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ORIGINAL ARTICLE

In anorexia nervosa, even a small increase in abdominal fat is responsible for the appearance of insulin resistance

A. Prioletta*, G. Muscogiuri*, G.P. Sorice*, A.P. Lassandro*, T. Mezza*, C. Policola*, E. Salomone*, C. Cipolla*, S. Della Casa*, A. Pontecorvi* and A. Giaccari*,†

Endocrinologia, Università Cattolica, Rome and †Don Gnocchi Foundation, Milan, Italy

Summary

Context The aim of treatment in patients affected by anorexia nervosa (AN) is weight recovery. However, during weight gain, anorectic patients' body composition is changed, with an increase in abdominal fat, particularly in the visceral compartment.

Objective We hypothesized that changes in body composition, particularly in abdominal fat, are responsible for the variability in insulin sensitivity (IS) in different stages of AN.

Design and Measurements We compared 20 anorectic patients in the acute stage, 19 in the weight-recovery stage and 21 controls. All subjects underwent an oral glucose tolerance test, hyperinsulinaemic euglycaemic clamp and dual energy X-ray absorptiometry to measure body composition.

Results The percentage of trunk fat was higher in weight recovery than in the acute phase (47·7 ± 8·4% *vs* 34·6 ± 7·6%; $P \leq 0{\cdot}01$) and in the control group (33·4 ± 7·6; $P < 0{\cdot}01$ *vs* weight recovery). Although the recovery group gained weight, their body mass index (BMI) was not statistically different from that of the acute group (14·4 ± 1·1 *vs* 13·6 ± 1·8 kg/m^2). Insulin sensitivity was lower in the weight-recovery group than the acute group (4·7 ± 1·5 *vs* 7·8 ± 1·6 mg/kg/min; $P < 0{\cdot}01$) and controls (7·7 ± 1·4 mg/kg/min; $P < 0{\cdot}01$). A linear negative correlation was found between IS and the percentage of abdominal fat in the weight-recovery and acute groups ($r = -0{\cdot}51$; $P = 0{\cdot}04$ and $r = -0{\cdot}53$; $P = 0{\cdot}04$ respectively), while IS did not correlate with BMI.

Conclusion Although weight-recovery represents the main aim of treatment in AN, refeeding is associated with an increase in abdominal fat which might be responsible of the onset of insulin resistance. As BMI and weight-recovery were associated with impaired IS, they cannot be considered the only aim of treatment of AN.

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Correspondence: Andrea Giaccari, Endocrinologia, Università Cattolica – Policlinico 'A. Gemelli', Largo A. Gemelli 8, 00168 Rome, Italy. Tel.: +39 063015 7094; Fax: +39 063015 6193; E-mail: giaccari@rm.unicatt.it

Introduction

Anorexia nervosa (AN) is a psychiatric eating disorder, affecting mostly female adolescents and is characterized by self-induced weight loss, body image distortion and obsessive fear of gaining weight. AN is characterized by two types of eating behaviours: restricting or binging–purging. The prevalence ranges from 0·3 to 1%,[1] and the all-cause mortality related to the disease is high.[2]

In the literature, there is a large amount of data regarding the endocrine and metabolic changes associated with AN, mostly representing physiological adaptation to starvation, and their reversal after refeeding, resulting in many being considered as indices of the acute phase of disease.[3,4] Nonetheless, weight loss and gain clearly affect fat content, and it is well known that fat content largely influences insulin sensitivity (IS). It is known that prolonged starvation causes changes in body composition with loss of fat and lean masses[5] while nutritional recovery may result in significant changes in the regional redistribution of fat mass, the abdominal area being the largest site of lipid storage.[6] As elegantly demonstrated by Mayer *et al.*[7] by magnetic resonance imaging, weight gain in AN is associated with an increase in visceral and intramuscular fat depots, closely resembling the detrimental fat distribution of patients affected by several metabolic disorders associated with insulin resistance. Nevertheless, previous studies of IS in patients with AN provided contradictory results, finding insulin action to be normal,[8] increased[9,10] or decreased[11]. These controversial results could be related to the different techniques used to evaluate IS, to the evaluation of insulin sensitive tissues on body mass index (BMI) rather than actual body composition and to different clinical conditions (losing or gaining weight) of the studied subjects.[8,10]

In the present study, we explored the hypothesis that, in anorectic patients, even small differences of abdominal fat, measured as trunk fat by dual energy X-ray absorptiometry (DEXA),[12] might be responsible for dramatic variations in IS.

Subjects and methods

Study subjects

Forty women with a restrictive type of AN, recruited between March 2008 and October 2010 in the Division of Endocrinology, were divided according to the stage of disease as 'weight-recovery

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group' ($n = 21$) and 'acute group' ($n = 19$) and compared with 20 control subjects matched for age but without any history of eating disorder, recruited from among the students and personnel of our University. Weight recovery was defined as an increase in weight more than 10% from the lowest reached weight. Weight recovery was attained through a multidisciplinary, re-educational programme of at least 12 weeks. The anorectic patients underwent medical and psychiatric evaluation: all of them met the diagnostic criteria for AN as presented in the 4th edition of the Diagnostic and Statistical Manual (DSM-IV).[13] All subjects, including controls, were Caucasian and ranged in age from 17 to 32 years.

None of the subjects included in the study had any allergies or had taken any medication for at least 3 weeks prior to the study. The weight of both groups (weight-recovery and acute) was stable for at least 15 days before the study (as assessed by clinical report), and the anorectic patients had not fasted during the 3 days before the study. The controls were weight stable at least for 3 months before the study. They were not dieting or performing regular exercise. All anorectic patients were amenorrhoeic, and the control subjects were within 5 days after the last menstrual bleed. All participants provided informed consent prior to participating in the study. The project was in accordance with the ethical standards of our responsible institutional committee on human experimentations.

Methods

Anthropometric parameters were determined according to standard procedures.[14] Height was measured, to the nearest 0·1 cm, with a wall-mounted stadiometer. Weight was recorded to the nearest 0·01 kg by using a calibrated computerized digital balance. BMI was calculated as the weight (kg) divided by the square of height (m^2).

All patients underwent an oral glucose tolerance test and, 1 week later, IS was tested by a hyperinsulinaemic euglycaemic clamp (HEC) with a basal continuous insulin infusion at a dose of 40 mU/m^2/min. The clamp test was performed after a 12-h overnight fast, as previously described [15–17]. Whole-body glucose uptake was assessed as milligrams of glucose metabolized per kilogram of body weight per minute during the steady state (2nd hour of the clamp).

Plasma glucose concentrations were determined by glucose oxidase technique, using a glucose analyzer (Beckman Instruments, Palo Alto, CA, USA). Insulin was measured by an enzyme chemiluminescence immunoassay (ECLIA; Roche Products Ltd, Modular E, Penzberg, Germany, intra-assay and interassay coefficients of variation were, respectively 4·5–7·2%, and 3·2–6·0%).

Body composition was determined by DEXA. All DEXA measurements were performed by trained staff in the Department of Medical Imaging using a DPX (Lunar Corp, Madison, WI, USA) total body scanner in fast scan mode. The total body scan takes approximately 10 min and involves a very low radiation dose of approximately 0·02 μSv. The precision for this technique *in vivo* as assessed at the hospital is 1·59% for percentage fat body mass and 0·82% for lean body mass. Fat mass was calculated for the whole body and for the trunk and leg regions. Trunk and leg fat were defined using standard regional settings as previously described by Ley *et al.*[12]

Statistical analysis

Statistical analysis was carried out using BIOSTAT 2008 5.4.0.0 (AnalystSoft Inc., Alexandria, VA, USA). Data are expressed as mean ± SD. After checking that variables were normally distributed, we performed one-way analysis of variance (ANOVA) to determine statistical differences in continuous variables between the two groups of anorectic patients, categorized on the basis of disease stage (acute and recovery group) and between these and the control group. A Tukey's HSD was used as a *post hoc* test. A linear correlation between variables, in the groups of anorectic patients, was described using Pearson's correlation coefficients.

The sample size was calculated using 'POWER AND SAMPLE SIZE' software, version 2.1.25 (Biostat, Englewood, NJ, USA). Based on the research design and strategy, sample size was calculated using Two Means Formula with 90% power of study. The minimum requirement for each group was 16 patients.

Results

Table 1 reports the clinical and biochemical characteristics of the two groups of anorectic patients divided according to the stage of disease: weight-recovery group (weight >10% above baseline), acute group and control group. The glucose tolerance test showed normal glucose tolerance in all subjects according to ADA criteria.[18]

The two groups of anorectic patients were similar in age and duration of disease; however, weight-recovery anorectic patients had lower glucose uptake and a higher percentage of fat mass in the trunk (all $P < 0·05$ or less). A linear negative correlation was found between glucose uptake and the percentage of trunk fat in weight-recovery and acute groups ($r = -0·51$; $P = 0·04$ and $r = -0·53$;

Table 1. General, anthropometric and metabolic parameters in acute, weight-recovery and control groups

	Acute	Weight-recovery	Control
N	20	19	21
Age (years)	27·7 ± 8·1	23·7 ± 7·26	23·8 ± 1·3
Duration of disease (months)	76·5 ± 30·2	59·3 ± 30·3	–
Weight (kg)	35·2 ± 4·1	39·3 ± 3·3	52·2 ± 8·5†,‡
Height (cm)	160 ± 8	170 ± 5	160 ± 9
Body mass index (kg/m^2)	13·6 ± 1·8	14·4 ± 1·1	20·1 ± 1·7†,‡
Total fat mass (kg)	3·4 ± 1·4	8·6 ± 2·1*	18·4 ± 3·7†,‡
Trunk fat mass (kg)	1·4 ± 1·7	4·1 ± 3·4*	5·8 ± 1·5‡
Trunk fat mass (%)	34·6 ± 7·6	47·7 ± 8·4*	33·4 ± 7·6†
Fat free mass (kg)	31·8 ± 2·7	30·7 ± 1·2	33·8 ± 4·8
Glucose uptake (mg/kg/min)	7·8 ± 1·6	4·7 ± 1·5*	7·7 ± 1·4†

Data are expressed as mean ± SD.
*$P < 0·05$ or less acute group *vs* recovery group.
†$P < 0·05$ or less, recovery *vs* control group.
‡$P < 0·05$ or less acute *vs* control group.

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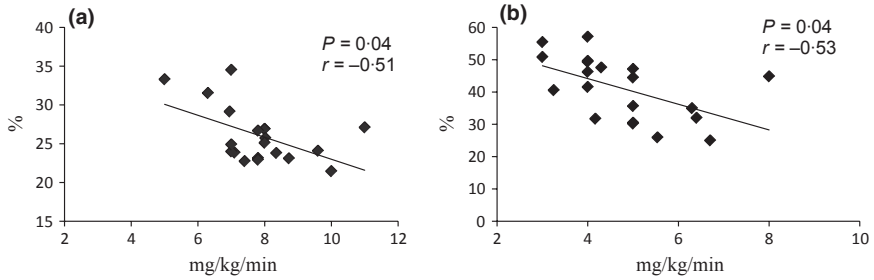

Fig. 1 Linear correlation between trunk fat mass percentage and glucose uptake in (a) acute group and (b) weight recovery group.

$P = 0{\cdot}04$ respectively) (Fig. 1) and in the entire group of anorectic patients ($r = -0{\cdot}51$; $P = 0{\cdot}04$; Fig. 2), while there was no correlation between glucose uptake and BMI ($r = -0{\cdot}08$; P = NS).

We compared the anorectic patients with age-matched controls and found that the weight-recovery group had lower (in absolute terms) but higher percentage of adipose tissue localized in the trunk than the controls ($47{\cdot}7 \pm 8{\cdot}4$ *vs* $33{\cdot}4 \pm 7{\cdot}6$; $P \leq 0{\cdot}01$) (Fig. 3, Panel a). In addition, the weight-recovery group displayed a lower whole-body glucose uptake than controls ($4{\cdot}7 \pm 1{\cdot}5$ *vs* $7{\cdot}7 \pm 1{\cdot}4$ mg/kg/min; $P \leq 0{\cdot}01$) (Fig. 3, Panel b). On the other hand, we did not notice any difference in terms of percentage of trunk fat mass and glucose uptake between the anorectic patients in the acute phase of disease and controls ($7{\cdot}8 \pm 1{\cdot}6$ *vs* $7{\cdot}7 \pm 1{\cdot}4$ mg/kg/min).

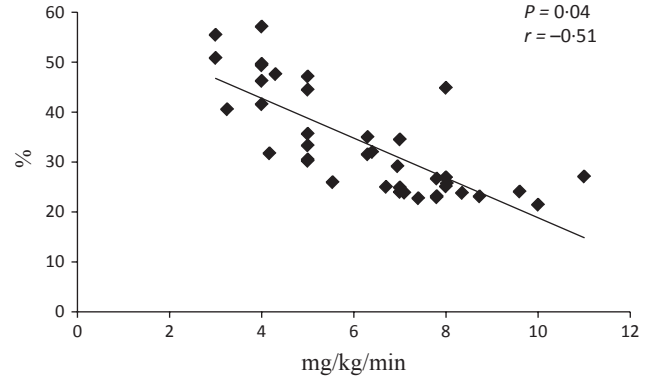

Fig. 2 Linear correlation between trunk fat mass percentage and glucose uptake in entire cohort of anorectic patients.

Discussion

Anorexia nervosa is a common psychiatric eating disorder associated with high morbidity and mortality[2] and with medical complications related not only to the acute alterations seen in the disease but also to the chronic complications that may persist into adult life.[19] Among these, the major fat loss during starvation and the (almost exclusive) restorage in the abdominal region[7,20] might cause severe metabolic derangements. As is well known, visceral adipose tissue is considered to be a major factor in the pathogenesis and clustering of important metabolic alterations (eventually resulting in the metabolic syndrome) and represents a cardiovascular risk factor in the long term. While overall adipose tissue is largely reduced in AN, the possible atrophy of subcutaneous fat pads (secondary to chronic under nutrition) might divert even small increases in fat content (secondary to refeeding) to other tissues, with large variations in fat content and distribution;[21] this should indeed cause severe insulin resistance.

Nevertheless, available data on IS in AN are conflicting.[5,8] These conflicting results may be partially related to different techniques used to measure IS (mostly indirect indices)[22,23] as well as to a possible wide range of body composition, which might reflect the variations in terms of lean and fat mass ratio[24,25] related to different phases of the disease.

Thus, we aimed to assess IS in anorectic patients by HEC, considered the gold standard to directly measure IS, and to correlate IS with fat distribution and stage of disease.

As acute changes in fluids and electrolytes might affect insulin effects on glucose metabolism, we decided to include in the study only patients with restrictive AN, excluding all binging-purging patients.

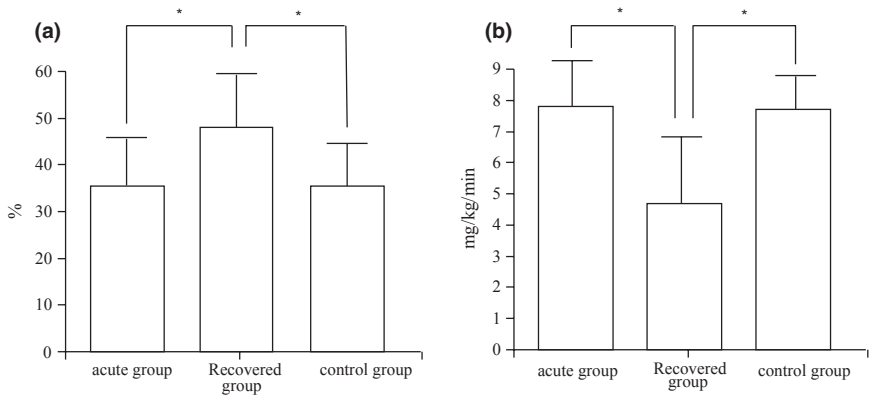

Fig. 3 Metabolic features of anorectic patients and controls. (a) Trunk fat tissue expressed as percentage of total fat mass (*$P < 0{\cdot}05$). (b) Insulin-stimulated glucose disposal calculated by hyperinsulinemic euglycaemic clamp as mg of glucose metabolized per kilogram of body weight per minute (M value; *$P < 0{\cdot}05$).

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In our study, we found a decrease in whole-body glucose disposal in the weight-recovery group as compared to the control and acute groups. We did not find any difference in terms of IS between acute group and controls. Although the study is cross-sectional, it appears logical to hypothesize that, in our restrictive anorectic patients, refeeding turned normal IS to insulin resistance.

Previous studies evaluating IS in AN patients have reported different results: Pannacciulli *et al.*[11] studied a cohort of adolescent and young adult women with AN, as compared with normal-weight healthy female controls, evaluating IS by our own method and found that anorectic patients had significantly lower insulin-stimulated glucose disposal. On the other hand, Zuniga-Guajardo *et al.*[9] found an increase in IS, again with the hyperinsulinaemic clamp. However, neither group investigated the relationship between IS and body composition and the type of AN (restrictive or binging-purging) was not reported or defined.

It might appear surprising that fat weight gain is larger than whole-body weight gain. However, it should be taken into account that whole-body weight is mostly determined by water and AN is characterized by large changes in water body. Therefore, measurement of fat mass can be considered as more reliable than whole-body weight.

We found that the increase in trunk fat percentage was strictly paralleled by a decrease in IS in the recovery group, as compared with controls. Furthermore, we correlated IS with percentage of trunk fat and BMI: interestingly, we found a significant correlation between whole-body glucose uptake and percentage trunk fat also when considering the entire AN cohort, while no correlation was found with BMI. As hypothesized (and well documented in several other metabolic disorders),[26] fat distribution, rather than BMI *per se*, is the major determinant of IS.

Our data support the hypothesis that IS decreases in the weight-gaining stage of the disease. In fact, anorectic patients with weight recovery showed an increased percentage of trunk adiposity that may be responsible for the onset of insulin resistance. These results closely resemble what is usually observed in lipodystrophic patients[27] in which the absence of subcutaneous fat depots diverts all lipid excess to other tissues, causing insulin resistance; our data allow us to hypothesize that anorectic patients lose most of the subcutaneous adipose tissue during the acute phase of disease, thus losing the ability to store (and eventually restore) fat in the subcutaneous compartment. Thus, during refeeding, the storage of fat occurs mainly in the visceral compartment and other tissues; these in turn will become responsible for the onset of insulin resistance.

It is clinically noteworthy that BMI is not a true index of the metabolic state in patients with AN, contrary to patients with metabolic syndrome and diabetes. Thus, to obtain a true metabolic index, the evaluation of body fat distribution appears to be more accurate than BMI, at least in these patients. The presence of trunk fat and insulin resistance in recovering (refeeding) anorectic patients leads us to conclude that a true and complete lifestyle change (including a balanced diet and appropriate physical exercise, with their consequences on fat distribution) is needed to consider this important disease cured.

Competing interests/financial disclosure

Nothing to declare.

Authors' contributions

Giaccari A and Prioletta A designed research, Salomone E., Cipolla C, Policola C and Lassandro AP conducted research, Mezza T and Sorice GP analyzed data, Della Casa S, Pontecorvi A and Muscogiuri G wrote the paper, Giaccari A and Prioletta A had primary responsibility for final content. All authors read and approved the final version of the manuscript.

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