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THE EFFECT OF FASTING AND PHYSICAL EXERCISE ON PLASMA LEPTIN CONCENTRATIONS IN HIGH-FAT FED RATS

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The aim of our study was to estimate the effect of fasting and physical exercise on a treadmill on plasma leptin concentrations in high-fat fed rats. Male Wistar rats were injected a low dose of streptozotocin (STZ) or buffer at 2 days of age and later fed a standard or high-fat diet (HFD). Plasma leptin was measured by RIA method in all the groups studied in basal conditions, after 48h fasting, a single bout of exhaustive exercise, and 4 weeks of exercise training. Plasma leptin concentrations were markedly elevated in the HFD and STZ/HFD groups compared to the control group. The significant correlation between plasma leptin and body weight was noted. Fasting and exercise training decreased plasma leptin in similar percentage in all the groups studied. The observed decrease was greater than expected from changes in body weight. We conclude that high-fat feeding results in an increase in plasma leptin levels in rats independently of plasma insulin or daily calorie intake. High-fat fed rats have maintained leptin response to fasting and exercise training. The reduction in plasma leptin after exercise training is partly independent on changes in body weight or plasma insulin.

Key words: *high-fat diet, leptin, fasting, exercise.*

INTRODUCTION

Leptin, the protein encoded by the *ob* gene and secreted by the adipocytes, regulates food intake and body weight in animals and in humans (1, 2). It acts *via* the central nervous system probably by inhibition of the neuropeptyde Y (NPY) synthesis (3). *Ob*/ob mice, with the mutation of the *ob* gene, have no detectable plasma leptin and are characterized by obesity, diabetes, reduced thermogenesis and physical inactivity. Leptin treatment of the *ob*/ob mice normalizes body weight and temperature and improves glucose tolerance (4). *Db*/db mice, phenotypically similar to the former, have mutation of the leptin receptor gene and become markedly hyperleptinemic and leptin resistant (5). The mutation of the leptin receptor gene is also present in the Zucker *fa*/fa rats (6).

In humans plasma leptin reflects body fat (7, 8). Obese people have elevated plasma leptin and it is supposed that they are leptin resistant (2). The mutation

of the leptin receptor gene has not as yet been found in humans and it is hypothesized that impaired leptin transport to the central nervous system plays a major role in human leptin resistance (9). Disturbances of leptin action are thought to play a pivotal role in human obesity. Regulation of leptin secretion is not fully understood. Insulin is supposed to be one of the important factors which increases leptin synthesis and secretion by the adipocytes (10, 11). Obesity is usually connected with insulin resistance and is also a potent risk factor for the development of type 2 diabetes mellitus, which is characterized by insulin resistance and impaired insulin secretion (12). However, the relationship between leptin and insulin resistance is as yet undefined.

There are numerous reports in the literature that high-fat feeding induces insulin resistance and increases body weight in rats (13, 14). The connection of high-fat feeding with the earlier neonatal low-dose streptozotocin (STZ) injection induces a state similar to type 2 diabetes mellitus (15, 16). There are a few reports that caloric restriction and physical exercise, which improve insulin sensitivity, also decrease plasma leptin in lean rats (17) and humans (18, 19). However, the data about their action on plasma leptin in insulin resistant states are conflicting. Therefore, the aim of the present study was to estimate the effect of 48h fast, a single bout of exhaustive exercise and prolonged exercise training on plasma leptin concentrations in high-fat fed rats with normal or slightly impaired β-cell secretory capacity.

MATERIALS AND METHODS

All the experiments were approved by the Ethics Commitee of The Medical School, Białystok.

Animals

All the studies were carried out on male Wistar rats at 11 weeks of age. The animals were maintained at about 20°C on a 12 h light-dark cycle. At 2 days of age rats were randomly assigned to the group which received STZ (45 mg/kg i.p.) in 50 µl citrate buffer (0,1 M.; pH 4,5) or citrate buffer only. All animals remained with their mothers until weaning at 4 weeks of age. Then they were allowed access to standard rat diet and water ad libitum. At 8 weeks of age, all the rats were randomly assigned to either high-fat (59% of calories as fat) or standard diet. The composition of the high-fat diet is given elsewhere (13, 16). All the food given was isocaloric (311 kJ per day). Four groups of rats were examined: I — buffer + standard diet — control; II — buffer + high-fat diet — the HFD group; III — STZ + standard diet — the STZ group; IV — STZ + high-fat diet — the STZ/HFD group. There were four subgroups generated in each of these groups. Each subgroup included 12 rats. Plasma leptin concentrations were assessed in basal conditions, after a 48 h fast, after a single bout of endurance exercise on a treadmill (mean running to exhaustion time 2 h 40 minutes, no significant changes between the groups) or after 4 weeks of exercise training on a treadmill. As plasma leptin levels show diurnal rhytmicity, all blood samples were collected at the same time (10 a.m.). Plasma glucose, insulin, triglycerides (TG) and non-esterified fatty acids (NEFA) were also determined. Insulin sensitivity index was calculated as the fasting insulin/glucose ratio and was assessed only in rats which did not receive STZ.

An intravenous glucose tolerance test (IVGTT) was performed on all the rats examined in basal conditions 4 days before the final experiments. 500 mg/kg glucose (40% solution) was administered *via* the tail vein. Glucose and insulin levels were measured at 0, 2, 5 and 10 minutes. Blood samples were collected by cutting the tail.

Before a single bout of exercise and exercise training the rats were adapted to a treadmill for 6 days of 10 minutes running. The treadmill was set at a rate of 20 m/min up a 10° grade. Rats were exercise trained 6 days a week from the 7th week of age. The training program was: first week — 1 h of running; 2nd week — 2 h of running; 3rd—4th week — 3 h of running daily. In exercise trained rats blood samples were collected after a 48 h rest.

Blood analysis

Plasma leptin was assessed by radioimmunoassay (RIA) (Linco Research, USA). The within-assay analytic coefficient of variation of the RIA kit ranged from 3.4 to 8.3%, and the between-assay coefficient of variation ranged from 3.0 to 6.2%. Plasma glucose was measured by enzymatic method using GOD-PAP Cormay GS-120 L Kit. Insulin levels were determined by RIA method with Rat insulin DLR-RI-13 K (DRG). Plasma TG were assessed by enzymatic method with Cormay TG Kit. Plasma NEFA levels were measured with colorimetric method described by Duncombe (20).

Statistical analysis

All analyses were performed using the Statistica program. To assess differences between groups paired Student's t-test was used. To examine correlations between variables simple and multiple regression analysis was performed. For all the analyses performed, a statistical significance was accepted at $p<0.05$.

RESULTS

Body weight

(*Table 1*). The HFD rats had markedly higher ($p < 0.01$) and the STZ rats lower ($p<0.005$) body weight than the controls. Fasting decreased body weight in the control and STZ groups ($p < 0.02$ in both groups) and a single bout of exercise did not change body weight. In those conditions the HFD rats still had higher body weight than the controls (fasting: $p < 0.002$; exhaustive exercise: $p < 0.02$). Prolonged exercise training decreased body weight in all the groups studied (control — 22%, $p < 0.02$; HFD — 34,7%, $p < 0.002$; STZ — 14,7%, p = NS; STZ/HFD — 11,4%; p = NS). There was no differences in body weight between groups after exercise training.

Table 1. Body weight (g). All the data are presented as mean ± SEM. Statistical significance: a — $p < 0.05$ in comparison to the control group in the same conditions, b — $p < 0.05$ in comparison to basal conditions within the same group.

	Basal	Fasting of exercise	A single bout of exercise	Exercise training
Control	316.17 ± 47.61	287.60 ± 14.26 b	280.33 ± 28.85	246.60 ± 26.63 b
HFD	353.17 ± 13.19 a	348.60 ± 6.35 a	321.27 ± 19.84 a	230.50 ± 18.36 b
STZ	254.31 ± 42.21 a	195.83 ± 49.96 b	242.11 ± 38.82	217.00 ± 23.95
STZ/HFD	282.44 ± 25.49	265.33 ± 24.44	269.20 ± 37.82	250.20 ± 18.86

Plasma leptin concentrations

(*Table 2*) were markedly elevated in the HFD and STZ/HFD groups compared to the control group ($p < 0.05$; $p < 0.005$; respectively). STZ injection did not markedly influence plasma leptin (control vs STZ and HFD vs STZ/HFD; p = NS). Fasting and exercise training decreased plasma leptin in all the groups studied (fasting: control — $p < 0.001$; HFD — $p < 0.05$; STZ — $p < 0.02$; STZ/HFD — $p < 0.01$; exercise training: $p < 0.001$; $p < 0.01$; $p < 0.005$; $p < 0.01$; respectively). The percentage of leptin decrease is shown in

Table 2. Plasma leptin concentrations (ng/ml). All the data are presented as mean ± SEM. Statistical significance: a — $p < 0.05$ in comparison to the control group in the same conditions, b — $p < 0.05$ in comparison to basal conditions within the same group.

	Basal	Fasting	A single bout of exercise	Exercise training
Control	2.18 ± 0.61	0.55 ± 0.1 b	2.08 ± 0.69	0.60 ± 0.14 b
HFD	5.64 ± 4.14 a	2.18 ± 0.84 ab	4.85 ± 1.77 a	1.62 ± 0.44 ab
STZ	2.34 ± 0.82	0.83 ± 0.32 b	1.80 ± 0.7	0.76 ± 0.13 b
STZ/HFD	5.93 ± 3.25 a	1.87 ± 0.87 ab	4.90 ± 1.37a	1.53 ± 0.94 ab

Table 3. A single bout of exercise did not influence plasma leptin. In all the conditions studied the HFD and STZ/HFD rats remained hyperleptinemic in comparison to the controls (fasting: $p < 0.02$ in both groups; a single bout of exercise: $p < 0.05$ in both groups; exercise training: $p < 0.02$; $p < 0.05$; respectively).

Table 3. Percentage of leptin decrease after 48 h fasting, a single bout of exercise and exercise training.

	Fasting	A single bout of exercise	Exercise training
Control	74.8	4.6	72.5
HFD	61.3	14.0	71.3
STZ	64.5	23.0	67.5
STZ/HFD	68.5	17.4	74.2

Plasma glucose and insulin during IVGTT

(*Fig. 1*). Plasma glucose was markedly increased in the STZ/HFD vs control group during all the tests ($p < 0.05$ at all points in the test) and in the HFD and STZ groups — at 5 and 10 minutes into the test ($p < 0.05$ in all cases). Plasma insulin was significantly higher in the HFD vs control group ($p < 0.05$ at all points in the test) and lower in the STZ and STZ/HFD groups ($p < 0.05$ at 2, 5 and 10 minutes).

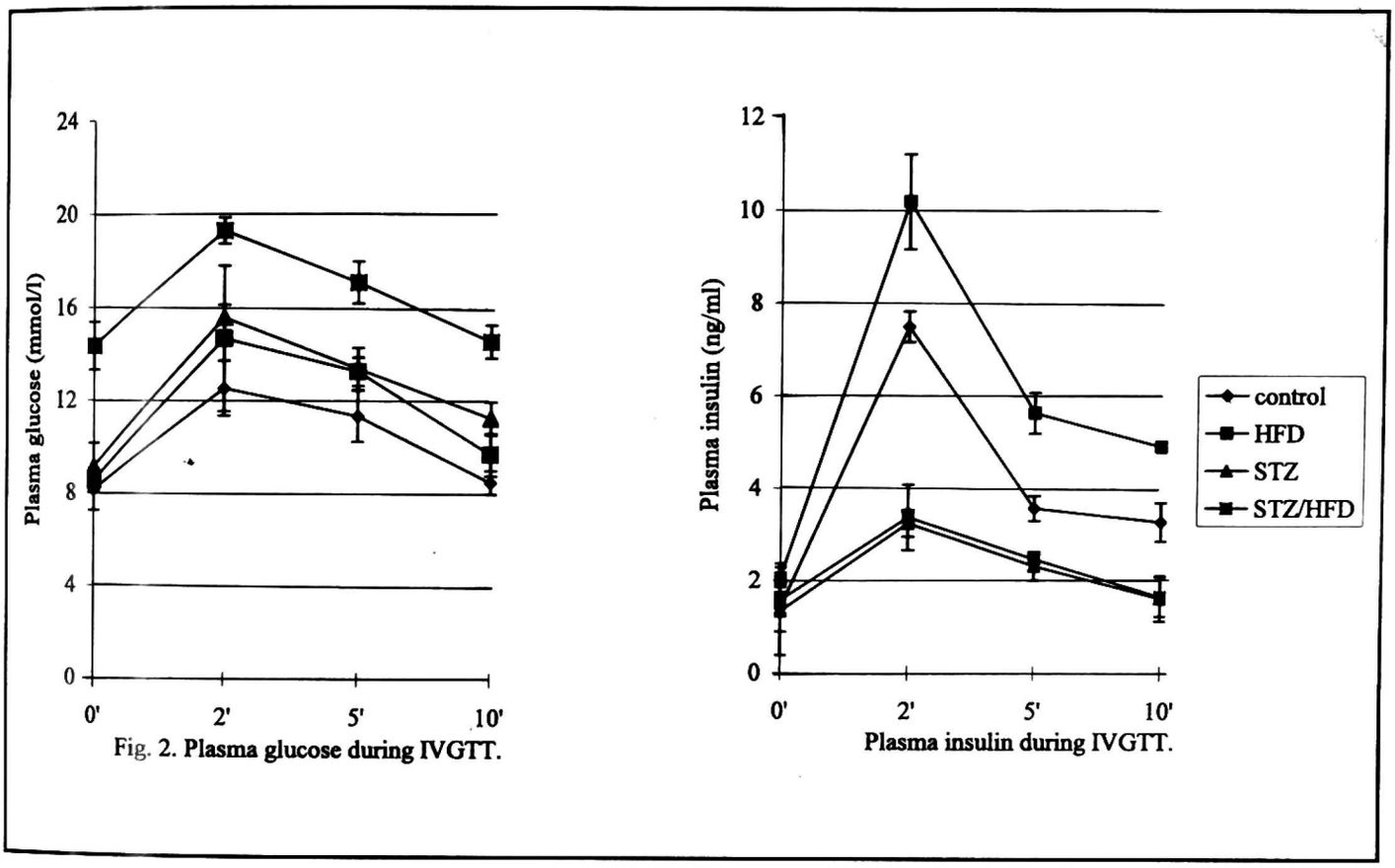

Fig. 1. Plasma glucose and insulin during IVGTT.

Plasma glucose concentrations

(*Table 4*) were markedly higher in the STZ/HFD group vs control ($p < 0.05$). Fasting and a single bout of exercise significantly decreased plasma glucose in all studied groups ($p < 0.05$ in all cases). Exercise training did not cause any changes in plasma glucose levels. In all examined conditions the STZ/HFD rats were hyperglycemic in comparison to the controls ($p < 0.05$ in all cases).

Table 4. Plasma glucose (mmol/l). All the data are presented as mean ± SEM. Statistical significance: a — $p < 0.05$ in comparison to the control group in the same conditions, b — $p < 0.05$ in comparison to basal conditions within the same group.

	Basal	Fasting	A single bout of exercise	Exercise training
Control	8.62 ± 1.06	6.54 ± 0.92 b	2.67 ± 0.84 b	7.29 ± 1.35
HFD	8.51 ± 1.17	5.04 ± 1.31 b	3.44 ± 0.64 b	8.50 ± 0.85
STZ	9.34 ± 1.03	6.85 ± 1.80 b	4.76 ± 0.71 b	7.62 ± 1.13
STZ/HFD	13.01 ± 2.55 a	8.33 ± 1.41 ab	4.92 ± 1.60 ab	12.64 ± 2.88 a

Plasma insulin

(*Table 5*) was higher in the HFD group vs control ($p < 0.01$). Fasting and a single bout of exercise caused a marked decrease in plasma insulin levels (fasting: control — $p < 0.005$; HFD — $p < 0.02$; STZ — $p < 0.02$; STZ/HFD

— $p < 0.02$; respectively; exercise: $p < 0.001$ in all cases). After fasting the HFD rats were still hyperinsulinemic compared to the control group ($p < 0.01$); while after a single bout of exercise there was no such relationship. Exercise training did not affect plasma insulin in any of the groups. Changes in insulin/glucose index were similar to those observed in fasting insulin levels.

Table 5. Plasma insulin (ng/ml). All the data are presented as mean ± SEM. Statistical significance: a — $p < 0.05$ in comparison to the control group in the same conditions, b — $p < 0.05$ in comparison to basal conditions within the same group.

	Basal	Fasting	A single bout of exercise	Exercise training
Control	1.40 ± 0.2	0.23 ± 0.05 b	0.10 ± 0.03 b	1.22 ± 0.23
HFD	2.10 ± 0.44 a	0.46 ± 0.11 ab	0.23 ± 0.09 b	1.84 ± 0.41 a
STZ	0.95 ± 0.21	0.11 ± 0.03 b	0.05 ± 0.01 b	1.03 ± 0.19
STZ/HFD	1.53 ± 0.42	0.17 ± 0.04 b	0.10 ± 0.04 b	1.17 ± 0.35

Correlations

A marked positive correlation was observed between plasma leptin and body weight in basal conditions ($r = 0.434$; $p < 0.01$) (*Fig. 2*). A similar

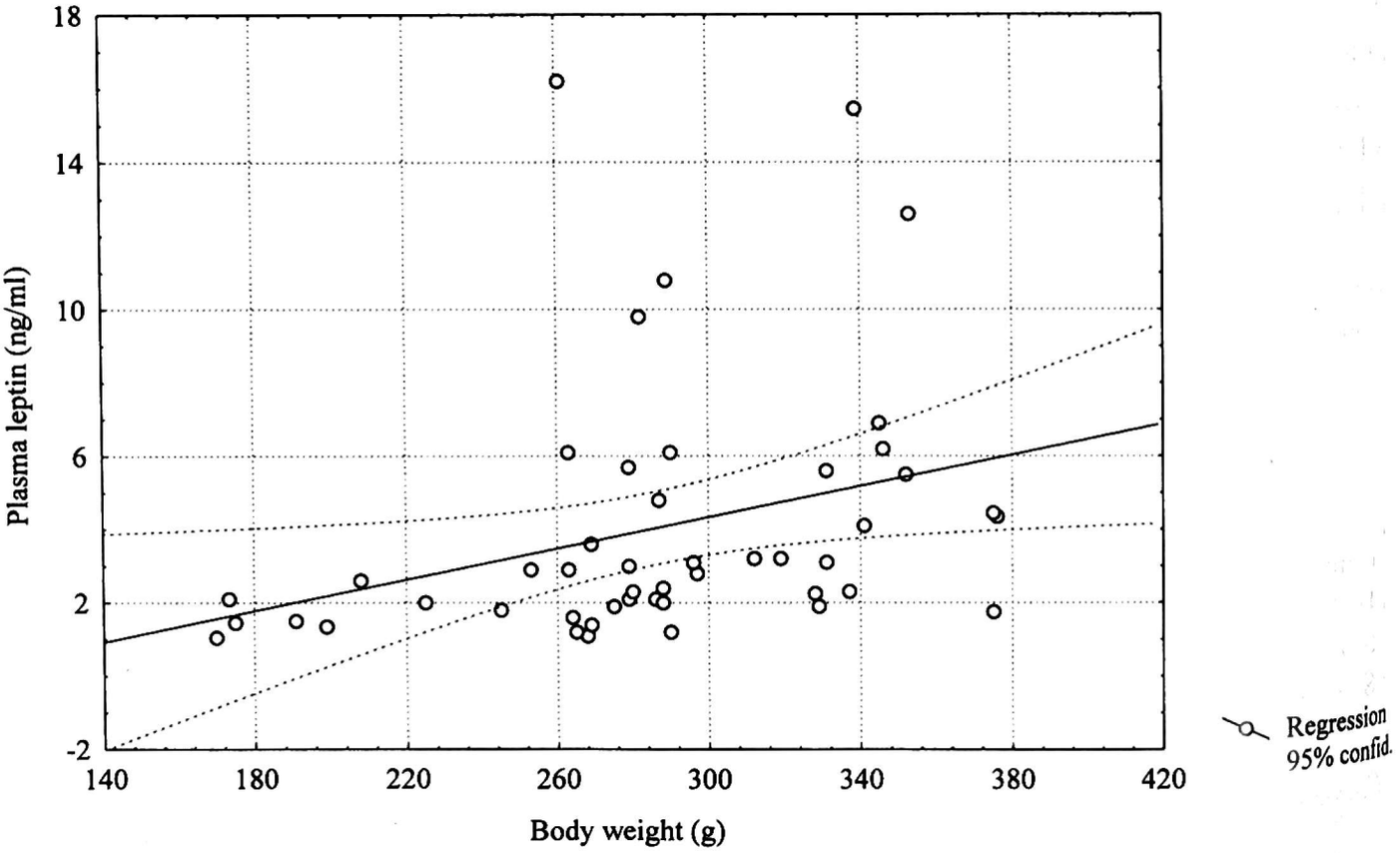

Fig. 3. Correlation between plasma leptin and body weight.

Fig. 2. Correlation between plasma leptin and body weight in basal conditions ($r = 0.434$; $p < 0.01$).

correlation was observed for the controls, HFD and STZ groups in basal conditions ($r = 0.43$; $p < 0.02$; $r = 0.58$; $p < 0.01$; $r = 0.67$; $p < 0.005$; respectively). No correlation was noted between leptin and insulin, glucose, insulin/glucose index, TG or NEFA.

DISCUSSION

In the present study the major determinant of plasma leptin levels in basal conditions is body weight, which may reflect body fat. This is consistent with the hypothesis, that in a steady-state energy balance, leptin is an index of adipose tissue amount (21). A similar relationship was demonstrated in rodents (22) and in humans (7, 8). In the present study a high-fat diet induced hyperleptinemia in rats. Increased leptin levels were previously found in diet-induced obesity in mice (22). However, the animals were fed ad libitum and the observed increase could be due to the excessive calorie intake. We demonstrated that high-fat food causes hyperleptinemia even if daily calorie intake is normal. Storlien *et al* previously reported that isocaloric high-fat food reduces thermogenesis and increases white adipose tissue accumulation (13). These changes are probably responsible for the slightly more rapid weight gain observed in the high-fat fed rats (15). Other authors have observed lesser sensitivity to exogenous leptin in rats with diet-induced obesity (23), suggesting that they are leptin resistant. Leptin resistance may also exist in the high-fat fed rats examined in the present study and may be responsible for the previously described reduced thermogenesis and energy expenditure after high-fat feeding (13). However, what the primary defect is, hyperleptinemia or leptin resistance, is as yet unknown.

Relative hypoinsulinemia caused by a low-dose STZ injection did not alter plasma leptin. No correlation between plasma leptin and insulin was observed. Other authors have found decreased *ob* gene expression in the STZ-treated rats (24, 25). However, we used a lower dose of STZ, and we administered it in the neonatal period, when β-cells are less sensitive for STZ (26). Therefore our treatment resulted in only impaired first-phase insulin secretion with no changes in basal plasma insulin values. Insulin is supposed to stimulate leptin synthesis and secretion. In rats insulin increases *ob* mRNA expression in white adipose tissue both *in vivo* (17, 27) and *in vitro* (28). Data obtained on humans are more conflicting. Some authors demonstrated an acute effect of insulin on plasma leptin (11); while others were unable to show any changes in plasma leptin levels after acute insulin administration (29—31). Kolaczynski *et al* showed a stimulating effect of insulin on the *ob* gene expression only during prolonged insulin infusion (30). Therefore they hypothesized that insulin

stimulates leptin synthesis only because of its trophic effect on the adipocytes (21, 30). We did not estimate the direct effect of insulin on leptin secretion. However, it seems to be an important finding, that high-fat food results in hyperleptinemia even if there is coexisting slight hypoinsulinemia after the STZ injection. There were no significant differences in circulating leptin levels between high-fat fed rats whether they received STZ or not. Perhaps, insulin secretion in the STZ/HFD group was still sufficient to stimulate white adipose tissue accumulation in conditions of dietary fat excess. Hyperleptinemia observed in that group shows that there is no direct relationship between insulin and leptin and that there are other factors involved in the regulation of leptin production. However, circulating plasma leptin levels may not exactly reflect the *ob* gene expression in adipose tissue as was demonstrated by Considine *et al* in obese humans (2). We can also hypothesize that the regulation of leptin synthesis and secretion may be disturbed in leptin resistant states. The STZ/HFD rats became diabetic, but it is unlikely that diabetes itself alters plasma leptin concentrations (11, 31). Our study does not provide a final explanation for the increased plasma leptin after a high-fat diet.

We assessed changes in circulating leptin levels during states of negative energy balance. 48h fast decreased plasma leptin values in all the groups studied. The observed decrease ranged from approx. 60% to 75% between groups and was accompanied by lowered plasma glucose and insulin while only a slight decrease in body weight was observed. Decreases in leptin mRNA or in plasma leptin values after fasting have been described by other authors in mice (22), rats (27, 32) and humans (2, 18, 33). The observed decreases are much higher than one may calculate from changes in body weight or body fat. These data suggest that in non-steady states of energy balance leptin might be a sensor of energy balance (21). We found no correlation between leptin and insulin, glucose or body weight in fasted rats. Data obtained by us and other authors (33) suggest that it is unlikely that insulin mediates leptin response to fasting. The mechanism of rapid changes in circulating leptin values, remains to be determined.

The percentage of the leptin decrease after fasting was similar in all the groups studied. Cusin *et al* (27) found that *ob* mRNA escapes downregulation by fasting in insulin resistant obese fa/fa Zucker rats. Impaired leptin response to fasting was also observed in the C57BL/6J mice fed with a high-fat diet (22) and in insulin resistant *Psammomys obesus* (Israeli sand rats) (32). The difference between our data and those described by other authors may come from the animal model used. However, our results are consistent with the results obtained from humans, which showed a similar reduction in plasma leptin in lean and obese subjects (18).

Another example of negative energy balance is physical exercise, which is characterized by increased energy expenditure. A single bout of exhaustive

exercise did not result in significant changes in plasma leptin in any of the groups examined. This might seem surprising as it is the state of extremely negative energy balance. However, mean running-to-exhaustion time was 2h 40 minutes and it is unlikely that plasma leptin values change so rapidly. Other authors have reported unchanged plasma leptin in response to acute exercise in humans (33, 34). It is still possible that a bout of exercise has a delayed effect on circulating leptin, which may be detectable several hours after the cessation of exercise.

The 4-week exercise training program decreased plasma leptin values in similar percentage in all the groups studied. Interestingly, it was not accompanied by changes in plasma insulin levels. We observed a slight decrease in body weight in the exercise trained rats, however, it achieved the statistical significance in the control and HFD groups only. The reduction in plasma leptin after exercise training may be the result of a negative energy balance, decreased fat mass, or both. In the present study we observed a decrease in plasma leptin ranging from approx. 67% to 75% between groups in the exercise trained rats, while the decrease in body weight varied from 11% to 35%. Therefore, we suppose that the effect of exercise training on plasma leptin cannot be attributable only to the changes in body weight. Although it is well-known that exercise training results in changes in body composition (i.e. decrease in fat mass and increase in muscle mass), it still seems unlikely that the observed profound reduction in plasma leptin values is due to a decrease in fat mass only. Data obtained by other authors are conflicting. Zachwieja *et al.* (35) demonstrated a significant decrease in adipose tissue mass, leptin mRNA expression and circulating leptin levels in rats resistant (S5B/P1) or sensitive (Osborne-Mendel) to diet-induced obesity and the observed changes were similar in both groups (35). Some authors have observed that the decrease in plasma leptin in humans after exercise training is no longer significant when the changes in body fat are taken into account (34, 36). However, in most of the studies cited, negative energy balance was at least partly compensated by increased calorie intake. This factor was ruled out in the present study by giving the animals the isocaloric diet. Hickey *et al* found a marked decrease in plasma leptin in exercise trained middle-aged women, while no alterations in fat mass was observed (37). Furthermore, Pasman *et al* demonstrated a significant correlation between the reduction in plasma leptin and number of hours of exercise training in obese males; which was independent of changes in body fat or insulin levels (38). The present study also shows that insulin is not the mediator in alterations in plasma leptin as leptin values decreased while insulin remained unchanged. What the mechanism is by which exercise training acts on plasma leptin, is at present unknown. As was observed in basal conditions and after fasting, the HFD and STZ/HFD rats remained hyperleptinemic after physical exercise in comparison to the control and STZ groups. This might

suggest that neither fasting nor physical exercise are sufficient to restore leptin sensitivity in the high-fat fed rats.

We conclude that: 1) high-fat feeding results in an increase in plasma leptin levels in rats independently of alterations in insulin concentrations or daily total calorie intake, 2) high-fat fed rats have maintained leptin response to fasting and exercise training, 3) the reduction in plasma leptin after exercise training is partly independent on changes in body weight or plasma insulin.

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