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Increased Insulin Sensitivity in Patients with Anorexia Nervosa: The Role of Adipocytokines

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Summary

Anorexia nervosa (AN) is characterized by self-induced starvation leading to severe weight and fat loss. In the present study, we measured fasting plasma levels of adiponectin, leptin, resistin, insulin and glucose in 10 women with a restrictive type of AN and in 12 healthy women (C). Insulin sensitivity was determined according to homeostasis model assessment of insulin resistance (HOMA-R). Plasma resistin, leptin and insulin levels were significantly decreased, whereas plasma adiponectin levels were significantly increased in patients with AN compared to the C. HOMA-R was significantly decreased in patients with AN compared to the C group. Plasma adiponectin and leptin concentrations negatively and positively correlated with the body mass index and percentage body fat in both groups. Plasma adiponectin levels were negatively related to plasma insulin levels in the AN group only. In conclusion, we demonstrated that AN is associated with significantly decreased plasma leptin and resistin levels, markedly increased plasma adiponectin levels and increased insulin sensitivity. Plasma leptin and adiponectin levels were related to the body size and adiposity. Hyperadiponectinemia could play a role in increased insulin sensitivity of patients with AN. Neither body size and adiposity nor insulin sensitivity are the major determinants of plasma resistin levels in AN.

Key words
Anorexia nervosa • Adipocytokines • Insulin • HOMA-R

Introduction

It has been clearly demonstrated that the adipose tissue (AT) is a complex system that participates in energy homeostasis by releasing numerous metabolically active substances and hormones in response to specific extracellular stimuli. The term "adipocytokines" was recently coined to describe the adipose-derived bioactive factors modulating the physiological function of other tissues as well as acting in a paracrine manner on adipose tissue metabolism. Leptin, adiponectin (ApN) and resistin are well known examples of adipocytokines that affect fuel homeostasis and insulin action (Brichard *et al.* 2003, Housa *et al.* 2006).

ApN is exclusively produced by adipocytes and has been found to be abundant in human circulation, with its plasma levels in the μg/ml range (Arita *et al.* 1999). ApN has been clearly demonstrated to have a variety of functions, including antiatherogenic, antiinflammatory, and insulin-sensitizing properties (Yamauchi *et al*. 2002,

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Chen MP *et al*. 2005). In contrast to all other adipocytokines known to date, plasma ApN concentrations were paradoxically found to be decreased, not increased, in human obesity and type 2 diabetes, conditions commonly associated with insulin resistance and hyperinsulinemia (Weyer *et al.* 2001, Anderlová *et al.* 2006). Studies focused on the effect of long-term malnutrition of patients with anorexia nervosa (AN) on plasma ApN levels brought rather contradictory results, because its plasma levels were found to be either increased (Delporte *et al.* 2003, Housová *et al*. 2005, Nedvídková *et al.* 2005), decreased (Tagami *et al.* 2004) or unchanged (Iwahashi *et al.* 2003) in these patients.

Many experimental and clinical studies have shown that leptin is a key player in the regulation of food intake and energy balance (Montague *et al.* 1997). Leptin levels can be influenced by such conditions as satiety, amount of adipose tissue or diurnal cycles. These influences are mediated mostly by sympathetic regulators, insulin, glucocorticoids, and glucose entry into adipocytes (Coleman and Herrmann 1999). Among these factors, insulin has been shown to promote ob gene expression in adipocytes and to increase plasma leptin levels in humans (Kolaczynski *et al*. 1996). Although markedly reduced plasma leptin levels in patients with AN are well known (Nedvídková *et al*. 2000, Dostálová *et al*. 2005), the relationship between low plasma leptin levels and a degree of insulin sensitivity in these patients has not been fully elucidated.

Resistin has been suggested to suppress the ability of insulin to stimulate glucose uptake in rodents (Steppan and Lazar 2002). However, the physiological significance of resistin in humans is less clear (Degawa-Yamauchi *et al.* 2003). In humans, the major sources of resistin are immune and endothelial cells rather than adipocytes and it is not yet clear whether this peptide plays a significant role in the development of insulin resistance (IR) (Vettor *et al.* 2005). On this basis, the role of resistin in human has been shifted towards inflammatory and immune processes (Kunnari *et al.* 2006). Similarly to ApN, data on plasma resistin levels in AN are not unified, with plasma resistin levels found to be either decreased (Brichard *et al.* 2003, Dostálová *et al*. 2006) or unchanged (Housová *et al.* 2005) in patients with AN.

The above mentioned alterations of circulating adipocytokines may have potential repercussions in the pathophysiology of AN. Anorexia nervosa is a psychiatric disorder characterized by chronic self-induced starvation and severe weight loss, mainly at the expense of adipose tissue. As expected, AN is associated with altered glucose and lipid metabolism, multiple endocrine perturbations and other dysfunctions such as immune defects (Delporte *et al.* 2003). Studies reporting a degree of insulin sensitivity (IS) in AN provided rather contradictory results. Although majority of studies have found increased IS (Hermans and Lambert 2002, Delporte *et al*. 2003, Misra *et al*. 2004), either decreased (Pannacciulli *et al*. 2003) or unchanged (Castillo *et al*. 1985). IS in patients with AN have also been observed.

As adipocytokines are suggested to influence IS and available data on plasma levels of ApN and resistin as well as on the degree of IS in AN are rather contradictory, the aim of the present study was to investigate the relationship between circulating adipocytokines leptin, resistin and ApN and plasma insulin and glucose levels in patients with AN. To determine the degree of IS in patients with AN, we used homeostasis model assessment of insulin resistance (HOMA-R) that is based on the maintenance of a negative feedback loop between liver and pancreatic beta cells regulating both fasting glucose and insulin concentrations.

Methods

Study subjects

Ten women with a restrictive type of AN (age 24.4±1.6 years; body mass index (BMI) 15.4±0.6 kg/m^2; percent body fat (% BF) 4.1±0.9) and twelve healthy age-matched women (C) (age 23.3±1.3 years; BMI 20.9±0.7 kg/m^2; % BF 19.5±2.6) were enrolled in the study. All subjects included in the study were non-smokers, had no allergies, and had been free of medication for at least three weeks prior to the study. Professional athletes were not included in the study. Control subjects had no history of obesity or malnutrition, hypertension, gastrointestinal disease, eating disorder or other psychiatric disorder, and had a normal physical examination and electrocardiogram (ECG). Blood tests confirmed normal blood count, liver and renal functions. Patients with AN were diagnosed according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994) after detailed medical and psychiatric evaluation. All patients with a restrictive type of AN were examined after two weeks of hospitalization on the Department of Psychiatry and were clinically stable and in relatively good health, except for their

Table 1. Anthropometric, hormonal and biochemical parameters of healthy control women (C; n =12) and of patients with anorexia nervosa (AN; n = 10).

	Controls (n = 12)	**Anorexia nervosa (n = 10)**
Age (*years*)	23.3 ± 1.3	24.4 ± 1.6
BMI (kg/m^2)	20.9 ± 0.7	15.4 ± 0.6*
% BF	19.5 ± 2.6	4.1 ± 0.9***
Adipocytokines		
Adiponectin (µg/ml)	28.0 ± 2.9	46.4 ± 5.0**
Leptin (ng/ml)	10.8 ± 1.1	1.6 ± 0.2***
Resistin (pg/ml)	312.2 ± 17.1	243.8 ± 21. 9*
Glucose homeostasis		
Glucose (*mmol/l*)	4.6 ± 0.1	3.9 ± 0.1
Insulin (*mIU/l*)	4.5 ± 0.2	2.1 ± 0.3*
HOMA-R index	0.89 ± 0.1	0.36 ± 0.1*

Values are means ± S.E.M.; n = number of subjects; BMI = body mass index; % BF = percent of body fat; HOMA-R = homeostasis model assessment of insulin resistance; *P <0.05, ** P <0.01, ***P <0.001 values significantly different from the C group.

eating disorder.

All healthy women were studied in day 7 to 10 of their menstrual cycle, whereas all patients with AN had amenorrhea. All subjects were asked to fast and drink only water on the night prior to the study. All participants provided written informed consent prior to participating in the study, which was approved by the Human Ethic Review Committee, Institute of Endocrinology, Prague, Czech Republic, and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Experimental procedures and blood sampling

All subjects were admitted to the Institute of Endocrinology at 07:00 h. After a short medical examination (blood pressure, heart and respiratory rates measurement, ECG), % BF was estimated by bioimpedance (TANITA, Japan). Blood samples for leptin, resistin, adiponectin, insulin and glucose evaluation were withdrawn at 08:00 h after 12 h of overnight fasting into chilled polypropylene tubes containing Na_2EDTA and antilysin. Plasma was immediately separated by centrifugation at 4 °C and stored at -30 °C until being assayed.

Analytical procedures

Plasma ApN and leptin were measured by commercial RIA kits (Linco Research, Inc., St. Charles, MO, USA). Sensitivity for ApN was 1 ng/ml, and the intra- and interassay variability was 1.78 % and 9.25 %, respectively. Sensitivity for leptin was 0.5 ng/ml and the intra- and interassay variability was 4.6 % and 8.7 %, respectively. Plasma resistin was measured by a commercial resistin RIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA). Sensitivity assay for resistin was 100 pg/ml and the intra- and interassay variability was 4.5 % and 7.4 %, respectively. Plasma insulin was measured by a commercial RIA kit (Immunotech, Prague, Czech Republic). Sensitivity was 0.5 µIU/ml and the intra- and interassay variability was 3.4 % and 4.3 %, respectively. Plasma glucose levels were measured on Cobas Integra 400 plus (Roche Diagnostics, GmbH, Mannheim, Germany). All assays were run twice in duplicate. Peripheral insulin resistance was determined using HOMA-R as previously described (Matthews *et al.* 1985) following the formula: fasting plasma insulin (mIU/l) x fasting plasma glucose (mmol/l) / 22.5.

Data analysis

Results are presented as mean ± S.E.M. Mann-Whitney test was used for group comparison. Correlations between parameters were examined using Spearman´s rank correlation coefficient. P<0.05 value denoted statistical significance.

Results

The anthropometric, hormonal and biochemical characteristics of the study subjects are shown in Table 1. BMI and fat mass were markedly reduced in AN compared to the control subjects. As shown in Table 1,

Table 2. A, B. The relationship of leptin, resistin and adiponectin (ApN) with anthropometric, biochemical, and hormonal parameters in patients with anorexia nervosa (A) and in healthy control women (B).

A.	**BMI**	**%BF**	**Insulin**	**Glucose**	**HOMA**	**Leptin**	**Resistin**	**ApN**
Leptin	R = 0.64	R = 0.82	R = 0.39	R = 0.53	R = 0.49	–	R = 0.24	R = 0.54
	P = 0.02	P = 0.04	P = 0.25	P = 0.10	P = 0.14		P = 0.52	P = 0.24
Resistin	R = 0.27	R = 0.17	R = 0.59	R = 0.39	R = 0.29	R = 0.24	–	R = – 0.38
	P = 0.37	P = 0.62	P = 0.09	P = 0.29	P = 0.15	P = 0.52		P = 0.29
ApN	R = – 0.62	R = – 0.49	R = – 0.47	R = – 0.62	R = – 0.54	R = 0.54	R = – 0.38	–
	P = 0.03	P = 0.01	P = 0.01	P = 0.09	P = 0.15	P = 0.24	P = 0.29	
B.	**BMI**	**%BF**	**Insulin**	**Glucose**	**HOMA**	**Leptin**	**Resistin**	**ApN**
Leptin	R = 0.52	R = 0.49	R = 0.31	R = 0.55	R = 0.38	–	R = 0.17	R = 0.16
	P = 0.01	P = 0.02	P = 0.27	P = 0.08	P = 0.16		P = 0.52	P = 0.54
Resistin	R = 0.26	R = 0.20	R = 0.36	R = 0.33	R = 0.34	R = 0.17	–	R = – 0.29
	P = 0.34	P = 0.46	P = 0.19	P = 0.23	P = 0.22	P = 0.52		P = 0.15
ApN	R = – 0.45	R = – 0.51	R = – 0.36	R = – 0.44	R = – 0.32	R = 0.16	R = – 0.29	–
	P = 0.03	P = 0.02	P = 0.23	P = 0.31	P = 0.15	P = 0.54	P = 0.15	

Statistical significance from Spearman rank correlation test. BMI = body mass index; %BF = percent body fat; HOMA = homeostasis model assessment of insulin resistance.

plasma ApN levels were significantly increased (46.4±5.0 vs. 28.0±2.9 μg/ml, P<0.01), whereas plasma leptin and resistin concentrations were significantly decreased (243.8±21.9 vs. 312.2±17.1 pg/ml for resistin, P<0.05; 1.6±0.2 vs. 10.8±1.1 ng/ml for leptin, P<0.001) in patients with AN compared to the C group. Plasma insulin levels were significantly decreased in AN compared to the C subjects (2.1±0.3 mIU/l vs. 4.5±0.24, P<0.05), whereas plasma glucose tended to be lower in AN, but this decrease did not reach the statistical significance (3.9±0.1 vs. 4.6±0.1 mmol/l). HOMA-R index was significantly lower in the AN group in comparison with the C group (0.36±0.06 vs. 0.89±0.05, P<0.05) (Table 1).

Plasma adiponectin and leptin concentrations correlated negatively and positively with BMI and % BF in both groups. Plasma ApN levels were negatively related to plasma insulin levels in the AN group, but not in the C group. We did not confirm significant relationship of resistin to any other parameters studied in either patients with AN or in the C group (Table 2 A,B).

Discussion

We found significantly decreased plasma leptin and resistin levels and markedly increased plasma ApN levels in patients with AN. HOMA-R index was significantly decreased in patients with AN compared to the C. Plasma ApN and leptin levels were related to the nutritional status, whereas plasma resistin levels were not. Plasma ApN levels were negatively related to plasma insulin levels in the AN group only. Neither resistin nor leptin were related to increased IS in patients with AN.

The conflicting results of previous studies addressing the issue of IS in patients with AN may be partly attributable to differences in the techniques for evaluating insulin action. Using hyperinsulinemic-euglycemic clamp, insulin-stimulated glucose disposal has been reported to be normal (Castillo *et al.* 1985), varied (Kiriike *et al.* 1990) or decreased (Pannacciulli *et al.* 2003) in patients with AN. Using a minimal model by frequently sampled intravenous glucose tolerance test, Fukushima *et al*. (1993) found significantly increased IS in patients with AN. In the present study, we found normal plasma glucose and reduced plasma insulin levels together with low HOMA-R index in patients with AN suggesting that AN is associated with increased IS. These results are in agreement with previously published studies in AN using HOMA-R to determine IS under basal conditions (Delporte *et al.* 2003, Misra *et al*. 2004, Housová *et al*. 2005). As AN is associated with many endocrine and metabolic disturbances, the cause of

increased IS in malnourished and hyperactive patients with AN might be multiple. Our finding of a negative relationship between plasma ApN and insulin has confirmed that increased IS in patients with AN can be related to hyperadiponectinemia found in these patients. Although Pannaciulli *et al.* (2003) hypothesized that hyperadiponectinemia might represent a compensatory mechanism for the reduced insulin-stimulated glucose metabolism in AN and Fasshauer *et al.* (2002) demonstrated that *in vitro* ApN gene expression is reversibly down-regulated by insulin, the insulin-sensitizing effect of ApN administration in mice has repeatedly been demonstrated (Yamauchi *et al.* 2001). Thus, increased IS in patients with AN is probably secondary to hyperadiponectinemia induced by loss of fat mass in these patients.

One possible factor that could contribute to increased IS in patients with AN through the up- or down-regulation of adipocytokine production might be the activity of sympathoadrenal system (Fasshauer *et al*. 2001), previously demonstrated by our group to be markedly increased *in vivo* in the subcutaneous abdominal adipose tissue of patients with AN (Barták *et al.* 2004). An excess of growth hormone and cortisol, higher plasma free fatty acids levels together with beta-cell dysfunction associated with AN may also account for the altered glucose tolerance.

Studies focused on plasma ApN levels in patients with AN provided rather conflicting results. In agreement with the most of previously published studies (Pannacciulli *et al*. 2003, Housová *et al.* 2005), we found significantly increased plasma ApN levels in low-weight patients with AN compared to age- and sex-matched controls. Paradoxically, Yang *et al.* (2001) observed plasma ApN increase following body weight loss in obese humans and the authors explained this fact by fatness reduction. Similarly, the extreme reduction of fatness may be one of causes of ApN upregulation in AN. As both *in vivo* and *in vitro* studies indicated that fat mass may exert a negative feedback on its own ApN production by synthetizing a factor that destabilizes ApN mRNA (Delporte *et al.* 2003), the disturbance of such a negative feedback could contribute to hyperadiponectinemia observed in AN. This view is further supported by the observation of a negative correlation between plasma ApN and % BF in patients with AN found in our study.

On the contrary to our results, Tagami *et al.* (2004) described significantly reduced plasma ApN levels in patients with AN compared to normal-weight controls and hypoadiponectinemia was reversed by weight recovery. Tagami *et al.* (2004) supposed that abnormal feeding behavior may affect some factors which down-regulate ApN, because they found equally low ApN levels in patients with AN and bulimia nervosa, regardless of their BMI. Although Iwahashi *et al.* (2003) did not observe any difference in ApN levels between AN and healthy women, this study pointed out the interesting fact that under the critical limit of body weight, the levels of ApN are lacking of inverse relationship to both BMI and % BF. Recently, Bosy-Westphal *et al.* (2005) reported that the gain in fat mass is not associated with reduction of hyperadiponectinemia in AN, suggesting that other factors than the loss of body fatness may influence plasma ApN levels in AN.

At present there is no obvious explanation for such a discrepancy in published data for circulating ApN levels in patients with AN. However, two studies that described decreased (Tagami *et al.* 2004) or unchanged (Iwahashi *et al.* 2003) ApN levels in AN were performed on a Japanese population and patients included were older than in our study.

Numerous studies have previously described an important role of leptin in the regulation of food intake and energy homeostasis (Friedman *et al*. 2003). This study supports previous reports (Dostálová *et al.* 2005) that anorectic patients have significantly lower leptin levels than normal, thus suggesting that this cytokine is not involved in the reduction of food intake associated with AN, but reflects total body fat mass. We confirmed a positive correlation between plasma leptin and BMI, % BF in patients with AN suggesting that the nutritional status is an important regulator of plasma leptin levels. However, we failed to find any relationship between reduced plasma leptin levels and enhanced IS in patients with AN. Although Misra *et al.* (2004) observed strong positive correlation between plasma leptin concentrations and IR in AN, the role of leptin in IS is still not fully understood (Schinner *et al.* 2005). However, we can not exclude a role of reduced leptin concentrations in determining the elevation of ApN plasma levels. It is well known that there are relevant interactions between leptin and other cytokines, these interactions could result in an additive, synergistic, or even antagonistic effect, thus suggesting the existence of positive and negative feedback systems dependent on cytokine-cytokine interactions (Loffreda *et al*. 1998).

Recently, resistin was identified in mouse

adipocytes (Hartman *et al.* 2002) and this molecule was found to be overexpressed in obesity (Steppan and Lazar 2002). However, the role of resistin in human IR has not been clearly established. In some studies, resistin has been supposed to play a role in whole-body IR (Silha *et al.* 2003, Miner 2004), while others described no relationship between circulating resistin levels and IR (Chen CC *et al.* 2005, Chen MP *et al.* 2005, Farvid *et al.* 2005). In healthy normal-weight women, weight loss was not associated with alterations in circulating resistin levels (Wolfe *et al.* 2004). In agreement with Brichard *et al*. (2003) and contrary to Housová *et al*. (2005), we found significantly decreased plasma resistin levels in patients with AN. Such a discrepancy might be partly explained by differences in the study design (different time of blood withdrawal) as well as by characteristics of studied probands. Age and gonadal status as well as re-nutrition period of studied patients with AN were not clearly described in the study of Housová *et al*. (2005) and all these points could influence obtained data. However, our results confirmed the results of Housová *et al*. (2005) that circulating resistin levels are not related to IS and do not appear to be closely related to the nutritional status in patients with AN. One possible explanation of low plasma resistin levels in AN could be the defective mononuclear-macrophage number and/or function and decreased systemic inflammatory state (Brichard *et al.* 2003, Dostálová *et al*. 2006).

Overall, we are aware of the fact that a weak point of this study is the low number of probands included in the study that could lead to masking of some correlations. It should be further noted that not only the sensitivity to insulin, but also plasma levels of studied adipocytokines might be influenced by 14-day nutritional rehabilitation of patients with AN, because the results of some studies suggested that glucose and insulin secretion are closely associated with body weight and eating behavior, and that these responses are reversible during nutritional rehabilitation (Tanaka *et al.* 2003). In general, the differences in the duration of nutritional rehabilitation together with wide range of individual persistence of the disorder of AN could contribute to a discrepancy in the published results.

In conclusion, we demonstrated that AN is associated with significantly decreased plasma leptin and resistin levels and markedly increased plasma adiponectin levels. Plasma leptin and adiponectin levels were related to the body size and adiposity. Hyperadiponectinemia in patients with AN could play a role in increased IS found in these patients. The role of reduced plasma resistin levels in anorexia nervosa needs further investigation. However, neither insulin sensitivity nor body size and adiposity are major determinants of plasma resistin levels in patients with AN.

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