Abstract:

Puberty is known as the period of life involving great morpho-physiological changes. The aim of this study was to analyze the behavior of lipids and lipoproteins during puberty.

Methods: The sample consisted of 570 male adolescents between 10 and 17 years. Weight, height and BMI were assessed. Total cholesterol (TC), HDL-C and triglyceride (TG) were determined by the enzymatic method, and the LDL-C was calculated. Participants were classified according to the maturation stages, from 1 to 5. The percentile criterion was
adopted for the distribution and identification of lipoprotein levels. The analysis of variance (ANOVA) and description tests with $p < 0.05$ were applied. Results: Participants had similar BMI $z$-score and physical activity habits in all groups. A significant reduction in TC and HDL-C concentrations between the start and the end of puberty was observed. LDL-C levels rose during stages 2 and 3 of development, decreasing at the end of the pubertal process. TG levels did not change significantly with pubertal stages. Conclusion: Lipid and lipoprotein concentrations tend to undergo changes during puberty in boys. The use of percentile values can be very useful to track variations in lipid and lipoprotein levels during the maturation process.

Keywords: maturation, lipid profile, lipoproteins.

**Background**

The evaluation of lipid profile in children and adolescents is important for early diagnosis of dyslipidemias, which are associated with the atherosclerotic process and becomes more evident in adulthood (BAO et al., 1997; FRIEDMAN et al., 2006). However, physiological changes are observed in the concentration of the lipid profile components, according to stage of sexual maturation (PORKKA et al., 1991). As a result, the scientific literature asks what would be the best cutoff points for diagnosis of dyslipidemias in the pediatric population, since the screening of children and adolescents has a low positive predictive value, but good negative predictive value (TASK FORCE, 2007; DANIELS, 2009).

The pubertal period brings important metabolic changes that may interfere in the adult health (MORRISON, 1978; GORAN and GOWER, 2001). It is a period of sexual maturation
marked by morpho-physiological changes (MALINA, 1994) that lasts for 4 to 5 years (TANNER, 1962), and can influence the lipid profile (PORKKA et al., 1997). Several studies have demonstrated increased dyslipidemia in obese and sedentary adolescents, ranging from 8% to 77% depending on the assessed lipoprotein (LEITE et al., 2008; JODIE et al., 2009). Lipoprotein abnormalities in European adolescents can be observed in studies of secular trends that report the increase of individuals with HDL-C levels below the recommended and with high TG concentrations (PORKKA et al., 1997; AGIRBASLI et al., 2012).

Although several authors emphasize that the development of cardiovascular disease can begin in childhood (BAO et al., 1997; FRIEDMAN et al., 2006), studies indicate the existence of a positive relationship between the beginning of sexual maturation and high TG and cholesterol levels in adulthood (KATON et al., 2009; PIERCE et al., 2010). Changes in the levels of lipoproteins during puberty differ between genders and may be responsible for dimorphisms between the sexes for cardiovascular diseases in adulthood, such as the decrease in HDL-C levels in boys and its stability in girls (BERENSON et al., 1997).

While some studies suggest that TG levels tend to remain unchanged (BERENSON et al., 1997; AZIZI, RAHMANI, MADJID, 2001), others point out to an increase in TG levels from the pre-pubertal to the pubertal period (JOLLIFE et al., 2007; HIRSCHLER et al., 2011). Recently, only a few studies in the literature focus on the biochemical changes in lipoproteins during childhood and adolescence (GIDDING, 2006; KOJIMA, 2005; TASK FORCE, 2007; DANIELS, 2009). There is the need for a better understanding of the lipoprotein behavior during puberty, which can lead to improved treatment and earlier diagnosis of cardiovascular disease risk. Therefore, this study aims to analyze the behavior of lipoproteins during the maturation process and identify cutoff points for diagnosis of dyslipidemia in male adolescents.
Methodology

Population and Sample

The random sample was composed of 662 male adolescents (10 to 17 years) from the CRIANSAÚDE program of the UFPR, which consisted of educational visits to the schools of Curitiba from 2010 to 2012. A total of 1,253,982 students were involved in this program, whose main goal was to educate adolescents about the importance of having an adequate diet and regular physical activity. During the visits, students were invited to participate in the study.

The study was approved by the ethics committee on human research at Clinical Hospital under the no. 1466.131/2007-06. The study was performed in accordance with the Declaration of Helsinki.

The sample calculation was based on the total number of students enrolled in each educational institution. A probabilistic analysis was performed, the sample size was evaluated according to the following criteria: a) total number of boys, b) confidence interval of 95%, c) 5% sampling error and 50% prevalence, since the prevalence of risk factors in this population is unknown (LUIZ and MAGNANINI, 2000). Foreseeing possible dropouts and loss of data during the evaluation period, as well as considering truancy rates of about 0.07% in the state of Paraná (IBGE, 2007), a total of 10% of assessed individuals for each gender was added, ensuring the representativeness of the sample. A minimum number of 650 individuals to represent the city of Curitiba was considered, but all students had equal opportunity to participate in the study. Parents and/or guardians received a term of informed consent for authorizing the adolescents participation.

Exclusion criteria were: failure to return the completed questionnaire; a family history of dyslipidemia or cardiovascular disease; TG levels greater than 400 mg/dl that could
influence the LDL-C calculation; type 1 diabetes mellitus or other health problem that could influence the assessments; the continuous use of medications that could affect the results of the blood biochemistry analysis; BMI z-score greater than 3, which is related to morbid obesity (SANTOS, 2001). Consequently, 92 adolescents were excluded from the final sample, whereas 570 met the inclusion criteria and were included in the study.

Some data were collected through the questionnaires send home to the parents/guardians about treatment of diseases and medications used by the, as well as family history of dyslipidemia, type 2 diabetes, cardiovascular disease and sudden death.

**Anthropometric measurements**

Height was measure by evaluating the greatest distance from the floor to the vertex of the head (DOCHERTY, 1996) via a portable vertical stadiometer (WCS®, Brazil) with a graduation of 0.1 cm. Body mass was measured with a Filizola® portable digital scale (Filizola, Brazil) with a resolution of 100 g. The technical errors of measurement and reliability were calculated (Mueller, 1988). The replicate measurements of anthropometric data were taken on 89 students. Reliability coefficients were 0.96 and 0.93 respectively.

In possession of this data, the BMI and the BMI z-score were calculated according to the CDC (2000). All variables were measured by expert professionals from the UFPR.

Self-assessment was used to determine sexual maturation, according to Tanner (1962). Study of Brazilian data on sexual maturation found 89% agreement between self-assessment and medical evaluation (MATSUDO and MATSUDO, 1994).

**Blood biochemical analysis**

Previously to collection of blood samples for determination of TC, HDL-C, LDL-C, TG and thyroid stimulating hormone (TSH), participants were instructed on the care measures preceding blood sampling, recommended by the Brazilian Society of Cardiology
fasting of at least 12 h; avoiding alcohol consumption for 3 days; avoiding excessive eating and physical activity the day before the test.

TC, HDL-C and TG determination was carried out through the automated enzymatic-colorimetric method, through an ABBOTT SPECTRUM®, CCX model apparatus. The LDL-C fraction obtained by the Friedewald equation: \( LDL-C = TC - HDL-C - TG/5 \) is recommended for TG concentrations below 400mg/dl (SANTOS, 2001). The TSH determination was carried out through the chemiluminescence method. TSH determination was used to exclude thyroid dysfunction as a cause of dyslipidemia and alterations in the anthropometric assessments. All blood samples were collect at the laboratory by a nurse.

The percentile classification system for lipoproteins in adolescents suggested by Jollife and Jassen (2006) was used to identify the distribution of lipoproteins during the maturation process: TC: borderline between 86th and 97th and high > 97th percentile; HDL-C: borderline between 26th and 13th and low > 13th percentile; LDL-C: borderline between 86th and 97th and high > 97th percentile; TG: borderline between 89th and 95th and high > 95th percentile. In the comparative analysis, the cutoff points for dyslipidemia recommended by the I Guideline for Prevention of Atherosclerosis in Childhood and Adolescence (SBC, 2005) for Brazilian adolescents was used: TC: borderline between 150 and169 mg/dl and high > 170 mg/dl; HDL-C > 45 mg/dl; LDL-C: borderline between 100 and129 mg/dl and high > 130 mg/dl; TG: borderline between 100 and 129 mg/dl and high > 130 mg/dl.

**Statistics**

Data were expressed as mean and standard deviation. Analysis of variance multivariate (MANOVA) was used to compare the groups and with age as covariate. The percentile frequency distribution was applied. Analyses were performed in the SPSS 13.0 software, with a significance level of p <0.05.
Results and Discussion

The participants of this study were classified according to the Tanner stages: 7% pre-pubertal, 80% pubertal and 13% post-pubertal. Table 1 shows the results of the variables according to the maturation stage.

Table 1. Characterization of the male sample according to the maturation stage.

Body mass and height increased over the maturation stages, corroborating results found in the literature (MALINA, 2000; ZEFERINO et al., 2003). However, there were no significant differences in the BMI z-score across the maturation process in the sample of this study. This index adjusts changes in BMI considering age and gender, which allowed the comparison of lipid profile across the different maturation stages, demonstrating that there was no variability in body mass between the groups beyond the expected for growth. Furthermore, in relation to the BMI classification, both obesity (15%) and overweight (20%) rates are consistent with the IBGE (2010) national rates, which correspond to 13% and 24%, respectively; therefore, participants are representative of the BMI profile for Brazilian adolescents.

The average TC and HDL-C levels decreased significantly at the end of puberty. LDL-C levels were higher during the beginning of puberty, in maturation stages 2 and 3, subsequently returning to levels similar to the first stage at the end of puberty. No significant differences were found for TG across the different stages.

In the literature, the maturation stages are gathered in two groups (pre-pubertal and pubertal), making it difficult to compare the results of this study, because the mean values were found by considering each maturation stage. However, Hirschler and colleagues (2011) found similar results when studying Argentine adolescents. The authors observed a
decreasing trend in TC and LDL-C levels, and the maintenance of HDL-C and TG levels in boys during puberty. Another study (KATON et al., 2009) with Mexican adolescents divided into pre-pubescent and pubescent showed a reduction in HDL-C, LDL-C and TG levels with the advance of sexual maturation in boys, regardless of being early or not. These results are similar to our study, except for TG.

The decrease in TC levels at the end of puberty may be due to the reduction of HDL-C (MORRISON et al., 1978; KATON et al., 2009). In a study involving Chinese adolescents, Chen and colleagues (2009) found that lipoproteins differ when analyzed before and after puberty for TC and LDL-C, noting that lipoprotein genetic influences range from 49% to 86% during puberty. However, after the pubertal period, environmental factors become more important, which would explain the post-pubertal decreases reported in the literature. In the lipid profile, the TG manifests a greater environmental than genetic influence, which would explain the lack of significance for TG found in this study, considering that the participants were from similar environments.

The monitoring of lipoproteins may lead to a better understanding of their behavior during the sexual maturation process, similarly to the growth curves for height, weight and BMI, already familiar to pediatricians and health workers. This could bring a new light to the clinical practice, in relation to what is considered a health risk. Table 2 shows the lipoprotein percentile distribution according to the classification system for adolescents by Jollife and Jassen (2006).

Table 2. Specific cutoff points by maturation stages and corresponding values according to Jollife and Jassen (2006).
The results suggest a variation of percentile values during the maturation process. It is observed that the TC cutoff values are reduced in the course of the maturation process. Regarding HDL-C, the borderline values (26th) show stability, but the values considered low (13th) decrease considerably with puberty. As for LDL-C, in pubertal stages 2 and 3, the concentrations increase for both the borderline (86th) and high (98th) values, although the borderline values fall from stages 4 and 5, and the high values remain on the rise. TG are higher in the pre-pubescent period and decline with puberty, stabilizing at half way into the maturation process.

Pinhas-Hamiel et al. (2007) and Hirschler et al. (2011) showed that in eutrophic children, the TC and LDL-C levels tend to decrease during puberty, corroborating this study. Jollife et al. (2007) showed that these lipoproteins decrease during adolescence, rising in adulthood.

As for HDL-C, borderline values shows little changes between the maturation stages, but reduced to the low cutoff point (13th) with a variation of 7mg/dl at the end of puberty. This decrease is concerning, because studies have shown that a 5mg/dl drop in HDL-C is associated with a 14% increase in risk of cardiovascular events (GOTTO, 2000). On the other hand, an increase of 1mg/dl is associated with cardiovascular risk reduction between 2% and 3% (BREWER, 2004).

Thus, reduced HDL-C levels should be carefully considered, because there is a high correlation with cardiovascular risk factors (WANG and PENG, 2011). The authors describe likely inflammation associated with excess body fat as responsible for the raising of cholesterol levels, justified by the reduction of expression ABCA1 and apo A-1, which consequently leads to the reduction of HDL-3, altering the performance of the reverse transport of esterified cholesterol (WANG and PENG, 2011).
According to Friedman and colleagues (2006), LDL-C shows sensitivity of cardiovascular changes in the order of 69% at 10 years of age, decreasing to 22% between 14 and 16 years. The authors also point out that this loss of sensitivity is related to the lack of longitudinal data on lipoprotein behavior to determine pediatric values that can infer complications in adulthood.

In this sense, the present study corroborates Friedman and colleagues (2006), as increases in the percentile values of LDL-C were observed with the advance of puberty, suggesting a possible need for cutoff points related to the pubertal stage. Generally, TG showed the lowest oscillation throughout the maturation process. Special attention should be given to TG during puberty and its outcomes in cardiovascular risk factors in adulthood, because as demonstrated by Chen et al. (2009), the variation in TG values is related to environmental factors.

When considering the cutoff points recommended for the lipid profile in the Brazilian population (SBC, 2005), the adolescents taking part in this research showed great TC concentration fluctuating between borderline values throughout puberty (graphics 1). For HDL-C, only half of the participants showed results above the recommended values, which declined with the advance of the maturation process (graphics 2). However, LDL-C concentrations were above the borderline values in individuals in the P2 and P3 pubertal stages (graphics 3). In turn, TG appears to remain constant throughout puberty in relation to the number of individuals with borderline or high values (graphics 4). The comparison with the Brazilian literature is compromised, since this is the first study attempting to identify changes in lipoproteins in relation to the pubertal stages and the cutoff points suggested for the Brazilian pediatric population.

The present study has some limitations regarding the cross analysis, data collection, utilization of self-report sexual maturation and the small sample of pre-pubertal individuals,
because the total pubertal data was not calculated, therefore suggesting further longitudinal studies involving a higher number of participants and clinical pubertal analyses. It should be emphasized that this study involves a number of overweight and obese individuals proportional to the national average rates for children and adolescents, as well as no difference in BMI z-scores between the five maturation stages, allowing to infer that the behavior of this sample is extrapolated to other Brazilian regions.

**Conclusion**

This study described the behavior of lipids and lipoproteins according to sexual maturation in individuals who had similar BMI z-scores. It was observed that TC and HDL-C levels decrease at the end of boys puberty, whereas LDL-C levels rise during stages 2 and 3 of sexual maturation, returning to values similar to those found in the pre-pubertal period at the end of puberty. TG did not change during the maturation process, thus demonstrating that the lipids and lipoproteins behave differently during boy’s puberty. It is suggested that the percentile values of cutoff point by sexual maturation can be of great value for clinical monitoring during adolescence.

Contribution of the authors:

LPGM - data collection, statistics, manuscript preparation

NL - manuscript preparation

DCM - data collection, manuscript preparation

ACKT - data collection, manuscript preparation

LMSB - data collection, manuscript preparation
All authors read and approved the final manuscript.

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Reference


Jolliffe CJ, Jassen I: **Development of age-specific adolescents metabolic syndrome criteria that are linked to the adult treatment panel and international diabetes federation criteria.** Journal of the American college of cardiology 2007, 49(8):891-98.


“The author(s) declare that they have no competing interests”
Table 1. Characterization of the male sample according to the maturation stage.

<table>
<thead>
<tr>
<th>Pubertal stage</th>
<th>1 (n=39)</th>
<th>2 (n=159)</th>
<th>3 (n=130)</th>
<th>4 (n=168)</th>
<th>5 (n=74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.45±0.95</td>
<td>12.17±1.42</td>
<td>12.91±1.34</td>
<td>14.71±1.51</td>
<td>15.83±1.27</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.05±15.63</td>
<td>47.26±14.91</td>
<td>49.14±12.16</td>
<td>61.28±14.27</td>
<td>63.92±10.57</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.49±0.09</td>
<td>1.51±0.11</td>
<td>1.55±0.10</td>
<td>1.69±0.10</td>
<td>1.72±0.07</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.65±1.41</td>
<td>0.64±1.45</td>
<td>0.52±1.16</td>
<td>0.33±1.20</td>
<td>0.23±0.98</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>160.12±30.89</td>
<td>162.16±30.23</td>
<td>161.64±29.16</td>
<td>154.32±29.33</td>
<td>146.81±34.30</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>46.28±10.32</td>
<td>46.05±8.90</td>
<td>46.56±10.80</td>
<td>44.26±10.98</td>
<td>41.19±9.30</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>96.12±28.70</td>
<td>104.51±32.59</td>
<td>105.24±32.90</td>
<td>96.76±29.71</td>
<td>91.33±28.82</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>102.50±58.45</td>
<td>88.81±53.69</td>
<td>84.22±46.40</td>
<td>84.93±43.52</td>
<td>87.90±42.10</td>
</tr>
</tbody>
</table>

a- different from stage 1; b- different from stage 2; c- different from stage 3; d- different from stage 4; e- different from stage 5.
Table 2. Specific cutoff points by maturation stages and corresponding values according to Jollife and Jassen (2006).

<table>
<thead>
<tr>
<th>Percentile</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75&lt;sup&gt;th&lt;/sup&gt;</td>
<td>86&lt;sup&gt;th&lt;/sup&gt;</td>
<td>97&lt;sup&gt;th&lt;/sup&gt;</td>
<td>26&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stage 1</td>
<td>172</td>
<td>212</td>
<td>224</td>
<td>38</td>
</tr>
<tr>
<td>Stage 2</td>
<td>187</td>
<td>191</td>
<td>221</td>
<td>40</td>
</tr>
<tr>
<td>Stage 3</td>
<td>182</td>
<td>192</td>
<td>222</td>
<td>38</td>
</tr>
<tr>
<td>Stage 4</td>
<td>169</td>
<td>182</td>
<td>217</td>
<td>37</td>
</tr>
<tr>
<td>Stage 5</td>
<td>168</td>
<td>181</td>
<td>213</td>
<td>36</td>
</tr>
</tbody>
</table>
Graph 1. Variations in TC levels during puberty

Graph 2. Variations in HDL-C levels during puberty

----- = borderline levels; ______ = high levels

______ = low levels
Graph 3. Variations in LDL-C levels during puberty

Graph 4. Variations in TG levels during puberty

------ = borderline levels; _____ = high levels
Additional files provided with this submission:

Additional file 1: Authors¿ contributions.doc, 1673K
http://www.biomedcentral.com/imedia/2876136999846041/supp1.doc