Cardiorespiratory Fitness, But Not Central Obesity or C-Reactive Protein, Is Related to Liver Function in Obese Children

Clarice Martins
High Education Institute from Maia

Ismael Freitas Jr.
Universidad Estadual Paulista

Andréia Pizarro, Luísa Aires, Gustavo Silva, Maria Paula Santos, and Jorge Mota
Porto University

Nonalcoholic fatty liver disease (NAFLD) is one of the most frequent complications associated with excess adiposity. Its pathogenesis is complex and there are multiple factors that may contribute to it. AIM: To analyze whether cardiorespiratory fitness (CRF), waist circumference (WC), and C-reactive protein (CRP) are associated with alanine aminotransferase (ALT) in children with obesity. METHODS: 79 overweight/obese children of both genders, 11–13 year-olds, with abnormal serum ALT from Porto public schools comprised the sample. Measurements included CRF (20-m Shuttle Run Test), WC (NHANES protocol), CRP and ALT (Cholestech LDX analyzer). Logistic regression adjusted for gender, maturation, and weight with ALT levels as dependent variable (risk vs. non risk), and WC (risk vs. non risk), CRP (risk vs. non risk), and CRF (fit vs. unfit) as independent variables. Level of significance was set at 95%. RESULTS: Logistic regression showed that obese fit children were less likely to have abnormal ALT values (OR=.031) CONCLUSION: In obese children, higher cardiovascular fitness appears to reduce the chance of decreased liver function.

Las enfermedades hepáticas no relacionadas con la ingesta de alcohol constituyen una de las afecciones mas frecuentes causadas por el exceso de adiposidad. Su patogenia es compleja ya que existen múltiples factores relacionados con su manifestación. OBJETIVO DEL ESTUDIO: Analizar el grado de relación entre

Martins is with Faculty of Sports - Research Centre in Physical Activity, Health and Leisure, High Education Institute from Maia, Porto, Portugal. Freitas is with Research Centre of Assessment and Exercise Prescription, Univ. Estadual Paulista, São Paulo, Brazil. Pizarro, Aires, Silva, Santos, and Mota are with Faculty of Sports, Research Centre in Physical Activity, Health and Leisure, Porto University, Porto, Portugal.
la aptitud cardiorrespiratoria (ACR), perímetro de la cintura (PC) y la proteína C-Reactiva (PCR) con los niveles de Alanina-Aminotransferasa (ALT) en niños obesos. METODOS: 79 niños de ambos sexos, de 11 a 13 años de edad, con sobrepeso/obesidad, que concurrían a las escuelas publicas en Porto y presentaban niveles anormales de ALT. Se midió la ACR (test de 20-m shuttle run), PC (protocolo de NHANES), la PCR y ALT (analizador Cholestech LDXR). Se realizó un análisis de regresión logística ajustada por el sexo, la maduración y el peso corporal tomando a los niveles de ALT como variable dependiente (riesgo Vs sin riesgo), el PC (riesgo Vs sin riesgo), la PCR (riesgo Vs sin riesgo) y la ACR (buena aptitud Vs pobre aptitud) como variables dependientes. El nivel de significación fue determinado al 95%. RESULTADOS: Las regresiones logísticas indicaron que los niños obesos con buen estado de forma física tenían menores probabilidades de tener valores anormales de ALT (OR=0.031). CONCLUSION: En niños obesos, un mayor rendimiento cardiorrespiratorio parece reducir el riesgo de disminución de la función hepática.

There is evidence of more obese children worldwide. Moreover, a concomitant rise in obesity-related comorbidities suggests that overweight and obesity track from childhood to adolescence and adulthood (24). Nonalcoholic fatty liver disease (NAFLD) is the most frequent complication associated with excess adiposity (16). It is characterized by pathological fat accumulation in the liver, which may lead to liver damage in the form of inflammation and fibrosis (23).

The pathogenesis of NAFLD is complex and comprises multiple factors. For example, obesity precursors, such as free fatty acids delivered from visceral adipose tissue, which drain directly into the portal vein and overload hepatocytes with lipids (2), play a key role in the pathogenesis of NAFLD (33). The association between obesity-related comorbidities and other surrogate markers of fatty liver disease such as alanine aminotrasferase (ALT), as commonly used in population studies (20), has also been assessed. Research using this technique has reported that abnormal serum ALT levels were associated with C-reactive protein (CRP; 11), a critical inflammatory marker in obese Italian children.

Cardiorespiratory fitness (CRF) has become a key outcome variable in clinical health settings and is an important determinant of changes in adiposity (3). Nonetheless, there is lack of information on the role and importance of fitness in the development of fatty liver. When low CRF is associated with overweight and obesity, there is an increased risk for the development of other comorbidities in youngsters (13). Thus, it is plausible that CRF may be important in the development of fatty liver in youth. However, few studies have examined this possibility in pediatric populations.

Despite that, other studies have examined this issue in adults. A lifestyle intervention study with overweight and obese adults showed a 3.5% reduction in body weight with diet and exercise therapy, and a 35% reduction in fatty liver indicators after 9 months associated with significant improvements in CRF (9).

In an adult based study, positive benefits of increasing CRF in fatty liver patients have been reported (7). However, this relation is not clear for all studies and it appears to be independent of body mass index (BMI) but not of visceral adiposity (9,12). For example, an intervention focused only on exercise, without diet, found around 50% reduction in ALT levels after 3 months (25). This reduction
was associated with a decreased waist circumference (WC), although no change in BMI was detected (25). Those findings suggested that abdominal obesity might play an important role in the development of fatty liver.

However, to the best of our knowledge, few studies have addressed the association between ALT with abdominal adiposity, inflammation markers and fitness in children with obesity. Therefore, the aim of this study was to analyze whether cardiorespiratory fitness, waist circumference, and C-reactive protein are associated with alanine aminotransferase in children with obesity.

**Methods**

**Sample and Study Design**

A total of 410 students from Porto public schools agreed to participate in the study. From those, 79 children aged 11–13 year-olds, who were defined as obese, according to Cole cut-points (4), with abnormal ALT levels (40 girls and 39 boys), comprised the sample.

The Regional Education Board approved the study protocol, and children, parents and schools agreed to participate. The nature, benefits, and risks of the study were explained to the volunteers, and a parent’s written informed consent was obtained before the study, consistent with the Helsinki Declaration. The evaluation methods and procedures were approved by the Scientific Board of the Faculty of Sports of the University of Porto.

Six Physical Education teachers, a nurse, and a medical doctor carried out all measures. Subjects were identified through an individual code number and a school code number. Fasting blood samples were taken followed by anthropometric evaluation. The children were then given breakfast followed by the determination of their maturational stage. Finally, the shuttle-run test was performed. All assessment took place between 8:00 and 11:00 a.m.

**Anthropometry**

Body height was measured to the nearest mm in bare or stocking feet with the adolescent standing upright against a Holtain Stadiometer. Weight was measured to the nearest 0.1 kg, lightly dressed and after having breakfast, using an electronic weight scale (Seca 708 portable digital beam scale). BMI was calculated from the ratio of body weight (kg) / body height (m²). Waist circumference (WC) was evaluated using the NHANES (31) protocol.

**Biological Assessment**

High sensitivity c-reactive protein (CRP) and Serum aminotransferase (ALT) was measured by taking a capillary blood sample from the right earlobe of participants after at least 12 hr of fasting. The blood samples were drawn in capillary tubes (33 μl, Selzer) coated with lithium heparin and immediately assayed using Cholestech LDX Analyzer. An analyzer daily optic check was performed and then, the sample was applied into a specific cassette, where the analyzer separated the plasma and the blood cells. All the Cholestech LDX cassettes were stored in the refrigerator before use.
The Cholestech LDX analyzer provides good agreement scores (88–99%), with laboratory measures for population-based screening for cardiovascular risk factors (22).

For statistical analysis, children were categorized according to abnormal serum ALT sex cut-offs (21), as they provide better sensitivity to detect chronic liver disease (21). For CRP, the cut-offs were set at the 2mg/L established value (6). Values higher than 10mg/L were excluded from the analysis.

Maturational Stage
Maturational stage was determined on an individual basis during physical examination. Each subject self-assessed his/her own stages of secondary sex characteristics. Stage of breast development in females and pubic hair in males was evaluated according to the criteria of Tanner (29). A previous study showed a high correlation ($r = .73$) between ratings on two occasions (three day interval) in a subsample of 50 selected subjects. Concordance between self-assessments of sexual maturity status and physician assessment ranged from 63% for girls and 89% for boys (14).

Cardiorespiratory Fitness (CRF)
CRF was predicted using the maximal multistage 20m Shuttle-Run Test according to procedures described by Léger & Lambert (10) as this measure shows good correlation with directly measured VO$_{2\text{max}}$ ($r = .80$) suggesting that it could be used as a measure of aerobic fitness in children (1). Children were then categorized as fit or unfit according to adopted age-adjusted criterion referenced health standards (Health Fitness Zone) for individual CRF using the Fitnessgram test battery (5). In this case children were classified as fit if boys and girls aged 11 did at least 23 and 15 laps, respectively. Children aged 12 had to complete 32 and 23 laps and children aged 13, 41 and 23 laps for boys and girls, respectively (5).

Statistical Procedures
Descriptive data are shown as means and standard deviation. Partial correlations adjusted for sex, biological maturation and weight were used to examine the correlations between ALT with WC, CRP and fitness (data not showed). Logistic regression analysis adjusted for sex, weight and maturation was used to analyze the influence of WC (model 1), CRP (model 2), and CRF (model 3) on ALT levels. The level of significance was set at 95%, and data were analyzed using SPSS (version 18.0 for Macintosh).

Results
Descriptive statistics for both CRF groups are presented in Table 1. Unfit participants were significantly heavier and presented higher values of BMI, WC and ALT than their fit counterparts.

Logistic regression (Table 2) adjusted for sex, maturation and weight showed that those classified as fit were less likely to be classified as at risk for abnormal levels of ALT ($OR = .031; p \leq .05$). No statistically significant associations were found for WC and CRP.
The aim of the current study was to analyze whether CRF, WC, and CRP are associated with ALT in children with obesity. Our results showed that although all participants were overweight or obese, those who were classified as fit presented statistically lower weight, BMI, WC and ALT. Furthermore, significant, negative correlations were observed between CRF with WC, ALT, and CRP. However, the major novel finding in the current study was that after adjustment for sex, weight and maturation, obese fit children showed significantly lower risk of demonstrating.

### Table 1  Anthropometric Variables, CRP and ALT Differences Between CRF Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fit (n = 33)</th>
<th>Unfit (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X (SD)</td>
<td>X (SD)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.1 (.33)</td>
<td>11.4 (.50)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.6* (6.93)</td>
<td>58.7 (11.60)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.5 (.06)</td>
<td>1.5 (.07)</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>23.6* (2.15)</td>
<td>25.0 (3.51)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>77.9* (7.47)</td>
<td>82.0 (9.35)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.5 (1.63)</td>
<td>1.8 (1.69)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>21.4** (3.53)</td>
<td>27.0 (4.42)</td>
</tr>
</tbody>
</table>

CRF = Cardiorespiratory fitness; BMI= Body Mass index; WC = Waist circumference; CRP= C-reactive protein; ALT= Serum alanine aminotransferase; *p ≤ 0.05; **p ≤ 0.01

### Table 2  Logistic Regression Adjusted for Gender, Maturation, and Weight with ALT Levels as Dependent Variable (Risk vs. Non-Risk), and Waist Circumference (Risk vs. Non-Risk), C-Reactive Protein (Risk vs. Non-Risk), and Cardiorespiratory Fitness (Fit vs. Unfit) as Independent Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC(cm)</td>
<td>Risk—ref</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(model 1)</td>
<td>Non risk</td>
<td>-0.39</td>
<td>0.67</td>
<td>0.153–2.967</td>
</tr>
<tr>
<td>CRP(mg/L)</td>
<td>Risk—ref</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(model 2)</td>
<td>Non risk</td>
<td>-1.18</td>
<td>0.31</td>
<td>0.042–2.230</td>
</tr>
<tr>
<td>CRF(laps)</td>
<td>Unfit—ref</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(model 3)</td>
<td>Fit</td>
<td>-3.49</td>
<td>.031</td>
<td>0.005–0.174</td>
</tr>
</tbody>
</table>

WC = Waist circumference; CRP= C-reactive protein; CRF= Cardiorespiratory Fitness; 95% Confidence interval; p = Significance value.

Discussion

The aim of the current study was to analyze whether CRF, WC, and CRP are associated with ALT in children with obesity. Our results showed that although all participants were overweight or obese, those who were classified as fit presented statistically lower weight, BMI, WC and ALT. Furthermore, significant, negative correlations were observed between CRF with WC, ALT, and CRP. However, the major novel finding in the current study was that after adjustment for sex, weight and maturation, obese fit children showed significantly lower risk of demonstrating...
abnormal ALT values. These outcomes are congruent with prior studies in adult populations, and show a negative association between CRF and fatty liver. This fact suggests that low CRF may have a pivotal negative role on hepatic metabolism (8,28).

There are two possible physiological mechanisms to explain the impact of CRF on fatty liver. The peripheral point of view emphasizes that lower CRF may have an indirect impact through peripheral factors such as insulin sensitivity or visceral adiposity (19). Exercise increases whole-body fatty acid oxidation owing to the increase in respiration rate within working skeletal muscle. Therefore, exercise may modulate liver fat via effects on hepatic mitochondrial biogenesis and capillarization leading to an augmentation in lipid oxidation (9).

Other studies showed that central and total obesity were lower in obese children with high CRF (15). Our study demonstrates an association between WC and CRF. However, several studies have presented contradictory results in similar populations (18). Moreover, total or visceral fat reduction is not always associated with reduced liver fat (28).

The second suggested mechanism highlights the intrinsic molecular/cellular role of CRF in fatty liver development (30). Indeed, it was observed that low-CRF rats presented hepatic mitochondrial content, function, and fatty acid oxidation significantly reduced compared with their high-CRF counterparts. This fact suggests that the elevated hepatic mitochondrial content and the raised fatty acid oxidation in high-CRF rats may provide a liver protection, as well as to show the importance of the specific hepatic molecular events (30).

To the best of our knowledge, the majority of prior studies addressing fatty liver and/or fat enzymes concentration and its relationship with CRF were carried-out within adult populations, and few studies have addressed this issue among children with obesity. Our outcomes are worthy of comment since there is a strong association between obesity and fatty liver in the youngsters (17), and preventive measures should be developed for the population targeted. Indeed, our findings illustrate that obese fit children, were less at risk for higher levels of ALT compared with their obese unfit peers, even after adjustments for age, maturity, and weight. Therefore, this outcome suggests there may be a potential protective effect of CRF against abnormal ALT values. This data on children with obesity support those found in adults showing that fit participants present greater improvement of liver enzymes function, including ALT, when compared with unfit (26). Furthermore, the results of the current study also agree with data reported in girls, which highlighted that regular exercise was significantly associated with improvement in serum ALT. The normal levels of serum ALT were obtained after one year of changes in lifestyle accompanied by a weight reduction of at least 5% (15). This fact might suggest that an important health promotion strategy would be to identify the children with obesity and try to increase their cardiorespiratory fitness.

As many lifestyle habits are established during childhood and adolescence, exercise habits may also be established during these formative years. Thus, one strategy to reverse the excessive amount of fatty liver even without targeting reduction of body weight may be to focus on lifestyle modification by increasing exercise (8). This suggestion is supported by research showing that both weight loss and regular exercise were associated with ALT reduction (27).

Nonetheless, some studies have found that although exercise remains fundamental to the management of fatty liver, intervention strategies should focus on exercises that promote increases in CRF, as a more efficient intervention strategy
(8,32). Therefore, the effect of a lifestyle intervention on fatty liver is not exclusively mediated by changes in total and visceral adiposity, but also by CRF, which has been indicated as an independent and best predictor of change in hepatic triglycerides (9). This target can be achieved by using different types of exercise, considering CRF as a reliable and easily quantifiable end-point measure of structured aerobic exercise (7).

The strength of the current study is that it was carried out within a specific obese population and gives additional information with regard to the association between CRF and fatty liver markers in such a population. However, some limitations should also be recognized. Firstly, this study employed a cross-sectional design with the intent to explore the associations between ALT with markers of fitness (CRF), central obesity (WC), and inflammation (CRP) in children with obesity. However, it is not possible to infer causal relationships with such a design and so, results should be analyzed with caution. Secondly, this study would benefit from additional data such as combined behavioral variables and social background characteristics. The assessment of other enzyme concentrations associated with liver function including gamma-glutamyltransferase, aspartate transaminase and alkaline phosphatase, might better predict fatty liver.

**Conclusion**

In obese children, higher cardiorespiratory fitness appears to reduce the chance of decreased liver function. Thus, health promotion strategies should focus on identifying the obese children with abnormal ALT levels and increase their cardiorespiratory fitness.

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**References**


