Insulin resistance and whole body energy homeostasis in obese adolescents with fatty liver disease

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nonalcoholic fatty liver disease; fat oxidation; 1H magnetic resonance spectroscopy; indirect calorimetry; oral glucose tolerance test.

CHILDSHOOD OBESITY is the most important nutritional disorder affecting US children (5), and its prevalence is increasing worldwide. In adolescents, as in adults, obesity is associated with abnormal glucose and lipid homeostasis. Intramyocellular lipid (IMCL) stores may play a role in modulating insulin sensitivity (24), and this relationship was also found in obese adolescents (30). Abnormal glucose tolerance in these youngsters is linked with altered partitioning of fat in the skeletal muscle and visceral fat with a strong association with insulin resistance, suggesting that these changes occur early in the natural history of obesity (36). Besides the abnormal IMCL accumulation, increased intrahepatic fat (IHF) accumulation has also been recognized as a typical finding in obese adolescents (16, 27). In adults, nonalcoholic fatty liver disease (NAFLD) was reported in association with impairment of the insulin-stimulated glucose metabolism, the suppression of endogenous glucose production, and the whole body lipolysis (15). Recently, the IHF content has been assessed as a continuous variable by means of 1H magnetic resonance spectroscopy (MRS) and was reported to be associated with hepatic insulin resistance in nonobese individuals (29). Because the pathogenesis and the potential metabolic effects of the ectopic fat accumulation within the liver are still obscure and have never been investigated previously in obese adolescents, this study was undertaken to explore three relevant issues: 1) the magnitude of hepatic fat content in severely obese adolescents, 2) the impact of the IHF content on metabolic features and on glucose homeostasis and insulin sensitivity and in parallel on insulin secretion, and 3) whether NAFLD might be related to an abnormal whole body energy metabolism.

MATERIALS AND METHODS

Subjects

The study participants were 54 severely (BMI >99th percentile for their age and sex) obese adolescents recruited from a cohort drawn from the Pediatric Metabolic Disease Clinic at Istituto Scientifico H San Raffaele. Age ranged from 11 to 18 yr, and thyroid and adrenocortical dysfunctions were excluded. The percentile of BMI was based on the curves for Italian children and adolescents (3). Pubertal stage of development was assessed by physical examination according to the criteria of the Tanner stage for breast and genital development in girls and boys; 13 were prepubertal and 41 were pubertal. All subjects were in good health and were taking no medications. History of hepatic disease (hepatitis B and hepatitis C) was excluded in all subjects. Their body weight was stable for ≥3 mo. Study subjects were segregated into two groups on the basis of the IHF content measured by means of 1H-MRS. One group had normal IHF content (<5% wet weight), and the other one a higher than normal IHF.

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content (>5% wet weight). Their anthropometric features are summarized in Table 1, and their laboratory characteristics are summarized in Table 2. Informed consent was obtained from all parents, and voluntary assent was obtained from the adolescents after explanation of purposes and nature of the study. The protocol was approved by the Ethics Committee of the Istituto Scientifico H San Raffaele.

Experimental Protocol

All study subjects were admitted for 3 days within the Division of Pediatrics of our Institute. On the basis of the possible effects of dietary macronutrient intake on substrate oxidation rates (31), the parents of these obese adolescents were instructed to administer a weight-maintaining diet containing at $\geq 250$ g of carbohydrates per day for 7 days before the admission. During the inpatient period, they received a standardized (50% carbohydrate, 20% protein, and 30% lipid) weight-maintaining diet and abstained from exercise activity. They underwent 1) a 3-h oral glucose tolerance test (OGTT) performed according to the criteria suggested by the American Diabetes Association (ADA) to establish glucose tolerance and to obtain parameters of insulin sensitivity and secretion, 2) indirect calorimetry to assess the resting (REE) and postglucose challenge energy expenditure and glucose and lipid oxidation, 3) dual-energy X-ray absorptiometry (DEXA) to assess body composition and 4) $^1$H MRS to assess the IHF content.

**Oral glucose tolerance test.** The OGTT was performed at 8:00–8:30 AM according to ADA recommendations (20). A teflon catheter was inserted into an antecubital vein for blood sampling; blood was obtained for the assessment of glucose, insulin, free fatty acids (FFAs), and C-peptide concentrations in the basal period and after the oral glucose load (75 g) at 30-min intervals for 180 min. Samples were also collected for the assessment of fasting plasma or serum concentration of total cholesterol, LDL cholesterol, triglycerides, creatinine, glycosylated hemoglobin (Hb A1c), aminotransferases, $\gamma$-glutamyl transpeptidase ($\gamma$-GT), thyroid-stimulating hormone (TSH), leptin, resistin, and adiponectin. A 24-h urinary collection period was performed to assess nitrogen and free cortisol excretion rate.

<table>
<thead>
<tr>
<th>IHF content</th>
<th>&lt;5% wet weight</th>
<th>&gt;5% wet weight</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (F/M)</td>
<td>(range: 0.98–4.98)</td>
<td>(range: 5.99–26.99)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age, yr</td>
<td>13.2±2.1</td>
<td>13.6±2.5</td>
<td>0.56</td>
</tr>
<tr>
<td>Height, cm</td>
<td>157±11</td>
<td>161±12</td>
<td>0.27</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81±16</td>
<td>96±26</td>
<td>0.048</td>
</tr>
<tr>
<td>BMI</td>
<td>32.7±4.6</td>
<td>36.2±5.8</td>
<td>0.054</td>
</tr>
<tr>
<td>Body fat, kg</td>
<td>53.5±9.7</td>
<td>43.3±13.7</td>
<td>0.067</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>43.3±6.1</td>
<td>44.9±7.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>41.2±10.6</td>
<td>46.3±13.2</td>
<td>0.22</td>
</tr>
<tr>
<td>Arm fat mass, kg</td>
<td>3.3±1.3</td>
<td>3.8±1.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Leg fat mass, kg</td>
<td>14.8±4.8</td>
<td>17.6±6.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Trunk fat, kg</td>
<td>15.8±4.6</td>
<td>20.2±6.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>127±12</td>
<td>126±8</td>
<td>0.87</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>74±10</td>
<td>73±6</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Values are means ± SD; independent-samples t-test (2-tailed). Hb A1c, glycosylated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; $\gamma$-GT, $\gamma$-glutamyl transpeptidase.

**Indirect calorimetry.** Indirect calorimetry was performed continuously for 30 min during the basal period and 1, 2, and 3 h after the administration of glucose with a ventilated hood system (SensorMedics 2900 metabolic measurement cart) to measure $V_O_2$ and $V_CO_2$. The mean coefficient of variation (CV) within the session for $O_2$ and $CO_2$ measurements was 5%.

**DEXA.** DEXA was performed with a Lunar-DPX-IQ scanner (Lunar, Madison, WI), as previously described (24). Three-compartment analysis was performed in the arms, trunk, and legs as previously described (24). Fat content is expressed as kilograms of fat mass and as percentage of soft tissues. For technical reasons, DEXA was performed in 45 study subjects (31 with IHF content <5% wet weight and 14 with IHF content >5% wet weight).

**Hepatic $^1$H-MRS.** $^1$H-MRS was performed at rest and with patients in the supine position with the use of a 1.5-T whole body scanner (Gyrosan Intera Master 1.5 MR System; Philips Medical Systems, Best, The Netherlands), using a conventional circular superficial coil (C1-coil), as previously described (22). Briefly, coronal and transverse imaging of the liver were obtained in all patients. Then, T1 in-phase and out-of-phase images were obtained to look for a potential loss of signal on out-of-phase images, indicating the presence of IHF accumulation. Then, an 8-cm$^3$ spectroscopic volume of interest was positioned within the right lobe, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. The voxel shimming was executed to optimize the homogeneity of the magnetic field within the specific volume of interest. Two $^1$H spectra were collected from the hepatic parenchyma in the same prescanning conditions using a PRESS pulse sequence (interpulse delay TR = 3,000 ms, spin echo time TE = 25 ms, 1,024 data points over a 1,000-Hz spectral width and 64 acquisitions) with and without suppression of the water signal. Areas of resonance from protons of water (4.8 ppm) and methylene groups in fatty acid chains of the hepatic triglycerides (1.4 ppm) were obtained with a time domain, nonlinear fitting routine using commercial software (VARPRO-MRUI).

**Analytical Determinations.**

Glucose concentration was measured with standard glucose oxidase method on a glucose analyzer (Beckman Coulter, Fullerton, CA). FFAs, triglycerides, total cholesterol, and HDL cholesterol were measured as previously described (22). LDL cholesterol was calculated...
lated using the Friedewald formula. Plasma levels of insulin (sensitivity 12 pmol/l; intra- and interassay CV <3.1 and 6% respectively; <1% cross-reactivity with C-peptide and proinsulin) and lep1tn (sensitivity 0.5 ng/ml; intra- and interassay CV <5 and 9% respectively) were measured with RIA (Linco Research, St. Charles, MO) according to the manufacturer’s assay protocols. Plasma C-peptide was measured using an RIA kit (Diagnostic Products, Los Angeles, CA). Serum resistin was measured by ELISA kit (BioVendor Laboratory Medicine, Brno, Czech Republic). The sensitivity of the assay was 0.2 ng/ml of sample. The intra-assay CV was <3.5% and the interassay CV <7%. Serum adiponectin was measured by ELISA kit (B-Bridge International, Sunnyvale, CA) with a sensitivity of 25 pg/ml. The intra-assay CV was <3.7% and the interassay CV <6%. Serum urea nitrogen, creatinine, aminotransferases, and γ-GT were measured using an enzymatic method on a Hitachi 747 (22). Hb A1c was measured by HPLC, TSH by immunofluorimetric method, and urinary free cortisol by immunoenzymatic method. Blood pressure was monitored for a 2-h period during mid-morning with 8–12 measures per study subject; means of all values for each subject were used.

Calculations

Insulin sensitivity and secretion. Insulin resistance was determined by two methods, the updated computer model homeostasis model assessment (HOMA)2 indexes (34) available from www.OCDerm.ox.ac.uk and the whole body insulin sensitivity index (WBISI) that was calculated using the modified formula of Matsuda and DeFronzo (17) and based on the assessments of plasma glucose and insulin in all seven of the blood samples collected during the 3-h OGTT:

\[
\text{WBISI (OGTT)} = \frac{10,000}{\gamma(G_0 \times I_0) \times (\text{mean } G_{30-180 \text{ min}}) \times (\text{mean } I_{0-180 \text{ min}})}
\]

where \( I_0 \) was the fasting insulin concentration (µU/ml), \( G_0 \) was the fasting glucose concentration (mg/dl), and \( G_{30-180 \text{ min}} \) and \( I_{0-180 \text{ min}} \) were the mean concentrations during the 3-h OGTT of glucose and insulin, respectively. This index was shown to correlate strongly with \( M \) values derived from the hyperinsulinemic euglycemic clamp in obese children by Yeckel et al. (39). To characterize the β-cell function, we calculated an index, \( \Phi (\muU/ml \text{ per mg/dl}) \), reflecting posthepatic insulin delivery rate; \( \Phi \) was calculated as the ratio between the area under the curve (AUC) of incremental insulin concentration and the AUC of incremental glucose concentration. The early insulin response, \( \Phi_{10} \) or insulogenic index, was calculated as the ratio between the AUC of incremental insulin concentration and AUC of incremental glucose concentration during the first 30 min of the oral glucose challenge, as was recently suggested by Jensen et al. (7) and reported to correlate well with the early insulin response obtained during a hyperglycemic clamp in children and adolescents (39).

Energy homeostasis. REE was calculated by Weir’s standard equation (35) from the \( O_2 \) and \( CO_2 \) production rates measured by indirect calorimetry (excluding the first 10 min of data acquisition) and from the urinary nitrogen excretion, as previously described (23).

IFH content. The percentage of IFH was calculated by dividing the integral of the methylene groups in fatty acid chains of the hepatic triglycerides by the sum of methylene groups and water \( 100 \). Signal decay due to spin-spin relaxation was calculated using mean \( T_2 \) relaxation times for water and fat of 50 and 60 ms, respectively, and the exponential relaxation equation \( I_m = I_0 \exp (-T_2/T_2) \), where \( I_m \) is the measured signal intensity obtained at the selected echo time \( T_e \), \( I_0 \) is the signal intensity immediately after the 90° pulse, and \( T_2 \) is the spin-spin relaxation time. Average \( T_2 \) relaxation times were used for these calculations (14, 33), as previously performed (32). These values represent a relative quantity of water and hepatic triglyceride fatty acid chain protons in the volume of interest. To convert these values to absolute concentrations expressed as percentage of fat by weight of volume, we used equations validated by Longo et al. (13).

Traditionally, liver fat content >50 mg/g (5% by wet weight and equivalent to 6.5% of ratio methylene/methylene + water \( \times 100 \) in our setting) is diagnostic of hepatic steatosis (6, 14), and study subjects were segregated in a group of individuals with normal IFH content (<5% wet weight) and another group with a higher than normal IFH content (>5% wet weight).

Statistical Analysis

Data in text, tables, and figures are means ± SD. Analyses were performed using the SPSS software (version 10.0; SPSS, Chicago, IL). Comparison between groups was performed using a two-tailed independent samples t-test, and a \( P \) value <0.05 was considered to be statistically significant. When parameters showed a skewed distribution (Kolmogorov-Smirnov test of normality), they were log transformed before the analysis [aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-GT, TSH, diastolic blood pressure, HOMA2-%S, and WBISI]. Comparison of the proportions of individuals with IFH content >5% wet weight between gender and pubertal stage was performed using Pearson’s \( \chi^2 \) test. Two-tailed Pearson’s correlation was performed to establish partial correlation coefficients between variables. The general linear model procedure for repeated measures was performed to detect significant effect of group by time interaction on plasma glucose, insulin, and FFA concentrations as well as on the whole body energy expenditure and on the respiratory quotient (RQ) during the OGTT. Stepwise regression analysis was performed (using \( F \) ratio-to-enter of 4 and \( F \) ratio-to-remove of 3.996) to assess the relevance of the association between variables. A prior power calculation analysis using an optimal allocation strategy indicated that 17 obese adolescents without excessive IFH content and 13 with excessive IFH content were required to provide a power of 87% to detect a 10% difference in RQ between groups, and 19 obese adolescents without excessive IFH content and 11 with excessive IFH content were required to provide a power of 86% to detect a 20% difference in WBISI between groups.

RESULTS

Intrahepatic Fat Content

IFH content ranged between 0.98 and 26.99% wet weight, and 16 (30%) individuals had an IFH content >5% wet weight and were defined as obese individuals with NAFLD; the remaining 38 subjects were defined as obese individuals with normal IFH content. We decided to use this dichotomous approach of segregating study subjects, because when we attempted to establish the effect of the IFH content as a continuous variable, segregating study subjects by tertiles of IFH content, we realized that the features of tertiles 1 and 2 were remarkably similar, and all individuals falling in these tertiles had a normal IFH content compared with those within tertile 3, all with IFH content >5% wet weight. IFH content was not different between sex (4.1 ± 3.9 vs. 6.6 ± 6.1% wet weight in females and males respectively, \( P = 0.078 \)). Also, the proportion of individuals with IFH content >5% wet weight was not different (21% in females and 37% in males; \( \chi^2 = 1.60, P = 0.20 \)). IFH content was not different between prepubertal and pubertal subjects (3.6 ± 3.4 vs. 6.0 ± 5.7% wet weight, \( P = 0.17 \)). Also, the proportion of individuals with IFH content >5% wet weight was not different (15% in prepubertal and 34% in pubertal, \( \chi^2 = 1.67, P = 0.19 \)).

Anthropometric and Biochemical Characteristics of Study Groups

The anthropometric features of these two groups are summarized in Table 1. Age, gender, pubertal stage (\( P = 0.57 \),
Table 3. Plasma glucose, insulin, and C-peptide, in the fasting state and during the OGTT, and fasting- and OGTT-derived surrogate indexes of insulin sensitivity and insulin secretion in obese children and adolescents with or without excessive hepatic fat accumulation

<table>
<thead>
<tr>
<th>IHF Content</th>
<th>&lt;5% wet weight</th>
<th>&gt;5% wet weight</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>4.61±0.50</td>
<td>4.94±0.44</td>
<td>0.014</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/l</td>
<td>126±66</td>
<td>138±36</td>
<td>0.29</td>
</tr>
<tr>
<td>Fasting plasma C-peptide, nmol/l</td>
<td>0.83±0.33</td>
<td>0.87±0.26</td>
<td>0.96</td>
</tr>
<tr>
<td>120-min Glucose, mmol/l</td>
<td>5.83±0.94</td>
<td>6.05±0.66</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean glucose, mmol/l</td>
<td>5.77±0.78</td>
<td>6.05±0.61</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean insulin, pmol/l</td>
<td>432±234</td>
<td>456±174</td>
<td>0.64</td>
</tr>
<tr>
<td>Φ</td>
<td>2.9±2.5</td>
<td>3.8±3.0</td>
<td>0.36</td>
</tr>
<tr>
<td>Φ30</td>
<td>1.9±1.5</td>
<td>2.0±1.0</td>
<td>0.85</td>
</tr>
<tr>
<td>HOMA2-%B</td>
<td>220±66</td>
<td>210±45</td>
<td>0.52</td>
</tr>
<tr>
<td>HOMA2-%S</td>
<td>46±18</td>
<td>37±11</td>
<td>0.02</td>
</tr>
<tr>
<td>WBISI</td>
<td>3.4±1.3</td>
<td>2.7±1.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD; independent-samples t-test (2-tailed). OGTT, oral glucose tolerance test; HOMA2-%B, homeostasis model assessment 2 for β-cell sensitivity; HOMA2-%S, HOMA 2 for insulin sensitivity; WBISI, whole body insulin sensitivity index.

BMI (P = 0.054), and total body fat content (P = 0.062) were not different between groups, although the trunk fat content was higher (P = 0.03) in those with NAFLD. Despite this, the percentage of body fat content was not different between groups. Systolic and diastolic blood pressures were not different between study groups. The lipid profile showed that adolescents with NAFLD were characterized by reduced HDL cholesterol (Table 2). Serum triglycerides and FFA were not different between groups. Even if within the normal range, Hb A1c was higher in adolescents with NAFLD. ALT was also significantly higher (P = 0.006) in adolescents with NAFLD than those without. Creatinine, leptin, adiponectin, resistin, TSH, and urinary cortisol excretion were not different between groups (Table 2).

OGTT: Insulin Sensitivity and Insulin Secretion

Within the entire cohort, one girl (with normal IHF content) showed impaired glucose tolerance. Fasting plasma glucose was significantly higher in adolescents with NAFLD (P = 0.014; Table 3). In contrast, plasma glucose during the OGTT was not different (lack of significant group by time interaction on plasma glucose, P = 0.17), as also reflected by the average plasma glucose during the OGTT and the plasma glucose at 120 min (Table 3). Fasting plasma insulin and C-peptide were not different in the fasting state (Table 3) or during the OGTT (lack of significant group by time interaction on plasma insulin, P = 0.15). HOMA2-%S (insulin sensitivity) and the OGTT-derived index of insulin sensitivity (WBISI) were reduced in obese adolescents with NAFLD compared with the obese adolescents with normal IHF content (P < 0.05; Table 3).

Energy Metabolism

The REE was higher in obese adolescents with NAFLD than those without (8.6 ± 1.8 vs. 7.5 ± 1.5 MJ/day, P = 0.043); this difference was linked to the different body size because the REE normalized for kilograms of lean body mass (LBM) was not different between groups (187 ± 38 vs. 191 ± 34 KJ·kg LBM⁻¹·day⁻¹, P = 0.81). OGTT-induced thermogenesis was not different between groups (%increment with respect to basal: 4 ± 2 vs. 7 ± 11%, P = 0.43). The fasting RQ was higher in adolescents with NAFLD than in those with normal IHF content (0.83 ± 0.08 vs. 0.77 ± 0.05, P < 0.01; Fig. 1A), reflecting a lower fasting lipid oxidation. During the OGTT, the RQ increased less in the adolescents with NAFLD compared with the increment observed in adolescents with normal
IHF content ($\Delta RQ$: 0.02 ± 0.05 vs. 0.10 ± 0.05, $P = 0.021$; Fig. 1B).

Correlation Analysis

Pearson correlation analysis showed that the IHF content was associated with the following circulating biochemical factors: ALT ($r = 0.79$, $P = 0.0001$), $\gamma$-GT ($r = 0.60$, $P = 0.0001$), TSH ($r = 0.29$, $P = 0.01$), fasting plasma glucose ($r = 0.24$, $P = 0.019$), HDL cholesterol ($r = -0.27$, $P = 0.048$), HOMA2-%S ($r = -0.29$, $P = 0.03$), and WBISI ($r = -0.25$, $P = 0.05$). A stepwise regression analysis including all these variables selected only ALT as the most relevant circulating marker associated with the IHF content. The IHF content was also associated with the fasting RQ ($r = 0.39$, $P = 0.006$); no association was found with trunk fat content, age, BMI, arm fat content, leg fat content, total fat content, the REE, or the REE normalized by kilograms of LBM.

Effects of BMI and Trunk Fat Content on the Correlation Between IHF Content with RQ, WBISI, and HOMA2-%S

The above-described difference of the RQ and WBISI observed between groups might have been influenced by the fact that the BMI and the trunk fat content were increased in those with excessive IHF content. In fact, the significant association observed between the IHF content and HOMA2-%S or WBISI were weakened when controlling for BMI ($P > 0.05$) or trunk fat content ($P > 0.1$). In contrast, RQ maintained its significant association with IHF when controlling for both BMI ($r = 0.40$, $P = 0.004$) and trunk fat content ($r = 0.37$, $P = 0.01$).

DISCUSSION

In this study, we used $^1$H-MRS to assess quantitatively and noninvasively the IHF content in obese adolescents. We found that, in obese adolescents, 1) NAFLD is a common feature affecting 30% of our population, 2) NAFLD is associated with a rearrangement of whole body substrate oxidative disposal, and 3) NAFLD is associated with an impairment of whole body insulin sensitivity and of glucose homeostasis when compared with obese adolescents with normal IHF content.

NAFLD in children and adolescents was first reported in the early 1980s (18). Its prevalence in obese adolescents, on the basis of the unexplained elevation of circulating ALT (16, 28) or the ultrasound (1), ranged between 12 and 25%. $^1$H-MRS is a noninvasive technique that can be used in the clinical setting, and its accuracy and safety make it an ideal methodology to assess and monitor changes in the IHF content (32). This study represents the first application of this technique to obese adolescents, and the higher prevalence (30%) found in our population may reflect the enhanced sensitivity of the technique.

A second finding of the present work is the impaired insulin sensitivity found in the obese adolescents with fasting and NAFLD. This conclusion is based on the findings that the OGTT-derived index of insulin sensitivity (HOMA2-%S and WBISI) were lower (Table 3) and Hb A1c (Table 2) and fasting plasma glucose (Table 3) were higher in the obese adolescents with NAFLD than in those with normal IHF content. On the basis of data in adult populations (11, 15, 26), the impairment of insulin sensitivity appeared to be less severe than expected, and this may be explained by three factors. First, the comparison was performed between adolescents with severe obesity (all with BMI >99th percentile); hence, the group without NAFLD was also characterized by a marked insulin resistance. Second, adolescence is a condition of insulin resistance (4), and the additional effect of NAFLD would be further difficult to be quantified. Third, on the basis of the assumption that the increased IHF content may affect selectively hepatic insulin sensitivity with a minor impact on the peripheral site, as suggested in both nondiabetic (29) and diabetic adult individuals (25), we cannot exclude that the sensitivity of HOMA2-%S and WBISI were not high enough to fully reflect the difference in magnitude of insulin resistance between the two groups, even if WBISI was shown to be tightly associated with the clamp-derived index of insulin sensitivity ($r = 0.78$) (39). The display of these markers of increased risk to develop diabetes in early adulthood, taking into account that the glucose tolerance status can deteriorate more rapidly in these obese adolescents than in adults (38), suggests that NAFLD should be a therapeutic target to be monitored. We must emphasize that the obese adolescents with NAFLD had slightly higher BMI and increased trunk fat content. Abdominal obesity was indicated to be related to the development of ectopic fat accumulation within the skeletal muscle (30, 36), and therefore, it may be the primary modulator of whole body peripheral insulin resistance. It is possible that the impairment of WBISI found in the obese adolescents with NAFLD may also be mediated by the increased abdominal fat content, as suggested by the fact that the association between the IHF and WBISI was weakened when corrected for the trunk fat content.

The third important observation arising from this work is that the obese adolescents with NAFLD were characterized by a rearrangement of fasting- and oral glucose challenge-induced whole body substrate oxidative disposal. The REE and the oral glucose-induced thermogenesis were not different between groups, but the obese adolescents without NAFLD showed a lower RQ, reflecting a higher contribution of the whole body fat oxidation to the REE than the adolescents with NAFLD (Fig. 1A). We have already reported higher fasting fat oxidation in overweight/obese but metabolically fit adults when compared with lean individuals matched for insulin sensitivity (23) and in the subgroup of adult first-degree relatives of type 2 diabetic parents with normal insulin sensitivity (12) when compared with the subgroup of insulin-resistant offspring subgroup. Kelley et al. (9) also reported an association between lower muscular RQ and insulin sensitivity in obese nondiabetic women, and Weiss et al. (37) showed a trend for higher fasting lipid oxidation in “obese-insulin-sensitive” adolescents compared with the obese insulin resistant. It is interesting to note that an impaired whole body lipid oxidation was not detected in NAFLD adult patients with type 2 diabetes (11, 25) and in nonobese, nonobese adult NAFLD patients in whom the whole body lipid oxidation appeared to be directly proportional to the intrahepatic fat content (2). Also, in our own cohort of 165 nondiabetic adult individuals, the IHF content is not associated with abnormality of whole body energy homeostasis (unpublished data). Therefore, this clear-cut association seems to be a peculiar finding of the adolescence years; it is possible that this defect may be observed at this early stage of life and not in the adulthood, because by that time many compensatory mechanisms may develop and compensate the reduced fatty acid flux through the oxidative disposal. We must keep in mind...
that the impaired whole body fat oxidation in the obese adolescents with NAFLD may not necessarily reflect an hepatic defect; we need to stress the fact that indirect calorimetry reflects whole body substrate oxidation, and it does not give specific hepatic insights. On the other hand, it was reported (5a, 19) that a specific abnormality of hepatic energy metabolism may be detected in overweight and obese subjects following fructose-dependent hepatic ATP depletion. In any case, the rearrangement of the oxidative substrate disposal observed in the fasting state was not related to the increased BMI or trunk fat content and appeared to be an independent abnormality of these obese adolescents with NAFLD.

Also, the homeostatic response of energy metabolism to the oral glucose challenge was different between groups; suppression of lipid oxidation was blunted in obese adolescents with NAFLD (Fig. 1B). The persistence of a predominant fat oxidation after glucose administration recalls the concept of “metabolic inflexibility” (10), which is recognized as a marker of skeletal muscle insulin resistance (8). Therefore, the lack of metabolic switch after the oral glucose challenge in adolescents with NAFLD may be determined by a more pronounced skeletal muscle insulin resistance compared with the obese counterpart without NAFLD, even if it is possible that the liver also contributes to this abnormality.

It should be kept in mind that a limitation of the present work is the lack of a control group of normal-weight adolescents. If this limitation is not important with respect to the segregation of study subjects in the two groups, because this is defined by the absolute IHF content (>5% wet weight), WBISI, but more importantly indirect calorimetry-derived (

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