Uric acid is associated with features of insulin resistance syndrome in obese children at prepubertal stage

M. Gil-Campos¹, C. M.ª Aguilera², R. Cañete¹ y A. Gil²


Abstract

Elevated plasma uric acid levels are associated with obesity and could be an expression of insulin-resistant state. The aim of the present study was to evaluate plasma uric acid in obese and normal-weight children exclusively at prepubertal stage and its relationship with anthropometric measurements, intake, and features of the insulin resistance syndrome. A study was performed in 34 obese and 20 normal-weight prepubertal children. Nutrient intake was determined using a 72 h recall questionnaire and a consumption food frequency questionnaire. Anthropometric parameters and fasting plasma lipids, glucose, insulin, leptin, adiponectin, tumour necrosis factor (TNF-α) and uric acid were measured. Multiple regression analysis was used to identify association of anthropometric parameters, nutrient intake and insulin resistance syndrome variables (arterial blood pressure, plasma glucose, insulin, homeostasis model assessment of insulin resistance index- HOMA- triacylglycerols and, HDL-cholesterol) with uric acid. Plasma uric concentration was significantly higher in the obese group than in the control group and when adjusted by sex, age and BMI was positively associated with tricipital skinfold and insulin resistance, and negatively with adiponectin. In multiple regression analysis, BMI, HDL-cholesterol and adiponectin were independent predictors of plasma uric acid. In conclusion, elevated levels of uric acid in obese children, compared with lean subjects, at the prepubertal period, seems to be an early metabolic alteration that is associated with other features of insulin resistance syndrome.

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Key words: Adipoquines. Childhood obesity. Insulin resistance syndrome. Uric acid.

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Resumen

Los niveles elevados de ácido úrico plasmáticos se asocian a la obesidad y pueden ser la expresión de un estado de resistencia insulínica. El objetivo de este estudio ha sido evaluar la concentración plasmática de ácido úrico en niños obesos y normales, exclusivamente en edad prepuberal, y determinar su relación con las medidas antropométricas, la ingesta dietética y los parámetros asociados al síndrome de resistencia insulínica. En el estudio se llevó a cabo en 34 niños obesos y 20 controles en edad prepuberal a los cuales se les estimó su ingesta dietética mediante el registro de un cuestionario de alimentos de 72 h y un cuestionario de frecuencia de consumo de alimentos y se determinaron, además de los parámetros antropométricos, la glucosa, la insulina, la leptina, la adiponectina y el factor de necrosis tumoral alfa (TNF-α) plasmáticos. Se realizó un análisis de regresión múltiple para identificar la asociación entre los niveles de ácido úrico y los parámetros antropométricos, la ingesta de nutrientes y las variables clásicas relacionadas con el síndrome de resistencia insulínica (hipertensión, glu cosa, insulina, índice de resistencia insulínica HOMA, tri glicéridos y, HDL-cholesterol plasmáticos), así como con leptina, adiponectina y TNF-α. La concentración plasmática de ácido úrico fue significativamente más elevada en los niños obesos que en los controles y cuando se ajustó por sexo, edad e índice de masa corporal, los niveles de ácido úrico se asociaron con el pliegue tricipital y la resistencia insulínica, y negativamente con la adiponectina. En el análisis de regresión múltiple, el índice de masa corporal, el HDL-colesterol y la adiponectina fueron predictores independientes del ácido úrico plasmático. En conclusión, los niveles elevados de ácido úrico en niños obesos en edad prepuberal, comparado con los de los niños normales, representan una alteración metabólica temprana asociada con la resistencia insulínica.

Palabras clave: Adipoquines. Obesidad infantil. Síndrome de resistencia insulínica. Ácido úrico.

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Introduction

Uric acid is the main product of purine metabolism and is formed from xanthine by the action of xanthine oxidase. Normal serum uric acid levels are generally 6.5-7 mg/100 ml for men, and 6-6.5 mg/100 ml for women, frequently expressed as mg %. The limit of uric acid solubility in extracellular fluids is 7.0 mg/dl, and patients with higher serum concentrations are considered hyperuricemic.3

Uric acid is primarily excreted via the kidney, where it is completely filtered at the glomerulus, fully reabsorbed into the proximal tubule, and then secreted (about 50% of the filtered load) and again reabsorbed. Elevated serum uric acid concentrations can result from an overproduction of uric acid but are usually the consequence of its low excretion. High serum uric acid levels are associated with alcohol intake, a purine-rich diet, compromised renal function, and obesity. Insulin increases both sodium and uric acid reabsorption.2 Therefore, increased serum uric acid levels may be an expression of an insulin-resistant state and metabolic syndrome (MS). This proposition is supported by evidence that higher serum uric acid levels correlate with a lower insulin-stimulated glucose uptake and a higher plasma insulin response to oral glucose loading.1

Although the cause of uric acid elevation has not yet been elucidated, it might be explained by a mechanism that involves high levels of extracellular adenosine (Ado), as stated by Bakker and his coworkers.1 Moreover, an elevated synthesis of fatty acid-acyl-CoA in peripheral tissues will result in a greater formation of AMP and hence in higher levels of uric acid.1 We have recently reported in the same obese population of the present work elevated plasma levels of fatty acids,6 which would condition higher levels of acyl-CoA in peripheral tissues and therefore a potential elevation of uric acid.

Elucidation of the relationship between serum uric acid and obesity or cardiovascular disease is complicated by the numerous variables (i.e., hyperlipidemia, hypertension, and hyperglycemia) reported to correlate with both cardiovascular disease and hyperuricemia.2 Elevated acid uric concentrations have even been described as an independent risk factor for non-alcoholic fatty liver disease. Furthermore, one study has indicated that uric acid may be a reliable indicator of “pre-metabolic syndrome” in obese youths.1 However, as far as we know, no data have been reported in obese children exclusively at prepubertal age. Thus, the aim of this study was to determine plasma uric acid in obese and lean prepubertal children and its relationships with anthropometric measurements, dietary intake, and parameters related to the insulin resistance syndrome (IRS).

Subjects and methods

Study Population

We studied a selected group of 34 obese (23 boys and 11 girls) Caucasian Spanish children, recruited from among children referred to our Paediatric Endocrinology Unit from primary care paediatric clinics. Inclusion criteria were: apparent good state of health, age between 6-12 years, and classification as prepubertal (Tanner I) based on Tanner criteria8 and validated by appropriate plasma sex hormone concentrations (results not shown). Exclusion criteria for both groups were: presence of pubertal development, disease, or malnutrition, use of medication that alters blood pressure or glucose or lipid metabolism, and consumption of hypocaloric diet. The groups (obese and normal-weight) were matched by age and sex.

Children were classified as obese if their body-mass index (BMI) exceeded the 97th percentile for their age and sex according to Spanish standards (BMI z score ≥2.0).1 Children were classified as normal-weight if their BMI was higher than the 5th and lower than the 75th percentile.6 Both obese and normal-weight children were also classified with IOFT reference6 showing a hundred per cent of concordance with the classification based on Spanish standards. The demographic and anthropometric characteristics of both groups are shown in table I.

The study was approved by the Human Investigation and Ethics Committees of the University of Granada and the Reina Sofia University Hospital of Cordoba (Spain). Written informed consent was obtained from parents and verbal approval from children.

Table I

Baseline demographic and anthropometric characteristics of the obese and normal weight prepubertal children

<table>
<thead>
<tr>
<th></th>
<th>Obese (n = 34)</th>
<th>Normal weight (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.4 ± 0.4</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.6 ± 2.7</td>
<td>29.1 ± 1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.7 ± 2.1</td>
<td>131.6 ± 1.8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29 ± 1.0</td>
<td>17 ± 0</td>
</tr>
<tr>
<td>Body mass index z score</td>
<td>4.05 ± 0.30</td>
<td>-0.99 ± 0.39</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>88.5 ± 2.2</td>
<td>59.5 ± 1.3</td>
</tr>
<tr>
<td>Tricipital skinfold (mm)</td>
<td>26.1 ± 0.8</td>
<td>8.7 ± 1.0</td>
</tr>
<tr>
<td>Subcapular skinfold (mm)</td>
<td>30.2 ± 1.1</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>34.2 ± 2.2</td>
<td>14.9 ± 2.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.

Comparisons of demographic and anthropometric variables between obese and control children, with adjustments for age and sex, were assessed with general linear models of variance. Significant differences between obese and controls: *P < 0.001.

Anthropometric, blood pressure measurements and dietary intake estimation

Anthropometric measurements children were bare-footed and in their underwear. Body weight (kg) was measured using a standard beam balance (Seca), preci-
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dition 100 g, range 0-150 kg. Height (cm): using a precision stadiometer (Seca), precision 0.2 cm, range 70-200 cm. Skinfold thicknesses (mm) were measured at the left side of the body to the nearest 0.1 mm with a skinfold caliper (Holtain, U.K., range 0-40 mm). The measurements were taken at the following sites: Triceps, halfway between the acromion process and the olecranon process at the back side of the arm; and subscapular, about 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body. Body fat mass was estimated from tricipital (TS) and subscapular, about 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body. Blood pressure was measured with a random-zero sphygmomanometer. The cuff size (bladder-size) was applied horizontally midway between the lowest rib margin and the iliac crest about the level of the umbilicus, at the end of gentle expiration.

Blood pressure was measured with a random-zero sphygmomanometer. The cuff size (bladder-size) was sufficiently long to surround at least two thirds of the upper arm. The centre of the inflatable part of the cuff (bladder) was positioned over the brachial artery of the inner side of the upper arm. The blood pressure was measured after resting with no change of position for at least 5 minutes, in a sitting position and using the right arm-unless there was a deformity.

Dietary intake was estimated using a previously validated food-frequency questionnaire and a 72 h dietary survey and a database for composition of Spanish foods13 (table II).

Sampling

Baseline blood samples were obtained from children while they were fasting, using an indwelling venous line to draw a 3-ml sample for the measurement of plasma levels of glucose, insulin, lipids, adipokines (leptin, adiponectin and tumour necrosis factor) and sex hormones (follicle stimulant hormone —FSH—, lutein hormone —LH—, estradiol, and testosterone). All samples were processed within 2 h of sampling and divided into aliquots for immediate analysis or long-term storage at -80 °C until their analysis.

Biochemical analysis

Glucose was analyzed by the glucose oxidase method using an automatic analyzer (Roche-Hitachi Modular PyD Autoanalyser, Roche Laboratory Systems, Mannheim, Germany), and plasma insulin and C-peptide were analyzed by radioimmunoassay with an automatic analyzer for microparticles (AxSYM, Abbott Laboratories, Chicago, IL, USA). Insulin resistance was calculated by means of the homeostasis model assessment (HOMA) index, defined by the equation HOMA = fasting glucose (G₀) (mM) x fasting insulin (I₀) (U/mL)/22.5.14 Sex hormones (FSH, LH, testosterone and estradiol) were measured by chemiluminescence using an automatic analyzer (Architect I4000, Abbott Laboratories Chicago, IL, USA). Plasma urate, triacylglycerols (TAG), high-density lipoprotein cholesterol (HDL-cholesterol), and apoprotein B (Apo B) levels were measured by means of an automatic analyzer (Roche-Hitachi Modular PyD Autoanalyser, Roche Laboratory Systems, Mannheim, Germany). Plasma adiponectin levels were measured by using a human adiponectin radioimmunoassay (RIA) kit (Cat.#HADP-61HK, Linco Research Inc. St. Charles, MO, USA), and plasma leptin and tumor necrosis factor-alfa (TNF-α) levels were measured by means of ELISAs (Cat.#KAC2282/KAC2281, Cat. #KHC3011C, Biosource Europe SA, Nivelles, Belgium).

Statistical analysis

The minimum sample size to detect a significant elevation of uric acid (type I error α = 0.05 and a type II error β = 0.2 -power 80%) was estimated to be 20 children per group, based on a minimal difference of 20% between obese and lean subjects and considering vari-
ances previously obtained for healthy children.

Data are expressed as means ± standard error of the mean (SEM). Variables with an abnormal distribution (insulin, HOMA) were log-transformed before analysis. General linear models of variance, adjusted for age and sex, were used to compare demographic, dietary intake and clinical variables between obese and control children. Pearson’s correlation coefficients were used to study correlations between uric acid and dietary intake of major nutrients, anthropometric, metabolic and adipokine variables. Since co-linearity may occur for BMI and these parameters associated with obesity, linear correlations were adjusted by age, sex and BMI.

Table II

Estimated daily nutrient intakes in obese and normal weight children at prepuberal age

<table>
<thead>
<tr>
<th>Nutrient intake</th>
<th>Obese (n = 34)</th>
<th>Normal weight (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/d</td>
<td>% of energy</td>
<td>g/d</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2,037 ± 65</td>
<td>1,601 ± 82</td>
</tr>
<tr>
<td>Proteins (g/d)</td>
<td>84 ± 3</td>
<td>16.5 ± 0.6</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>222 ± 10</td>
<td>43.6 ± 2.0</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>93 ± 4</td>
<td>41.1 ± 1.8</td>
</tr>
<tr>
<td>Saturated (g/d)</td>
<td>30.6 ± 1.3</td>
<td>15.5 ± 0.6</td>
</tr>
<tr>
<td>Monounsaturated (g/d)</td>
<td>47.1 ± 2.6</td>
<td>20.8 ± 1.1</td>
</tr>
<tr>
<td>Polyunsaturated (g/d)</td>
<td>15.3 ± 1.3</td>
<td>6.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.

Comparison of nutrient’s intake between obese and control children, with adjustments for age and sex, were assessed with general linear models of variance. Significant differences between obese and controls: *P < 0.05; **P < 0.001.
In addition, multiple regression analyses were performed to identify predictor variables of uric acid associated with the classical features of metabolic syndrome. SPSS software (version 13.0, SPSS Inc. Chicago, IL, USA) was used for the analyses.

**Results**

BMI, TS, SS, WC, and percentage fat mass were significantly higher in the obese group compared with the control group (table I). In addition, obese children had a significant higher intake of energy and major nutrients compared with normal-weight children (table II).

Table III shows the blood pressure and the biochemical parameters related to the IRS in obese and normal-weight children. SBP, DBP, insulin, HOMA, C-peptide, TAG, Apo B and leptin were higher in the obese group compared with the control group, whereas HDL-cholesterol and adiponectin were lower and TNF-α were similar. Serum creatinine and glomerular filtration rate were in the normal range in both groups (result not shown). Plasma uric acid concentrations were significantly higher in the obese children than in the normal-weight controls (table III).

There was a positive association between plasma uric acid levels and anthropometric parameters, with TS showing the strongest correlation (table IV). In relation to diet, uric acid levels were correlated with the intake of energy, proteins, carbohydrates, and monounsaturated and polyunsaturated fats (table IV). However, when nutrient intakes were adjusted by sex, age and BMI no significant correlations with uric acid were found. SBF, DBP as well as plasma insulin and C-peptide concentrations were positively correlated with plasma uric acid levels and hence with the HOMA index. Likewise, uric acid was positively correlated with TAG and apo B and negatively correlated with HDL-cholesterol. Moreover, uric acid was positively correlated with leptin and negatively correlated with adiponectin and TNF-α (table IV).

Since univariate correlations between uric acid and any other parameters such as blood pressure, plasma lipids and glucose metabolism parameters may just explained by the fact that uric acid concentrations as well as those parameters are both related to BMI, we performed linear regression analyses adjusted by age, sex and BMI. With this approach, only TS, HOMA and adiponectin were significantly correlated with plasma uric acid (table V).

In a backward stepwise multiple correlation analysis that included the known features of IRS (BMI, SBP,
Table V

Correlations between different anthropometric and metabolic variables and plasma uric acid levels in obese and normal weight prepubertal children adjusted by sex age and BMI, and backward stepwise multiple regression analysis of uric acid and features of metabolic syndrome

<table>
<thead>
<tr>
<th>Predictor variables of plasma uric acid associated with the IRS*</th>
<th>β standardized coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.368</td>
<td>0.014</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>-0.358</td>
<td>0.017</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>-0.382</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Obese (n: 34) vs controls (n: 20).
*For multiple regression analysis adjusted values for sex and age of BMI, systolic blood pressure, plasma glucose, insulin, triacylglycerols and HDL-cholesterol were included as classical characteristics of the IRS (R² of the model: 0.425). When adiponectin was included into the model, R²: 0.547.
HOMA: homeostasis model assessment index; IRS: insulin resistance syndrome.

The association between obesity and hyperuricemia is well documented in both adults and mixed populations of adolescents and children; in adults, hyperuricemia is considered a common obesity- and MS-related “lifestyle” disorder and might be useful as an indicator of metabolic derangement in obesity from early adolescence onwards.15-18

In normal-weight children, plasma uric acid increases significantly at the end of puberty, due to a lower renal clearance of it. However, in obese children renal clearance decreases at the beginning of puberty, with a progressive increase in plasma uric acid as puberty advances, which may at least in part be due to uric acid overproduction.19 The present study suggests that obesity in prepubertal age may bring forward changes in uric acid metabolism which are usually observed at puberty.

There have been reports that 23-25% of obese children present at least three MS components.20 Uric acid is one of the non-traditional risk factors for MS.21 However, few studies have addressed the relationship of serum uric acid levels with obesity and the IRS in children and adolescents22-24 and no reports have been published exclusively at the prepubertal period. In a study of hyperuricemia in normal-weight children, Oyama et al, 200622 found an incidence of 8.8% in boys and 0.6% in girls. Another study found that 80.4% of severely obese children had at least one MS complication, including elevated uric acid.23 Results obtained in the present study show a correlation between uric acid levels in obese prepubertal children and some risk factors for the MS (table IV). In another study in adults, uric acid also appeared to be correlated with obesity, blood pressure, TAG, insulin, HOMA and adiponectin, underscoring its impact on the IRS25 and it has also been suggested that hyperuricemia in obesity can be affected by fat mass distribution,26 testosterone,27 or leptin.28 In the present study with obese children at early age, adiponectin, a well known adipokine involved in the regulation of insulin signalling and a key factor in IRS and cardiovascular disease,29 was independently associated with uric acid, which emphasised the potential role of this hormone in IRS and subsequently in uric acid elevation.

A potential mechanism to explain the increased levels of plasma uric acid in plasma is the rise in intracellular Ado, derived of the higher AMP concentrations associated to increased synthesis of fatty acid-acyl-CoA in peripheral tissues.3 As stated before, we have found elevated plasma levels of fatty acids in obese children, which would condition higher levels of acyl-CoA in peripheral tissues and therefore a rise in plasma uric acid. Thus, we propose that in early obesity increased levels of uric acid are related to elevated plasma fatty acids (fig. 1). The mitochondrial adenine nucleotide translocator (ANT) serves to supply the mitochondrial matrix with cytosolic ADP and also to supply the cytosol with ATP produced from ADP in the mitochondrial matrix. Hence, inhibition of ANT by elevated intracellu-
lar LCFA-CoA concentrations, derived from the obese state, would result in an excess of cytosolic ADP and a parallel decrease in cytosolic ATP concentrations. Consequently, cytosolic adenylate kinase shifts towards production of one molecule of ATP and one molecule of AMP from two molecules of ADP. This rise in cytosolic AMP concentration increases dephosphorylation of AMP to Ado. This increase in Ado impairs equilibrative transport from the extracellular space because of a lower concentration gradient, resulting in higher plasma Ado and urate concentrations. Moreover, an elevated synthesis of fatty acid-acyl-CoA in peripheral tissues will result in a greater formation of AMP and hence in higher levels of uric acid.

Various hypotheses have been proposed for the role of uric acid in the early pathogenesis of primary (idiopathic) hypertension and vascular disease. In animal experiments, modest increases in plasma uric acid produced subtle glomerulotubular damage that activated the renin-angiotensin system and increased blood pressure, all reversed by removal of the hyperuricemic stimulus. Elevated serum uric acid also plays a part in endothelial dysfunction, both directly and indirectly via xanthine oxidase activity, which is elevated in atherosclerotic plaques. Oxidative stress produced by the generation of oxygen free radicals, limiting the availability of nitric oxide, reduces endothelial-regulated vascular relaxation. Moreover, free radicals formed in hyperuricemia also stimulate lipid peroxidation, which might be responsible for increasing carotid intima-media thickness. Other potential mechanisms by which hyperuricemia and/or elevated xanthine oxidase activity might produce vascular damage include increased platelet adhesiveness, smooth muscle cell proliferation, and stimulation of inflammatory responses.

Studies in children have related uric acid to blood pressure. Children with primary hypertension are frequently obese and have elevated serum uric acid levels. Consequently, some authors have proposed that obese and hypertensive children should be screened for MS components in order to initiate early prevention measures against cardiovascular disease. Based on the present study findings in prepubertal obese children in which arterial pressure was significantly higher than in the controls and positively correlated with uric acid levels, we propose that plasma uric acid should also be assessed for this purpose.

In conclusion, plasma uric acid elevation in prepubertal obese children is associated with IRS features and BMI, HDL-cholesterol and adiponectin are independently associated with uric acid levels. We suggest that uric acid is an early marker of the excess of body weight and a key link in MS in prepubertal obese children.

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There are no conflicts of interest.
References


