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Insulin sensitivity, metabolic flexibility, and serum adiponectin concentration in women with anorexia nervosa

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Abstract

Anorexia nervosa (AN) is an eating disorder resulting in sustained low weight and marked decrease in fat mass. The lack of adipose tissue observed in lipodystrophies is accompanied by insulin resistance. It remains unclear if the same phenomenon would be present in AN. The objective of the study was to estimate insulin sensitivity, oxidative and nonoxidative glucose metabolism in insulin-stimulated conditions, metabolic flexibility, and serum adiponectin concentration in women with AN. We examined 21 women with AN and 24 healthy normal-weight female controls. Euglycemic hyperinsulinemic clamp, indirect calorimetry, and the measurement of serum adiponectin concentration were performed in all the subjects. We did not observe differences in insulin sensitivity, oxidative and nonoxidative glucose metabolism in insulin-stimulated conditions, and metabolic flexibility between AN and control subjects. Serum adiponectin was higher in AN women in comparison with control group ($P = .002$). Women with AN have normal insulin sensitivity because of the preserved response of glucose oxidation, nonoxidative glucose metabolism in response to insulin, and normal metabolic flexibility. High adiponectin concentration and normal insulin sensitivity in anorectic women suggest that in AN the adipocytes are still capable of functioning at the level that is sufficient to prevent the metabolic consequences.

1. Introduction

Anorexia nervosa (AN) is an eating disorder that usually begins in adolescence and is characterized by determined dieting, often accompanied by compulsive exercise, resulting in sustained low weight [1]. In this disorder, similarly to the syndromes of lipodystrophy, one observes the significant loss of adipose tissue.

The lipodystrophies are a heterogeneous cluster, having in common a lack of fat tissue [2]. In all cases of lipodystrophies, the metabolic consequences are similar, namely, insulin resistance and hypertriglyceridemia [2]. Obesity is also associated with insulin resistance and increased risk of type 2 diabetes mellitus. The resemblance between the metabolic abnormalities of these 2 extreme states of adiposity underscores the importance of fat tissue in energy homeostasis.

The crucial step in the development of insulin resistance is impaired ability of insulin to stimulate glucose uptake and oxidation and inhibit lipid oxidation. It might also be related to an impaired insulin-stimulated nonoxidative glucose metabolism, which mainly reflects muscle glycogen synthesis. In lean healthy individuals, in fasting conditions, skeletal muscle oxidizes mostly fatty acids, with the relative suppression of glucose oxidation. Insulin stimulates glucose oxidation and inhibits lipid oxidation. The capacity of skeletal muscle to switch from lipid to glucose oxidation in response to insulin is called *metabolic flexibility*. When this ability is impaired, we have the condition of *metabolic inflexibility*, which is proposed to play a role in the development of insulin resistance [3].

Adipose tissue is known to express and secrete a variety of bioactive peptides, known as *adipocytokines*. One of these adipocytokines is adiponectin. The expression of adiponectin is highly specific to adipose tissue [4]. However, adiponectin is paradoxically decreased in human obesity [4]. On the other hand, its circulating level is strongly reduced in patients with generalized lipodystrophy [5]. Both in obesity and in syndromes of lipodystrophy, hypoadiponectinemia is

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associated with insulin resistance [4,5]. Several studies indicate that adiponectin has insulin-sensitizing effects [6,7] and could be involved in glucose and lipid metabolism [8].

Metabolic abnormalities in anorectic patients are not clear. Insulin-stimulated glucose disposal has been reported to be normal [9], enhanced [10-13], or decreased [14,15] in anorectic subjects. Both hypoadiponectinemia [13] and hyperadiponectinemia [10,12,15] were observed in the patients with AN.

Therefore, in the present study, we aimed to estimate insulin sensitivity, glucose and lipid oxidation, metabolic flexibility, nonoxidative glucose metabolism, and serum adiponectin concentration in women with AN.

2. Subjects and methods

2.1. Participants

We examined 21 women with AN (body mass index [BMI] <18 kg/m^2) and 24 healthy normal-weight female controls (BMI <25 kg/m^2). All women with a restrictive type of AN met the criteria defined in the revised *Diagnostic and Statistical Manual of Mental Disorders* [16] and were recruited from the Department of Endocrinology, Diabetology, and Internal Medicine, Medical University of Bialystok, and from the Psychosomatic Clinic of Medical University of Bialystok. The mean duration of the disease was 17.22 ± 11.39 months (range, 6-36 months). All anorectic women without history of weight regain had been weight stable for at least 1 month before the study. They also were weight stable before receiving multidisciplinary treatment. All patients with AN had amenorrhea. Control subjects were recruited from the medical staff and students. Control group was age and height matched. This population of young and healthy women with BMI within the reference range had no history of eating disorders and significant weight loss. All subjects were white. Smokers were not included in the study. All subjects were without serious diseases (other than AN) and were not taking any drugs known to affect glucose, lipid metabolism, and body composition. Before entering the study, physical examination, anthropometric measurements, and appropriate laboratory tests were performed. The BMI and percentage of body fat were assessed by bioelectric impedance analysis using the Tanita TBF-511 Body Fat Analyzer (Tanita, Tokyo, Japan). Analyses were performed after an overnight fast. All subjects underwent an oral glucose tolerance test with determination of glucose and insulin concentrations, and all had normal glucose tolerance according to the World Health Organization criteria. The study protocol was approved by the Ethics Committee of Medical University of Bialystok. All the subjects gave written informed consent before entering the study.

2.2. Insulin sensitivity

Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique according to DeFronzo et al [17], as previously described [7].

2.3. Indirect calorimetry

Resting energy expenditure (REE) and the whole-body glucose and lipid oxidation were measured with indirect calorimetry using the ventilated hood technique (Oxycon Pro; Viasys-Erich Jaeger, Hochberg, Germany). The measurements were taken while the subjects were lying in the supine position at baseline (in the fasting state) and during the last 30 minutes of the clamp. The average gas exchange recorded over the two 30-minute periods was used to calculate the rates of glucose and lipid oxidation [18]. Total glucose metabolism was calculated from the clamp. The nonoxidative glucose metabolism was calculated in insulin-stimulated conditions by subtracting glucose oxidation from total glucose metabolism. The change in respiratory quotient (ΔRQ) in response to insulin was used as a measure of metabolic flexibility.

2.4. Laboratory analyses

Fasting blood samples were also taken from the antecubital vein before the beginning of the clamp for the determination of lipid parameters and adiponectin.

Plasma glucose, serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were analyzed immediately as previously described [7].

Before estimation of concentrations of adiponectin, insulin, and free fatty acids (FFAs), the samples were kept frozen at −80°C. Serum insulin was measured with the monoclonal immunoradiometric assay (IRMA; Medgenix Diagnostics, Fleunes, Belgium) with a sensitivity of 1 μIU/mL and with intraassay and interassay coefficients of variation less than 2.2% and 6.5%, respectively. Plasma FFA concentrations were measured by the colorimetric method [19]. The concentration of serum adiponectin was measured using the radioimmunoassay method (Linco Research, St Charles, MI) with detection limit of 1 ng/mL and with intraassay and interassay coefficients of variation less than 6.3% and 9.5%, respectively.

2.5. Statistical analysis

The statistics were performed with the STATISTICA 7.0 program (StatSoft, Krakow, Poland) using the nonparametric tests. The differences between the studied groups were estimated with the Mann-Whitney *U* test. Relationships between variables were assessed with Spearman rank *R* analysis. Statistical significance was accepted at a P value < .05.

3. Results

Clinical characteristics of the studied groups are given in Table 1. There were no significant differences in age between studied groups. The BMI and percentage of body fat were significantly lower in women with AN compared with healthy

Table 1
Anthropometric, biochemical, and metabolic characteristics of the studied groups

	AN group (n = 21)	Control group (n = 24)
Age (y)	22.43 ± 5.17	24.12 ± 4.76
Body weight (kg)	42.78 ± 4.85*	57.34 ± 6.60
BMI (kg/m^2)	15.56 ± 1.55*	21.02 ± 2.11
Waist (cm)	61.05 ± 3.55*	71.04 ± 6.12
% Body fat	12.93 ± 4.15*	25.56 ± 7.66
Fasting glucose (mg/dL)	72.56 ± 9.31*	80.86 ± 8.11
Postload glucose (mg/dL)	76.86 ± 22.69	80.62 ± 15.13
Fasting insulin (μIU/mL)	6.50 ± 2.32*	12.55 ± 6.06
Postload insulin (μmol/L)	24.94 ± 15.24*	42.46 ± 22.47
Fasting FFA (μmol/L)	277.12 ± 125,16*	590.22 ± 339.04
FFA 120-min clamp (μmol/L)	111.14 ± 77.18	189.84 ± 118.38
Total cholesterol (mg/dL)	174.62 ± 30.54	185.04 ± 40.15
Triglycerides (mg/dL)	79.58 ± 37.43	68.02 ± 28.19
HDL cholesterol (mg/dL)	58.91 ± 12.58	61.55 ± 11.82
LDL cholesterol (mg/dL)	99.48 ± 27.22	104.67 ± 42.33
M (mg × kg^{-1} × min^{-1})	9.02 ± 2.46	8.63 ± 2.64
Adiponectin (μg/mL)	23.03 ± 8.70*	15.75 ± 5.95
Nonoxidative glucose metabolism (mg × kg^{-1} × min^{-1})	7.50 ± 2.60	6.73 ± 2.53
ΔRQ	0.05 ± 0.08	0.06 ± 0.06

Data are presented as mean ± SD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; M, insulin sensitivity index; ΔRQ, the change in respiratory quotient in response to insulin as a measure of metabolic flexibility.

* $P < .05$ vs control subjects.

controls (both Ps < .0001). Fasting glucose and insulin concentrations were significantly lower in AN subjects in comparison with controls (P = .003 and P = .00008, respectively). Furthermore, postload insulin concentration was significantly lower in AN in comparison with control subjects (P = .005). Fasting plasma FFA concentration was significantly lower in AN than controls (P = .005) (Table 1).

Insulin sensitivity and nonoxidative glucose metabolism were not different between AN and healthy controls. (Table 1). The RQ and the glucose and lipid oxidation in baseline and hyperinsulinemic states were also similar in AN and control women (Fig. 1). As a consequence, metabolic flexibility was not different between AN and control women (Table 1). The REE was lower in women with AN; however, this difference disappeared when the REE was normalized for body weight (Fig. 1).

Serum adiponectin level was higher in AN women in comparison with control subjects (P = .002) (Table 1).

Insulin sensitivity was highly correlated with nonoxidative glucose metabolism (r = 0.77, P = .0002) in AN. Furthermore, in the analysis of within-group correlations in AN group, adiponectin level positively correlated with lipid oxidation in the baseline state (r = 0.54, P = .022).

4. Discussion

We demonstrated normal insulin sensitivity and metabolic flexibility in AN women. Of note is the fact that we examined a homogeneous group of women with AN. As mentioned, all women had history of weight loss, had amenorrhea, and remained untreated until the time of examinations. In addition, all participants had stable weight for at least 1 month before participation in our study; so the effect of acute changes in body weight on estimated parameters can be ruled out.

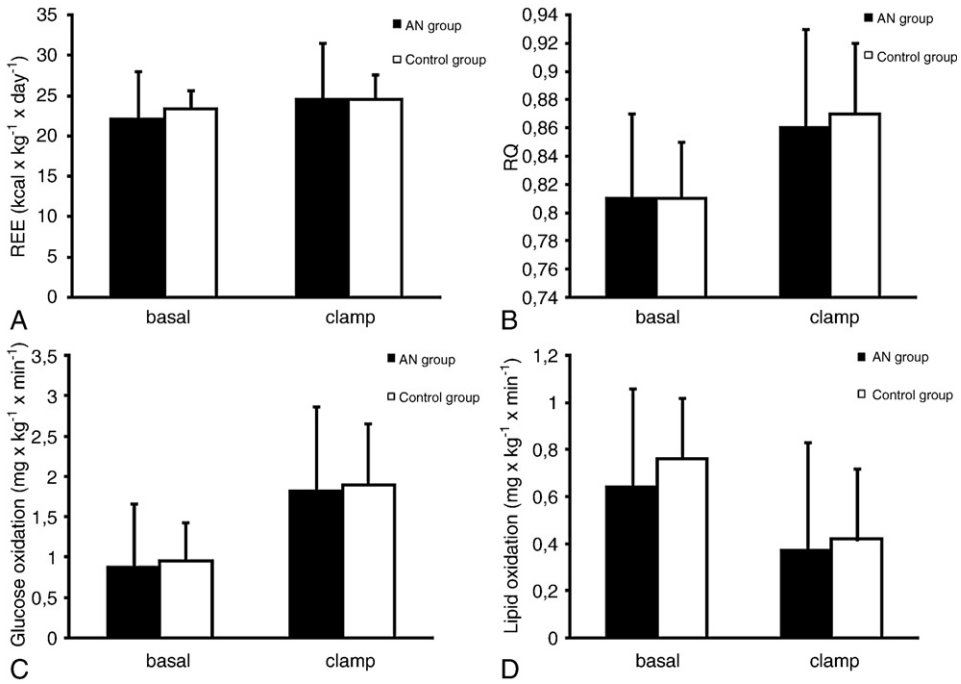

Fig. 1. Resting energy expenditure (A), RQ (B), glucose oxidation (C), and lipid oxidation (D) in AN and control groups before and during the clamp.

As mentioned, the data regarding insulin sensitivity in AN are conflicting. Some investigators reported increased insulin sensitivity in anorectic women [10-13]. The inconsistent results of these studies may be attributable to differences in the techniques for evaluating insulin action. These authors assessed insulin resistance by homeostasis model assessment (HOMA) [10,12,13] or by the Bergman minimal model method [11]. Both glucose and insulin, which are used in the calculation of HOMA index, are secreted in a pulsatile manner, which might affect the reproducibility of this index. In addition, HOMA reflects mainly hepatic insulin resistance, whereas euglycemic clamp estimates mainly peripheral (skeletal muscle) insulin sensitivity. In the minimal model approach, there is a risk of hypoglycemia in insulin-sensitive individuals (quite probable in anorexia), which might bias the results. In our study, we used euglycemic hyperinsulinemic clamp technique, which is generally recognized as the reference method in measuring insulin sensitivity and, in our opinion, gives the most reliable results. In 2 previous studies involving 11 anorectic women in each, clamp-derived insulin-stimulated glucose disposal was reported to be decreased [14,15] in AN group compared with normal-weight controls. However, our data on normal insulin sensitivity in AN are in agreement with the finding of a recent study reporting similar insulin sensitivity in anorectic subjects and healthy female controls [9]. In this study, insulin-stimulated glucose disposal was also measured by the glucose clamp technique.

In AN, similarly to lipodystrophy, one observes the inadequate adipose tissue mass. It remains unclear whether metabolic consequences in AN are similar to these observed in lipodystrophy. Our data indicate that normal oxidative and nonoxidative glucose metabolism in insulin-stimulated conditions and normal metabolic flexibility contribute to normal insulin sensitivity in AN women. We also found positive relationship between insulin sensitivity and nonoxidative glucose metabolism in AN group. Other investigators also noticed normal glucose oxidation in anorectic women [14,15]. Metabolic flexibility was not estimated in these studies.

It is generally accepted that nonoxidative glucose metabolism reflects mostly muscle glycogen synthesis. Some studies indicate that nonoxidative pathway of glucose metabolism is resistant to the action of insulin in AN [14,15]. It has been hypothesized that insulin resistance in anorectic patients could be a compensatory response to energy deprivation during severe starvation [15]. On the other hand, abundant glycogen granules have been reported in skeletal muscle of anorectic patients [20]. Furthermore, remarkable accumulation of glycogen in hepatocytes was observed in patient with AN [21]. Adaptation to starvation may spare glucose utilization and stimulate hepatic glycogenesis [21]. Glycogen storage may be a potential reservoir of glucose especially under fasting conditions [21].

We reported higher serum adiponectin concentration in patients with AN compared with healthy normal-weight female controls. Other investigators also found increased adiponectin levels in AN [10,12,15]. In contrast, Tagami at al [13] found decreased circulating adiponectin concentrations in anorectic female subjects. However, in this study, the concentrations of adiponectin after weight recovery increased to the normal level despite a relatively small increase in BMI, suggesting that there might be an optimal fat mass for adiponectin secretion [13]. It has been hypothesized that during the development of obesity the feedback mechanism could suppress the production of adiponectin [15]. The lack of such negative feedback could contribute to hyperadiponectinemia in AN. Extremely low circulating adiponectin concentrations may be observed in rare cases of generalized lipodystrophy [5]. These data do not support the hypothesis about negative feedback mechanism on adiponectin production. Hypoadiponectinemia, both in obesity and lipodystrophy, may be due to intrinsic and profound defects of adipocyte function. Our study and other reports [10,12,15] suggest that in AN low adipose tissue mass is sufficient for adiponectin secretion.

Many authors observed a positive correlation between adiponectin concentration and insulin sensitivity in human obesity [4], in type 2 diabetes mellitus [6], and in lean nondiabetic offspring of type 2 diabetes mellitus subjects [7]. This positive relationship was not observed in anorectic women in our study. This result was similar to some other studies [10,12] regarding the adiponectin level in relation to insulin sensitivity in AN. However, we found high serum adiponectin level together with normal insulin sensitivity in the AN group. The lack of significant correlation between adiponectin and insulin sensitivity could be due to the limited number of AN women and/or to the narrow range of insulin sensitivity index values.

We observed a positive correlation between adiponectin and lipid oxidation in the baseline state in AN women. So far, none of the studies has examined the association between adiponectin and glucose and lipid oxidation in AN. Salmenniemi et al [8] showed that the rates of both oxidative and nonoxidative glucose disposal during hyperinsulinemia similarly increased with increasing adiponectin concentrations. They also showed correlation between adiponectin and lipid oxidation in insulin-stimulated condition, whereas Yokoyama et al [22] observed significant correlation only between adiponectin and nonoxidative glucose disposal. These studies examined different groups of individuals, that is, offspring of type 2 diabetes mellitus subjects [8] or, in large part, patients with type 2 diabetes mellitus [22]. It was demonstrated that adiponectin increased glucose uptake because of enhancement of glucose transporter 4 translocation through activating adenosine monophosphate–activated protein kinase [23]. Adenosine monophosphate–activated protein kinase might also increase insulin sensitivity through stimulation of FFA oxidation in skeletal muscle [24], thus preventing intramyocellular lipid accumulation. An impaired basal FFA oxidation is a feature of metabolic inflexibility. Thus, our data suggest a potential mechanism through which

high adiponectin associates with normal insulin sensitivity and metabolic flexibility in AN.

Our data show that women with AN have normal insulin sensitivity, assessed by the euglycemic hyperinsulinemic clamp technique, because of the preserved response of glucose and lipid oxidation and nonoxidative glucose metabolism to insulin. Therefore, AN women appear to demonstrate normal metabolic flexibility. Furthermore, we demonstrated markedly increased serum adiponectin concentrations in patients with AN. These results indirectly indicate that in anorectic patients the adipocytes are still capable of functioning at the level that is sufficient to prevent the metabolic consequences.

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