$$_{max}$.

**Fig. 14.** Plasma levels of glucose and fatty acids (FFA) in group B subjects under basal conditions and immediately following exercise (at VO$_2$$_{max}$) and after 2 h of rest session in tests before and 24 h after blood donation. Mean ± SEM of 12 tests on 12 subjects. Asterisk indicates significant change as compared to the basal level. Cross indicates significant decrease as compared to the value recorded at VO$_2$$_{max}$.
Blood glucose level showed a small but significant elevation at the VO₂\text{max} in both groups of subjects before and after meal or before and after blood donation. Plasma FFA level showed significant elevation only 2 h after exercise in both groups of subjects (Figs. 13 and 14).

DISCUSSION

This study provides further evidence that strenuous exercise fails to affect the plasma level of leptin measured both during physical activity at VO₂\text{max} and after two hours of the resting session. This finding is in keeping with previous reports showing no alteration of plasma leptin after exercise and energy expenditure such as occurring after 2—3 h treadmill run (20 miles) or after 2 h of strenuous pedalling on cycloergometer at the level of 75% of VO₂\text{max} (8, 9). These results are in apparent disagreement with recent study of Duclos et al. (10) who observed significant fall in plasma leptin after 2 h of strenuous exercise followed by 2 h of resting session, when also the rise in free fatty acids was noticed, to be elevated and correlated negatively with a decrease in plasma leptin. It is not excluded that with the prolongation of exercise to 2 h and waiting 2 more hours under resting conditions we also could observe the fall in plasma leptin but this requires additional studies.

The major finding of this report is the observation that the whole blood donation (450 ml), that induced ~7% decrease in hematocrit, greatly reduced (by about 30%) the basal plasma leptin and its response to exercise, while feeding significantly enhanced the plasma levels of leptin during exercise at VO₂\text{max} being accompanied by a significant rise of major gut hormones such as gastrin, CCK and PP with concurrent fall in plasma insulin.

The rise in plasma leptin concentrations after feeding is reported here for the first time in humans but it was expected based on previous studies in dogs (14) and rats (19—21) showing an increase in plasma leptin after feeding suggesting that this postprandial rise in the hormone level may originate from the stomach rather than from adipocytes. We reported recently in dogs (14) that plasma leptin rose also after sham-feeding and ordinary feeding, being accompanied by an elevation of plasma gastrin and CCK that could be responsible for the mobilisation of leptin from the gastric stores. It agrees with the observation that the rise in plasma leptin after feeding or administration of CCK or gastrin was accompanied by the reduction in immunoreactive leptin content in the stomach (19, 21). Leptin was detected by immunocytochemistry in human stomach (22) and identified by analysis of gene expression at mRNA and protein levels in gastric mucosa after its damage and found to contribute to the protection of this mucosa against acute damage, partly through scavenging of reactive oxygen species (20). Although the postprandial rise in
plasma leptin observed in this study on humans may not be due to mucosal
damage and may not be large enough to exert the systemic effects, the
paracrine action of locally released leptin in the gastric mucosa in response to
feeding and local injury (21) might contribute to the strengthening of the
integrity of this mucosal lining by removal of oxygen reactive species and
increase in gastric mucosal blood flow as demonstrated in rats (20). It remain to
be established whether gastric release of leptin by feeding is due to the action of
concurrently released gut hormones such as gastrin and CCK but the fact that
exogenously applied CCK and gastrin in rats (19, 20) or pentagastrin and
secretin in humans (23) enhanced plasma leptin to similar extent to that
attained after feeding, favours the concept of gut hormone mediation of
elevated plasma leptin detected after food intake.

As expected, acute exercise to exhaustion, that represents an oxidative
stress, provoked a sudden release of so called stress-related hormones including
catecholamines, cortisol and growth hormone. All these hormones were found
in this study to rise during exercise at 100% of VO$_{2\text{max}}$ and to return to
preexercise level after 2 h of resting session. The profound stimulatory influence
of acute exercise on these stress hormones was described previously after
exercise by numerous reports (24—30) but our study provides for the first time
an evidence that gut hormones such gastrin and CCK are also released by
strenuous exercise, possibly due to an alteration of gastric emptying and gastric
adaptive relaxation but this requires further study. The observed rise in PP and
catecholamines after exercise, reported also previously (24, 25), may result from
increased activity of the autonomic nervous system, particularly its
vagal-cholinergic and sympatho-adrenergic divisions with its cardiovascular,
respiratory and metabolic responses.

The major finding of this study is the observation that whole blood
withdrawal in amount of ~7% of total blood volume (450 ml), greatly reduced
plasma growth hormone and cortisol responses to exercise without affecting
plasma norepinephrine elevation. The mechanism of this decline in stress
hormone response to exercise requires further study but it seems to be
unrelated to blood glucose level but accompanied by a significant rise in
plasma free fatty acids (FFA). This is in keeping with the observation of Ducklos
et al. (10) showing negative correlation between plasma FFA concentration
and plasma leptin levels and the finding of Rentsch and Chiesi (31)
demonstrating the reduction in leptin mRNA expression in adipocytes. Since in
our experiments, the rise in plasma FFA found 2 h after exercise was similar
before and after meal or before and after blood withdrawal, FFA do not seem
to be the major factor contributing the respective rise (after feeding) and fall
(after blood donation) of plasma leptin. Based on our data it seems rational to
attribute the rise in plasma leptin after feeding to the mobilisation of gastric
leptin storage by gastrin and CCK, while the fall of leptin after blood donation
could be ascribed to deficient stimulation of stress hormones such as growth hormone and cortisol as well as the impairment of vagal-cholinergic system expressed by reduced response of PP (16).

It is of interest that plasma levels of insulin showed a marked and significant decrease immediately after exercise. It is not clear whether this insulin fall, that is negatively correlated with the rise in plasma norepinephrine occurring immediately after exercise, could be causally related. As it is well established, however, that the excessive sympatho-adrenergic stimulation attenuates the pancreatic B cell activity and release of insulin (32), it is reasonable to assume that the sudden rise in plasma norepinephrine is responsible for the fall in insulin release though it is also possible that locally released leptin in the pancreas suppresses insulin expression and release, particularly when sympatho-adrenergic system is activated (32), as proposed previously (33—35). An alternative explanation for the postexercise fall in insulin level could be a direct inhibitory action by excessively released PP on insulin producing B cells of pancreatic islets (36).

In summary, the major finding of this report is the observation that the whole blood donation (450 ml), that induced ~7% decrease in heematocrit, greatly reduced (by about 30%) the basal plasma leptin and its response to exercise, while feeding significantly enhanced the plasma levels of leptin during exercise at 100% of VO_{2max} being accompanied by the significant rise of major gut hormones such as gastrin, CCK and PP with concurrent fall in plasma insulin.

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