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Serum uric acid in children and adolescents

Valores de referência do ácido úrico em crianças e adolescentes

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ABSTRACT

Introduction: Uric acid (UrA) is a product of purine catabolism, and hyperuricemia (hUrA) is associated with risk factors for cardiometabolic diseases. Objective: To evaluate the concentration of UrA in children and adolescents. Methods: Cross-sectional study with 623 eutrophic students (5 to 15 years old, aged 9.9 ± 2.7 years, 52% girls). Blood was collected (fasting 12-14 h) for analysis of laboratory parameters, and blood pressure and anthropometric measures were verified. UrA was stratified according to sex and age ranges (5 to < 10, ≥ 10 to < 13 and ≥ 13 to 15 years, male; and 5 to < 9, ≥ 9 to < 12 and ≥ 12 to 15 years, female), and the percentiles 2.5 (2.5th) and 97.5 (97.5th) were calculated. Results: The mean UrA was 3.7 ± 1.03 mg/dl (boys) and 3.58 ± 0.91 mg/dl (girls) (p = 0.0113). Considering the age ranges, the mean UrA was increasing and higher for boys (p = 0.0024, for the 3rd age range). For girls, the UrA increased progressively and significantly in the age ranges (p ≤ 0.005). According to the 97.5th, there was statistical difference only in the third range between sexes (p = 0.002). For comparisons between age ranges, UrA 97.5th also increased for boys and girls (p ≤ 0.05). According to the 97.5th, 26 students presented hUrA. Conclusion: According to the results, stratification by age ranges and sex, in addition to the 97.5th as concentration threshold, was important for evaluation of serum levels of UrA in children and adolescents.

Key words: uric acid; child; adolescent; reference values; hyperuricemia.

INTRODUCTION

Uric acid (UrA) is an organic compound, product of purine catabolism, through the catalytic effect of xanthine oxidase. Variation in serum UrA level is multifactorial and comes under the influence of environmental and genetic factors. The excretion of approximately two-thirds is through the kidneys; the remaining one-third, through the intestinal tract. Despite the apparent antioxidant activity of UrA, the results of some studies highlighted the association of hyperuricemia (hUrA) with several risk factors for cardiometabolic diseases (CMD), including hypertension, diabetes, endothelial dysfunction, obesity, and metabolic syndrome, both in adults and in children and adolescents.

In childhood and adolescence, the presence of hUrA can pose a serious risk of CMD, and it can persist or become more evident until adult life. For this reason, early identification is important for the adoption of prevention measures in the youth population. However, although the reference values for children and adolescents are established, the guidelines of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) suggest the determination of the reference interval by obtaining the 95 percentile (95th) value for the different populations, also considering their peculiarities, age and sex, especially among children.

Thus, the objective of the present study was to assess the serum UrA concentration in children and adolescents, who were stratified by age ranges and by sex. The 95th percentile was calculated to obtain the hUrA threshold for the participating students.
This is a cross-sectional study covering 1,813 students, what accounts for 67.7% of the total of students regularly enrolled in elementary education (1st to 9th year) in public schools of the municipalities of Botuverá, Major Gercino, and Guabiruba, in Santa Catarina (SC), Brazil. Participants were students self-reported Caucasians, who did not suffer from any disease, did not use any medication, and were aged between 5 and 15 years.

For calculation of the 2.5th and 97.5th percentiles of UrA, students with overweight, obesity, increased waist circumference (WC), altered blood pressure, insulin resistance, hypertriglyceridemia, and low levels of high-density lipoprotein cholesterol (HDL-C) were not included. Therefore, the students considered eutrophic amounted to 623 (299 boys and 324 girls).

Blood samples (12-14 h fast) were collected from the median antecubital vein (10 ml) in tubes with no additives or with sodium fluoride and ethylenediaminetetraacetic acid (EDTA), which were immediately centrifuged (1500 × g, 10 min). It is worth noting that nowadays fasting is not required for assessing lipid profile(13). However, upon the study conduction, the new standardization had not taken place yet. Serum and plasma were stored at -20°C during less than three months for further biochemical analysis.

Methodology

Serum biochemical analytes, total cholesterol (TC), triglycerides (TG), glucose, and UrA were measured by enzymatic colorimetric methods (oxidase/peroxidase); creatinine was determined by the Jaffe’s method after reaction with picrate ions in an alkaline medium by a two-point kinetic reaction; HDL-C, by a homogeneous method (Biotécnica, Varginha-MG), at an automated equipment (BTS 370 PLUS BioSystems, Connecticut-EUA) according to the instructions by the manufacturer. Low-density lipoprotein cholesterol (LDL-C) was calculated according to the formula by Martin et al. (2013)13: LDL-C = TC – HDL-C – (TG/×), with “×” being the factor resulting from non-HDL-C concentrations. Insulin and high-sensitivity C-reactive protein (hsCRP) were measured by an enzyme-labeled chemiluminescent immunometric assay, with an Immulite 2000 analyzer (Siemens Healthcare, United Kingdom).

Insulin resistance (IR) was evaluated by the homeostatic model assessment of insulin resistance index (HOMA-IR), using the formula: serum insulin (μU/ml) × serum glucose (mg/dl)/40513.

Blood pressure (BP) was recorded using cuff and sphygmomanometer, with students on the seated position(15). Initially, a five-minute rest was observed, and then two measures were taken with a five-minute interval, choosing the lowest measure.

Weight (kg) and height (m) were measured using a scale with capacity of 200 kg and precision of 100 g and stadiometer with a capacity of 2 m and precision of 0.5 cm (Welmy, São Paulo-SP). Body mass index (BMI) was calculated according to the formula: BMI = weight (kg)/height (m²). Waist circumference (WC) was determined using the measure between the lower rib and the upper border of the iliac crest, with flexible inelastic measuring tape, as described by Taylor et al. (2000)(10).

The cut-off points recommended by I Diretriz Brasileira para a Prevenção da Aterosclerose em Crianças e Adolescentes were observed for lipid parameters (TC > 150 mg/dl and HDL-C < 45 mg/dl), glucose (≥ 100 mg/dl) and HOMA-IR (> 3.16)(17). For UrA, the 97.5th percentile stratified by sex and age range (5 to < 10, ≥ 10 to < 13 and ≥ 13 to 15 years, male; and 5 to < 9, ≥ 9 to < 12 and ≥ 12 to 15 years, female) was considered. For the classification of pre-hypertension and stages 1 and 2 hypertension, the cut-off points stratified by sex, age and height17 were considered. The BMI percentile was used for the classification of overweight (85th-94th percentile) and obesity (≥ 95th percentile), according to the BMI Percentile Calculator for Child and Teen of the Centers for Disease Control and Prevention (CDC)(18), while high WC was verified according to the reference table of Taylor et al. (2000)(10), by both sex and age.

The study was approved by the Human Research Ethics Committee of Universidade Federal de Santa Catarina (UFSC) (CAAE: 26960914.6.0000.0121), and participants and their legal guardians signed the consent form. Subjects who reported the use of medication or did not present the consent form were excluded from the study.

Statistical analysis

Categorical results are quantitatively presented as mean and standard deviation (SD), or median and interquartile range. For statistical analysis of the variables, data were first tested for normality (Kolmogorov-Smirnov). Transformation was used for quantitative data without normal distribution. Student’s t test or Mann-Whitney test were applied when data were normally distributed or not, respectively. For evaluation of UrA in the age ranges, the paired t test was used for mean analysis, the 2.5th and
97.5th percentiles, intragroup and between boys and girls. For establishing reference limits for UrA by the 95th percentile, the interval between 2.5th and 97.5th percentiles was used, based on the "robust method", as recommended for a sample size smaller than 120.

All analyses were performed in the software MedCalc version 12.3.0.0 (MedCalc Software bvba 1993-2012, Broekstraat 52, 9030 Mariakerke, Belgium).

**RESULTS**

In the 623 eutrophic analyzed students (mean age 9.9 ± 2.7 years), 52% were females. Alteration in renal function was not observed by means of measurement of serum creatinine (results not shown). The mean concentration of UrA found in the population of students assessed was 3.63 ± 0.97 mg/dl. In the group stratification by sex, there was a statistically significant difference for UrA (p = 0.0113), glucose (p = 0.0488) and insulin (p = 0.0063), demonstrated in Table 1.

The mean serum concentration of UrA was increasing in the different age ranges in both sexes, and significant intragroup differences were observed for all ranges (Table 2). According to sex, boys presented mean UrA higher than girls in all ranges, but with significant difference just in the third age range (p = 0.0024; Table 2).

In relation to the 97.5th percentile, a statistically significant difference was verified between boys and girls just in the third age range (p = 0.0024). Based on intragroup comparisons, there was significant increased UrA concentration for girls in the third age range compared with the first (p = 0.0001) and second ranges (p = 0.008), while for the boys the difference occurred among all age ranges (first vs. second: p < 0.05; first vs. third: p < 0.0001; second vs. third: p < 0.001).

Using the 97.5th percentile as UrA cut-off value, 26 students presented hUrA.

### TABLE 1 – Biodemographic and laboratorial characteristics of 623 eutrophic students

<table>
<thead>
<tr>
<th></th>
<th>General</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>9.9 ± 2.7</td>
<td>9.8 ± 2.7</td>
<td>10.1 ± 2.7</td>
<td>0.132</td>
</tr>
<tr>
<td>UrA (mg/dl)</td>
<td>3.63 ± 0.97</td>
<td>3.7 ± 1.03</td>
<td>3.58 ± 0.91</td>
<td>0.0113</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>89.8 (84-95.8)</td>
<td>90.4 (84.7-97)</td>
<td>89 (83.3-94.9)</td>
<td>0.0488</td>
</tr>
<tr>
<td>Insulin (µM/ml)</td>
<td>3.4 (2.2-5.88)</td>
<td>2.93 (2.19-5.1)</td>
<td>3.91 (2.27-6.97)</td>
<td>0.0063</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.8 (0.49-1.4)</td>
<td>0.7 (0.5-1.16)</td>
<td>0.89 (0.49-1.61)</td>
<td>0.0839</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>162.5 (146.5-184.1)</td>
<td>160.7 (146.9-182.5)</td>
<td>163.7 (146.3-184.6)</td>
<td>0.4297</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>54.8 (49.9-60.7)</td>
<td>53.5 (48.8-61.1)</td>
<td>54.4 (49.4-60.1)</td>
<td>0.5826</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>92.5 (77.7-110.5)</td>
<td>92.5 (77.7-110)</td>
<td>95.3 (78.3-111.6)</td>
<td>0.6904</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>61.5 (49.7-73.5)</td>
<td>60.2 (47.8-73.4)</td>
<td>62.2 (52.3-77.1)</td>
<td>0.0586</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>0.32 (0.21-0.64)</td>
<td>0.31 (0.22-0.69)</td>
<td>0.32 (0.21-0.63)</td>
<td>0.397</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>43 (32-65)</td>
<td>44 (30-65.5)</td>
<td>41 (22-66)</td>
<td>0.1977</td>
</tr>
</tbody>
</table>

Results are quantitatively presented as mean and standard deviation (SD), or median and interquartile range.

UrA: uric acid; HOMA-IR: homeostatic model assessment of insulin resistance index; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; hsCRP: high-sensitivity C-reactive protein; BMI: body mass index; *students with overweight or obesity, increased waist circumference, high blood pressure, insulin resistance, hypertriglyceridemia and decreased HDL-C levels were excluded.

### TABLE 2 – Limits for the reference interval at the 95th percentile and mean UrA concentrations (mg/dl) in eutrophic children and adolescents by sex and age range

<table>
<thead>
<tr>
<th>Age range</th>
<th>2.5th</th>
<th>97.5th</th>
<th>Mean ± SD</th>
<th>2.5th</th>
<th>97.5th</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>148</td>
<td>1.57</td>
<td>5.14</td>
<td>105</td>
<td>1.56</td>
<td>5.01</td>
</tr>
<tr>
<td>Second</td>
<td>97</td>
<td>1.83</td>
<td>5.48</td>
<td>112</td>
<td>1.65</td>
<td>5.3</td>
</tr>
<tr>
<td>Third</td>
<td>54</td>
<td>1.86</td>
<td>6.86</td>
<td>107</td>
<td>2.02</td>
<td>5.58</td>
</tr>
</tbody>
</table>

Results are presented in 2.5th, 97.5th and mean and SD. Age range: male – first: 5 to < 10; second: ≥ 10 to < 13; third: ≥ 13 to 15; female: first: 5 to < 9; second: ≥ 9 to < 12; third: ≥ 12 to 15. *p = 0.0024 in comparison with the respective percentile and the mean of the male group; †p < 0.008 in comparison with the other age ranges of the female group; **p < 0.0005 in comparison with the other age ranges of the respective group; ‡p < 0.001 and ††p < 0.05 in comparison with the first age range of the male group. *p < 0.0001 in comparison with the first age range of the male group.

UrA: uric acid; SD: standard deviation.
In the present study, the evaluation of UrA in the 623 eutrophic students, from a population of 1,813 individuals (aged 5-15 years), stratified by age ranges and sex, resulted from the exclusion of those presenting alterations in parameters associated with hUrA. Among them, excess body weight, evaluated by BMI and increased WC, whose association is linked to insulin resistance and higher leptin production, with both reducing UrA renal excretion\(^\text{(19)}\). From the current analysis were also excluded students with insulin resistance, hypertriglyceridemia, and low HDL levels, besides high blood pressure, because these components, as well as increased WC, are used for classification of metabolic syndrome and associated with hUrA\(^\text{(5-5)}\).

The use of age ranges has as indicative the three intimately related interactive processes that occur in children and adolescents: growth, maturation, and development\(^\text{(20, 21)}\). According to the review by Rogol \textit{et al.} (2002)\(^\text{(22)}\), in the pre-pubertal period or childhood, development is a relatively stable process, with growth being more accentuated because of growth hormone axis (GH) and insulin-like growth factor type 1 (IGF-1), with similarities in the secretion pattern between boys and girls. In fact, in the current study, the first and second age ranges showed no significant difference in the UrA concentration between boys (3.41 mg/dl and 3.68 mg/dl, respectively) and girls (3.38 mg/dl and 3.52 mg/dl, respectively), as described by Rogol \textit{et al.} (2002)\(^\text{(22)}\).

On the other hand, puberty is a period of dynamic development marked by body changes, more specifically in size, shape and composition, characterized by strong sexual dimorphism with onset approximately at 13 years on boys and 11 years on girls\(^\text{(22)}\). In the assessed students, a significant elevation was observed in boys (4.41 mg/dl) and girls (3.85 mg/dl) in the third range, in comparison with the other age ranges, corresponding to 13 years in the boys and 12 years in the girls. Adeli \textit{et al.} (2015)\(^\text{(23)}\) observed the difference beginning at 13 years (5.7 mg/dl and 4.1 mg/dl, males and females, respectively), although those authors considered the median in the interval between 13 and 79 years in both sexes.

Shatat \textit{et al.} (2012)\(^\text{(24)}\) found an average of 5.14 ± 1.45 mg/dl of UrA in 1,912 students aged 13-18 years in the United States of America, a figure higher than the observed in our study for the third range (4.02 ± 1.03 mg/dl, result not shown). It is noted that the authors included in the evaluated population those whose characteristics were the exclusion criteria in our study. However, similarly to our research, those authors observed increased mean UrA in relation to age and sex. Similar results were reported by Adeli \textit{et al.} (2015)\(^\text{(25)}\); and Stapleton \textit{et al.} (1978)\(^\text{(26)}\) had already reported that the serum value of UrA increases with age during childhood and differs between sexes in puberty.

The World Health Organization (WHO) defines adolescence as the interval between 10 and 19 years\(^\text{(25)}\), while in Brazil the Estatuto da Criança e do Adolescente (ECA – Child and Youth Statute) considers child the individual younger than 12 years, and adolescents, those in the 12-18 age group\(^\text{(20)}\). Tanner’s classification, widely used for pubertal staging, uses 8 years as initial age\(^\text{(27)}\). The review by Lourenço and Queiroz (2010)\(^\text{(28)}\) describes the occurrence of thelarche on average at 9.7 years, and menarche at 12.2 years, while testicular enlargement starts at 10.9 years\(^\text{(28)}\).

Based on our results, mean serum concentrations of UrA increased in each age range (3.41, 3.69 and 4.41 mg/dl, males; and 3.38, 3.52 and 3.85 mg/dl, females, respectively, in the first, second and third ranges), but with significant difference between sexes just in the third range. Lockith \textit{et al.} (1988)\(^\text{(29)}\) and Burritt \textit{et al.} (1990)\(^\text{(30)}\) verified that up to age 9 there was no significant variation in serum UrA concentration between sexes, with difference appearing just at age 10; while Adeli \textit{et al.} (2015)\(^\text{(23)}\), in the Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) study, observed such difference from 13 years. Thus, the considerations found in the literature and the age of the population of students we assessed (5-15 years), besides the values found in our study, were essential for the adoption of the age ranges.

By contrast, IFCC highlights that in order to employ biomarkers for screening or diagnosis, one must consider, among other factors, the reference value to be used, and those should be evaluated in populations without pathological alterations, besides age ranges and sex. In the child population, especially, the interfering factors can make even major alterations in analyte concentrations, as, for instance, ethnicity in the face of world diversity. Nevertheless, because of temporal progression, factors connected with organic maturity, immune and hormonal, nutritional and metabolic responses may contribute more significantly\(^\text{(31, 32)}\). In this context, establishing the reference interval in the child population for certain laboratory parameters, considering population and region, besides age and sex, can be fundamental\(^\text{(12, 32)}\).

Accordingly, the UrA 97.5\textsuperscript{th} percentile of the population here evaluated and the values of the first range (5.14 mg/dl for males.
and 5.01 mg/dl for females) and second range (5.48 mg/dl, males and 5.3 mg/dl, females), although increasing, did not present statistically significant differences in the comparison between boys and girls. Our results are similar to that observed in the study by Colantonio et al. (2012)\(^{33}\), in which values were the same (4.9 mg/dl) for boys and girls. At the same time, in that study there was no stratification in different ranges, that is, the interval was 1-12 years, for both sexes. In the third age range, 13 years for boys (6.86 mg/dl) and 12 years for girls (5.58 mg/dl), values were lower than those in the study by Colantonio et al. (2015)\(^{35}\), 7.6 mg/dl and 5.9 mg/dl, for boys and girls, respectively. In the study by Adeli et al. (2015)\(^{31}\), 5.7 mg/dl and 4.1 mg/dl, respectively, for boys and girls, values were lower than our finding, but the authors considered the range aged 13-70 years.

The process of human development is marked by numerous transformations up to the 20 years of age. Biological changes have universal character and represent a phenomenon common to all individuals in that phase of life\(^{22}\). Guedes (2011)\(^{34}\) highlights that chronologic age is not always coinciding with biological age, which depends on the maturational stage, and the beginning of puberty tends to be earlier in the male sex\(^{22}\). The use of age ranges and the 97.5th percentile as cut-off for individuals here evaluated identified higher number of students with hUrA (26 individuals; 5.6%) in comparison with those described by Asayama et al. (2003)\(^{9}\) (< 6 mg/dl) (12 individuals), by Feig and Johnson (2003)\(^{17}\) (< 5.5 mg/dl) (22 individuals) and by Oyama et al. (2006)\(^{10}\) (< 7 mg/dl) (just one student with hUrA). Yet, Ferraz and Delgado (1988)\(^{40}\) detected 29 individuals with hUrA, using the cut-off points < 6 mg/dl, for males and < 5 mg/dl, for females.

Therefore, in the present study, the serum concentration of UrA in children and adolescents was observed to undergo growing alteration associated with age and sex, as verified in other studies\(^{25, 28, 29}\) and it seems to be more evident when stratified in age ranges, according to our results. Besides, the adoption of the 97.5th percentile for this population revealed important hUrA prevalence.

**STRENGTHS AND WEAKNESSES**

The current study presents limitations, as the use of chronologic age as time delimiter, for age is not a good indicator of biological maturity, was not chosen because of its difficult to use (self-assessment or assessment by a medical professional, this one can cause embarrassment and non-participation of children). Besides that, food profile and socioeconomic status of the students, which can contribute to serum UrA value\(^{35}\), were not evaluated. It must be highlighted, though, that the evaluated municipalities are not in an underdeveloped region, and undernourished students were not found, including because all schools serve meals that are prescribed by dietitians. By the same token, the exclusion of students that presented altered factors associated with hUrA, in spite of reducing the number of participants, can be considered a strength of the study, because really healthy students were evaluated.

**CONCLUSION**

The presented results suggest that stratification by age range and sex, besides the 97.5th percentile as threshold for hUrA determination, is fundamental for evaluation of UrA in children and adolescents. The new threshold permitted relevant identification of hUrA in the children and adolescents assessed. However, studies with new populations and with higher representativeness can contribute to confirm findings.

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RESUMO

Introdução: O ácido úrico (AUr) é um produto do catabolismo das purinas, e a hiperuricemia (hAUr) associa-se a fatores de risco para doenças cardiometabólicas. Objetivo: Avaliar a concentração de AUr em crianças e adolescentes. Métodos: Estudo transversal com 623 estudantes eutróficos (5 a 15 anos de idade; 9,9 ± 2,7 anos; 52% meninas). Foram coletados sangues (jejum 12-14 h) para analise de parâmetros laboratoriais e foram aferidas pressão arterial e medidas antropométricas. AUr foi estratificado segundo sexo e faixas etárias (5 a < 10, ≥ 10 a < 13 e ≥ 13 a 15 anos, masculino; 5 a < 9, ≥ 9 a < 12 e ≥ 12 a 15 anos, feminino) e foram calculados os percentis 2,5 (2,5th) e 97,5 (97,5th). Resultados: A média de AUr foi de 3,7 ± 1,03 mg/dl (meninos) e 3,58 ± 0,91 mg/dl (meninas) (p = 0,0113). Considerando as faixas etárias, a média de AUr foi crescente e superior nos meninos (p = 0,0024, para terceira faixa). Nas meninas, o AUr aumentou progressiva e significativamente nas faixas etárias (p ≤ 0,005). Segundo o 97,5th, houve diferença estatística somente na terceira faixa entre os sexos (p = 0,002). Nas comparações entre faixas etárias, o 97,5th do AUr também aumentou para meninos e meninas (p = 0,05). Segundo o 97,5th, 26 estudantes apresentaram hAUr. Conclusão: A estratificação por faixas etárias e sexo, além do 97,5th como limiar de concentração, foi importante para avaliação da concentração sérica do AUr em crianças e adolescentes.

Unitermos: ácido úrico; criança; adolescente; valores de referência; hiperuricemia.

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