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EFFECT OF MODERATE INCREMENTAL EXERCISE, PERFORMED IN FED AND FASTED STATE ON CARDIO-RESPIRATORY VARIABLES AND LEPTIN AND GHRELIN CONCENTRATIONS IN YOUNG HEALTHY MEN

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Background: Although hormonal responses to exercise performed in fed state are well documented, far less in known about the effect of a single exercise bout, performed after overnight fasting, on cardio-respiratory responses and hormones secretion. It has been reported that recently discovered hormones as leptin and ghrelin may affect cardiovascular responses at rest. However, their effect on the cardiovascular responses to exercise is unknown. Aims: This study was designed to determine the effect of overnight fasting on cardio- respiratory responses during moderate incremental exercise. We have hypothesised that fasting / exercise induced changes in plasma leptin / ghrelin concentrations may influence cardiovascular response. Material and Methods: Eight healthy non-smoking men (means ± SE.: age 23.0 ± 0.5 years; body mass 71.9 ± 1.5 kg; height 179.1 ± 0.8 cm; BMI 22.42 ± 0.49 kg · m^{-2} with VO_2max of 3.71 ± 0.10 l · min^{-1}) volunteered for this study. The subjects performed twice an incremental exercise test, with the increase of power output by 30 W every 3 minutes. Tests were performed in a random order: once in the feed state - cycling until exhaustion and second, about one week later, after overnight fasting - cycling until reaching 150 W. Results: In the present study we have compared the results obtained during incremental exercise performed only up to 150 W (59 ± 2 % of VO_2max) both in fed and fasted state. Heart rate measured during exercise at each power output, performed in fasted state was by about 10 bt · min^{-1} ($p = 0.02$) lower then in fed subjects. Respiratory quotient and plasma lactate concentration in fasted state were also significantly ($p<0.001$) lower than in the fed state. Pre-exercise plasma leptin and ghrelin concentrations were not significantly different in fed and fasted state. Exercise induced increase in hGH was not accompanied by a significant changes in the studied gut hormones such as ghrelin, leptin, and insulin, except for plasma gastrin concentration, which was significantly ($p = 0.008$) lower in fasting subjects at the power output of 150 W. Plasma [IL-6] at rest before exercise performed in fasted state was significantly ($p = 0.03$) elevated in relation to the fed state.

This was accompanied by significantly higher ($p = 0.047$) plasma noradrenaline concentration. Plasma IL-6 concentration at rest in fed subjects was negatively correlated with plasma ghrelin concentration ($r = - 0.73$, $p < 0.05$) and positively correlated with plasma insulin concentration ($r = 0.78$, $p < 0.05$). Significant negative correlation ($r = - 0.90$; $p < 0.05$) was found between plasma insulin and ghrelin concentration at rest in fed subjects. Conclusions: We have concluded that plasma leptin and ghrelin concentrations have no significant effect on the fasting-induced attenuation of heart rate during exercise. We have postulated that this effect is caused by increased plasma norepinephrine concentration, leading to the increase in systemic vascular resistance and baroreceptor mediated vagal stimulation. Moreover we believe, that the fasting-induced significant increase in plasma IL-6 concentration at rest, accompanied by higher plasma norepinephrine concentration and lower RQ, belongs to the physiological responses, maintaining energy homeostasis in the fasting state.

Key words: *Interleukin-6, growth hormone, norepinephrine, gastrin, ghrelin, leptin, insulin, heart rate, oxygen uptake*

INTRODUCTION

It is well known that physical exercise affects secretion of several hormones (see *e.g.* 1-3) and accelerates cardio-respiratory function (4-6). Most of the reported data on this topic are collected during exercise performed in fed state. Although physical exercise is often undertaken by man after overnight fasting, surprisingly little is known about combined effects of brief fasting and exercise of moderate intensity on cardio-respiratory variables and their connection with hormone secretion.

It has been reported, that food intake may influence cardiovascular regulation at rest both in healthy (7, 8) as well as in cardiac transplant recipient patients (9). Therefore, it has been postulated that the effect of food intake (fasting / fed state) on the cardio-respiratory responses may be mediated both *via* neural and hormonal pathways (7-9). Moreover, it has been reported that exercise tolerance of patients with *angina pectoris* falls following pre-exercise meals (10). Other researches postulate that recently discovered appetite behaviour hormones such as leptin (11) known as "satiety hormone" and ghrelin (12) known as "hunger hormone" may affect cardiovascular activity (13-17). This hypothesis is supported by the discovery of leptin (18) and ghrelin (19) receptors in human myocardium and vascular system. Recently it has been reported that injected leptin can activate sympathetic nervous system (20, 21) and injection of ghrelin acutely decreases heart rate in rabbits (22).

Little however is known about the effect of plasma leptin and ghrelin concentrations on cardio-respiratory responses during exercise in fed / fasted humans. Some studies have shown a decreases in plasma leptin concentration after single bout of exercise (23), whereas others have reported no effect of exercise on plasma leptin concentration (24-27). Plasma ghrelin concentration, however, seems

to be rather resistant to physical exercise (28, 29). On the other hand, food consumption did not increase leptin levels in man (30), whereas immediate suppressive effect of meal on ghrelin secretion has been observed (16). Additionally, post-prandial relationship between insulin and leptin seems to be questionable (30), while between insulin and ghrelin, on the contrary, is possible (16, 31).

The effect of fasting on exercise induced increase in heart rate in humans remains ambiguous. Some authors have reported no effect of fasting on heart rate during exercise (32, 33), whereas, others observed its attenuation (10, 34) or even potentiation (35).

To our best knowledge no study have been performed to relate the exercise induced changes in cardio-respiratory variables to plasma leptin and ghrelin concentrations during exercise performed in fasting and fed state. If indeed increase in plasma ghrelin concentration decreases heart rate, than fasting-induced increase in plasma ghrelin concentration should be accompanied by decreases in heart rate during exercise. This hypothesis was tested in a group of healthy young physically active males subjected to moderate intensity exercise (up to 60 % of VO_2max), corresponding to self paced exercise intensity of normal daily morning physical activities (36, 37).

MATERIAL AND METHODS

Subject characteristics

Eight healthy non-smoking men (means ± SE : age 23.0 ± 0.5 years; body mass 71.9 ± 1.5 kg; height 179.1 ± 0.8 cm; BMI 22.42 ± 0.49 kg · m^{-2}; VO_2max of 3.71 ± 0.1 l · min^{-1}) experienced in laboratory tests, were subjects in this study. Local University Ethic Committee approved this project and supervised its realisation. Informed consent was obtained from each man subjected to this study. Basic blood variables of the studied subjects determined at rest are presented in *Table 1*.

Table 1. Basic blood variables determined at rest in the tested subjects (n = 8).

	Ht (%)	**[Hb]** (g · dl^{-1})	**RBC** (M · μl^{-1})	**WBC** (k · μl^{-1})	**[Na^+]** (mmol · l^{-1})	**[K^+]** (mmol · l^{-1})	**[Cr]** (µmol · l^{-1})
Min	40.4	13.0	4.58	3.42	141	4.49	85.5
Max	52.0	17.1	5.91	7.46	145	4.83	103.1
Mean ± SE	46.2 ± 1.4	15.4 ± 0.5	5.30 ± 0.16	5.35 ± 0.44	143 ± 0.4	4.62 ± 0.05	92.3 ± 2.3

Ht - haematocrit value, [Hb] - haemoglobin concentration, RBC - erythrocyte count, WBC - leukocyte count, [Na^+] - plasma sodium concentration, [K^+] - plasma potassium concentration, [Cr] - plasma creatinine level.

Experimental protocol

The subjects performed twice an incremental exercise test with one week break in between. The maximal incremental exercise test - until exhaustion - was performed after a standard breakfast

(containing ~ 30 g of protein, ~ 120 g of carbohydrates, ~ 30 g of fat and ~ 870 kcal), ingested 2 hour prior to the test. The sub-maximal incremental exercise test - up to 150 W - was performed in the morning hours in fasted state (*i.e.* after overnight fasting).

Exercise protocol

The incremental exercise tests were performed on the cycloergometer Ergo-Line GmbH & Co KG 800s, (Bitz, Germany). Before the test, 6-minute resting period was allowed, to determine the resting stage of the cardio-respiratory parameters, as well as to withdrawal the blood samples. The maximal exercise test started at power output of 30 W, followed by gradual increase amounting to 30 W every 3-minute and it was continued until exhaustion, as described previously (38). The pedalling rate was 70 rev $\cdot$ min^{-1}. The sub-maximal incremental exercise test was performed at the same ergometer. The initial phase of this test was identical as for the maximal incremental test (as described above), but the sub-maximal incremental test was stopped after 12 minutes *i.e.* after completing the stage of 150 W (in order to avoid hypoglycemia). In the present study we have applied moderate intensity exercise (up to 60 % VO_2max), reflecting normal morning physical activity and corresponding to the exercise protocols used in clinical testing (see 36, 37).

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. The catheter was connected with the extension set using a "T" Adapter SL Abbot Ireland (a tube 10 cm in length). Immediately before the blood samples were taken for an appropriate analysis, samples of 1 ml of blood were taken in order to eliminate the blood from the catheter and the T-set. Venous blood samples 5 ml each were withdrawn *via* catheter, in seating position: (1) at 5 min before the onset of exercise, (2) after completing the stage of 90 W, (3) after completing the stage of 150 W, (4) at the VO_2max (the end of the maximal exercise test - only) and (5) 30 minutes after the finishing each test. A part of each sample (about 1 ml of blood) was used for the immediate measurements of blood gases (PO_2 and PCO_2), blood hydrogen ion concentration $[H^+]_b$ and haematocrit. Subsequently, the remaining blood samples were centrifuged. The obtained samples of plasma were stored at a temperature of minus 25 °C for lactate $[La]_{pl}$, hormone and cytokine measurements.

Measurements

Cardio-respiratory variables

Gas exchange variables in this test were measured continuously breath-to-breath starting 6 min prior to the exercise - until the test was stopped, using Oxycon Champion, Mijnhardt BV, (Bunnik, The Netherlands), calibrated as previously described (38). Heart rate was determined continuously from the ECG curve registered by the Hellige SMS 181 unit, (Freiburg, Germany).

Haematological blood variables

PO_2 and PCO_2 as well as $[H^+]_b$ were determined using a Ciba-Corning 248 Analyser (England). The blood bicarbonate concentration $[HCO_3^-]_b$ was calculated by this unit. Plasma lactate concentration $[La]_{pl}$ was measured using an automatic analyser Vitros 250 Dry Chemistry System, Kodak, (Rochester, NY (USA). Serum sodium $[Na^+]$ and potassium $[K^+]$ concentration were determined using a flame photometer Ciba Corning 480 (Halstead, Essex, England). Serum creatinine level [Cr] was determined by kinetics method based on reaction with picric acid using an automatic analyser Hitachi 912 (Hitachi Co., Japan). Haemoglobin concentration [Hb], haematocrit

value [Ht], erythrocyte count (RBC) and leukocyte count (WBC) were determined using an automatic haematological analyser Cell Dyn 3700 Abbott Lab (Abbott Park, IL, USA).

Hormones, glucose and IL-6 detection

Concentrations of all hormones in the plasma were detected using the unit 1272 Clinigamma, LKB WALLAC, Finland. Plasma GH was assayed by radioimmunoassay using HGH-IRMA kit (Polatom, Otwock, Swierk, Poland). The within-assay coefficient of variations was 2.1, 3.4, and 3.4 % at 7.7, 16.3 and 24.5 μIU · ml^{-1} and the between-assay coefficient of variation was 2.7, 5.1 and 1.1 % at 7.7, 15.9 and 22.8 μIU · ml^{-1}. The limit of quantification of the method was 0.5 μIU · ml^{-1} (1 μIU · ml^{-1} = 3.125 · 1 ng · l^{-1}).

Plasma norepinephrine was measured using Noradrenalin ELISA-kit (IBL, Hamburg, Germany) according to the producers instruction. The detection limit was 20 pg · ml^{-1} for plasma and the intra- and inter-assay variations were 7.9% and 14.9%, respectively.

Plasma leptin and ghrelin were assessed using human leptin and ghrelin kits bought from R & D Systems, Inc. Minneapolis, USA. The detection limit for leptin was 7.8 pg · ml^{-1} and intra- and inter-assay variations were 3.0 - 3.3 % and 3.5 - 5.4 % The detection limit for ghrelin 12 pg · ml^{-1} and intra- and inter-assay variations were 5 - 10% and 7 - 14 %.

Plasma amidated gastrin (G-17 and G-34) concentration was measured by radioimmunoassay using antiserum 4562 that is directed against C-terminal sequence of the α-amidated gastrin (G-17 and G-34). This antiserum was kindly provided by Professor Jens Rehfeldt (Department of Clinical Biochemistry, University of Copenhagen, Denmark) and employed in final dilution of 1:500,000. The sensitivity of the amidated gastrins was about 4.9 pmol · l^{-1}. The intra- and inter-assay variations were 5.4 and 7.5 %, respectively.

Plasma insulin concentration was measured using kit Insulina-RIA-Prop (Polatom, Otwock-Swierk, Poland) in accordance with manufacture's instructions. The detection limit was 2.5 mU · ml^{-1} and the intra- and inter-assay variations were 4 % and 4.8 %.

Plasma glucose concentration was measured enzymatically using the analyzer Hitachi 917, Roche, USA, with the glucose GOD-PAP assay, Behringer Mannheim Systems, Germany. The detection limit was 0.11 mmol · l^{-1} and the measuring range has amounted to 0.11-25 mmol · l^{-1}.

Plasma interleukin-6 concentration (IL-6) was measured by enzyme-linked immunosorbent assay (ELISA) using Quantikine HS human IL-6 (high sensitivity) kit from R&D Systems (Minneapolis, USA) according to the manufacture's instruction. The detection limit was 0.039 pg · ml^{-1} with intra- and inter-assay variations less than 10%. The measuring range was 0.156 to 10 pg · ml^{-1}.

Statistics

The data are presented as mean ± SE. The statistics in this study were performed by the means of the statistical packet StatXact 6.0, using Wilcoxon sign rank test for dependent samples, as well as by the means of the statistical packet STATISTICA 6.0, using two-way ANOVA. The figures were prepared using the packed STATISTICA 6.0.

RESULTS

Cardio-respiratory variables:

The results of oxygen uptake (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ) and heart rate (HR) obtained in fed and fasted state, at

rest and at each stage of the incremental exercise test - up to 150 W are presented in *Figs 1, 2, 3* and *4*, respectively. The data in the figures are presented as mean ± SE for 8 subjects. The VO_2 during exercise at the power output between 30-150 W, performed in fed and fasted state, were not significantly different (two-way ANOVA). The VCO_2 during exercise in the same range of power output, performed in fed state was significantly higher ($p = 0.013$, two-way ANOVA) than in fasted state. The RQ during exercise at the power output between 30-150 W performed in fed state was also significantly higher ($p = 0.0002$, two-way ANOVA) than in fasted state. The HR during exercise at the power output between 30 - 150 W performed in fed state was significantly higher ($p = 0.002$, two-way ANOVA) than in fasted state.

Plasma measurements:

Plasma concentrations of lactate, IL-6, hGH, leptin, ghrelin, gastrin, insulin, glucose and norepinephrine, were obtained for 8 subjects, both in fed and fasted state: (1) at 5 min before the onset of exercise, (2) after completing the stage of 90 W and (3) after completing the stage of 150 W. Additionally, only in fed state, data were obtained at the end of the maximal exercise test (at the power output at which VO_2max was reached).

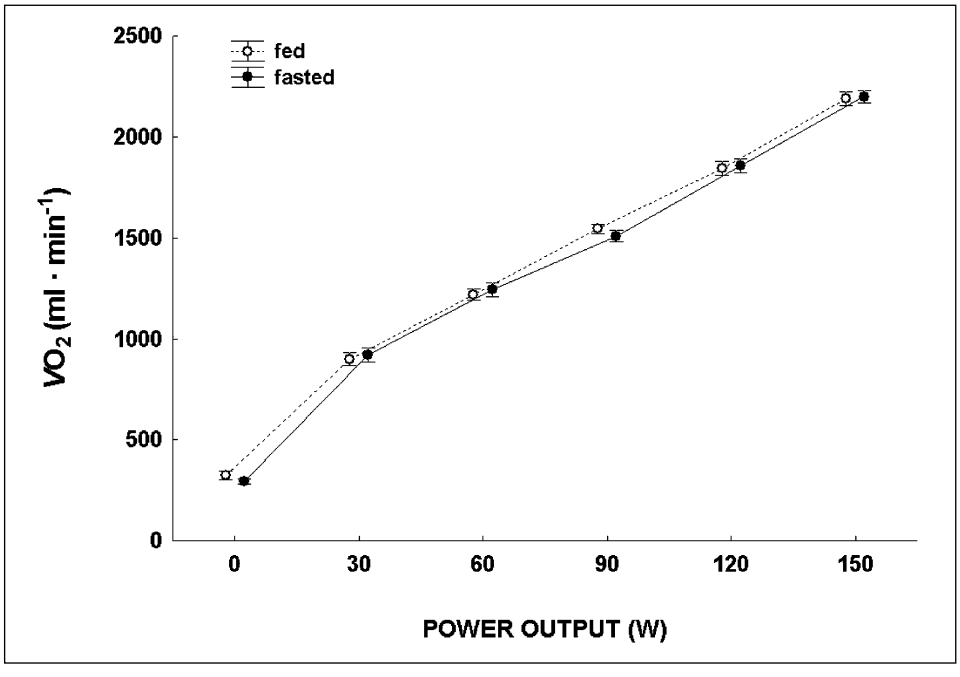

Fig. 1. Mean (± SE) values of oxygen uptake (VO_2) during incremental exercise performed in fed (o) and fasted state (•).

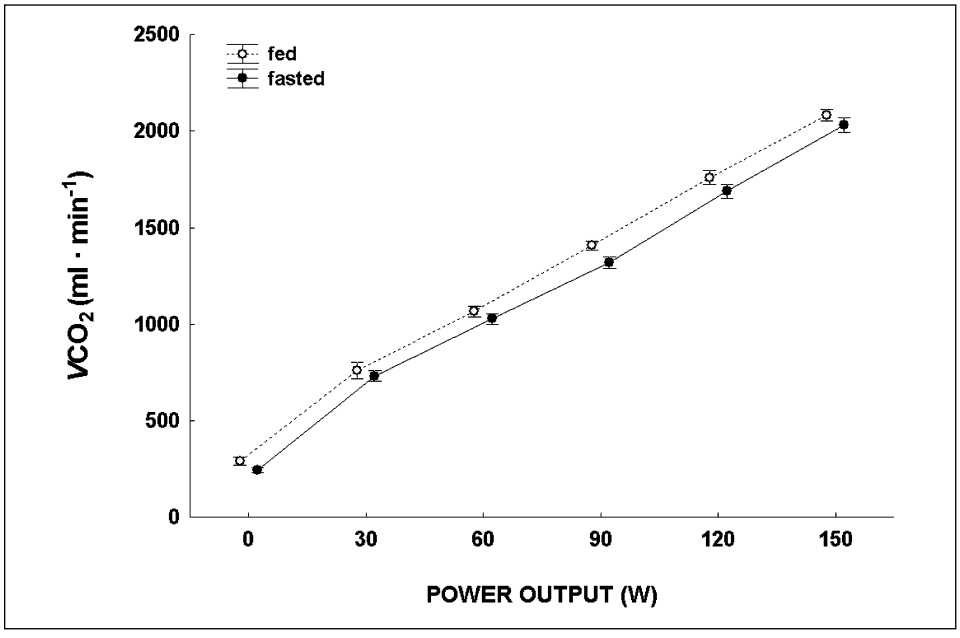

Fig. 2. Mean (± SE) values of carbon dioxide production (VCO_2) during incremental exercise performed in fed (o) and fasted state (•). Two-way ANOVA (p = 0.013).

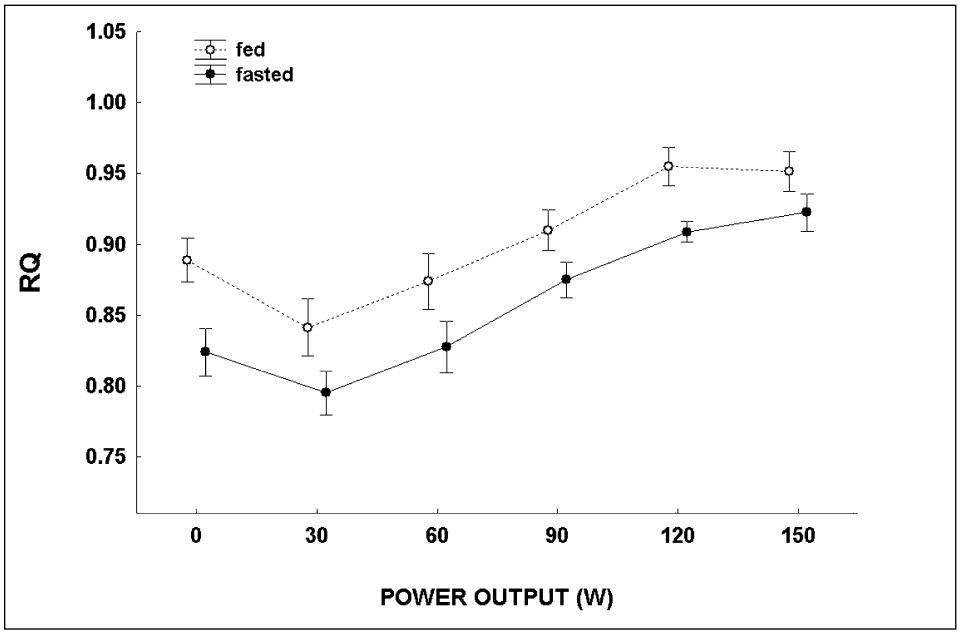

Fig. 3. Mean (± SE) values of respiratory quotient (RQ) during incremental exercise performed in fed (o) and fasted state (•). Two-way ANOVA (p = 0.0002).

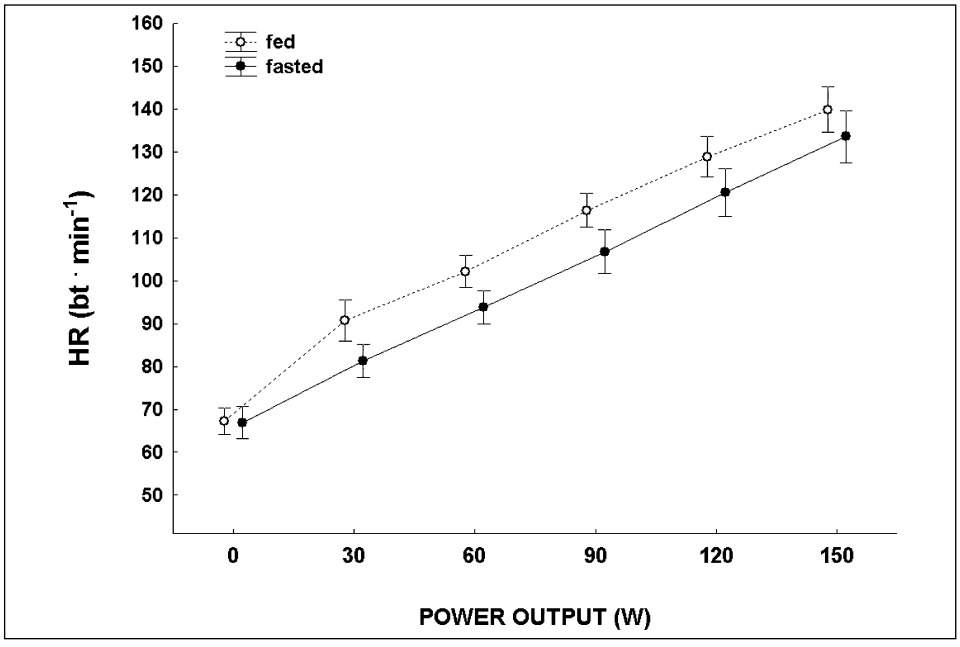

Fig. 4. Mean (± SE) values of heart rate (HR) during incremental exercise performed in fed (o) and fasted state (•). Two-way ANOVA (p = 0.002).

Plasma lactate

Plasma lactate concentration during exercise at the power output between 30-150 W performed in fed state was significantly higher (p = 0.0000, two-way ANOVA) than in fasted state. Moreover, in fed subjects plasma lactate concentration during cycling at 150 W was significantly higher in relation to rest (p = 0.016) (*Fig. 5*).

IL-6

At rest plasma IL-6 concentration in fed state has amounted to 0.288 ± 0.040 pg · ml^{-1} and it was significantly lower (p = 0.03) than in fasted state amounting to 0.650 ± 0.180 pg · ml^{-1}. At the power output of 90 W as well as 150 W the IL-6 concentration was not significantly different in both states and in relation to the resting values (*Fig. 6*).

hGH

At rest plasma hGH concentration in fed state was not significantly different from its level determined in fasted state. Plasma hGH concentration at the power output of 90 W in fed state was significantly higher than at rest (p = 0.016). At the power output of 150 W the hGH concentration in fed state has amounted to 14.85 ± 4.67 ng · ml^{-1} and in fasted state it rose to 12.83 ± 3.62 ng · ml^{-1}. At this

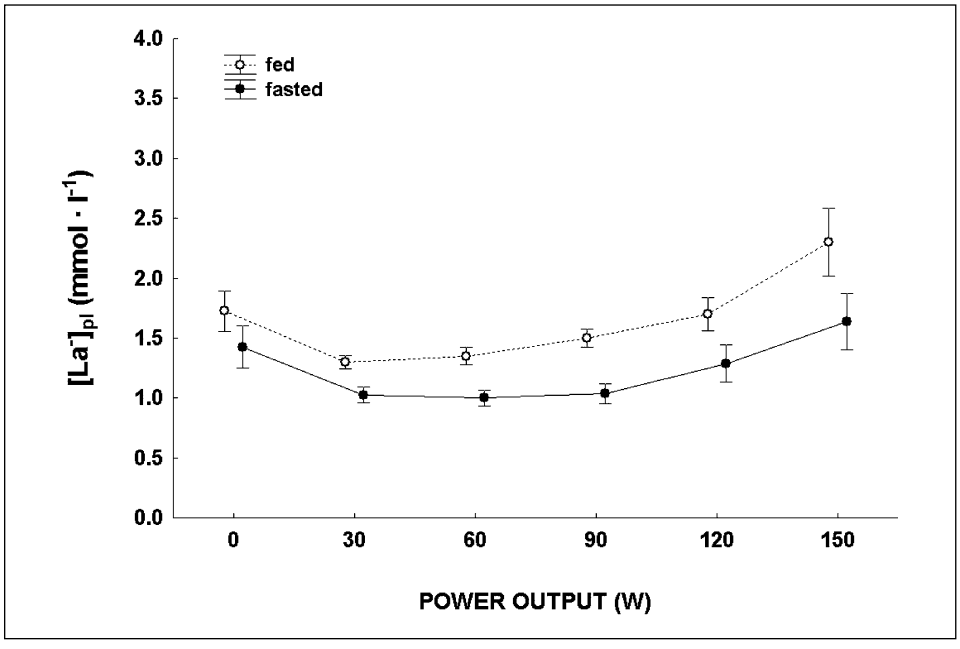

Fig. 5. Mean (± SE) values of plasma lactate concentration $[La^-]_{pl}$ during incremental exercise performed in fed (o) and fasted state (•). Two-way ANOVA (p = 0.000).

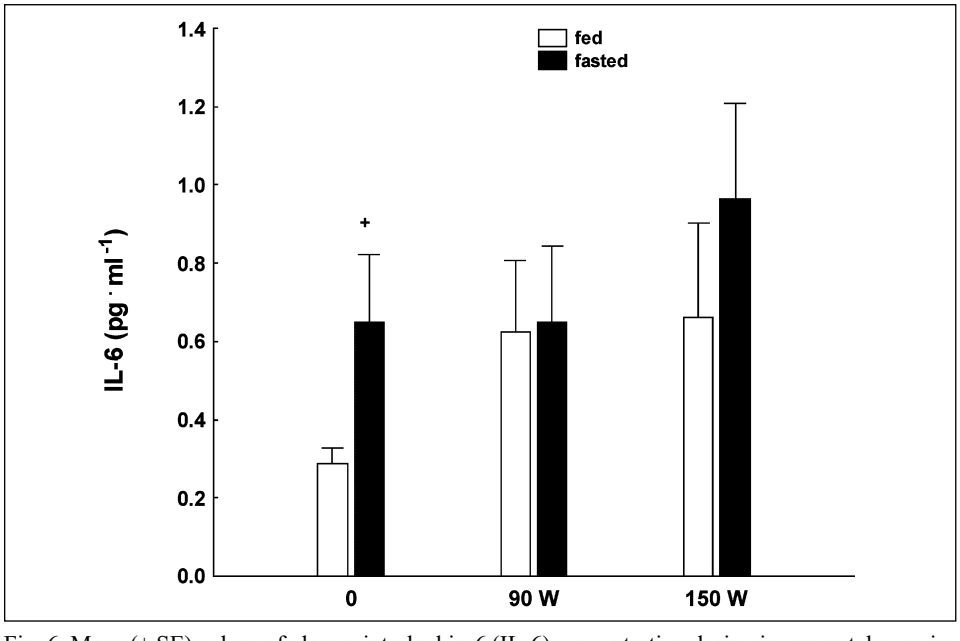

Fig. 6. Mean (± SE) values of plasma interleukin-6 (IL-6) concentration during incremental exercise performed in fed (□) and fasted state (■). + - significantly different from fed (Wilcoxon sign rank test for dependent samples, p = 0.03).

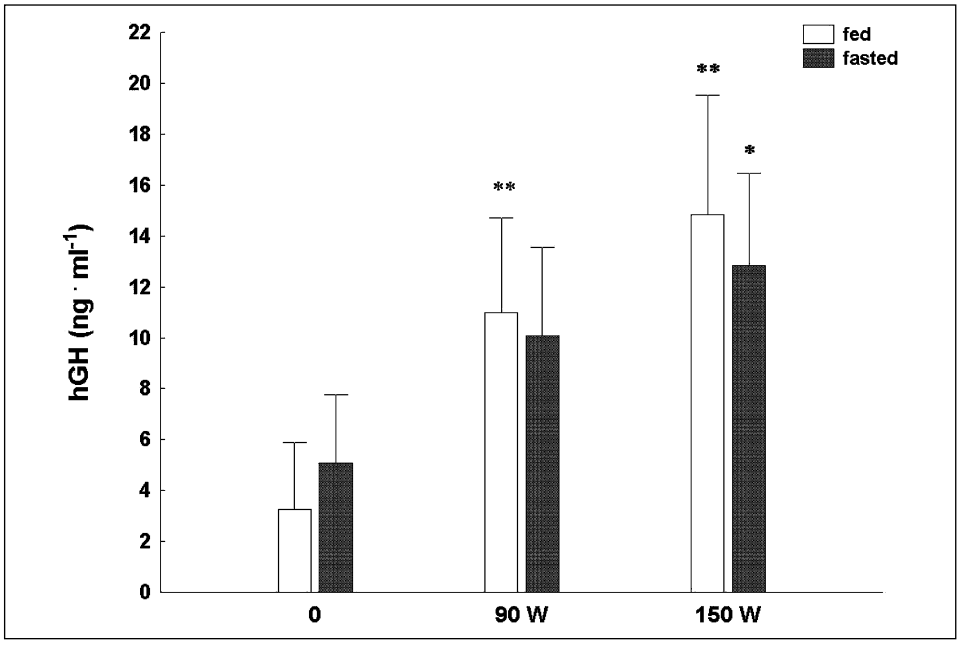

Fig. 7. Mean (± SE) values of plasma human growth hormone (hGH) concentration during incremental exercise performed in fed (□) and fasted state (■). Significantly different from rest (Wilcoxon sign rank test for dependent samples, ** p = 0.016 * p =0.04).

stage of power output the hGH concentrations were also not significantly different in both states, but in relation to rest - were significantly elevated (p = 0.016, p = 0.04, respectively in fed and fasted state) (*Fig. 7*).

Ghrelin

At rest plasma ghrelin concentration in fed state was not significantly different from its level determined in fasted state. The increase of power output in both states did not cause any significant changes in ghrelin concentration in relation to its resting level (*Fig. 8 A*).

Leptin

At rest plasma leptin concentration in fed state was not significantly different from its level determined in fasted state. The increase of power output in both states did not cause any significant changes in leptin concentration in relation to its resting level (*Fig. 8 B*).

Gastrin

At rest plasma gastrin concentration in fed state was not significantly different from its level determined in fasted state. At the power output of 90 W the plasma

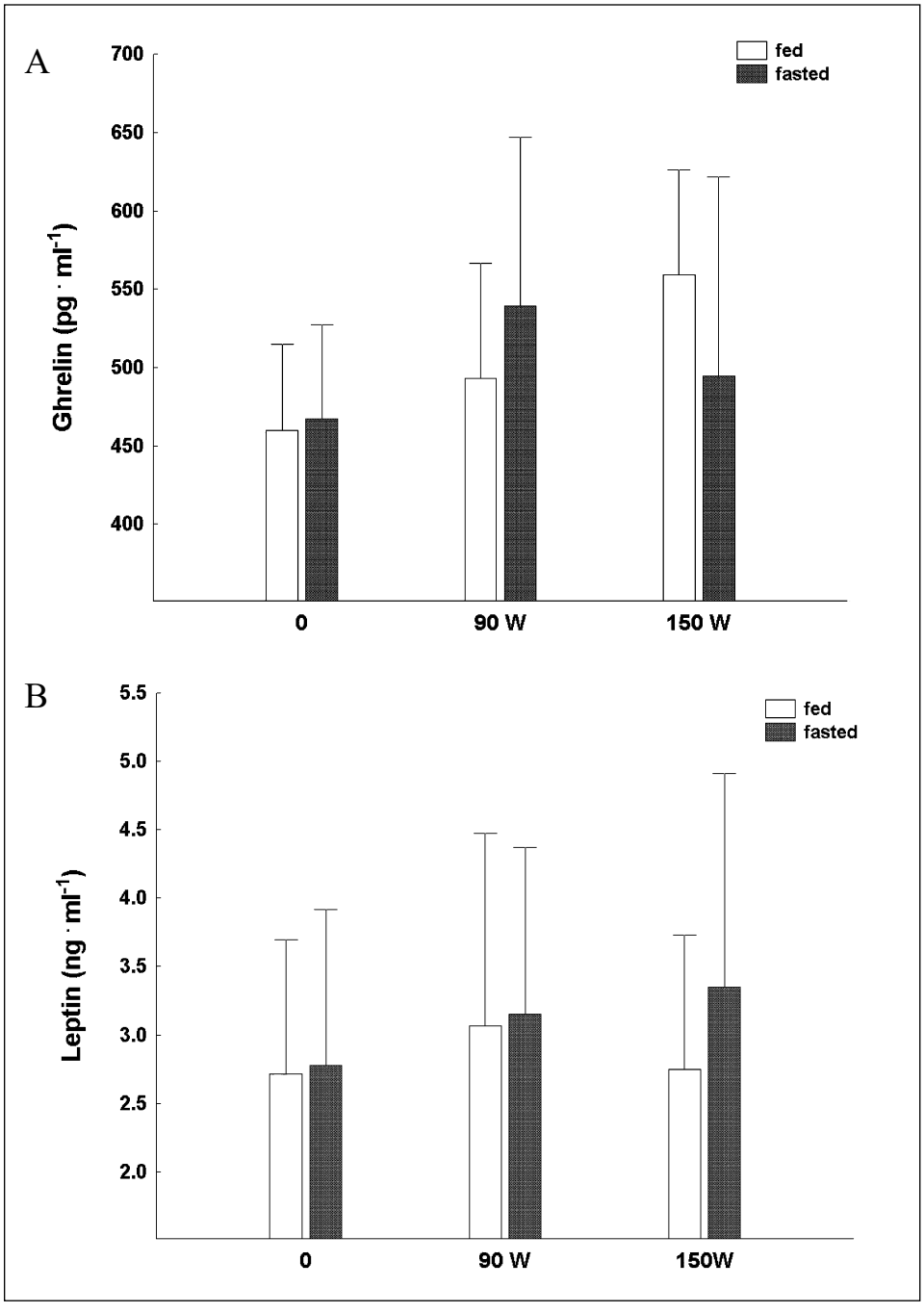

Fig. 8. Mean (± SE) values of plasma grehlin (panel A) and leptin (panel B) concentration during incremental exercise performed in fed (□) and fasted state (■).

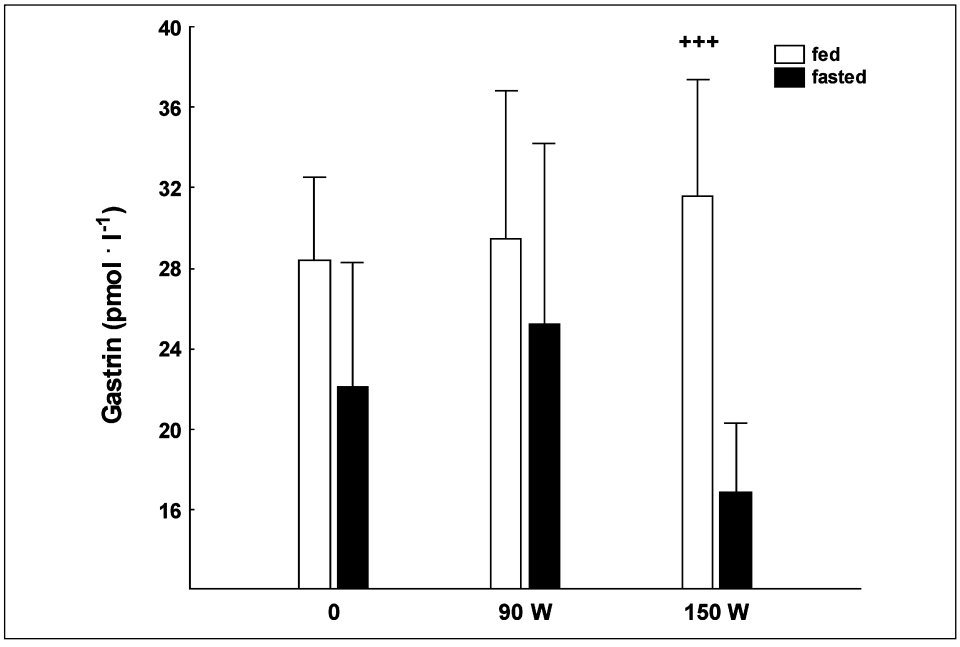

Fig. 9. Mean (± SE) values of plasma gastrin concentration during incremental exercise performed in fed (□) and fasted state (■). +++ - significantly different from fasted (Wilcoxon sign rank test for dependent samples, p = 0.008).

gastrin concentration in fed state was 28.49 ± 7.38 pmol · l^{-1} and it was not significantly different than in fasted state amounting to 25.26 ± 8.93 pmol · l^{-1}. At the power output of 150 W the gastrin concentration in fasted state amounting to 16.90 ± 3.43 pmol · l^{-1} and it was significantly (p = 0.008) lower than in fed state amounting to 31.59 ± 5.80 pmol · l^{-1} (*Fig. 9*).

Insulin

At rest plasma insulin concentration in fed state was not significantly different from its level determined in fasted state, amounting to 12.76 ± 2.21 μU · ml^{-1}. No significant effect of the performed exercise was found, when compared to the values measured at rest, both in fed and fasted state (*Fig. 10 A*).

Glucose

At rest plasma glucose concentration in fed state was not significantly different from its level determined in fasted state. At the power outputs of 90 W plasma glucose concentration in the fed state rose to 5.25 ± 0.27 mmol · l^{-1} and it was significantly higher (p = 0.039) than at rest. In fasted state plasma glucose concentration has amounted to 5.05 ± 0.17 mmol · l^{-1}. This increase was no significant in relation to the resting value. Level of glycemia at this power output

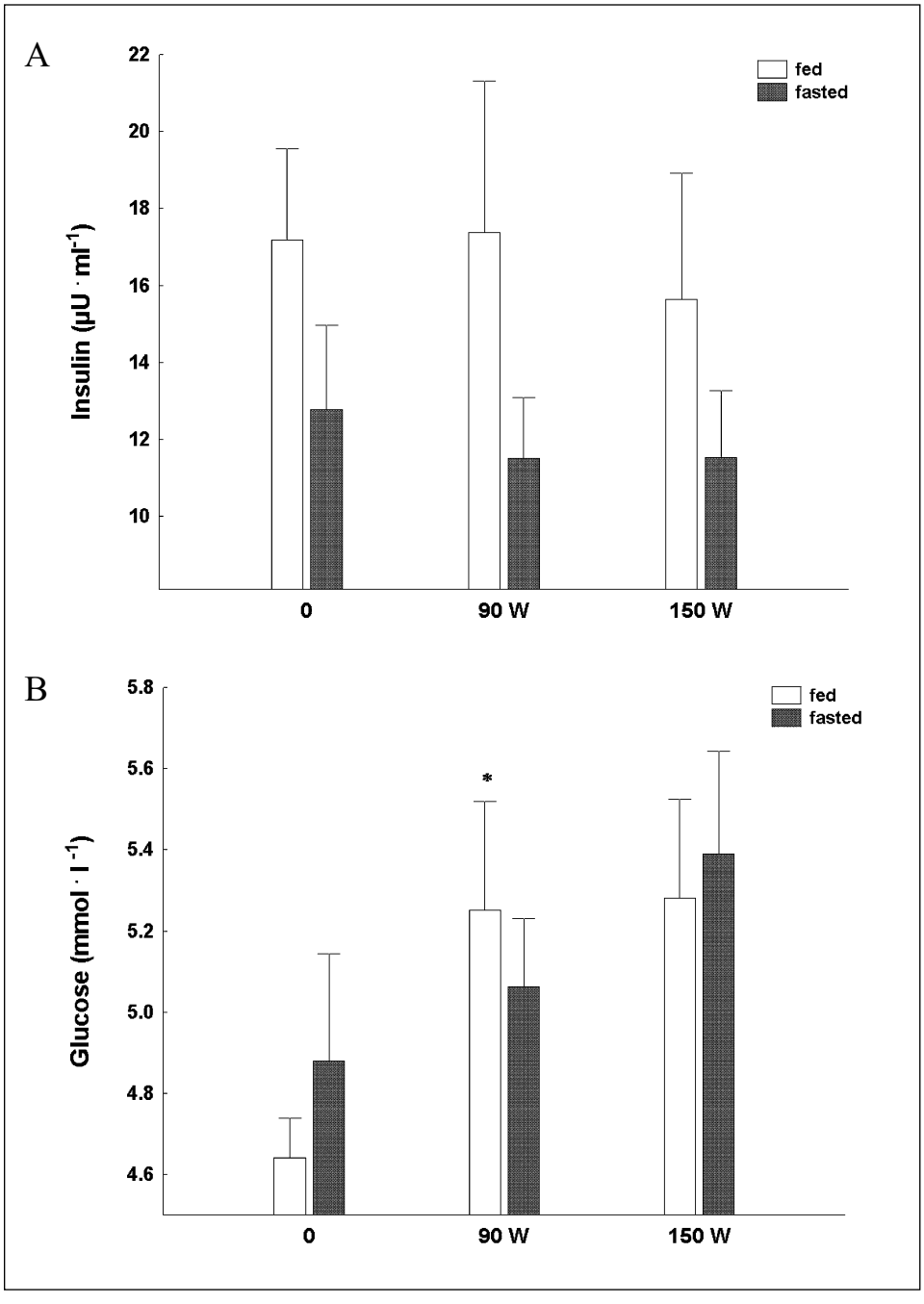

Fig. 10. Mean (± SE) values of plasma insulin (panel A) and glucose (panel B) concentration during incremental exercise performed in fed (□) and fasted state (■). Significantly different from rest (Wilcoxon sign rank test for dependent samples, * p = 0.039).

was not significantly different in fed and fasted state. Similarly, at the power output of 150 W no difference in plasma glucose concentration was found between fed and fasted state (*Fig. 10 B*).

Norepinephrine

At rest plasma norepinephrine concentration in fed subjects has amounted to 1426 ± 70 ng · l^{-1} and it was significantly lower (p = 0.047) than in fasted state, amounting to 2842 ± 414 ng · l^{-1}. At the power output of 90 W plasma norepinephrine concentration in fed subjects has amounted to 1253 ± 96 ng · l^{-1} and it was significantly lower (p = 0.016) than in fasted state amounting to 4225 ± 885 ng · l^{-1}. At the power output of 150 W plasma norepinephrine concentration in fasted state amounting to 2798 ± 442 ng · l^{-1} was also higher than in the feed state (1584 ± 166 ng · l^{-1}), however this difference did not reach statistical significance (p = 0.11) (*Fig. 10 B*).

Correlations

We have found significant negative correlation (r = - 0.90; p < 0.05) between plasma insulin and ghrelin concentration at rest in fed subjects (n = 8) (see *Fig. 12*).

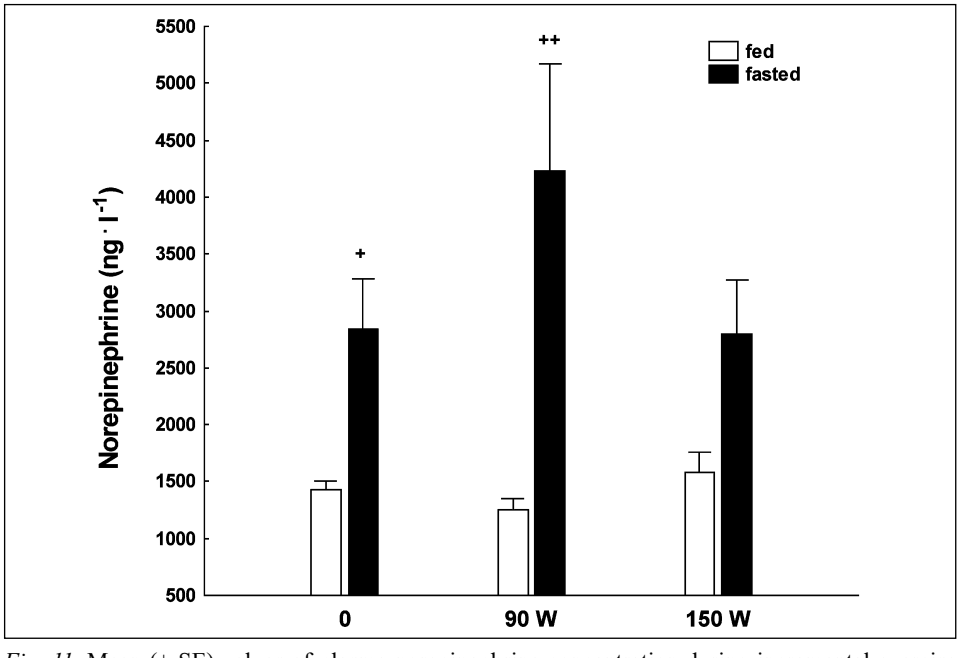

Fig. 11. Mean (± SE) values of plasma norepinephrine concentration during incremental exercise performed in fed (□) and fasted state (■). + - significantly different from fasted (Wilcoxon sign rank test for dependent samples, p = 0.047) at rest, and ++ - significantly different from fasted (Wilcoxon sign rank test for dependent samples, p = 0.016) at 90 W.

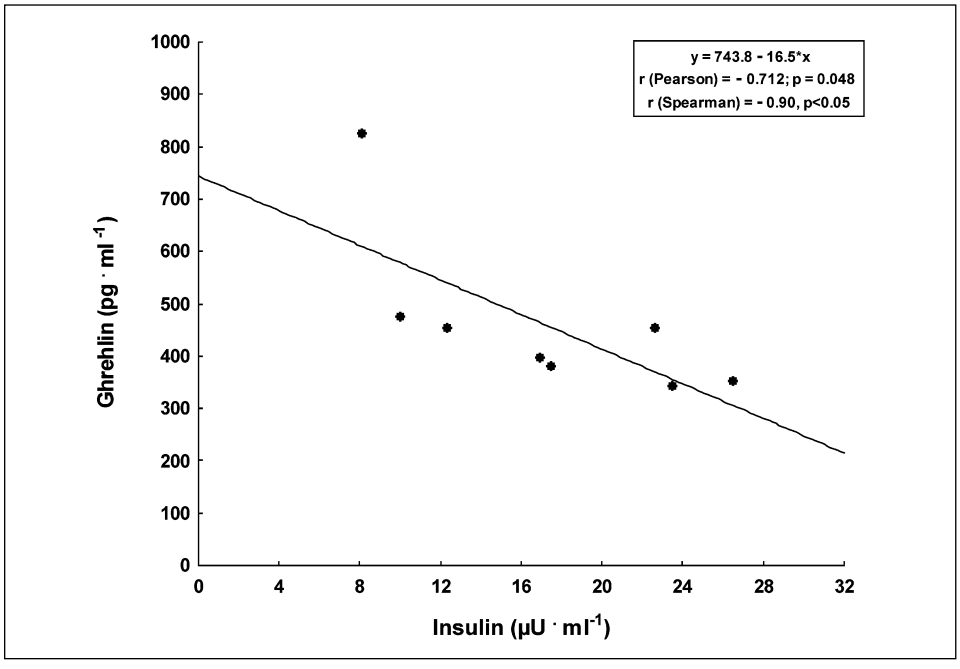

Fig. 12. Relationship between plasma insulin and ghrelin concentration at rest in fed subjects.

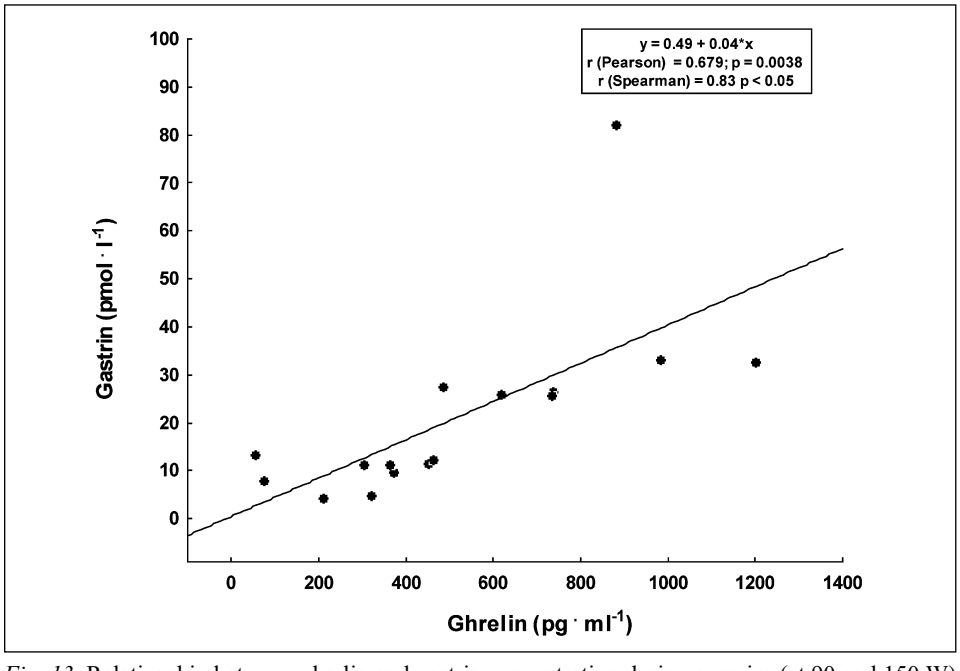

Fig. 13. Relationship between ghrelin and gastrin concentration during exercise (at 90 and 150 W) in fasted subjects (n = 8 + 8).

Significant positive correlation ($r = 0.83$; $p < 0.05$) was found between plasma ghrelin and gastrin concentration during exercise (at 90 W and 150 W) in fasted subjects (n = 8 + 8), see *Fig. 13*.

DISCUSSION

Despite of a significant effect of overnight fasting on cardio-respiratory responses to exercise such as: lower heart rate, lower carbon dioxide production, lower respiratory quotient and lower plasma lactate concentration, we have found no major effect of this combined intervention on the studied plasma hormone concentrations including grehlin, leptin, and insulin, except for norepinephine, which was significantly higher in the fasting state.

Our results regarding respiratory quotient (RQ) are in agreement with the data reported in the literature, showing that overnight fasting significantly decreases RQ during exercise, and indicating relative increase in lipid oxidation (39-41). Overnight fasting acutely induces changes in energy substrates utilization resembling the effect of endurance training (42-45). Far less attention has been paid to the effect of overnight fasting on heart rate during exercise. Moreover, in none of the previous reports the effect of exercise, performed after overnight fasting, was related to the hormones involved in the control of food intake (*i.e.* leptin, ghrelin, gastrin and insulin concentrations).

We have observed significant attenuation of the exercise induced increase in heart rate during exercise performed in fasting state. It was by about 10 bt . min^{-1} lower than in the control study (see *Fig. 4*). This effect was observed systematically at each power output up to 150 W. Our results are in agreement with previous observations (10, 34), and contradict some others (32, 33, 35).

The effect of attenuation of heart rate, during exercise performed after overnight fasting, fallaciously resembles the training induced decrease in HR after endurance exercise. On the other hand it is well know that fasting decreases endurance capacity (see *e.g.* 34, 42). Therefore, the effect of fasting induced attenuation of heart rate during exercise should be taken into consideration when assessing human exercise capacity applying the heart rate / power output relationship, using *e.g.* Astrand test (see 47). We have calculated that in case of our subjects this effect could overestimate the VO_2max predicted from the Astrand-Ryhming nomogram as much as by 17 %.

Based on the literature data concerning complementary, yet antagonistic, effect on the heart rate at rest of leptin (14, 17) and ghrelin (20), one would expect that the attenuation of the heart rate during exercise performed in the fasted state should be related to the changes in plasma leptin and ghrelin concentrations. However, despite of significant attenuation of the heart rate during exercise performed in the fasted state (see *Fig. 4*), no effect of the performed exercise on the plasma leptin and ghrelin concentrations was found

(see *Fig. 7* and *8*). Moreover, we have found no effect of the overnight fasting on pre-exercise plasma leptin and ghrelin concentrations. This may be due to relatively short duration of fasting (48, 49).

The most likely explanation of the effect of attenuation of heart rate during exercise performed in fasting state is the observed significant increase in plasma norepinephrine concentration (see *Fig. 11*), leading to the increase in systemic vascular resistance, loading of arterial baroreceptors and causing vagal stimulation (for review see 50, 51).

Interesting, and unexpected finding of this study was that pre-exercise plasma IL-6 concentration in fasting state was significantly elevated in relation to fed state (*Fig. 6*). Plasma IL-6 concentration at rest in fed subjects was negatively correlated with plasma ghrelin concentration ($r = -0.73$, $p < 0.05$) and positively correlated with plasma insulin concentration ($r = 0.78$, $p < 0.05$). It has been hypothesised that IL-6 produced by contracting skeletal muscle (52, 53) partly mediates the hepatic glucose output (54). Our results suggest, that similar mechanism may operate at rest while fasting. We postulate that the significant increase in plasma IL-6 concentration at rest in the fasting state is a defence response to maintain glucose homeostasis. Concomitantly, in our study we observed increase in plasma IL-6 concentration in the fasting state accompanied by lower RQ, indicating higher fat oxidation. This may suggest that fasting-induced increase of this cytokine concentration stimulates lipolysis and fat oxidation in fasting, as recently observed by van Hall *et al.* (55) after infusion of rhIL-6.

During the applied incremental exercise of rather moderate intensity, we did not observed a significant increase in plasma IL-6 concentration both in fed as well as in fasted state (*Fig. 6*). This indicates that during this exercise of rather low intensity (up - to 60 % of VO_2max) and of short duration - lasting 12 minutes - the maintenance of glucose homeostasis both in fed and fasted state (see *Fig. 10 B*) was not IL-6 dependent. In view of the literature data muscle glycogen depletion (53, 56) and type II muscle recruitment (57) are the most likely stimulus for exercise induced increase muscle IL-6 realise. This is rather unlikely, that our exercise protocol, lasting only 12 minutes and not exceeding 60 % VO_2max, applicable for rehabilitation and therapeutic purposes, could cause substantial glycogen depletion in type II muscle fibres recruitment, both in fed or fasted subjects.

However, in the control study, *i.e.* in fed state, at the end of the incremental exercise test - until exhaustion, we have observed significant ($p < 0.02$) - 4 fold - increase in plasma IL-6 concentration - up to 1.29 ± 0.32 pg $\cdot$ ml^{-1}, in relation to the pre-exercise level (0.29 ± 0.04 pg $\cdot$ ml^{-1}). This increase was even more pronounced at 30 minutes after finishing this exhaustive exercise, where the plasma IL-6 concentration reached 2.07 ± 0.01 pg $\cdot$ ml^{-1} (7 - fold increase in relation to rest value). Unfortunately, we can not compare these results with

exhaustive exercise performed in fasted state, most frequently ending with hypoglycaemia - as experienced before.

No difference in oxygen uptake during exercise performed in fed and fasted state was found (see *Fig. 1*), therefore, this is rather unlikely that lower HR observed in fasted state was accompanied by lower muscle blood flow. We postulate that the lower HR during exercise performed in fasted state was possible due to a lower need for perfusion of the intestinal vascular bed in fasted state. What would this effect have on the plasma gut hormones realize is not clear, but in the present study in the fasting state, we have observed tendency towards lower plasma gastrin concentration at rest and during exercise in relation do fed state, reaching significance at 150 W (*Fig. 9 A*). This could be caused by lower splanchnic blood flow and greater vagotony in the fasting state.

We have observed expected tendency towards increase in plasma insulin concentration in the fed subjects (*Fig. 10 A*), however we could not noticed the well known exercise-induced depression of insulin secretion, perhaps due to applying rather moderate intensity exercise of short duration. In the present study, we have found significant negative correlation between plasma insulin and ghrelin concentration ($r = - 0.91$, $p < 0.05$) at rest in fed subjects. The data presented in *Fig. 12 A* are in agreement with the well established fact that in fasting plasma insulin decreases and ghrelin increases (16, 17, 58).

In fed subjects only at rest we have observed significant negative correlation between plasma glucose and plasma leptin concentration ($r = - 0.81$, $p < 0.05$). This is in agreement with previous findings showing that leptin ("satiety" hormone) has an additive effect with insulin causing normalization of glycaemia (see 59).

In the present study, as previously (26), in the fed state we have observed higher concentrations of plasma gastrin both at rest as well as during exercise (see *Fig. 9*). Moreover, we have found significant negative correlation in fed subjects between plasma gastrin and leptin concentration at rest ($r = - 0.53$, $p < 0.05$) as well as during exercise performed in fed ($r = - 0.55$, $p < 0.05$) and in fasted state ($r = - 0.58$, $p < 0.05$). Negative correlation between gastrin and leptin could be explained by the decrease in gastric acidity by leptin and secondary change in plasma gastrin, whose release from the G-cells is controlled by gastric acidity.

In the fasted subjects only, significant positive correlation ($r = 0.83$, $p < 0.05$) between ghrelin and gastrin concentration during exercise was found (see *Fig. 13*). The relationship between ghrelin and gastrin is quite interesting as remains in close agreement with our recent studies showing that ghrelin stimulates the release of gastrin as well as gastric acid secretion (60) and our previous results in animals are in keeping with the observed rise in plasma gastrin accompanying the increment in plasma ghrelin. We have also found that both plasma gastrin and ghrelin concentration determined during exercise in the fasted subjects was negatively correlated with respiratory quotient ($r = - 0.53$, $p < 0.05$ and $r = - 053$, $p < 0.05$).

As others before (1, 3, 27, 61, 62) we have observed significant exercise-induced increase in plasma hGH concentration (*Fig. 7*). It has been previously suggested, that increase in plasma ghrelin concentration may cause increase in hGH level (63, 64). Ghrelin is known for its potent hGH releasing activity through the stimulation of GH secretagogue (GHS) receptor type 1a (GHRS-R-1a), that are concentrated in hypothalamus-pituitary unit and other central and peripheral tissues. In the present study, significant increase in plasma hGH concentration has occurred without any changes in plasma ghrelin suggesting that secretion of hGH during exercise of moderate intensity is independed of plasma ghrelin concentration, which is in agreement with the previous study (28). Moreover, these authors postulated that exercise induced hHG concentration may suppress / inhibit ghrelin secretion, as observed in patients with GH deficiency after hGH infusion (28). In the previous study a significant relationship between plasma lactate and plasma GH concentration was reported (3, 27, 65, 66). In the present study we have observed similar increase in plasma hGH concentration during exercise, however in fasted state the same increase in hGH concentration during exercise was accompanied by significantly lower plasma lactate concentration. This provides further support to the concept, that the exercise-induced increase in plasma GH concentration is not closely dependent upon the increase in plasma La concentration (see *e.g.* 27, 67).

In conclusion: Incremental exercise of moderate intensity - up to 60 % of VO_2 max, performed in fasted state, causes about 10 bt · min^{-1} lower HR at each power output than the same exercise performed in fed state. This effect is independent of plasma leptin and ghrelin concentrations, which were not affected by the overnight fasting and the applied exercise. We postulated that attenuation of heart rate during exercise, performed in the fasting state, is caused by increased plasma norepinephrine concentration, leading to the increase in systemic vascular resistance and baroreceptor mediated vagal stimulation. Moreover we believe, that the fasting-induced significant increase in plasma IL-6 concentration at rest, accompanied by higher plasma norepinephrine concentration and lower RQ, belongs to the physiological responses, maintaining energy homeostasis in the fasting state.

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