Increased visceral adipose tissue is associated with increased circulating insulin and decreased sex hormone binding globulin levels in massively obese adolescent girls

M. De Simone*, A. Verrotti**, L. Iughetti***, M. Palumbo*, G. Farello*, E. Di Cesare****, R. Bernabei*****, T. Rosato*, S. Lozzi*, and S. Criscione*

*Department of Experimental Medicine, Institute of Pediatrics and ****Department of Radiology, University of L'Aquila, L'Aquila; **Department of Medicine, Division of Pediatrics, University of Chieti, Chieti; ***Pediatrics Chairs, University of Modena, Modena; *****Hospital "S. Salvatore", L'Aquila, Italy

ABSTRACT. The current study was designed to examine the relationship between body fat distribution, as evaluated by anthropometry and magnetic resonance imaging (MRI), and circulating insulin, sex hormone and SHBG levels in obese adolescent girls. Twenty-nine obese adolescent girls, aged 12.6-16.9 years with a mean BMI of 30.51±1.86 participated in this study. All girls had breast stage B4-5 and pubic hair stage P4-5. Percent obesity and BMI as indices of being overweight were calculated; the waist-to-hip ratio (WHR) and the waist-to-thigh ratio (WTR) were calculated to obtain two anthropometric indices for the pattern of body fat distribution. The areas of visceral (VAT) and subcutaneous adipose tissue (SAT) were evaluated by MRI at the L4-L5 level. Serum concentrations of total T, DHEAS, 17β-estradiol, progesterone and SHBG were measured. Plasma glucose and insulin concentrations were evaluated during an oral glucose tolerance test. WHR was the only anthropometric parameter that was significantly associated with the area of VAT. Insulin level showed correlation with both WHR and the area of VAT; no correlation was found between insulin levels and WTR. Both WHR and VAT were negatively correlated with serum DHEAS level and positively correlated with T level. There were strong negative correlations between serum SHBG level and the area of VAT and WHR. Inverse correlation was found between serum SHBG level and insulin. Serum 17β-estradiol and progesterone levels showed no significant correlation with all the patterns of body fat distribution. SAT was not significantly correlated with both anthropometric parameters and any of the sex hormones evaluated. We can draw two main conclusions. Firstly, in massively obese adolescent girls, the WHR seems to be a good indicator for the accumulation of VAT, and abdominal obesity, rather than adiposity *per se*, appears to be related to biochemical complications. Secondly, increased upper body adiposity and, in particular, the intra-abdominal fat area are associated with increased insulin levels in massively obese adolescent girls. The associated reductions in SHBG and DHEAS levels represent an early general risk factor for the development of metabolic and cardiovascular diseases in this population, as previously described for obese adult women.

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INTRODUCTION

Several studies suggest that body fat distribution and intra-abdominal fat location, in particular, play a major role in determining metabolic perturbations antecedent to cardiovascular disease in adults. These perturbations include high plasma concentration of triglycerides, hyperinsulinemia, increased propensity for Type 2 diabetes mellitus and hypertension (1-8). Previous studies have shown that women with abdominal obesity have reduced plasma levels of sex hormone binding globulin (SHBG) and increased lev-

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Correspondence: Dr. Michele De Simone, Dipartimento di Medicina Sperimentale, Clinica Pediatrica, Università de L'Aquila, Via Vetoio, Coppito II, 67100 L'Aquila, Italy.

E-mail: udesi@tin.it

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els of free testosterone (9-12). Furthermore, decreased levels of SHBG, an indirect index of androgenicity, are also associated with glucose intolerance and hyperinsulinemia (9). This suggests that the relationship between circulating SHBG, glucose and insulin may be mediated by obesity and body fat distribution. However, there have been few investigations of this issue within the pediatric and, in particular, the pubertal population.

Anthropometric indices [waist girth, waist-to-hip circumference ratio (WHR), skinfold thickness and skinfold ratios] are commonly used to estimate visceral fat accumulation in obese adults. In large-scale epidemiological studies, WHR is the preferred index of fat distribution because it is the least obtrusive measure, is more reliable than skinfold thickness and can readily be measured and reported by the subject (13). Evidence suggests that WHR is useful in predicting atherosclerosis risk in adults.

However, in the case of childhood obesity it is unclear whether WHR or the trunk-to-extremity skinfold ratio are valid indicators of visceral fat accumulation (14, 15). While anthropometry can distinguish between central and peripheral adiposity, it does not differentiate between visceral and subcutaneous fat depots. Computed tomography (CT) provides a more reliable technique for the measurement of adipose tissue distribution and assessment of subcutaneous and, in particular, deep fat depots but, for ethical reasons, it is inappropriate for use in children. However, magnetic resonance imaging (MRI) offers a non-invasive alternative method to CT (16-18) in this population.

The purpose of this investigation was therefore twofold: 1) to validate the accuracy of anthropometric indices, as supposed to MRI indices, of body fat distribution; and 2) to evaluate the influence of the visceral fat depot on metabolic and hormonal parameters in a group of massively obese adolescent girls.

MATERIALS AND METHODS

Subjects

Data were obtained from a homogeneous group of 29 massively obese adolescent girls, aged 12.6-16.9 years (mean: 14.19±1.05), with body weight >50% that predicted for their height, and body mass index (BMI) above the 97th centile for their age according to Cachera *et al.* (19). All girls were of breast stage B4-5 and pubic hair stage P4-5 according to Marshall and Tanner's classification (20). Their age at menarche ranged from 10.9 to 14 (mean 12.14±0.73) years. All girls had regular monthly menstrual cycles and the study was performed during the first 8 days of their cycles.

Subjects with metabolic and endocrine disorders and genetic syndromes associated with obesity were excluded.

Written informed consent was obtained from the girls' parents or guardians prior to study entry. The study was approved by the Ethical Committee of the University of L'Aquila.

Anthropometry

Body weight was measured to the nearest 0.1 kg with a balance scale, and height was measured to the nearest 0.1 cm with Harpenden's stadiometer with subjects lightly dressed and without shoes. Four skinfold thicknesses (tricipital, bicipital, subscapular, suprailiac) were measured with Harpenden's caliper by the same investigator. Waist circumference was measured midway between the lower rib margin and the iliac crest, and hip circumference was measured at the widest point over the great trochanters. Both circumferences were measured in the standing position and at the end of a gentle expiration. The maximum thigh circumference was also measured. The WHR and the waist-to-thigh ratio (WTR) were calculated to obtain two indices of body fat distribution.

MRI

MRI scans (Ansaldo Elettronica Biomedicale, Italy) were performed with a 0.2 T magnetic field (500 Mhz) and spin echo sequence (echo time 20 ms); the section thickness was 8 mm.

Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were evaluated at the L4-L5 level. Computed calculation for each defined area was then performed with manual region of interest (ROI).

Biochemical analysis

Following admission to the clinic, subjects were placed on a 3-day weight maintaining diet which provided 50% of calories as carbohydrate, 30% as fat and 20% as protein. An oral glucose tolerance test (OGTT) was then performed using a glucose load of 1.75 g/kg of body weight. The maximum administered dose of glucose was 75 g. The tests were started between 08:00 and 09:00 h and serial blood samples for glucose and insulin determination were collected at 0, 30, 60, 90, 120 and 180 min post-dose.

Plasma glucose levels were measured by an enzymatic assay using the Beckman glucose analyzer (Beckman Instruments Inc., Palo Alto, CA, USA). The intra-assay coefficient of variation (CV) was 1.5%. Plasma insulin was assessed by a double antibody radioimmunoassay (Sorin Biomedica, Italy). The intra- and inter-assay coefficients of variation (CVs)

were 6.6 and 6.2% respectively. The results were expressed as the sum of baseline and post-dose values.
Serum levels of total testosterone were measured with commercial solid phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA); the intra- and inter-assay CVs were 5.5 and 8.8%, respectively.
Levels of dehydroepiandrosterone sulfate (DHEAS) were determined using a commercial radioimmunoassay (Bio Mérieux, France); the intra- and inter-assay CVs were 4.3 and 6.1%, respectively.
Serum levels of 17β-estradiol and progesterone were measured using a specific ELISA method (Boehringer Mannheim Immunodiagnostics).
Serum levels of SHBG were determined by radioimmunoassay (Farmos Diagnostica, Oulunsalo, Finland). The inter- and intra-assay CVs for this method did not exceed 10%.

Statistical analysis

Descriptive statistics were used with data presented as mean±SD.
Linear regression analysis, performed with the Statgraphic Program (Graphics Software Systems, Inc.) on an IBM PC system, was used to assess the relationship between variables.
Correlations for the simple regression analysis were considered statistically significant at $p<0.05$.

RESULTS

The mean overweight (%) of participants was 64.24±8.40, ranging from 50 to 68.
The mean BMI (kg/m^2) was 30.51±1.86, ranging from 27 to 34.
The mean values of VAT (cm^2) and SAT (cm^2) were 64.39±16.66, ranging from 35.14 to 89.7, and 354.28±62.59, ranging from 222.6 to 480.0, respectively.
Other anthropometric parameters, metabolic and hormonal characteristics of the subjects are summarized in Table 1.
Correlation coefficients between the MRI parameters, selected anthropometric variables and circulating sex hormone levels are presented in Table 2. These data demonstrate that the WHR was the only anthropometric parameter that was significantly associated with the area of the VAT (r=0.811, $p<0.0001$). VAT was not significantly correlated with BMI, percentage of obesity, sum of four skinfold thicknesses and WTR (p not significant).
As regards skinfold thickness, the only significant correlation was between the suprailiac skinfold and SAT (r=0.498, $p<0.001$).
No significant correlations were noted between SAT and any of the anthropometric variables (p not significant).
Plasma insulin sum during OGTT (353.41±104.36 μUI/ml, ranging from 122 to 500), but not plasma glucose sum (491.85±76.92 mg/dl, ranging from 230.9 to 599.0), showed significant correlations with both the WHR and the area of VAT (r=0.677, $p<0.0001$; r=0.704, $p<0.00001$, respectively).
No significant correlation was observed between plasma insulin sum and WTR (p not significant).
Both WHR and area of VAT were negatively correlated with serum DHEAS level (r=-0.691, $p<0.00001$; r=-0.940, $p<0.00001$, respectively) and positively correlated with serum total testosterone level (r=0.806, $p<0.00001$; r=0.993, $p<0.00001$, respectively). Serum 17β-estradiol and progesterone levels showed no significant correlation with these parameters (p not significant). However, there were strong negative corre-

Table 1 - *Anthropometric data and ormonal profile.*

Variables	Average	Median	SD	Range
Skinfold thicknesses				
tricipital (mm)	26.93	27.0	1.99	23-30
bicipital (mm)	20.82	20.0	2.00	18-26
subscapular (mm)	39.89	39.0	2.47	34-45
suprailiac (mm)	34.48	35.0	4.44	26-42
sum skinfolds (mm)	97.69	99.0	6.42	85-107
WHR	0.97	0.98	0.07	0.81-1.06
WTR	1.46	1.46	0.10	1.21-1.63
Total T (ng/dl)	38.45	36	20.55	10-68
DHEAS (μg/dl)	136.06	155	87.58	33-290
17β-estradiol (pmol/l)	135	132	20.38	99-176
Progesterone (nmol/l)	1.86	1.6	0.75	0.93-3.2
SHBG (nmol/l)	28.41	25	10.8	16-51

WHR: waist-to-hip ratio; WTR: waist-to-thigh ratio.

Table 2- *Correlation coefficients between magnetic resonance imaging parameters, anthropometric indices and hormonal profile.*

Variables	WHR	WTR	VAT (cm^2)	SAT (cm^2)
BMI (kg/m^2)	-	-	-0.173	-0.273
Overweight (%)	-	-	0.179	0.171
Skinfold thicknesses				
tricipital (mm)	-	-	-0.103	0.167
bicipital (mm)	-	-	-0.101	-0.105
subscapular (mm)	-	-	0.031	0.226
suprailiac (mm)	-	-	-0.109	0.498*
sum skinfolds (mm)	-	-	0.361	-0.049
WHR	-	-	0.811**	0.003
WTR	-	-	-0.144	0.024
Sum glycemia (mg/dl)	0.252	-0.199	0.152	0.227
Sum insulinemia (μU/ml)	0.677**	-0.008	0.704***	0.079
Total T (ng/dl)	0.806***	-0.100	0.993***	-0.143
DHEAS (μg/dl)	-0.691***	0.154	-0.940***	-0.201
17β-estradiol (pmol/l)	-0.005	-0.094	-0.068	0.097
Progesterone (nmol/l)	-0.188	-0.316	0.097	-0.158
SHBG (nmol/l)	-0.552*	-0.001	-0.476*	0.166

*$p<0.001$; **$p<0.0001$; ***$p<0.00001$. WHR: waist-to-hip ratio; WTR: waist-to-thigh ratio; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue.

lations between serum SHBG level and the area of visceral fat and WHR (r=-0.476, $p<0.001$; r=-0.552, $p<0.001$, respectively).
No significant relationships were found between the other indices of body fat distribution (WTR, SAT) and serum sex hormone levels.
A statistically significant inverse correlation was found between serum SHBG level and plasma insulin sum (r=-0.749, $p<0.00001$) (Fig. 1).

DISCUSSION

We can draw two main conclusions from our study. Firstly, in massively obese adolescent girls, the WHR seems to be a good indicator for the accumulation of VAT, and abdominal obesity, rather than adiposity *per se*, appears to be related to biochemical complications. However, as in adults,

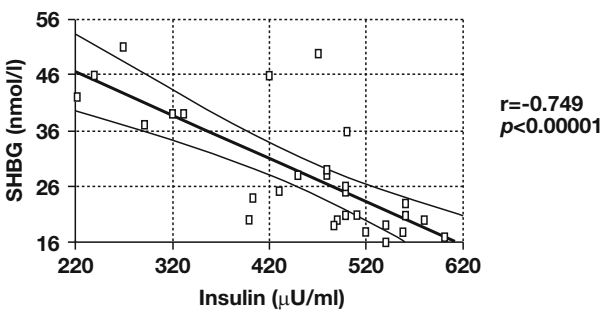

Fig. 1 - Relationship between serum insulin levels and SHBG values.

the relationships between VAT and anthopometry in childhood and adolescence are difficult to assess. Secondly, increased upper body adiposity and, in particular, the intra-abdominal fat area are associated with increased insulin and decreased SHBG levels in massively obese adolescent girls.

Anthropometric indices and MRI measures

In our massively obese girls, the WHR was the best single anthropometric measurement associated with area of VAT (as determined by MRI). This result contrasts with the findings of other authors. Goran *et al.* (21) established that there was no significant correlation between VAT and WHR in young children; de Ridder *et al.* (16) reported that conventional anthropometric measurements were not predictive of the amount of intra-abdominal fat in normal weight girls; in a study, Brambilla *et al.* (18) failed to find any significant relationship between WHR and area of VAT (as determined by MRI). One possible explanation for these discrepancies is the large variations in sex ratio, age, pubertal stage and adiposity of the subjects in these various studies. These characteristics may have affected the degree of association between WHR and MRI measurements. In fact, an important feature of our study was the homogeneity of the subjects with regard to age, pubertal stage and adiposity; moreover, each girl was massively obese. Different results would most likely be obtained in a population sample of different lower body weight, also perhaps in boys. This observation further emphasizes the importance of ensuring sample homogeneity when

studying the association between anthropometry and MRI-derived measurements.
The area of VAT was not significantly correlated with BMI, percentage of obesity and sum of four skinfold thicknesses. Therefore, simple evaluation of body weight excess *per se*, as well as BMI and skinfold thickness does not appear to provide an accurate assessment of the visceral fat depot. The BMI is not sensitive to body fat distribution; in fact, the two components of this index (height and body weight) suggest its possible limitations in predicting the amount of visceral fat in severe obesity: firstly, independence from height is a desirable characteristic in any indicator of obesity; secondly, BMI is influenced to almost equal extents by the lean and the fat tissue compartments of the body.
As regards skinfold tickness, the existing calipers were insufficiently large to accommodate some skinfolds in our massively obese girls; this methodologic consideration probably limited the usefulness of these anthropometric measurements as indicators of the visceral fat depot in our study. This was particularly true for the subscapular and triceps folds. Accordingly, the only significant correlation was between the suprailiac skinfold and SAT.

Fat distribution, metabolic and hormonal parameters

Other interesting results of this study were the relationships between the anthropometric indices of body fat distribution, MRI measures and metabolic and hormonal parameters. WHR and amount of VAT were correlated with the insulin sum during OGTT, but not with the glucose sum. No significant correlation was found between the insulin sum and other anthropometric indices. These results are in agreement with those of Pedersen *et al.* (22), who reported that metabolic disturbance was linked to the WHR but not to indices of overweight or adiposity.
Moreover, serum SHBG levels were negatively correlated with both WHR and VAT; an inverse correlation was also found between serum SHBG levels and the insulin sum. The negative correlation between serum SHBG with upper body adiposity has been described previously in pre- and post-menopausal women (23, 24).
Studies suggested that while overall adiposity is associated with increased pancreatic insulin secretion, increased upper body adiposity is associated with decreased hepatic clearance of insulin (25). In addition, hepatic clearance of insulin shows a positive correlation with SHBG levels (26). This association supports the observation that a low SHBG level is a predictor for the development of Type 2 diabetes mellitus (27, 28).
Our results also established that WHR and VAT were negatively correlated with DHEAS and positively correlated with total testosterone level. Several studies have been performed to investigate the role of androgens in the metabolic disturbances that characterize abdominal obesity in women, but the relationship between circulating androgen levels and adiposity is still controversial in obese women. Using CT, several authors (29, 30) reported a negative independent correlation between serum levels of total testosterone and the amount of VAT. In contrast, using MRI, Leenen *et al.* (31) recently reported a weak positive association between serum testosterone level and VAT. Moreover, serum DHEAS level was shown to be inversely related to BMI in a group of patients, some of whom had a very high BMI (11). In other studies no statistical associations were found between DHEAS levels and body fat distribution (30, 32). An explanation for these apparent discrepancies may lie in the different methods used to evaluate body fat distribution or visceral fat accumulation.
DHEAS improves insulin sensitivity and lipoprotein profile (33-35) and it has a protective role in metabolic and cardiovascular diseases. In fact, recent evidence suggests that DHEAS exerts multiple anti-atherogenic effects and that hyperinsulinemia may reduce serum DHEAS levels by decreasing its production and enhancing its clearance. The pattern of decreased SHBG with decreased DHEAS levels is closely linked to the accumulation of visceral fat and plasma insulin levels and represents an a early general risk factor for the development of metabolic and cardiovascular diseases in this population, as previously described for obese adult women.
The strong negative correlation between total testosterone and VAT shows that DHEAS may have opposite effects to those of testosterone. This result is in contrast to the findings of De Pergola *et al.* (36), who demonstrated an inverse correlation between testosterone and VAT (as evaluated by ultrasonography) in pre-menopausal obese women.
All data concerning 17β-estradiol and progesterone seem to exclude an important role of these hormones in altering body fat accumulation and distribution in obese adolescent girls.
In conclusion, moderate weight loss may reduce the amount of visceral adipose tissue and plasma insulin levels and result in a concomitant increase in SHBG and DHEAS levels; therefore, it may well be a worthwhile therapeutic goal in the treatment of massively obese adolescent girls.

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