Concurrent reductions of serum leptin and lipids during weight loss in obese men with type II diabetes

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Halle, Martin, Aloys Berg, Ulrich Garwers, Dominik Gratwwohl, Werner Knisell, and Joseph Keul. Concurrent reductions of serum leptin and lipids during weight loss in obese men with type II diabetes. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E277–E282, 1999.—The aim of the study was to examine the effects of weight reduction by exercise and diet on metabolic control in obese subjects with insulin resistance, particularly investigating if changes in serum leptin concentrations were directly associated with improvements in metabolic control. Twenty obese men (48 ± 8 yr; body mass index 32.1 ± 3.9 kg/m²) with previously diagnosed type II diabetes mellitus were assigned to a 4-wk intervention program of exercise (2,200 kcal/wk) and diet (1,000 kcal/day; 50% carbohydrates, 25% protein, 25% fat; polyunsaturated-to-saturated fatty acid ratio 1.0). Intervention induced significant reductions in body weight and serum leptin levels, and improvements in lipoprotein profile and glucose control. Reductions in leptin levels were directly associated with reductions in serum triglycerides and cholesterol, a finding that was independent of improvements in glucose control. These data show that serum leptin concentrations can be reduced with caloric restriction and exercise in male patients with type II diabetes, and they suggest a direct relationship between leptin and lipoprotein metabolism that is not solely due to weight reduction.

LEPTIN, THE PRODUCT OF the ob gene, is an adipocyte-derived hormone that has been shown to regulate body weight and thermogenesis in rodent models of obesity (4, 11, 28). It has been reported that diet-induced weight loss is accompanied by significant reductions in circulating leptin levels in humans (6). However, the effect of exercise on systemic leptin levels in humans has only recently been reported in healthy individuals (13, 31), an effect, however, that could only be demonstrated in females (13, 31) but not in male subjects (13). Although chronic alterations in energy balance induced by exercise or weight loss have a profound effect on carbohydrate and lipid metabolism (9, 15, 21), reductions in fasting insulin concentrations by exercise training have only recently been linked to reductions in serum leptin levels (13), and the association between serum leptin levels and lipids is virtually unknown. Thus the purpose of this investigation was to determine the influence of a 4-wk intervention program of exercise and low-caloric diet on body weight, systemic leptin levels, and metabolic control in male obese subjects with insulin resistance, particularly focusing on the relationship between serum leptin levels and lipoproteins.

METHODS

Patients with type II diabetes mellitus were referred by their general practitioners or diabetic specialists to the hospital for improvement of metabolic control. Upon reporting to the hospital, these patients were asked whether they were willing to participate in an intervention study. The patients had to fulfill the following criteria: age between 30 and 60 yr, stable weight, body mass index (BMI) >27 kg/m², sedentary lifestyle, and diagnosis of diabetes mellitus that at the time of inclusion in the study was not treated with oral antidiabetic medication. Exclusion criteria were medication with insulin, oral antidiabetic medication or lipid-lowering drugs, hypertension resistant to pharmacological treatment, history of proliferative retinopathy, ischemic heart disease, peripheral vascular disease, or orthopedic problems limiting exercise training. Before the intervention program, an exercise stress test was performed for detecting possible exclusion criteria for the study, such as signs of ischemic heart disease, a low exercise capacity of <100 W, hypertension, or arrhythmias during exercise. In addition, maximal heart rate was registered. Laboratory screening parameters (white and red blood cell count, platelet count, sodium, potassium, creatinine, aspartate, and alanine aminotransferase) had to be within normal limits. In addition, a thorough physical examination was performed before the patients were assigned for participation in the study. The study had been approved by an institutional review committee, and all subjects gave informed consent before participating in the study.

The Intervention Program

Twenty men (age 48 ± 8 yr, body weight 96.4 ± 18.5 kg, BMI 32.1 ± 3.9 kg/m²) fulfilled all criteria listed above and were included in this prospective longitudinal intervention study. For the time of the study, the patients were closely medically followed by the hospital staff. The intervention program included daily exercise and a low-caloric diabetic diet. Medication was not changed during the study.

Exercise program. The exercise program included individual exercise performed on a bicycle ergometer for 30 min on 5 days/wk at an individual intensity of 70% maximal heart rate (3,100 kcal/wk). Heart rate during the program was monitored by heart rate telemetry (Polar, Pacer). An additional energy expenditure of 1,100 kcal/wk was achieved by exercise in groups [2-h hiking tours one time/wk (800 kcal), swimming, water games, and stretching two times/wk for 30 min (300 kcal)]. A total energy expenditure of 2,200 kcal/wk was achieved by physical exercise. The adherence to the exercise program was reinforced and monitored daily by the exercise staff.

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Diet. All patients consumed the same diet, which was especially prepared. Diet consisted of a 1,000-kcal diabetic diet with a carbohydrate content of ~50%, a fat content of 25% and a protein content of 25%. The ratio of polyunsaturated to saturated fatty acids was 1.0. The amount of fiber in the diet was ~10 g/day. The patients were encouraged not to eat additional food and were asked daily regarding this matter.

Clinical Chemistry

After an overnight fast of 12 h, blood was drawn in the morning on the second day after admission to the hospital for laboratory analysis. An identical procedure was repeated 28 days afterward for comparison of the laboratory values.

Lipids and apolipoproteins. Very low density lipoprotein [VLDL; density (d): <1.006 g/ml], intermediate density lipoprotein [IDL; d: 1.006–1.019 g/ml], low-density lipoprotein (LDL; d: 1.019–1.063 g/ml), and high-density lipoprotein (HDL; d: 1.063 to 1.210 g/ml) were prepared by density gradient ultracentrifugation (1, 24). Cholesterol and triglycerides were measured by automated enzymatic methods (Boehringer Mannheim). The apolipoproteins (apo) A-I, B, and A-II were measured by endpoint nephelometry (Boehringer, Marburg, Germany). The within-assay coefficient of variation for lipoproteins was <4% for cholesterol and <4.5% for apolipoproteins.

Insulin, fructosamine, and free fatty acids. Fasting insulin concentrations were determined by an ELISA (Boehringer Mannheim), and free fatty acids were determined by an enzymatic colorimetric method (Wako Chemicals). Fructosamine was also determined by a commercial enzymatic test (Enzymotest; Hoffmann-La Roche). Variation coefficient within assay and between assays was <6%. Glucose was measured during the day before and 2 h after meals, and the “glucose profile” is equivalent to the mean of six glucose measurements/day (see text).

Leptin. Serum leptin levels were determined using a commercialRIA (Linco Research, St. Louis, MO). The variation within the assay was <8% and between assays <6%.

Statistical Analysis

Before statistical analyses were performed, each parameter was tested for normality by the Kolmogorov-Smirnov test. In addition, Q-Q plots (expected against observed values) were made. Parameters that were not normally distributed (serum triglycerides, VLDL cholesterol) were logarithmic transformed to reduce the skew of the distribution. To assure a normal distribution, these transformed parameters were then again tested for normality.

The data from all 20 patients obtained before the intervention program and 4 wk afterward were compared by the t-test for paired samples.

In addition, Pearson’s correlation coefficient was determined between baseline leptin levels and baseline parameters of metabolic control (fasting insulin and fructosamine, glucose profile, lipids, and lipoproteins). For this procedure, logarithmically transformed data were used for triglycerides and VLDL cholesterol. In addition, a stepwise multivariate regression analysis was performed in which baseline values for BMI, leptin, glucose profile, fasting insulin, and fructosamine were entered, with baseline lipids and lipoproteins being the dependent variables.

Pearson’s correlation coefficient was also determined for the relationship between changes of leptin (Δleptin) and improvements of glucose control (Δglucose profile, Δfasting insulin, and Δfructosamine) and lipoproteins during intervention. This was followed by a stepwise multivariate regression analysis in which changes of obesity parameters (ΔBMI, leptin) and changes in glucose control (Δglucose profile, Δfasting insulin, and Δfructosamine) were entered, with the changes in lipids and lipoproteins being the dependent variables.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL). Changes of parameters during intervention were termed Δvalues. These were calculated as values after intervention minus paired values before intervention. Data are expressed as means ± SD. All P values of <0.05 were considered to indicate statistical significance.

RESULTS

During the 4 wk, none of the subjects had to be withdrawn from the study or dropped out because of other reasons. Medication (2 patients on angiotensin converting-enzyme inhibitors) was not changed during the study. The daily adherence to the exercise program was >90%, and adherence was similar for dietary measures (85%). The 4-wk intervention program induced a reduction of body weight from 96.4 ± 18.5 to 92.3 ± 17.9 kg or expressed as BMI a reduction from 32.1 ± 3.9 to 30.7 ± 3.9 kg/m². Significant improvements in carbohydrate and lipid metabolism and serum leptin concentrations were observed also (Tables 1 and 2).

Correlation analysis between baseline leptin and other parameters before intervention showed a signific
significant relationship to BMI (r = 0.58, P = 0.008), fasting insulin (r = 0.58, P = 0.007), serum cholesterol (r = 0.58, P = 0.007), serum apoB (r = 0.51, P = 0.022), serum triglycerides (r = 0.66, P = 0.002), and VLDL cholesterol (r = 0.59, P = 0.007). Multivariate regression analysis revealed that baseline leptin levels were the only predictor for baseline serum triglycerides (R² = 0.52, P < 0.001). VLDL cholesterol was determined by leptin only (R² = 0.47, P = 0.001). For the regression model for baseline serum cholesterol, fructosamine (P = 0.004), glucose (P = 0.019), body weight (P = 0.002), and leptin (P < 0.001) were included as significant predictors (R² = 0.77). Baseline LDL cholesterol levels were only determined by fructosamine (R² = 0.24, P = 0.035), and HDL cholesterol levels were determined by BMI only (R² = 0.44, P = 0.002). Other variables were excluded from the regression models.

Correlation analyses between changes of leptin during intervention (Δleptin) were correlated with improvements in serum cholesterol (r = 0.68, P < 0.001; Fig. 1A), serum triglycerides (r = 0.70, P < 0.001; Fig. 1B), and triglyceride-rich particles such as VLDL cholesterol (r = 0.72, P < 0.001) but not with changes in LDL, LDL, and HDL cholesterol or serum apolipoprotein values such as apoB, apoAI, and apoAl. No association was observed between changes of leptin levels and improvements of glucose control (Δglucose profile, Δfructosamine, and Δinsulin).

Multivariate regression analysis revealed that changes in serum triglycerides (Δlog-transformed triglycerides) were only dependent on changes in leptin (Δleptin) concentrations (P = 0.004) and Δfructosamine (R² = 0.56; P = 0.014). ΔBMI, Δglucose profile, and Δfasting insulin were excluded from this model as they did not add any additional significance to the relationship. The analysis with original not logarithmically transformed triglyceride data showed that only Δleptin predicted Δserum triglyceride levels (R² = 0.52, P < 0.001). Changes in total cholesterol during intervention were also only dependent on Δleptin concentrations (R² = 0.56, P < 0.001). ΔVLDL cholesterol (log transformed) was also only determined by Δleptin values (R² = 0.52, P < 0.001). The regression model for changes in untransformed VLDL cholesterol values included Δfructosamine in addition to Δleptin as an equivalent predictor for ΔVLDL cholesterol concentrations (R² = 0.67, P < 0.001). ΔIDL cholesterol was only determined by Δfructosamine (R² = 0.49, P = 0.001) and not by Δleptin. In this model, Δleptin was excluded as the last variable. ΔFructosamine predicted Δserum apoB (R² = 0.60, P = 0.006), and Δglucose profile determined Δserum apoAI (R² = 0.24, P = 0.027). Change in BMI was always excluded from the regression models as a predictor for changes in lipids or lipoproteins.

**DISCUSSION**

Diet-induced weight loss and exercise training have been shown to reduce systemic leptin concentrations (6, 13, 18). To our knowledge, no study has examined the extent to which systemic leptin levels may be altered by exercise and diet in a group of obese men with type II diabetes mellitus. Our findings support the idea that a nonpharmacological approach in the treatment of type II diabetes is capable of significantly improving glucose and lipid metabolism (8, 10). The results additionally reveal that serum leptin concentrations can be substantially reduced in almost all male diabetic subjects investigated. This is in agreement with observations that reductions of serum leptin levels by ~20% can be induced by exercise training in healthy nonobese subjects (13, 18), although this effect could previously only be documented in females but not in males despite...
an identical intervention program (13). Therefore, the present study is the first, to our knowledge, indicating that weight loss induced by exercise and diet is associated with reductions of serum leptin levels in males also. Moreover, it is also one of the first two studies investigating patients with metabolic disturbances such as insulin resistance and dyslipoproteinemia with respect to leptin metabolism. Preliminary data by Ryan and Elahi (31) investigating four diabetic women had indicated that reductions in body fat mass and improvements in aerobic capacity by exercise training over 6 mo can reduce leptin concentrations in women with metabolic disturbances (31). The present data confirm this preliminary report in a larger male population.

Four weeks of intervention induced a significant concurrent reduction of serum leptin levels and an improvement of glucose control and dyslipoproteinemia in obese men with type II diabetes. Leptin has been reported to be directly related to fasting plasma insulin concentration and insulin resistance in cross-sectional studies (6, 7, 22). However, in humans, it appears to be relatively stable in response to short-term hyperinsulinemia or hyperglycemia (7, 19, 31). For baseline values, we also observed a close relationship between changes of serum leptin concentrations and improvements of glucose control during intervention. Instead, we observed a close relationship between leptin and triglyceride-rich lipoproteins. Baseline leptin levels alone predicted baseline serum triglyceride levels by >50%. Moreover, changes of leptin during intervention were closely related to changes in total cholesterol, serum triglycerides, and VLDL cholesterol, relationships that were independent of changes in glucose control or body weight (Fig. 1, A and B). These data suggest that leptin seems to be more closely related to serum triglycerides than to glucose control. Therefore, the relationship between leptin and insulin resistance might be a secondary result of elevated concentrations of serum fatty acids or triglycerides (2, 20). This has not been considered as a confounding factor in previous studies.

The relationship between the concurrent reductions of leptin and lipids cannot be readily explained. As triglyceride-rich particles are directly secreted by the liver and then catabolized by lipases such as hepatic triglyceride lipase or lipoprotein lipase, a direct metabolic influence of leptin on lipoprotein metabolism or lipase activities may be proposed. Very recent data have addressed this issue in leptin-deficient ob/ob mice. The administration of leptin in these animals acutely caused a rapid stimulation of long-chain fatty acid synthesis (5) and an increase of plasma triglycerides by 31% (25). In contrast, long-term administration of leptin markedly decreased fatty acid synthesis in these animals (5). Similarly, overexpression of leptin in normal rats also induced a depletion of triglyceride content in hepatic and skeletal muscle and pancreatic cells (33). This is explained by a leptin-induced increased fat oxidation confined to the intracellular compartment, as serum levels of triglycerides remained unchanged in this setting (33, 34). This effect seems to be directly induced by leptin and has been shown to be independent of caloric intake or hypothalamic regulation (34). In addition to fat oxidation, leptin may also have a direct influence on hepatic lipid metabolism (23). In obese Zucker rats that carry a mutation in the leptin receptor gene, hypertriglyceridemia is one of the phenotypic characteristics. In contrast to lean Zucker rats, these obese animals show a reduced basal expression of the hepatic LDL receptor, which can explain part of the serum lipid abnormalities in these animals (23).
Overview, the data strongly support the notion of a direct influence of leptin on lipid metabolism. However, so far the studies have primarily investigated leptin and leptin receptor-deficient animals and have focused on the intracellular lipid metabolism. Furthermore, data on the systemic influence of leptin are still equivocal (30, 33). In addition, other mechanisms such as the influence of exercise and diet on the expression of tumor necrosis factor-α (TNF-α) in adipocytes and muscle cells have to be considered (12, 14, 17). Both leptin and TNF-α are directly related and regulated by the same mechanism, the peroxisome proliferator-activated receptor family, which is also important for fatty acid metabolism and insulin sensitivity (26, 35). Therefore, the primary mechanisms that account for the simultaneous changes between leptin and lipids during intervention by exercise and diet observed in our patients with type II diabetes have yet to be elucidated.

Unfortunately, from our study, we cannot differentiate which of the three factors, exercise training, diet, or weight reduction, is the most important factor influencing leptin concentrations. Although previous studies have indicated that weight loss might be the most important factor reducing leptin levels (6, 18, 29), others have also observed a decrease in leptin concentration by exercise training in subjects with stable weight (13). Moreover, it was shown in obese men that the number of endurance exercise hours per week is also directly associated with a reduction in plasma leptin levels independent from changes in body fat percentage and insulin (27). Our results reveal a relationship between BMI and leptin concentration before intervention but fail to show a correlation between changes of leptin and changes of BMI during intervention. This dissociation during the time of intervention has been observed before (27, 32). It has been proposed that long-term hypocaloric diet may uncouple the relationship between body fat and leptin (32). However, this dissociation may also be caused by the fact that body weight and BMI, as measured in the present study, are inaccurate measures of body fat mass. In addition, correlations are always minor when the range for a variable is small, as was the case for BMI and leptin. Furthermore, diet may also play a significant role. Fasting alone has been reported to reduce serum leptin concentration by 64–72% within 1–2 days, despite virtually no change in body fat mass (3). In addition, a hypocaloric diet without additional exercise has also been shown to reduce leptin concentrations by >50% after 4 wk of intervention (16). Future studies may differentiate between short- and long-term diet-induced versus exercise-induced weight reduction, also focusing on the localization of fat depots as well as fat cell size and fatty acid oxidation. Whether the uncoupling of the relationship between body fat mass and serum leptin concentration will be important for the weight course after intervention remains to be shown. In summary, this study has shown that the assumption that leptin levels can only be reduced by exercise-induced weight loss in females but not in males may only be confined to a healthy population. Our data have shown that the combination of daily exercise and a hypocaloric diet can reduce leptin levels in male obese subjects with insulin resistance. Most interestingly, we found that reductions in leptin levels were closely related to improvements in lipid metabolism, a finding that was mainly independent of changes in glucose control and body weight. This observation is new and certainly worthwhile to be examined in future studies.

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