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Impact of percent body fat on oral glucose tolerance testing: A cross-sectional study in 1512 obese children

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ABSTRACT. ***Background:*** **Although an association between insulin resistance (IR) and body adiposity has been reported in obese children, this relationship has not been studied as thoroughly as in adults.** ***Aim:*** **We evaluated the association between oral glucose tolerance testing (OGTT) and percent body fat (PBF) in a sample of 1512 obese children followed at a Pediatric Obesity Clinic.** ***Subjects and methods:*** **Six hundred and twenty-eight male and 884 female obese children aged 6 to 18 yr were consecutively enrolled into the study. OGTT was performed with administration of 1.75 g of glucose per kg of body weight (up to 75 g). PBF was estimated through bioelectrical impedance analysis (BIA) using a population-specific formula recently published by our group. Multivariable median regression was used to evaluate the association between 4 outcomes [glucose area under the curve (AUC), insulin AUC, insulin sensitivity index (ISI), and insulinogenic index (IGI)] and gender, age or pubertal status and PBF.** ***Results:*** **Median PBF was 52% (range 26 to 70%). After correction for age and gender, a 10% increase of PBF was associated with a decrease of –0.50 [95% confidence interval (CI): –0.65 to –0.35] units of ISI and an increase of 0.15 units of IGI (95%CI 0.07 to 0.24).** ***Conclusions:*** **In obese children, PBF is inversely associated with IR and directly associated to β-cell response as detected by OGTT.**
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INTRODUCTION

Insulin resistance (IR), i.e. the decreased response of tissues to insulin-mediated cellular actions, is commonly associated with obesity. However, not all obese individuals are insulin resistant and IR may occur in non-obese adults as well as in children (1). IR is determined primarily by the response of skeletal muscle to insulin, but body adiposity is estimated to be responsible for 25% of this effect in adults (1, 2).

Although an association between IR and body adiposity has been reported in obese children (3), this relationship has not been studied as thoroughly as in adults. The need of invasive and not readily available methods to obtain reliable assessments of both IR (e.g. euglycemic hyperinsulinemic clamp) and body composition [e.g. dual-energy X-ray absorptiometry (DXA)] has probably contributed to this lack of systematic evaluation. Most pediatric studies have evaluated the association of IR with adiposity using the homeostasis model assessment (HOMA) index as surrogate measure of IR and body mass index (BMI) as surrogate measure of body fat (1).

Although BMI is a predictor of cardiometabolic disease in adults and childhood BMI tracks adult BMI, it is not an accurate indicator of body composition and this is especially true in pediatric age (4). Among the methods for the assessment of body composition, DXA has gained increased popularity in recent years (5). However, DXA involves exposing the body to ionizing radiation so that indirect methods are commonly calibrated against DXA to develop prediction equations suitable for use in epidemiological studies (6). With this aim in mind, we have recently developed a prediction equation for the assessment of body composition in obese children based on bioelectrical impedance analysis (BIA) (7).

In the present study, we evaluated the association between IR and β-cell function as detected by OGTT and percent body fat (PBF) estimated from BIA in a sample of 1512 obese children followed at our Pediatric Obesity Clinic.

MATERIALS AND METHODS

Study design

Obese children and adolescents of Italian ancestry followed as inpatients at the Division of Auxology of the Istituto Auxologico Italiano (Piancavallo, Verbania, Italy) between January 2004 and January 2009 were consecutively enrolled into the study if they were 18-yr-old or younger. They were studied just before commencing an inpatient nutritional rehabilitation program at our Division. As determined by an interview performed by a dietitian, none of the children was following a diet at the time of study or was engaged in any kind of systematic physical activity. Obesity was diagnosed in the presence of a BMI≥95th percentile for gender and age using Italian reference data (8). Genetic or syndromic obesity and treatment with any drug were reasons for exclusion from the study. The study protocol was approved by the local Ethics Committee and parental consent was obtained.

Key-words: Body composition, children, dual-energy x-ray absorptiometry, obesity, oral glucose tolerance testing.

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Clinical examination

Pubertal status was classified as pre-pubertal (stage 1), early pubertal (stages 2 and 3) or late pubertal (stages 4 and 5) according to Tanner (9). Weight and stature were measured following standard procedures (10). BMI was calculated as weight (kg) / stature (m)^2. SD scores (SDS) of weight, stature and BMI were calculated using Italian reference data (8).

Laboratory evaluation

Glucose tolerance was assessed by means of OGTT with administration of 1.75 g of glucose per kg of body weight (up to 75 g) (11, 12). Glucose and insulin were measured at 0, 30, 60, 90, and 120 min during OGTT. Glucose was measured using standard laboratory methods and insulin using a chemiluminescent immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). Diabetes mellitus (DM) was defined as fasting glucose ≥126 mg/dl or 120-min OGTT glucose ≥200 mg/dl; impaired fasting glucose (IFG) as fasting glucose between 100 and 126 mg/dl; and impaired glucose tolerance (IGT) as 120-min OGTT glucose between 140 and 200 mg/dl (11, 12). The area under the curve (AUC) of insulin (IAUC) and glucose (GAUC) during OGTT was calculated using the trapezoid rule. The insulin sensitivity index (ISI) was calculated as suggested by Matsuda & De Fronzo (13) and the insulinogenic index (IGI) as suggested by Phillips et al. (14).

Body composition assessment

Whole body impedance (Z) was measured using a multifrequency impedance-meter (Human IM Plus II, DS Medica, Milan, Italy). Measurements were performed according to international guidelines (15). The impedance index (ZI) was calculated as stature (cm)^2 / Z at 50 kHz (Ω). Fat-free mass (FFM) (kg) was estimated from ZI by using a population-specific formula developed by our group in children strictly comparable to those studied here (7). Fat mass (FM) (kg) was obtained by subtracting FFM from body weight (BW) and PBF by dividing FM per BW.

Statistical analysis

Most continuous variables were not normally distributed and all are reported as 25th, 50th (median), and 75th percentiles. Categorical variables are reported as the percentage or number of subjects with the characteristic of interest. Multivariable median regression was used to evaluate the association between 4 outcomes (IAUC, GAUC, ISI, and IGI) and PBF (16). Sex (dichotomous, 0 = female, 1 = male), age (continuous, yr) or pubertal status (discrete, 5 Tanner stages), and PBF (continuous, 10% change) were used as covariates. GAUC (continuous) and IAUC (continuous) were used as covariates in the models whose outcomes were IAUC and GAUC, respectively. Multivariable fractional polynomials were used to take into account non-linear associations of the continuous predictors with the outcomes (17). Statistical significance was set to a *p*-value <0.05 and all tests were two-tailed. Statistical analysis was performed using Stata 12.0 (Stata Corp, College Station, TX, USA).

RESULTS

Six-hundred and twenty-eight male and 884 female children and adolescents aged 6 to 18 yr were consecutively enrolled into the study. Their measurements are given in Table 1 and their pubertal status is reported in Table 2.

Table 1 - *Measurements of the 1512 children.*

	Males (no.=628) P_{50} (P_{25} ; P_{75})	Females (no.=884) P_{50} (P_{25} ; P_{75})
Age (yr)	15 (13 ; 16)	15 (13 ; 17)
Weight (kg)	102.8 (86.5 ; 118.9)	91.6 (81.0 ; 103.8)
Weight (SDS)	2.92 (2.42 ; 3.43)	3.07 (2.51 ; 3.66)
Stature (m)	1.68 (1.58 ; 1.75)	1.60 (1.55 ; 1.65)
Stature (SDS)	0.25 (–0.43 ; 1.01)	0.10 (–0.56 ; 0.82)
BMI (kg/m^2)	36.2 (32.5 ; 40.4)	35.5 (32.1 ; 39.7)
BMI (SDS)	3.03 (2.57 ; 3.54)	2.92 (2.56 ; 3.31)
Body fat (kg)	50 (41 ; 62)	48 (40 ; 58)
Percent body fat (%)	50 (46 ; 55)	53 (49 ; 57)
GAUC	13950 (13020 ; 15338)	13605 (12473 ; 14940)
IAUC	8156 (6039 ; 11732)	6868 (5051 ; 9773)
ISI	3.7 (2.7 ; 5.1)	4.4 (3.1 ; 5.9)
IGI*	1.4 (0.9 ; 2.1)	1.1 (0.8 ; 1.7)

*7 negative values were omitted. P_{50}: 50th percentile; P_{25}: 25th percentile; P_{75}: 75th percentile; SDS: SD score; BMI: body mass index; GAUC: area under the curve of glucose during oral glucose tolerance testing; IAUC: area under the curve of insulin during oral glucose tolerance testing; ISI: insulin sensitivity index; IGI: insulinogenic index.

Table 2 - *Pubertal status of the 1512 children.*

	Males		Females	
Pubertal stage (Tanner)	No.	%	No.	%
1	64	7.2	80	12.7
2	30	3.4	99	15.8
3	62	7.0	142	22.6
4	193	21.8	126	20.1
5	535	60.5	181	28.8
Total	884	100.0	628	100.0

Table 3 - *Glucose status of the 1512 children as detected by fasting glucose and oral glucose tolerance testing.*

	No.	%
Fasting glucose		
Normal glucose status	1508	99.7
Impaired fasting glucose	4	0.3
Diabetes mellitus	0	0.0
Total	1512	100.0
Oral glucose tolerance testing		
Normal glucose status	1299	85.9
Impaired glucose tolerance	206	13.6
Diabetes mellitus	7	0.5
Total	1512	100.0

The frequency of altered glucose status is reported in Table 3. The difference between fasting glucose and OGTT in detecting altered glucose status is striking as there were 206 cases of IGT *vs* 4 cases of IFG and 7 cases *vs* 0 cases of diabetes. However, among the 4 children with IFG, 3 had also IGT.

Table 4 reports the median regression models used to evaluate the association between the 4 outcomes of in-

Table 4 - *Association between the outcomes of interest and percent body fat (median regression analysis).*

	Model 1A GAUC	Model 1B GAUC	Model 2A IAUC	Model 2B IAUC	Model 3A ISI	Model 3B ISI	Model 4A IGI	Model 4B IGI
IAUC	2931.28[c] [2345.91;3516.65]	2972.38[c] [2399.64;3545.12]						
Male gender	247.89 [–17.77;513.56]	220.79 [–56.33;497.92]	1120.46[c] [560.72;1680.21]	1144.95[c] [655.83;1634.06]	–0.71[c] [–0.90;–0.52]	–0.75[c] [–1.02;–0.49]	0.24[c] [0.14;0.34]	0.21[c] [0.11;0.31]
Age_term1	36.26 [–18.59;91.11]		16.13 [–100.79;133.06]		57.14[c] [39.64;74.64]		–0.02 [–0.04;0.01]	
PBF/10	–102.19 [–310.54;106.16]	–97.73 [–299.38;103.92]	435.43 [–8.03;878.89]	428.16[a] [69.15;787.16]	–0.50[c] [–0.65;–0.35]	–0.53[c] [–0.72;–0.33]	0.15[c] [0.07;0.24]	0.15[c] [0.07;0.22]
Tanner stage 2[d]	—	–30.78 [–612.19;550.62]	—	927.92 [–107.52;1963.35]		–0.03 [–0.59;0.53]		0.13 [–0.08;0.33]
Tanner stage 3[d]	—	372.05 [–150.69;894.79]	—	470.90 [–461.62;1403.42]		–0.62[a] [–1.13;–0.12]		0.11 [–0.08;0.29]
Tanner stage 4[d]	—	236.99 [–243.52;717.50]	—	209.32 [–647.86;1066.50]		–0.32 [–0.79;0.15]		–0.06 [–0.23;0.11]
Tanner stage 5[d]	—	120.83 [–324.16;565.82]	—	606.26 [–186.65;1399.16]		–0.27 [–0.70;0.16]		–0.01 [–0.17;0.15]
GAUC	—	—	0.72[c] [0.58;0.86]	0.72[c] [0.61;0.84]				
Age_term2[e]	—	—			24.76[c] [17.05;32.47]			
Intercept	13800.85[c] [13631.06;13970.64]	13668.00[c] [13240.34;14095.65]	7151.10[c] [6796.19;7506.01]	6646.72[c] [5889.64;7403.79]	4.26[c] [4.13;4.39]	4.73[c] [4.32;5.15]	1.13[c] [1.07;1.20]	1.13[c] [0.98;1.29]
N	1512	1512	1512	1512	1512	1512	1505[f]	1505[f]

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$; [d]vs Tanner stage 1; [e]Degree 2 fractional polynomial (–0.5;0). [f]7 subjects with negative values were omitted. Values are regression coefficients and 95% confidence intervals (in brackets). GAUC: area under the curve of glucose during oral glucose tolerance testing; IAUC: area under the curve of insulin during oral glucose tolerance testing; ISI: insulin sensitivity index; IGI: insulinogenic index; PBF: percent body fat.

terest (GAUC, IAUC, ISI, and IGI) and gender, age or pubertal status, PBF, and IAUC or GAUC depending on the model.

GAUC was not associated with PBF in any model and IAUC was associated with PBF only in the puberty model (Models 1A, 1B, 2A, and 2B). However, the effect sizes were very similar for the age and puberty models (435 *vs* 428 units of IAUC) and the difference was explained by narrower confidence intervals (CI) associated with the puberty model. ISI was negatively associated with PBF and IGI was positively associated with PBF in all models. In detail, a 10% increase of PBF was associated with a decrease of –0.50 (95%CI: –0.65 to –0.35, Model 3A) units of ISI and with an increase of 0.15 (95%CI 0.07 to 0.24, Model 4A) units of IGI. Other variables being equal, males had higher values of IAUC, lower values of ISI and higher values of IGI. Neither age nor pubertal status were associated with GAUC, IAUC or IGI. However, age was associated with PBF in a non-linear way (Model 3B). As expected (1), children in Tanner stage 3 had higher IR as detected by lower values of ISI [when BMI was replaced for PBF (data not shown), a 5 kg increase of BMI was associated to a decrease of ISI of the same entity of that associated to an increase of 10% of PBF but to an increase of IGI that was 50% lower].

DISCUSSION

Besides being an harbinger of DM, IR is associated with a clustering of cardiometabolic risk factors in both children and adults (1). Even if there is a need for more long-term population studies (18), there is increasing evidence that insulin may be an independent predictor of cardiovascular mortality (1, 19). IR is determined primarily by the response of the skeletal muscle to insulin and body adiposity is responsible for 25% of this effect in adults (1, 2). Much less data are available in children (1, 3), partly because of the invasiveness of the reference methods used to assess IR and body composition.

In the present study, we evaluated the relationship between body adiposity estimated by BIA and OGTT in a sample of 1512 obese children followed at a Pediatric Obesity Clinic. We found that PBF was inversely associated with ISI and directly associated with IGI after correction for age or puberty and gender. On the other hand, there was no association between GAUC and PBF and an association between IAUC and PBF was apparent only when puberty was modeled instead of age. Even if age and pubertal status were highly correlated (Spearman's ρ=0.79, bootstrapped 95%CI =0.77 to 0.81), this confirms that there is some advantage in modeling puberty instead of age as IR is concerned (1).

In agreement with other studies, we found a greater number of subjects with altered glucose status detected by OGTT as compared to fasting glucose (20). Adult studies have shown that IGT is a risk factor not only for DM but also for cardiovascular disease (21). Although the prognostic significance of IGT in children is much less clear than in adults (1), we believe that the information provided by OGTT in our children is clinically important, especially in view of the fact that they were all obese and thus at higher risk of perturbations of glucose homeostasis.

The limitations of this study should be kept in mind. First,

we used a surrogate measure of IR (OGTT) which, albeit commonly accepted, has to undergo further validation studies (1, 22). A possibly more critical issue is the relatively low reproducibility of OGTT (1, 20). Second, we used an indirect method (BIA) to assess body composition. However, we were careful to employ a population-specific formula internally developed by our group and did inference only at the population level (6, 7). Third, all our children were Europids and of Italian ancestry and because of that we could not consider the effect of the ethnic group, which is recognized as an important predictor of IR (1). Fourth, longitudinal studies of body fat changes are expected to shed more light on the relationship between body composition and IR as compared to the cross-sectional design employed here.

In light of the available knowledge, the inverse association of PBF with ISI and the direct association of PBF with IGI that we found in our obese children can be interpreted to mean that the magnitude of the insulin response to OGTT minimizes the effect of increasing adiposity on glucose response (23). Beta-cell function as detected by IGI was in fact preserved and able to compensate with an increase of insulin to maintain glucose concentrations within normal ranges.

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