Physical Activity is related to Fatty Liver Marker in Obese Youth, Independently of Central Obesity or Cardiorespiratory Fitness

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Abstract
Nonalcoholic fatty liver disease (NAFLD) is one of the most frequent complications associated with excess adiposity and has been identified as the leading cause of liver disease in pediatric populations worldwide. Because cardiorespiratory fitness (CRF) is related to physical activity (PA) levels, and increased PA plays a protective role against NAFLD risk factors, the aim of this study was to analyze the association between PA and a fatty liver marker (alanine aminotransferase - ALT) in obese children and adolescents, independently of central adiposity or CRF. 131 obese children (83 girls, 7-15 year-olds) involved in a PA promotion program comprised the sample. Measurements included anthropometric and body composition evaluations (DEXA), biological measurements (venipuncture), CRF (progressive treadmill test), PA (accelerometry), and maturational stage (Tanner criteria). The associations between ALT with PA intensities, central obesity, and CRF were calculated by three different models of linear regression, adjusted for potential confounders. Level of significance was set at 95%. RESULTS: ALT was negatively associated with MVPA (β = -0.305), and CRF (β = -0.426), and positively associated with central obesity (β = 0.468). After adjustment for central obesity the negative and statistically significant association between ALT with MVPA (β = -0.364) and CRF (β = -0.550) still persists while a positive and significantly correlation was shown between ALT and SB (β = 0.382). Additional adjustment for CRF (Model 3) showed significant associations for all the PA intensities analyzed including light activity. PA at different intensities is associated to a fatty liver marker in obese children and adolescents, independently of central adiposity or CRF.

Key words: Physical activity, fatty liver, obese youth, cardiorespiratory fitness, central obesity.

Introduction
Over the last two decades, the rise in the prevalence of overweight and obesity may explain the number of risks for obesity-related metabolic and endocrine derangements, and have been leading to early incidence of obesity-related diseases that used to be exclusive to adulthood.

Nonalcoholic fatty liver disease (NAFLD) is one of the most frequent complications associated with excess adiposity (Papandreou et al., 2008) and has been identified as the leading cause of liver disease in pediatric populations worldwide (Barshop et al., 2008). It is characterized by pathological fat accumulation in the liver, which may lead to liver damage in the form of inflammation and fibrosis (Siegel and Zhu, 2009). Furthermore, NAFLD predisposes subjects to other pathological conditions, such as metabolic syndrome and type 2 diabetes (Targher et al., 2005; Yki-Jarvinen, 2005).

Weight loss via lifestyle modification remains the most common and well-established fundamental therapy for reducing hepatic fat (Dixon et al., 2004) since low levels of physical activity (PA) seems to be associated with many NAFLD risk factors, such as high cholesterol, diabetes, and metabolic syndrome (Muros Molina et al., 2011; Skaaby et al., 2012).

In a previous study we investigated whether cardiorespiratory fitness (CRF) was associated with fat liver (alanine aminotransferase - ALT) in obese children. Our findings suggested that there might be a potential protective effect of CRF against abnormal ALT values (Martins et al., 2013). Because CRF is related to PA levels, and increased PA plays a protective role against NAFLD risk factors, we hypothesized that it might be an association between PA and NAFLD in obese children and adolescents, independently of central adiposity.

Recent studies suggested that increased PA might have a beneficial effect on NAFLD (Golbidi et al., 2012; Kwak et al., 2014). Nonetheless, to the best of our knowledge, no studies investigated this association in youth populations. Therefore, the aim of this study was to analyze the association between PA and a fatty liver marker (ALT) in obese children and adolescents, independently of central adiposity or CRF.

Methods

Participants and data collection
This is a cross-sectional baseline study, which is part of a school-based intervention PA program carried out in 5 primary and 2 middle-high public schools from a suburban setting from Oporto-Portugal. Over a period of 6 months, 159 students volunteered to attend a PA program, twice a week. For this cross-sectional study, the sample was comprised by 131 children (83 girls, 7-15 year-olds) involved in the program who: (1) did all the baseline testing procedures; (2) were not attending any other formal sports or PA program; and (3) were classified as obese, according to age-gender specific body mass index (BMI) cut-off points (Cole et al., 2000).
For the purpose of this study, the sample size was calculated using the G*Power software 3.1.9.2. Hypothesizing an effect size (Cohen’s d) of 1.0 for a required power of 95% at p<0.05, a sample size of at least thirty subjects was required. Therefore, a larger sample size of 131 subjects should provide adequate statistical power for the analysis.

The Regional Education Board approved the study protocol, and students, 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 Scientific Board of the Research Unit from The Faculty of Sports of Oporto University approved the evaluation methods and procedures used.

One week before the evaluation week, all the subjects were familiarized with the testing protocols, procedures and equipment. Six Physical Education teachers, three pediatric nurses, and a medical doctor carried out all measures, which took three consecutive mornings. Participants were identified through an individual code number and a school code number, and divided in three groups, according to their convenience. One group was evaluated per day. Fasting blood samples were taken between 08:00am and 9:00am. The children and adolescents were then given breakfast, followed by the evaluation of the anthropometric, body composition, maturational, and cardiorespiratory fitness variables, between 09:30 am and 11:00 am. At the end of the evaluation protocol, the accelerometers were given to the students. Participants’ parents were recommended to encourage their children to maintain the same standard meal.

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 protocol (Kuczmarski, 1996). A bony landmark is first positioned and marked. The examiner, positioned to the right of the participant, palpated the upper hip bone to locate the right iliac crest. Just above the uppermost lateral border of the right iliac crest, a horizontal mark was drawn, and then crossed with a vertical mark on the mid-axillary line. The measuring tape was placed in a horizontal plane around the abdomen at the level of this marked point on the right side of the trunk. The plane of the tape was parallel to the floor and the tape was held snug, but did not compress the skin. The measurement was made at a normal minimal respiration.

Biological measurements
Blood samples were collected by three pediatric nurses. A mean inter-observers and intra-observers coefficient of variation (CV) of 2.4 and 1.2% respectively, was observed. Blood was collected from the antecubital vein between 8:00 and 09:00 a.m., after at least ten hours of fasting. Blood samples were obtained on a fasting basis and processed within 2h of collection. Blood was obtained by venipuncture, to EDTA containing tubes. Following centrifugation, plasma was separated, aliquot and immediately stored at -80ºC until the end of the week, when it was assayed. To determine ALT concentrations, a routine automated technology (ABX Diagnostics) was used. The biochemical evaluation of all participants was conducted in the same laboratory.

Physical activity (PA) measurement
PA was objectively assessed by accelerometers (wGT3x, Actigraph, Florida) during 7 consecutive days. Data was stored in raw mode in samplings of 30Hz. With a specific software (ActiLife, version 6.9, Actigraph, Florida), data was reduced into one-minute periods (epochs), organized into daily physical activity and analyzed after data collection. Wear and nonwear time was determined according to Choi et al. (2011) algorithm. Time periods with at least 10 consecutive minutes of zero counts recorded were excluded from analysis assuming that the monitor was not worn. A minimum recording of 8-hours/day (480-minutes/day) was the criteria to accept daily PA data as valid. Individual’s data were only accepted for analysis if at least three-week days and one weekend day were successfully assessed. The main outcomes of reduced accelerometer data were: total physical activity [total PA (counts/min/day)], time in sedentary behavior [SB (min/day)], light physical activity [LPA (min/day)], moderate physical activity [MPA (min/day)], vigorous physical activity [VPA (min/day)] and moderate to vigorous physical activity [MVPA (min/day)].

International recommendations (Trost et al., 2011, Evenson et al., 2008b) cut-points were used to determine time spent in PA of different intensities. The following counts intervals were considered: 0-100 for SB, 101-2295 for LPA, 2296-4011 for MPA, and ≥4012 for VPA (Evenson et al., 2008a) For the analysis we used MVPA, defined as the sum of MPA and VPA.

Cardiorespiratory fitness
To assess the maximal oxygen uptake (VO2max), all subjects were submitted to a continuous progressive treadmill exercise. Participants were instructed to walk/run until exhaustion, according to a standardized exercise protocol (Eiberg et al., 2005a; 2005b). The exercise protocol started at 4 km·h⁻¹ without inclination and maintained for 3 minutes so that subjects could adapt to the treadmill. After 3 min, speed was increased to 8 km·h⁻¹. When 5 min were completed, the inclination was raised to 3%. After 7 and 9 min, the inclination was increased to 6% and 9%, respectively. If the subjects were able to endure more, the speed was increased to 9 km·h⁻¹ after 1 min and then 10 km·h⁻¹ after 13 min.

Oxygen uptake was measured directly with Oxygen™ Pro Metabolic Cart (Jaeger™, Höchberg, Germany). Respiratory variables were recorded breath-by-breath, which in turn were averaged over a 5-seconds periods, yielding a “fair” representation of the change in
VO₂ during incremental exercise (Astorino, 2009). Before each individual test, oxygen and carbon dioxide analyzers were calibrated according to the manufacturer’s instructions. Directly measured VO₂max or peak VO₂ was the main variable determined using the open-circuit method. Directly measured VO₂max was considered when a plateau in the VO₂ curve was detected, defined as an increase in a VO₂ of less than 2 ml/kg·min⁻¹ with a concomitant increase in speed stage. If a VO₂ plateau was absent (Docherty, 1996), the peak VO₂ was taken and defined as the highest oxygen uptake achieved during exercise at exhaustion (Armstrong and Welsman, 2007). For practical reasons, from now on, this paper will refer to the highest VO₂ values achieved as VO₂max. Exhaustion was confirmed when: (1) subjects desired to stop or demonstrated an inability to maintain the required running pace despite strong verbal encouragement; (2) maximal heart rate was greater than 85% of age-predicted maximal heart rate (220-age); (3) the respiratory exchange ratio (RER) was greater than 1.0 at the end of the test; (4) the participants showed symptoms of discomfort and/or signs of high sweating, facial flushing and grimacing (Docherty, 1996).

Careful control was taken concerning technical and environmental variables that might have had some influence on the results, so that highly reliable metabolic measures could be obtained.

### Central adiposity
Regional (arms, legs, trunk, and head) body fat mass was measured using a whole-body DEXA scanner (Hologic QDR-2000, software version V5.67A, Hologic Inc., Bedford, MA). The standard procedures described in the literature for DEXA measurements were employed (Paradisi et al., 1999, Wong et al., 2002). The coefficient of variation of the instrument was < 1% for in vivo measurements. The body regions were delineated by means of specific anatomical landmarks, and central fat mass was calculated as the trunk fat mass.

### 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 (Tanner, 1962). A previous study showed a high correlation (r=0.73) between ratings on two occasions (three day interval) in a sub-sample of 50 selected subjects. Concordance between self-assessments of sexual maturity status and physician assessment ranged from 63% for girls and 89% for boys (Mota et al., 2002). This information was used as a co-variable in the statistical procedures.

### Statistical analysis
All variables were checked for normality and homogeneity, and appropriately transformed when necessary. Descriptive statistics (mean and range values) were used in order to characterize the participants.

Independent sample t-test was used to analyze differences between genders for all the measured variables. To analyze how variables correlate, three different models for Partial Correlations were calculated. The first one analyzed the association between ALT with different intensities of PA, central adiposity, and CRF, adjusted for potential confounders (gender and maturational stage). In the second model, we adjusted the analyses for central obesity. In the third one we added a further adjustment for CRF. The associations between ALT with PA intensities, central obesity, and CRF were calculated by linear regression and followed the same three models used for calculating the partial correlations.

Analyses were performed with the statistical software package (SPSS 20.0 for Macintosh) and level of significance was set at p ≤ 0.05.

### Results
Descriptive statistics and mean comparisons between gender groups are presented in Table 1. Boys were significantly more active (MVPA) than girls are, while girls showed to spend significantly more minutes in light and sedentary activities (p < 0.05) boys. No other statistical significant differences were found-out.

Partial correlations and multiple linear regressions showed the associations between ALT with different intensities of PA, CRF, and central obesity (Table 2). The associations were adjusted respectively for gender and maturation (Model 1) as well as with further adjustments for central obesity (Model 2) and CRF (Model 3), as described in table 2. ALT was negatively associated with

### Table 1. Sample characteristics. Data are means (±SD).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Female (n = 83)</th>
<th>Male (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.80 (3.50)</td>
<td>10.33 (3.62)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49.01 (21.59)</td>
<td>48.73 (24.13)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.43 (.13)</td>
<td>1.44 (.16)</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.83 (6.37)</td>
<td>22.10 (6.34)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>78.11 (18.92)</td>
<td>77.50 (19.94)</td>
</tr>
<tr>
<td>Central fat mass (kg)</td>
<td>7.85 (4.52)</td>
<td>7.15 (5.12)</td>
</tr>
<tr>
<td>VO₂max (ml·kg⁻¹·min⁻¹)</td>
<td>41.82 (8.04)</td>
<td>44.90 (10.61)</td>
</tr>
<tr>
<td>MVP (mean min⁻¹)</td>
<td>50.65 (22.30)</td>
<td>65.38 (28.52)*</td>
</tr>
<tr>
<td>SB (mean min·day⁻¹)</td>
<td>562.78 (82.78)</td>
<td>575.19 (108.26)*</td>
</tr>
<tr>
<td>LPA (mean min·day⁻¹)</td>
<td>377.12 (62.56)</td>
<td>339.33 (91.20)*</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>27.50 (8.43)</td>
<td>28.12 (9.04)</td>
</tr>
</tbody>
</table>

*Independent sample t-test between gender groups; BMI= Body Mass index; WC=Waist circumference; VO₂max= Maximal oxygen consumption; MVP= moderate-to-vigorous physical activity; LPA= Light physical activity; SB = sedentary behavior; ALT= Serum alanine aminotransferase. * p ≤ 0.05.
complete diagnosis of NAFLD, we observed that this lence of high ALT level. Despite our study didn’t make a found between the different PA intensities with the preva-

The main finding of this study was the inverse association between PA and ALT remained statistically significant even after adjustment for the effects of central obesity and CRF. The data found in partial correlations are strongly supported by multiple linear regression analysis. The most important finding was, indeed, the fact that SB was positively associated with ALT, while LPA and MVPA were negatively associated with ALT.

Discussion

The main finding of this study was the inverse association between the different PA intensities with the prevalence of high ALT level. Despite our study didn’t make a complete diagnosis of NAFLD, we observed that this association between PA and ALT remained statistically significant even after adjustment for the effects of central obesity and CRF, which were considered possible links between PA and NAFLD (Kwak et al., 2014).

For developing NAFLD, visceral adiposity is known to play a major role by secreting hormones, adipokines, and free fatty acids, which drain directly into the portal vein and overload hepatocytes with lipids (Bugianesi et al., 2005). The role of weight reduction, though a moderate one, in NAFLD therapy is well-known, since a decrease in visceral fat leads to a reduced portal free fatty acid supply, favoring changes in the secretion of adipokines (Tilg and Hotamisligil, 2006). Nonetheless, Hallsworth and colleagues (Hallsworth et al., 2011) observed the effect of exercise on NAFLD even without changes in body weight.

When considering PA, specific literature has long been describing it as a central point of a healthy lifestyle, with its well-established protective role in cardiovascular and metabolic diseases. However, its importance in NAFLD still requires scientific support and elucidation (Zelber-Sagi et al., 2008). Our findings stress the fact that PA (MVPA or LPA) as well as lower sedentary behavior, was associated with lower ALT level independent of the effect of potential mediators, such as central obesity and CRF.

Previous studies in adult population have recommended some possible action mechanisms for PA on NAFLD: firstly, subjects engaging in regular PA tended to have a healthier diet in terms of fat composition (Zelber-Sagi et al., 2008); secondly, the association of PA with adipokynes, which are all implicated in the development of hepatic steatosis (Bajaj et al., 2004; Muse et al., 2004), may explain, at least in part, PA association with NAFLD. Finally, we could speculate that the amount of daily activities, as well as the reduction in SB, can have a significant health effect by increasing energy expenditure, even without promoting fitness benefits. Fundamentally, a positive energy balance underlies the development of obesity. More SB contribute to a positive energy balance, by decreasing energy expenditure (Mitchell et al., 2013).

Similarly to our findings in children and adolescents, a recent epidemiological study with 3718 adult subjects showed an inverse association between various types of PA and diagnosed NAFLD, even after adjusting for the effects of visceral fat (Kwak et al., 2014). A study with adult population demonstrated a significant reduction in ALT levels in patients with NAFLD who adhered to an aerobic exercise program, regardless of weight loss (Sreenivasa Baba et al., 2006). Other studies with adult population have also shown an association between PA and hepatic fat independent of obesity or visceral obesity (Bae et al., 2012; Hallsworth et al., 2011). Nonetheless, there are divergent results in the literature with adult population and further studies are needed to elucidate this association. A study aimed to analyze the association between PA and NAFLD (Zelber-Sagi et al., 2008) demonstrated that habitual leisure-time PA, may play a protective role in NAFLD, however, this association appears to be mediated by a reduced rate of abdominal obesity.

Two recent investigations reported the association between PA and fatty liver in youth. Fintini and colleagues (Fintini et al., 2012) observed that youth with MVPA (r = -0.519), and CRF (r = -0.547), and positively associated with central obesity (r = 0.575). After adjustment for central obesity the negative and statistically significant correlation between ALT with MVPA (r = -0.517) and CRF (r = -0.548) still persist while a positive and significant correlation was shown between ALT and SB (r = 0.430). Additional analysis with adjustment for CRF (Model 3) showed statistically significant associations for all the PA intensities analyzed including light activity. The data found in partial correlations are strongly supported by multiple linear regression analysis. The most important finding was, indeed, the fact that SB was positive and significantly associated with ALT, while LPA and MVPA were negatively associated with ALT.

Table 2. Partial correlations and linear regressions showing estimating results with ALT as dependent variable and central obesity, CRF, and PA intensities as independent variables.

<table>
<thead>
<tr>
<th>Dependent Variable ALT</th>
<th>Independent Variables</th>
<th>r</th>
<th>β (95%(CI))</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Central obesity</td>
<td>.575*</td>
<td>.468 (.000 ; .001)</td>
<td>.001*</td>
</tr>
<tr>
<td></td>
<td>CRF</td>
<td>-.547*</td>
<td>-.426 (.001 ; .008)</td>
<td>.005*</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>.226</td>
<td>.260 (-.006 ; .052)</td>
<td>.260</td>
</tr>
<tr>
<td></td>
<td>LPA</td>
<td>-.124</td>
<td>-.222 (-.058 ; -.011)</td>
<td>.181</td>
</tr>
<tr>
<td></td>
<td>MVPA</td>
<td>-.519*</td>
<td>-.305 (-.197 ; -.009)</td>
<td>.033*</td>
</tr>
<tr>
<td>Model 2</td>
<td>CRF</td>
<td>-.501*</td>
<td>-.550 (-.120 ; -.002)</td>
<td>.428</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>.430*</td>
<td>.382 (.001 ; .009)</td>
<td>.019*</td>
</tr>
<tr>
<td></td>
<td>LPA</td>
<td>-.222</td>
<td>-.372 (-.013 ; .086)</td>
<td>.140</td>
</tr>
<tr>
<td></td>
<td>MVPA</td>
<td>-.571*</td>
<td>-.364 (-.362 ; -.034)</td>
<td>.019*</td>
</tr>
<tr>
<td>Model 3</td>
<td>SB</td>
<td>.520*</td>
<td>.421 (.001 ; .010)</td>
<td>.021*</td>
</tr>
<tr>
<td></td>
<td>LPA</td>
<td>-.460*</td>
<td>-.424 (-.343 ; -.022)</td>
<td>.027*</td>
</tr>
<tr>
<td></td>
<td>MVPA</td>
<td>-.511*</td>
<td>-.492 (-.454 ; -.039)</td>
<td>.022*</td>
</tr>
</tbody>
</table>

p ≤ 0.05; ALT= serum alanine aminotransferase; PA = physical activity; CRF = cardiorespiratory fitness; SB = sedentary behavior; LPA = light physical activity; MVPA = moderate – to – vigorous physical activity; Model 1 adjusted for gender, and maturational stage; Model 2 adjusted for gender, maturational stage and central obesity; Model 3 adjusted for gender, maturational stage, central obesity, and CRF.
NAFLD had significantly lower total energy expenditure, spent less time in moderate PA and more hours in sedentary activities compared with their healthy controls. Magger and colleagues (2010) in a study with Canadian youth with hepatic steatosis reported that they spent more than 65% of daily leisure time in sedentary activities such as watching television or playing computer-video games. Nonetheless, our findings are difficult to discuss since there are a few recent studies in the literature that investigated PA and fatty liver in youth.

So, even without promoting a CRF increase, or a fat reduction, the increase in PA and/or reduction in SB time could promote health benefits associated to ALT levels. Given the fact that plasma ALT concentration is considered the best biochemical predictor of hepatocellular lipid content and a marker of fat liver accumulation (Devries et al., 2008), we could speculate that low levels of PA, as well as high SB might accentuate the chance of having NAFLD.

Therefore, increased PA and reduced SB should be stimulated for children and adolescents with altered ALT. In terms of primary prevention of fatty liver, the assessment of daily PA is an important tool for targeting candidates for future intervention. However, further prospective studies are needed to assess this finding, once several limitations of our study should be considered. Firstly, this study employed a cross-sectional design, and 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, as gamma-glutamyltransferase, aspartate transaminase and alkaline phosphatase, and the assessment of adipocytokines might better predict fatty liver and its associations.

**Conclusion**

Evidence from the present analysis supports the idea that PA at different intensities is associated to a fatty liver marker in obese children and adolescents, independently of central adiposity or CRF.

**Acknowledgements**

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**References**


Key points

- In a previous study our group observed that there might be a potential protective effect of cardiorespiratory fitness (CRF) against abnormal ALT values; 
- Considering that CRF is related to physical activity (PA), and increased PA plays a protective role against fatty liver, we hypothesized that it might be an association between PA and fatty liver in obese youth, independently of central adiposity or CRF; 
- No other study has investigated these associations in obese youth; 
- Our findings stresses the fact that moderate-to-vigorous and light physical activities, as well as lower sedentary behavior, is associated with lower fatty liver marker, independent of the effect of potential mediators, such as central obesity or CRF.

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