Association of nonalcoholic fatty liver disease with cardiovascular risk factors in obese adolescents: The role of interdisciplinary therapy

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KEYWORDS: Cardiovascular risk factors; Interdisciplinary intervention; Non-alcoholic fatty liver disease; Obesity

BACKGROUND: Obesity is associated with several cardiovascular risk factors, including nonalcoholic fatty liver disease (NAFLD). These risk factors can induce changes in the arteries such as an increase in the carotid intima-media thickness (cIMT), which contributes to the early development of atherosclerosis.

OBJECTIVE: To determine whether NAFLD is associated with an atherogenic lipid profile, inflammatory markers, or cIMT in obese adolescents and to compare the effects of therapeutic lifestyle changes in NAFLD and non-NAFLD groups.

METHODS: A total of 79 obese adolescents were divided into two groups: 33 NAFLD and 46 non-NAFLD. They were submitted to an interdisciplinary therapy involving diet exercise and psychological support during the course of 1 year. The cIMT and estimates of fat mass (liver, intra-abdominal, and subcutaneous) were determined ultrasonographically. Body composition, glucose, lipid profile, and adipokines were analyzed before and after the therapy.

RESULTS: At baseline, only in the NAFLD group was the homeostasis model assessment of insulin resistance positively correlated with cIMT and triglyceride/high-density lipoprotein cholesterol ratio. Therapy was associated with an increase in adiponectin concentrations and reduced visceral fat, cIMT, leptin, and plasminogen activator inhibitor-1 concentrations, as well as the ratios of total cholesterol/
Obesity is associated with several traditional and emergent cardiovascular risk factors, including nonalcoholic fatty liver disease (NAFLD), insulin resistance, and an atherogenic lipid profile, which is shown by increasing lipoprotein ratios such as total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C), low-density lipoprotein cholesterol/high-density lipoprotein cholesterol (LDL-C/HDL-C), and, more recently, triglycerides/high-density lipoprotein cholesterol (TG/HDL-C).1–5 These risk factors in early life can induce changes in the arteries that contribute to the development of atherosclerosis in adulthood.6

A subclinical measure of early atherosclerosis is an increase in the carotid intima-media thickness (cIMT)7 as assessed by high-resolution ultrasound (US). Previous research by our group showed that a change in the homeostasis model assessment of insulin resistance (HOMA-IR) index was an independent predictor of a reduction in the cIMT in obese adolescents.8 Insulin resistance appears to play the key role in the genesis of NAFLD, suggesting a possible interplay among insulin resistance, atherosclerosis and NAFLD.8–10

NAFLD and the severity of liver injury and inflammation have been reported to be strongly associated with endothelial dysfunction, increased atherogenic lipid profile, and early atherosclerosis in children.2,11 Indeed, the low-grade increase in markers of inflammation commonly present in obesity is also typically linked to NAFLD. Studies in animals suggest that NAFLD may represent a potential mediator of the systemic inflammation.12

Previous research with therapeutic interventions successfully reducing NAFLD has been associated with changes in the predictive factors of metabolic syndrome, inflammatory markers, and hepatic transaminases.13,14 Our group has previously reported that in obese adolescents, those with NAFLD had significantly greater values for their anthropometric measures, insulin resistance, and metabolic variables. After 1 year of interdisciplinary intervention, these parameters were significantly improved in the patients with NAFLD. However, the patients with NAFLD continued to have significantly greater values of body weight, visceral fat, insulin concentration, HOMA-IR, TG, alanine aminotransferase (ALT), and γ-glutamyl transferase (GGT) compared with the non-NAFLD patients.15

On the basis of these data, we can hypothesize that the NAFLD is associated with cardiovascular risk factors and it could impair the improvement in the insulin resistance, atherogenic lipid profile, and the inflammatory and subclinical markers of atherosclerosis after an interdisciplinary weight-loss therapy.

Thus, the aim of this study was to verify whether NAFLD is associated with atherogenic lipid profile, inflammatory markers, and cIMT in obese adolescents with and without NAFLD and to compare the effects of producing a loss in fat mass in both groups on a measure of early atherosclerosis.

Materials and methods

Study subjects

A total of 131 postpubescent obese adolescents were recruited for interdisciplinary therapy that lasted for 1 year. At the beginning of the year, the project was published in newspapers and magazines from São Paulo to recruit adolescents. The inclusion criteria for the postpubertal stage were Tanner stage five, between 15 and 19 years of age, and obese (ie, body mass index [BMI] ≥95th percentile, according to the Centers for Disease Control and Prevention) but healthy enough to perform physical activities and have the desire and availability to participate in the program for 1 year.16,17 Individuals were excluded if they had an identified genetic disease, chronic alcohol consumption, previous use of drugs, such as glucocorticoids and psychotropics, or were pregnant. Of the 131 participants, we excluded those who did not complete therapy and evaluation at 1 year. Reasons for failure to complete included having found work, changes in school hours, lack of motivation, and lack of money for transportation. A total of 79 obese adolescents (33 NAFLD and 46 non-NAFLD) were evaluated in this study.

The ultrasonic definition of fatty liver was based on previously reported diagnostic criteria, and the detected liver steatosis was classified as grade I (liver attenuation slightly less than spleen), grade II (more pronounced difference between the liver and spleen and intrahepatic vessels not observed or with a slightly higher attenuation than liver), or grade III (markedly reduced liver attenuation with a sharp contrast between the liver and intrahepatic vessels).18,19 In the present study, the NAFLD group presented with some liver steatosis diagnosed by US, and all
of the patients presented with normal alanine aminotransferase levels, which was diagnosed as simple steatosis.

Informed parental consent and the adolescents’ assent to participate as volunteers in the weight-loss therapy were obtained. This study was conducted in accordance with the principles of the Helsinki Declaration, formally approved by the Ethics Committee of the Federal University of São Paulo (Number: 0135/04), and registered at clinicaltrials.gov (NCT01358773). All the stages of the volunteers’ recruitment and treatment are illustrated in Figure 1.

**Anthropometric measurements and body composition**

Volunteers were weighed while wearing light clothing and barefoot on a Filizola scale to the nearest 0.1 kg. Height was measured to the nearest 0.5 cm with a wall-mounted stadiometer (Sanny, model ES 2030; São Paulo, SP - Brazil). BMI was calculated as body weight divided by height squared (wt/ht²). Body composition was measured by air displacement plethysmography in a BODPOD body composition system (version 1.69; Life Measurement Instruments, Concord, CA).

**Measurements of visceral and subcutaneous fat**

US measurements of the visceral and subcutaneous fat were taken. All abdominal ultrasonography procedures were performed by the same blinded diagnostic imaging specialist, who used a 3.5-MHz multifrequency transducer (broad band) before and after the intervention.

US-determined subcutaneous fat was defined as the distance between the skin and external face of the rectus abdominis muscle, and visceral fat was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta. The intraexamination coefficient of variation for US was 0.8%.

**Measurements of cIMT**

Using high-resolution US equipment (Logic 5 and Logic 7, General Electric) with a 7–14 MHz linear array transducer, the same experienced radiologist who was blinded to the participants’ laboratory values and risk factor levels before and after intervention measured cIMT. The intraexamination coefficient of variation for the cIMT was 4.36%. Patients were examined while in the supine position with their neck in hyperextension. The protocol involved repeated measurements of the right and left common carotid far wall at 2 cm proximal to the bulb bifurcation. On a longitudinal B-mode image, the far wall of the common carotid artery appears as 2 bright, parallel lines separated by a hypoechoic space. The distance between the leading edge of the first bright line on the far wall (lumen–intima interface) and the leading edge of the second bright line (media–adventitia interface) indicates the cIMT of the

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**Figure 1** Diagram of the interdisciplinary therapy. BMI, body mass index; ECG, electrocardiography; NAFLD, nonalcoholic fatty liver disease.
far wall, as previously described. Three measurements of the left and right common carotid were taken, and the average of the maximal measures of each side represented the cIMT in this study.

**Serum analysis**

Blood samples were collected at the outpatient clinic at approximately 8 a.m. after an overnight fast. TC, TG, HDL-C, very-low-density lipoprotein, and the hepatic transaminases (ALT, aspartate aminotransferase, and GGT) were analyzed using a commercial kit (CELM, Barueri, Brazil). LDL-C was calculated using the Friedewald formula (LDL-C = TC – [TG/5 + HDL-C]). To identify any individuals with abnormal lipid profiles, we used the reference values from the I Guideline for Preventing Atherosclerosis in Childhood and Adolescence. The reference values of the hepatic transaminases were analyzed as previously described by de Piano et al. (2010). The ratios of the lipoprotein levels (TC/HDL-C, LDL-C/HDL-C, and TG/HDL-C) were calculated because these ratios have been described in the literature as predictors of cardiovascular disease and metabolic syndrome in adults and more recently also in children.

Insulin resistance was assessed by HOMA-IR, which was calculated as the product of fasting blood glucose and immunoreactive insulin (I): (fasting blood glucose [mg/dL] × I [mU/L]/405). The cut-off of HOMA-IR adopted for the adolescents was 3.16. The coefficient of variation for the insulin test was 3.3%.

The leptin, adiponectin, and plasminogen activator inhibitor-1 (PAI-1) concentrations were measured using a commercially available enzyme-linked immunosorbent assay kit from R&D Systems (Minneapolis, MN), according to the manufacturer’s instructions.

**Research design**

The interdisciplinary weight-loss therapy included clinical, nutritional, and psychological interventions, physiotherapy and combined exercise training (aerobic plus resistance training) (Fig. 1). All measurements were performed before and after 1 year of intervention.

**Clinical therapy**

To address the health and clinical parameters, the obese adolescents visited the endocrinologist once each month. The medical follow-up and treatment were based on an initial patient and family history, physical examination, and intervention in any health problems that the patient developed during the course of the therapy.

**Nutritional therapy**

The energy intake was set at levels recommended for subjects with low levels of physical activity of the same age and gender, following a balanced diet. Once a week, the adolescents received dietetics lessons covering the topics related to a healthy eating pattern. All patients received individual nutritional consultations during the intervention program. At the beginning of the study and at 12 months into the program, a 3-day dietary record was collected. Although the degree of underreporting may still be substantial, this method is validated for the assessment of dietary consumption. These dietary data were transferred to a computer by the same dietician, and the nutrient composition was analyzed with a PC program developed at the Federal University of São Paulo–Paulista Medicine School (Nutwin software for Windows, version 1.5, 2002).

**Psychological therapy**

Psychological treatment plans were established on the basis of validated questionnaires and taking into account some of the psychological problems caused by obesity, as described in the literature, including depression, eating disorders, anxiety, decreased self-esteem, and body-image disorders. The interdisciplinary therapy consisted of a weekly 1-h group session. Individualized psychological therapy was recommended when depression, eating disorders, and anxiety symptoms were identified.

**Exercise protocol**

The combined exercise-training program was performed 3 times per week and included 30 minutes of aerobic training plus 30 minutes of resistance training per session. The subjects were instructed to reverse the order of the exercises (aerobic and resistance) at each training session.

The aerobic training consisted of running on a motor-driven treadmill (Model TR 9700HR; LifeFitness, São Paulo, Brazil) at a cardiac frequency intensity representing ventilatory threshold 1 (±4 bpm) according to the results of an initial oxygen uptake test. The exercise program was based on guidelines from the American College of Sports Medicine, 2009.

The resistance training also was designed based on American College of Sports Medicine recommendations. The exercises targeted each of the main muscle groups. After an introductory period (2 weeks for adaptation to the training to learn the movements), the training load was adjusted, and each 8-week volume and intensity were adjusted inversely, decreasing the number of repetitions from 15–20 to 10–12 and 6–8, respectively, for 3 sets. All sessions were rigorously supervised by an experienced physiologist.

**Physiotherapy**

The adolescents participated in an intervention with 2 physical therapists once a week. The themes of these interventions were global postural re-education, isostretching, diaphragmatic breathing, hydrotherapy, balance, and stretching. Individual consultations also were performed if...
the patient had any injuries. The interventions were conducted in a room next to the gym that was suitable for this type of intervention.

**Statistical analysis**

The statistical analyses were performed using STATISTICA (version 7.0 for Windows). The Gaussian distribution of the variables (including the Δ values) was verified with a Kolmogorov–Smirnov test. Variables with a normal distribution were expressed as the mean ± SD, whereas variables without a normal distribution were expressed as a median [lower and upper quartiles] in the descriptive Tables 1 and 2.

Nonparametric methods were used when appropriate (for ALT; GGT; HOMA-IR; TG; and adiponectin concentrations; TC/HDL-c, TG/HDL-c, and leptin/adiponectin ratios). Comparisons between measurements before and after intervention were performed with paired Student t-tests and Wilcoxon signed-rank test for variables with and without a normal distribution, respectively. The independent Student t and Mann-Whitney U tests were performed to compare the magnitude of change (Δ) between groups.

Pearson’s correlation coefficients were calculated to assess possible relationships between normally distributed variables and to select meaningful variables to perform a logistic regression analysis to identify possible predictors of NAFLD development in obese adolescents. P-values less than .05 were considered significant.

**Results**

At baseline, the NAFLD group was found to have a significantly different body composition, with greater total body mass, BMI, fat body mass, and visceral fat (Table 1). Those with NAFLD also presented with the greatest mean HOMA-IR value, ALT, insulin, and PAI-1 concentration (Tables 1 and 2). HOMA-IR was positively correlated with cIMT (Fig. 2) and TG/HDL-c ratio (r = 0.45, P = .008) in the NAFLD group.

After the interdisciplinary weight loss therapy both groups presented a significant reduction in total body mass, BMI, fat body mass, and visceral fat (Table 1). The group mean decreased for measures of cIMT, HOMA-IR, and concentrations of leptin and PAI-1. HOMA-IR was strongly related to the prevalence of NAFLD (odds ratio: 9.95, 95% CI: 2.52–38.34).

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**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>1 Year</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>17 ± 2</td>
<td>17 ± 2</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>Total body mass, kg</td>
<td>100.17 ± 15.81</td>
<td>89.50 ± 16.06†</td>
<td>10.67 ± 2.11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>35.17 ± 5.46</td>
<td>30.96 ± 4.85</td>
<td>4.21 ± 0.65</td>
</tr>
<tr>
<td>Fat body mass, kg</td>
<td>49.68 ± 11.19</td>
<td>39.48 ± 10.38</td>
<td>10.20 ± 2.70</td>
</tr>
<tr>
<td>Visceral fat, cm</td>
<td>3.03 ± 0.71</td>
<td>2.66 ± 1.20</td>
<td>0.37 ± 0.44</td>
</tr>
<tr>
<td>Subcutaneous fat, cm</td>
<td>4.06 ± 1.31</td>
<td>3.16 ± 0.69</td>
<td>0.89 ± 0.62</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>89.95 ± 6.22</td>
<td>91.30 ± 7.19†</td>
<td>1.35 ± 0.84</td>
</tr>
<tr>
<td>Insulin, μg/mL</td>
<td>4.96 ± 0.71</td>
<td>4.96 ± 0.71</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>14.6 ± 4.97</td>
<td>12.97 ± 4.97</td>
<td>1.65 ± 0.37</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>18.2 ± 3.1</td>
<td>16.5 ± 2.8</td>
<td>1.70 ± 0.74</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>35.78 ± 5.46</td>
<td>34.57 ± 5.96</td>
<td>1.21 ± 0.45</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyl transferase; NAFLD, nonalcoholic fatty liver disease; PAI-1, plasminogen activator inhibitor-1; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; cIMT, carotid intima–media thickness; HOMA-IR, homeostasis model assessment of insulin resistance. The data are expressed as the means ± SD or medians [lower and upper quartiles]. P-values less than .05 were considered significant.

The statistical analyses were performed using STATISTICA (version 7.0 for Windows). The Gaussian distribution of the variables (including the Δ values) was verified with a Kolmogorov–Smirnov test. Variables with a normal distribution were expressed as the mean ± SD, whereas variables without a normal distribution were expressed as a median [lower and upper quartiles] in the descriptive Tables 1 and 2.
Table 2  Traditional and emergent cardiovascular risk factors analyzed before and after the interdisciplinary therapy

<table>
<thead>
<tr>
<th></th>
<th>Without NAFLD (n = 46)</th>
<th>With NAFLD (n = 33)</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 Year</td>
<td>Δ</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.26 ± [2.28–3.89]</td>
<td>2.10 ± [1.40–3.08]</td>
<td>−0.83 ± [−1.50 to 0.43]</td>
</tr>
<tr>
<td>Total cholesterol,</td>
<td>168 ± 30</td>
<td>155 ± 29</td>
<td>−12 ± 21</td>
</tr>
<tr>
<td>mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C, g/dL</td>
<td>103 ± 26</td>
<td>91 ± 23</td>
<td>−11 ± 18</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45 ± 9</td>
<td>47 ± 10</td>
<td>2 ± 6</td>
</tr>
<tr>
<td>VLDL, mg/dL</td>
<td>20 ± 9</td>
<td>17 ± 9</td>
<td>−3 ± 9</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>3.56 ± [3.10–4.45]</td>
<td>3.29 ± [2.88–3.68]</td>
<td>−0.46 ± 0.58</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>2.40 ± 0.84</td>
<td>2.03 ± 0.66</td>
<td>−0.36 ± 0.50</td>
</tr>
<tr>
<td>TG/HDL-C ratio</td>
<td>2.03 ± [1.55–2.58]</td>
<td>1.56 ± [1.28–2.12]</td>
<td>−0.49 ± 1.19</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>46.10 ± 26.52</td>
<td>24.51 ± 13.03</td>
<td>−15.06 ± [−25.61 to 5.46]</td>
</tr>
<tr>
<td>ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>11.95 ± 7.99</td>
<td>8.00 ± 7.54</td>
<td>−3.58 ± 5.81</td>
</tr>
<tr>
<td>cIMT, mm</td>
<td>0.41 ± 0.06</td>
<td>0.35 ± 0.05</td>
<td>−0.06 ± 0.07</td>
</tr>
</tbody>
</table>

cIMT, carotid intima-media thickness; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance index; LDL-C, low-density lipoprotein-cholesterol; NAFLD, nonalcoholic fatty liver disease; PAI-1, plasminogen activator inhibitor 1; TC, total cholesterol; TG, triglycerides; VLDL, very-low density lipoprotein.

Reference values: HOMA-IR (>3.16),27 HDL-C (>45), LDL-C (<100), TC (<150), and TG (>100).24

The data are expressed as the means ± SD or medians [lower and upper quartiles].

*Difference between the baseline and after 1 year (P < .05) obtained by Student t and Wilcoxon signed-rank tests.
†Difference between both groups at the baseline condition (P < .05) obtained by Student t and Wilcoxon signed-rank tests.
‡Comparison between Δ (P < .05) obtained by independent Student t and Mann-Whitney U tests.
Figure 2  Correlation between homeostasis model assessment of insulin resistance (HOMA-IR) and carotid intima-media thickness (carotid IMT) in obese adolescents with nonalcoholic fatty liver disease (NAFLD) (n = 33; r = 0.35; P = .04).

Table 3 Logistic regression analysis to predict the NAFLD development in obese adolescents

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral fat, cm</td>
<td>0.68</td>
<td>0.46</td>
<td>1.01</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.65</td>
<td>0.42</td>
<td>0.98</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>0.92</td>
<td>0.45</td>
<td>1.88</td>
</tr>
<tr>
<td>TG/HDL-C ratio</td>
<td>0.99</td>
<td>0.65</td>
<td>1.53</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>0.95</td>
<td>0.89</td>
<td>1.02</td>
</tr>
<tr>
<td>cIMT, mm</td>
<td>106.06</td>
<td>0.01</td>
<td>752354.4</td>
</tr>
</tbody>
</table>

CI, confidence interval; cIMT, carotid intima-media thickness; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance index; LDL-C, low-density lipoprotein-cholesterol; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PAI-1, plasminogen activator inhibitor 1; TG, triglycerides.
Financial disclosures

We thank the patients who participated in the study and the following funding sources that supported the CEPE-GEO Interdisciplinary Obesity Intervention Program: AFIP, FAPESP (2008/53069-0, 2011/50356-0, 2011/50414-0), FAPESP (CEPID/Sleep #9814303-3 S.T), CNPq, CAPES (2011/2566), CENESP, FADA, and Universidade Federal de São Paulo.

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