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ORIGINAL CONTRIBUTION

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Determinants of plasma adiponectin levels in patients with anorexia nervosa examined before and after weight gain

Summary *Objective* To examine the determinants of adiponectin levels (i) in 23 women with anorexia nervosa (mean BMI 15.0 ± 1.2) and 43 healthy normal weight females (mean BMI 22.3 ± 2.3; cross-sectional design) as well as (ii) after six and twelve weeks of weight gain in subgroups of 18 and 11 anorectic patients (mean weight gain 5.8 kg; longitudinal design). Plasma adiponectin and leptin concentrations were measured and their relationships to body composition (fat mass by bioelectrical impedance analysis and anthropometrics), different hormones and metabolic parameters (insulin, ACTH, cortisol, glucose, FFA, lipid profile) were investigated. *Results* In anorectic patients, adiponectin levels were higher (+29 %) and leptin levels were lower (–75 %) than in control subjects. There was a high variance in adiponectin levels in patients ranging from 2.6 to 18 nM. Combining patients and controls, an inverse linear correlation was observed between adiponectin levels and fat mass ($r = -0.36$, $p < 0.05$), while a positive exponential relation was found between leptin levels and fat mass ($r = 0.82$, $p < 0.001$). In anorectic patients, there were no significant correlations between adiponectin and hormonal or metabolic parameters. Weight gain resulted in increasing leptin (+0.17 ± 0.12 nM; $p < 0.001$) and a nonsignificant decrease in adiponectin concentrations (–1.12 ± 2.51 nM). Changes in leptin levels were mainly explained by a gain in fat mass ($r = 0.85$, $p < 0.001$). In contrast, changes in adiponectin levels were closely linked to initial adiponectin levels ($r = -0.84$, $p < 0.001$) but not to changes in fat mass or BMI. *Conclusion* Cross-sectionally serum adiponectin concentration followed a linear inverse function with fat mass when patients and controls were combined. Longitudinally gain in fat mass was not associated with changes in adiponectin levels suggesting other yet unidentified influences on adiponectin secretion in anorexia nervosa.

Key words adiponectin – anorexia nervosa – body composition – weight gain

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Introduction

Plasma levels of adiponectin, an adipokine which is specifically secreted by adipocytes [1], are reduced in obese subjects [2] whereas elevated levels are reported in patients with anorexia nervosa by some (3–5, for a review see 6) but not all authors [7]. In obesity weight loss increases adiponectin concentrations [8]. Data on changes in plasma adiponectin levels with weight gain in undernutrition are confined to two studies [4, 7]. In a Japanese study initial plasma adiponectin levels in 31 fe-

males with anorexia nervosa were significantly lower than in normal weight controls and increased to normal after weight recovery [7]. In one case report of a severely malnourished patient, plasma adiponectin level was also low [4]. It gradually increased with increasing BMI up to a BMI of about 16 kg/m^2 and subsequently decreased with further weight gain. Since an endocrine dysfunction of severely reduced adipose tissue has been proposed to explain this observation [4], our study sets out to investigate whether a certain threshold for fat mass exists that determines the direction of change in adiponectin levels in underweight patients. Besides fat mass, plasma levels of Insulin and glucocorticoids are analyzed as potential determinants of adiponectin levels in anorexia nervosa.

Methods

Twenty-three women meeting criteria for anorexia nervosa based on the Diagnostic and Statistical Manual of Mental Disorders IV [9] were enrolled in a multidisciplinary psychotherapy and nutritional treatment program at the Psychosomatic Clinic Bad Bramstedt, Germany. No patient had a medical condition (other than anorexia nervosa) or received hormonal or other medication known to affect body composition. All patients had CRP levels under 5 mg/l. Body composition and serum hormone concentrations were assessed within the first days after referral in all patients (T_0). Follow-up was 43 ± 5 days (T_0–T_1) and 84 ± 11 days (T_0–T_2). A control group consisted of forty-three normal-weight, healthy women, who were recruited from students of nutritional sciences and examined at the Institute of Human Nutrition, University of Kiel, Germany. The study protocol was approved by the local ethical committee. All patients or parents of underage patients gave informed written consent.

Standard procedures were used to assess body height and weight (SECA, model 2200, Vogel & Halke, Hamburg, Germany). Bioelectrical Impedance Analysis was performed as described previously [10] (Body Composition Analyser, model TVI-10^{TM}, Danninger Medical Technology, Inc. Detroit/USA). Fat mass was calculated with a standard software package (bodycomp, Danninger Medical). Fat mass assessed by BIA and skinfold measurements (sum of biceps, triceps, subscapular and suprailiacal skinfolds) showed a good correlation ($r = 0.89$ for patients and controls and $r = 0.76$ for patients only). However, the correlation between changes in fat mass$_{BIA}$ and the sum of skinfolds was weaker ($r = 0.48$; $p = 0.02$). Because phase angle increased from 4.8° at T0 to 5.1° at T1 ($p < 0.05$) and 5.4 at T2 ($p < 0.01$) the discrepancy between BIA and anthropometric data is likely due to an increase of fat-free mass hydration (i. e. an increase of extracellular water) with weight gain.

Blood samples were obtained between 7:00 and 8:00h after an overnight fast. Plasma was immediately frozen at –80 °C until analysis. A RIA procedure was used to measure adiponectin serum levels. Adiponectin antiserum was generated in rabbits using a C-terminal fragment of adiponectin (aa 189–202; Biotrend Cologne, Germany) coupled to hemocyanin. A working antibody titer of 1:1000 was subsequently used. The tracer generated by the iodogen method was HPLC purified on a C18 column. Full length adiponectin or an adiponectin fragment was used as a standard. Antibody-bound fraction and free peptide were separated by 2 % dextrancharcoal. There was parallelism between diluted high adiponectin serum samples and the standard curve. The maximal inter- and intra-assay CV was 6.5 %. Plasma total leptin concentrations were measured by RIA using a commercial kit (Linco Research St. Charles, MO). Insulin was determined by ELISA (DAKO Diagnostics Cambridgeshire, UK). Insulin resistance was assessed by homeostasis model assessment (HOMA) calculated as fasting plasma insulin (µU/ml) x glucose (Mm)/22.5 [11]. ACTH was measured from EDTA-plasma (LUMI test^R, coated tube system, Brahms Diagnostik GmbH, Germany). Cortisol was analyzed by Electro Chemiluminescence Immuno Assay (ECLIA, Elecsys®, Roche Diagnostics Mannheim, Germany).

Statistical analyses were performed by SPSS for Windows (8.0, SPSS Inc., Chicago U. S.A). Data are presented as means ± SD. Pearson's correlation was calculated to test for relationships among different parameters. Mann-Whitney U-test was used for comparisons between groups, Wilcoxon test for intra-individual, longitudinal changes of one parameter. P-value < 0.05 was considered statistically significant.

Results

Cross-sectional data

In patients, weight, BMI, fat mass and leptin levels were lower and mean adiponectin levels were higher than in control subjects (Table 1). However, there was a high variation in adiponectin levels among patients ranging from 2.6 to 18 nM. Adjusting adiponectin levels for the inverse relationship to fat mass resulted in similar values in patients and controls (Table 1). Combining patients and controls, there was an inverse linear relationship between plasma adiponectin and fat mass (Fig. 1), while the positive relationship between leptin and fat mass was exponential (Fig. 1). In patients mean levels for hormones and metabolic parameters were: ACTH 27.5 ± 16.4 ng/l; cortisol 673.2 ± 234.5 nM; glucose 3.90 ± 0.43 mM; HOMA-index 0.83 ± 0.46; FFA 0.36 ± 0.27 mM; triglycerides 1.04 ± 0.52 mM; cholesterol 5.15 ± 0.82 mM. There were no significant correlations

Table 1 Nutritional status and plasma levels of adipocytokines and insulin in female patients with anorexia nervosa (at baseline, T0 and during follow up, delta T1 and T2) and healthy female controls. Data are means ± SD

n	Patients T0 23	Controls 43	ΔT1* 18	ΔT2** 11
Age, years	24.1 ± 5.9	24.7 ± 3.3	–	–
Weight, kg	42.3 ± 4.4	63.1 ± 7.8[a]	5.8 ± 2.3[b]	10.3 ± 3.3[c]
BMI, kg/m^2	15.0 ± 1.2	22.3 ± 2.3[a]	2.1 ± 0.9[b]	3.5 ± 1.2[c]
Σ skinfolds, mm	24.2 ± 9.4	51.9 ± 12.7[a]	15.1 ± 9.5[b]	33.6 ± 6.7[c]
Fat mass, kg	5.8 ± 2.9	20.9 ± 5.0[a]	4.2 ± 2.3[b]	7.8 ± 2.3[c]
Fat mass, %	13.2 ± 5.9	32.6 ± 4.2[a]	7.0 ± 4.1[b]	12.1 ± 3.7[c]
Adiponectin, nM	6.70 ± 3.25	5.19 ± 1.56[a]	−1.12 ± 2.51	−1.15 ± 2.37
Adiponectin adj[d], nM	5.62 ± 3.22	5.67 ± 1.59	−3.17 ± 2.77[b]	−2.72 ± 1.60[c]
Leptin, nM	0.13 ± 0.08	0.53 ± 0.26[a]	0.18 ± 0.13[b]	0.42 ± 0.38[c]
Leptin adj[e], nM	4.38 ± 1.28	4.54 ± 2.48	1.56 ± 1.52[b]	2.41 ± 3.62[c]
Insulin, pM	34.10 ± 15.97	39.79 ± 16.32	6.39 ± 22.23	12.01 ± 12.64[c]

* T1 minus T0; ** T2 minus T0; [a] difference between patients and controls by Mann Whitney test; [b] difference between patients T0 and T1 by Wilcoxon test; [c] difference between patients T0 and T2 by Wilcoxon test; [d] adiponectin levels adjusted for fat mass; [e] leptin levels adjusted for fat mass

with adiponectin and any of the measured parameters at T0 as well as between the differences T0–T1 or T0–T2 (data not shown).

Longitudinal data

During follow-up patients gained an average of 5.8 kg body weight mainly consisting of fat mass (4.2 kg). With weight gain, there was a significant rise in plasma leptin levels (Table 1) that was related to gain in fat mass (Fig. 1). However, a decrease in adiponectin levels after weight gain was not significant. Fat mass adjusted adiponectin levels significantly decreased in patients during weight gain (T0–T1 and T0–T2) because of increased fat mass and nearly unchanged adiponectin levels (Table 1). Changes for mean levels of hormones and metabolic parameters in patients from T0 to T1 and from T0 to T2 were: ACTH +11.26 ± 18.9 ng/l ($P < 0.05$) and +7.25 ± 19.5 ng/l; cortisol +65.39 ± 141.0 nM and −23.18 ± 157.3 nM; glucose +0.30 ± 0.49 mM and +0.33 ± 0.49 mM; HOMA-index −0.30 ± 0.69 and −0.44 ± 0.44; FFA −0.12 ± 0.37 mM and +0.22 ± 0.19 mM; triglycerides −0.02 ± 0.34 mM and −0.12 ± 0.25 mM; cholesterol +0.36 ± 0.92 mM and −0.32 ± 0.12 mM, respectively.

The small changes in adiponectin levels highly correlated with initial adiponectin levels (T0) but showed no significant relation to changes in fat mass measured by BIA, the change in the sum of skinfolds or BMI as well as to changes in hormonal and metabolic parameters.

Discussion

Our cross-sectional data confirm the finding of three other recent studies reporting higher plasma adiponectin levels in patients with anorexia nervosa when compared with healthy normal weight controls [3–5]. By contrast, in a recent Japanese study, plasma adiponectin levels in 31 anorectic females were significantly lower than in normal weight controls and increased to normal levels after recovery [7]. One previous study examined only one anorectic patient during weight gain [4]. These authors also reported increasing adiponectin levels up to a BMI of 16 kg/m^2 with a gradual decrease with further increases in BMI. Our data do not support such a threshold. Combining all patient data (T0 + T1 + T2) we observed an inverse relation between adiponectin levels and the whole range of BMI ($r = 0.32$) and a nonsignificant decrease of mean plasma adiponectin levels with weight gain (T0–T1) in patients (Table 1, Fig. 1). However, cross-sectionally as well as longitudinally, the variance in plasma adiponectin levels was high in our patients (see Results).

The etiology of a higher adiponectin secretion with lower fat mass is not clear. Since the expression of adipogenic genes was suppressed in the development of obesity a feedback inhibitory route has been suggested [12]. However, in our anorectic patients variation in basal adiponectin levels was high (Table 1, Fig. 1) and showed no significant correlation to fat mass or BMI. Moreover, increasing fat mass with weight gain was not significantly related to changes in adiponectin levels. However, adipose tissue consists of adipocytes and nonfat cells like stromovascular cells. These may also function in cytokine secretion. It was shown only recently that the vast majority of TNFα released by adipose tissue was due to the nonfat cells [13].

TNFα levels are elevated in patients with anorexia nervosa and remain high even during recovery [14]. Since the current paradigm is that TNFα is a direct inhibitor of the synthesis by adipocytes of leptin as well as adiponectin [15], TNFα levels might contribute to the variance in these cytokines in anorexia nervosa. Nevertheless, it is tempting to speculate that the effect of TNFα

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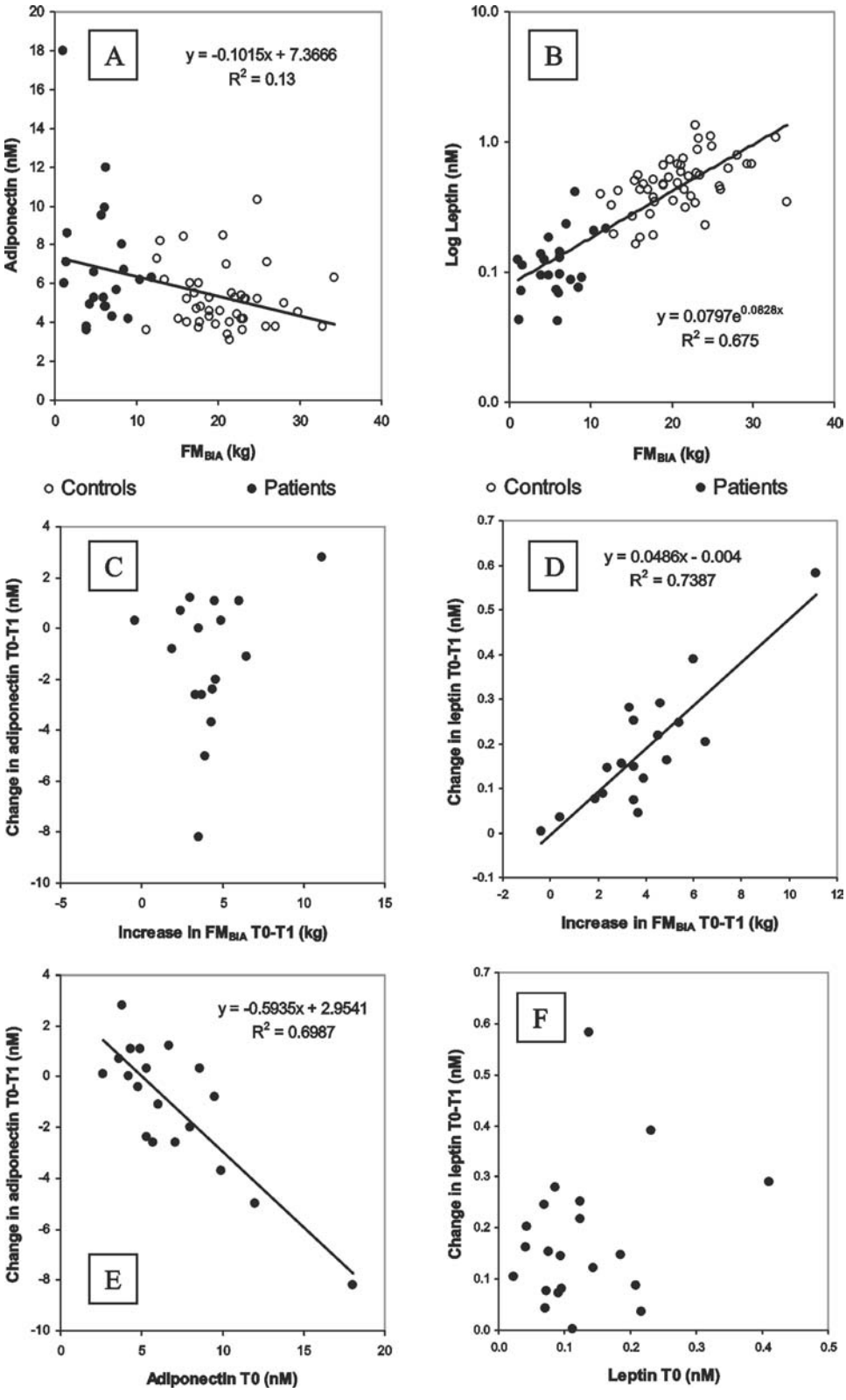

Fig. 1 **A**, **B** Plasma adiponectin and log transformed leptin levels in 23 patients and 43 controls were plotted against fat mass (FM, kg) assessed by bioelectrical impedance analysis (BIA). **C**, **D** For anorectic patients, changes in plasma adiponectin and leptin levels from T0 to T1 were potted vs. changes in fat mass. **E**, **F** For anorectic patients, changes in plasma adiponectin and leptin levels from T0 to T1 were potted vs. changes in initial adiponectin and leptin levels (T0)

should apply to adiponectin as well as leptin and might thus only explain reduced leptin secretion but not elevated adiponectin levels in these patients.

Besides fat mass, insulin was implicated in the regulation of adiponectin expression in humans [16]. However, we found no significant correlation between insulin and adiponectin levels in patients and controls. There was also no correlation between adiponectin and ACTH or cortisol levels in patients although glucocorticoids have been shown to decrease adiponectin gene expression and secretion [17, 18]. Therefore we may deduce that other yet unidentified determinants explain the variance in adiponectin levels at low fat mass in weight stable anorectic patients as well as in anorectic patients at increasing fat mass.

While the pathophysiologic role of adiponectin due to its anti-atherogenic and anti-inflammatory properties [for a review see 19] seems to be confined to diminished levels in obesity, the physiologic relevance of high adiponectin levels in anorexia nervosa is unclear. Adiponectin levels in these patients have been reported to be positively correlated with insulin sensitivity [3]. In our underweight patients, plasma adiponectin was not related to glucose, HOMA index, or any parameter of the lipid profile. However, since we only analyzed these parameters in the patient group, a narrow range of values might explain missing correlations. Our data suggest that fat mass, insulin and glucocorticoids do not explain the variance in adiponectin levels in weight stable and weight gaining patients with anorexia nervosa.

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