**Differential mediating effects of uric acid and metabolic factors in the association between body composition and blood pressure in children and adolescents**

Deyse Magdinier DUTRAa, Divanei ZANIQUELIb, Rafael de Oliveira ALVIMc, \*Marcelo Perim BALDOd, Elis Aguiar MORRAb, Eliane Rodrigues de FARIAa, José Geraldo MILLb, Carolina Perim de FARIAa

aPost-graduation Program in Nutrition, Federal University of Espirito Santo, ES, Brazil.

bCardiovascular Investigation Clinic, Federal University of Espirito Santo, ES, Brazil.

cDepartment of Physiological Sciences, Federal University of Amazonas – UFAM, Manaus, AM, Brazil

dDepartment of Pathophysiology, Montes Claros State University – UNIMONTES, Montes Claros, MG, Brazil/ Department of Medicine, Centro Universitário, UniFIPMOC, Montes Claros, MG, Brazil

**Short title:** Body composition and blood pressure in children

**The authors declare that they have no competing interests.**

Word count: 2,806

Number of Tables: 2

Number of figures: 3

Number of supplementary digital content files: 0

**\*Corresponding author:**

Marcelo P. Baldo, PhD, Department of Pathophysiology, Montes Claros State University

Av Dr Rui Braga, S/N, Vila Mauriceia – 39401-089 – Montes Claros, MG, Brazil.

Email: marcelobaldo@ymail.com

**ABSTRACT**

**Background:** Studies have reported a close association between elevated uric acid (UA) levels and high blood pressure. During somatic growth it is difficult to distinguish the independent effect of UA and other metabolites on blood pressure from their contribution in mediating the increase in blood pressure caused by body composition changes.This study sought to investigate whether UA along with other metabolites take part as independent mediating pathways in the association between body composition components and blood pressure.

**Methods:** Across-sectional study was conducted in 928 children and adolescents aged 6-18 years. Structural equation modelling was performed to test direct pathways between SBP and body composition parameters, but also through the mediator effect of the UA and a latent variable comprising different metabolic parameters.

**Results:** In girls, a mediating pathway through UA was not significant, but the association between fat mass (FM) and muscle mass (MM) with SBP was mediated by the cluster of metabolic factors. In boys, both MM and FM were associated with SBP through a mediating pathway via UA. FM had a strong association with the cluster of metabolic factors but it did not affect SBP as a mediator.

**Conclusion:** Our study showed an independent association between UA levels and SBP in boys and between the cluster of metabolic factors and SBP in girls. This results indicate that the increase of uric acid may affect blood pressure levels early and even in the absence of obesity. Whether insulin regulates blood pressure independently during growth needs to be further investigated.

**Keywords:** Blood pressure,Uric acid, Body composition, Lipids, Insulin, Children

**INTRODUCTION**

The typical increase in blood pressure that parallels the somatic growth is part of an orchestrated biological program encompassing a gradual increase in body height and maturation of the musculoskeletal system [1]. Although body mass index (BMI) has been consolidated as the most important predictor of blood pressure, BMI may overestimate the effect of adiposity as a cause for increased blood pressure as it is not able to discriminate fat mass and fat-free mass. Indeed, our group has recently shown that muscle mass (MM) is the strongest somatic growth indicator that mediates the increase in systolic blood pressure (SBP) with aging in children and adolescents [2].

It has been well documented that obesity in children and adolescents may cause metabolic disorders such as increased triglycerides (TG) and decreased high-density lipoproteins (HDL-c) [3], glucose disturbances [4], and elevated uric acid (UA) level [5]. However, UA level increases sharply in the onset of puberty, mainly in boys, and the increase in muscle mass ha­­­­s shown to be the main determinant of UA elevation, even after controlling for total fat mass (FM) [6].

Cross-sectional and longitudinal studies have reported a close association between elevated UA levels and high blood pressure [7-9]. However, at an early age, it is difficult to distinguish the independent effect of UA and other metabolites on blood pressure from their contribution in mediating the increase in blood pressure caused by body composition changes. Hence, given to these complex interactions, we hypothesized that changes in body composition would increase blood pressure directly but also through independent pathways mediated by UA and a cluster of metabolic factors. Thus, this study was designed to investigate whether UA along with other metabolic factors take part as independent mediating pathways in the association between body composition components and blood pressure.

**METHODS**

**Study design and population**

This cross-sectional study was carried out with data from two samples of children and adolescents living in the same metropolitan region. From July 2016 to February 2017, 269 students of nine public schools aged 8-14 years, attended the Cardiovascular Investigation Clinic located at the Federal University of Espírito Santo to be submitted to clinical and laboratory exams. A second sample was investigated from June 2018 to November 2019, in which 858 volunteers (aged 6-18 years), assisted in a social project, attended a clinic specially constructed within the premises of the social project. The social project is established from a partnership between the public sector, the mining company VALE, and the community, and it is directed to school children and adolescents (6-18 years) living in the vicinity of “Estação Conhecimento”. The majority of the families living in that region belong to lower socioeconomic classes. All participants were also regularly enrolled in public schools of the municipality and attended the project in a half period of the workdays to receive additional classes and engage in sport and cultural activities. A merged database with 1,154 children and adolescents aged 6-18 years was prepared for the present analysis. After excluding 226 individuals with missing data, the final sample consisted of 928 children and adolescents. No exclusion was necessary based on the presence of severe cardiovascular disease or other limiting health conditions. For both samples, informed written consent and assent were obtained from parents or legal guardians following the Center for Health Sciences Ethics Committee (register numbers: 53609716.0.0000.5060 and 30385014.8.0000.5060).

**Anthropometry**

Weight was measured using an electronic scale with 0.05 kg precision (Toledo, Brazil) in barefoot individuals using only undergarments and after bladder emptying. Height was obtained in a wall-mounted stadiometer (Seca Stadiometer − Seca GmBH & Co, Hamburg, Germany) with 0.1 cm precision. Waist circumference was measured at the top of the iliac crest and waist-to-height ratio (WHtR) obtained as the ratio between waist circumference and height, both in centimeters. WHtR was preferred over waist circumference as an indicator of abdominal obesity to avoid the need for statistical correction by body height. Body composition, including total FM and MM, was assessed by multi-frequency bioelectrical impedance analysis (MF-BIA8, InBody 230, Bioespace, South Korea).

**Biochemical examination**

A blood sample was obtained by venipuncture after overnight fasting (8-14 h) and sent to a central laboratory (Laboratório Tommasi − Vitória, Brazil) to determine serum concentrations of glucose, insulin, total cholesterol and its fractions, triglycerides, and uric acid. All dosages were performed with commercially available kits. Plasma glucose concentration was obtained by enzymatic hexokinase method (ADVIA 1200, Siemens®) and fasting insulin by immunoenzymatic assay (Centaur Siemmens®). Total cholesterol by cholesterol oxidase method (enzymatic colorimetric), HDL-c by homogeneous colorimetric, without precipitation, and TG by glycerol-phosphate peroxidase method according to Trinder (ADVIA 1200, Siemens®). Triglycerides-to-HDL-c ratio (TG/HDL) was calculated as the ratio between TG and HDL-c.

**Blood pressure measurement**

Blood pressure was measured in the left arm by using an automatic validated device (Omron 705CP, Intellisense, Japan) after a resting period of 5 minutes in the sitting position. Three-to-four measures were taken with 1-minute intervals. The value of the first measure was discarded, and the clinic blood pressure was calculated as the average of two measures with a difference less than or equal to 5 mmHg.

**Statistical analysis**

The assumption of normality of the main variables of the study was tested with the Kolmogorov-Smirnov test. SBP, FM, and MM for age and sex quartiles were obtained by dividing the sample into six age groups as follows: 6 to <8, 8 to <10, 10 to <12, 12 to <14, 14 to <16, and 16 to 18. Analysis of variance (ANOVA) with Tukey’s multiple comparisons was performed to compare normally distributed variables and Kruskal-Wallis with Dunn’s multiple comparisons to compare non-normally distributed variables between the groups divided into quartiles of SBP.

Analysis of covariance (ANCOVA) was used to test the association between SBP and the quartiles of FM/MM adjusting for covariates. We used Pearson’s product-moment correlation to provide an overview of the interrelations of the variables candidate to explain the variability of SBP.

Structural equation modeling (SEM) was performed to identify the pathways between body composition parameters and the increase in SBP in children and adolescents using a mixed model with a latent variable (unobserved) and other observed variables. A hypotheticalmodel (Figure 1) was developed for the direct pathways between SBP and body composition parameters (MM, FM or WHtR), but also through the mediator effect of the UA and a latent variable comprising different metabolic parameters (TG/HDL, insulin, and fasting glucose). To define a good model fit, we used fit indices such as the root mean square error of approximation (RMSEA) lower than 0.08, comparative fit index (CFI) and Tucker-Lewis index (TLI) higher than 0.9, and the standardized root mean square residual (SRMR) lower than 0.1. The SEM analysis was performed using the R software (version 3.5.1) with semPlot (path diagrams and visual analysis of various SEM packages' output, v. 1.1) and Lavaan (latent variable analysis, v. 0.6‐3) packages. Statistical significance was set at P< 0.05 for all analyses.

**RESULTS**

A total of 928 children and adolescents aged 6-18 years (57.2% of boys) were enrolled in this study. The characteristics of the participants according to the quartiles of SBP are shown in Table 1. In boys, height, WHtR, FM, MM, glucose, insulin, and UA were higher in the 4th quartile compared with the 1st quartile of SBP. In girls, WHtR, FM, MM, and insulin were higher in the 4th quartile compared with the 1st quartile of SBP.

The impact of UA and other metabolic factors in the association between body composition parameters and SBP was tested. Boys and girls were divided into four groups according to quartiles of FM or MM. In the crude analysis, girls in the 4th quartile of FM showed higher SBP than their counterparts in the 1st quartile. However, no difference in the SBP was detected between the quartiles of FM after adjusting for UA and the cluster of metabolic factors (Figure 2A). In boys, SBP was higher in the 4th quartile of FM compared with the 1st, 2nd, and 3rd quartiles even after adjusting for UA and the cluster of metabolic factors (Figure 2B). When divided into quartiles of MM, girls in the 4th quartile showed higher SBP than those in the 1st and 2nd quartiles in both crude analysis and adjusted for UA. Even after adjusting for metabolic factors, SBP was higher in girls of the 4th quartile compared with 1st quartile (Figure 2C). In boys, even in the adjusted analyses, SBP was higher in the 4th quartile of MM than in the other quartiles (Figure 2D).

An overview of the multicollinearity between variables potentially associated with SBP is shown in Table 2. The variables were subsequently included in the SEM analysis based on a significant correlation with SBP either in boys or in girls. Age was not included because it is highly correlated with MM. WHtR and FM were also highly correlated with each other but since adiposity distribution could distinctly influence the dependent variables, separate analyses were conducted for WHtR and FM.

The next step was to investigate how body composition indicators would affect SBP. Thus, we tested the hypothetical model in which increases in SBP elicited by changes in body composition could be partially explained by the mediating effects of UA and the cluster of metabolic factors (Figure 1). We detected a significant total association (the sum of all direct and mediated effects) between body composition parameters with SBP in boys (0.536, P<0.001) and girls (0.379, P<0.001). In girls, a strong direct association with SBP was showed for MM, but not for FM. Also, although a mediating pathway through UA was not found statistically significant, the association between FM and MM with SBP was significantly mediated by the cluster of metabolic factors (Figure 3A). In boys, the MM was the only variable that was directly associated with SBP. However, both MM and FM were associated with SBP through a mediating pathway via UA (Figure 3B). Besides, the FM had a strong association with the cluster of metabolic factors but it did not affect SBP as a mediator.

To investigate if different markers of adiposity would have the same effect on SBP, we replaced the FM (total adiposity) by the WHtR (central adiposity). In girls, the association between body composition and SBP was explained only by the direct effect of MM, without mediation by UA or metabolic factors (Figure 3C). In boys, the MM is associated with SBP through a direct pathway but also mediated by UA. The WHtR is associated with SBP only via the mediation of UA (Figure 3D).

**DISCUSSION**

The main finding of this study is that the association between body composition and blood pressure in children and adolescents has a complex design, and also has a sex-specific mediating component. Although MM has a strong direct effect on blood pressure in both boys and girls, the cluster of metabolites serve as an independent mediator in the association between body composition and SBP in girls, while UA mediates such association in boys.

Researchers of a proficuous line of experimental research have defended the hypothesis that a uricase gene mutation leading to a more elevated uric acid level during hominoid evolution was originally a survival advantage to maintain blood pressure under a restricted-salt diet, but the switch of a low to high-salt diet by the modern civilization triggers a renal maladaptation that might be the genesis of hypertension [10]. Experimental studies have shown that elevated uric acid leads to renal damages such as tubulointerstitial injury and renal fibrosis, arteriolopathy of the afferent arteriole, and arteriolopathy of preglomerular vessels [11-13]. In a cross-sectional study, Feig and Johnson [14] did not report significant correlation between uric acid and glomerular filtration rate in children (6-18 years) with primary hypertension. However, they found that 89% of the children with primary hypertension had UA >5.5 mg/dL, whereas all of the 40 normotensive controls had UA bellow this value.

In this study, we investigated the feasibility of UA as a mediator in the association between body composition and blood pressure in a population of predominantly normotensive (92.3%) children and adolescents. In fact, the remaining hypertensive individuals in the sample had higher UA levels but also higher BMI, FM, MM, and WHtR. Besides, the fact that UA is associated with SBP in normotensive boys supports the assumption that elevated UA levels may precede the development of hypertension [15].

Of our knowledge, this is the first study to show that UA has a direct effect on SBP in boys, but not girls. Indeed, UA increases less in girls than in boys in the onset of puberty [6], probably because of the uricosuric effect induced by the estrogens [16,17] and this may prevent the early influence of UA on the blood pressure regulation. The prospective analysis conducted by Alper Jr. et al. [18] suggested that elevated UA levels are associated with increased blood pressure beginning in childhood and with higher blood pressure into adulthood in both sexes. However, childhood UA along with childhood BMI and other independent variables were included as predictors of adult systolic and diastolic blood pressure in a multiple regression analysis, which makes it difficult to attribute causation or confusion to the independent predictors.

Contrary to what was seem for the UA, we showed that a cluster of metabolic factors mediates the association between body composition and SBP in girls, but not boys. Also, this mediating effect was observed only when FM was used as the adiposity marker. This is a novel finding and must be viewed with caution. Among the metabolites composing the cluster, insulin was the most strongly correlated with SBP in girls (r= 0.28). This observation supports results from the CARDIA Study [19] showing that insulin levels are associated with blood pressure in a healthy young population. Besides, the authors reported a strong association between insulin levels and several lipid measurements. Indeed, hyperinsulinemia leads to increased synthesis of fatty free acid in adipose tissue, that once transferred to the liver induces increased plasma TG and decreased HDL-c [20]. On the other hand, several mechanisms such as increased renal sodium reabsorption, activation of the sympathetic nervous system, alteration of transmembrane ion transport, and hypertrophy of resistance arteries have been proposed to explain the association between insulin and blood pressure [21]. However, these mechanisms are implicated in pathophysiological pathways involved in the relationship between insulin resistance and hypertension, and in general, are not independent of the presence of comorbidities such as obesity. A population-based study corroborates this assumption by showing that abdominal adiposity explained almost entirely the association between obesity, fasting insulin, insulin sensitivity, and blood pressure [22]. Besides, in a prospective cohort of healthy children and adolescents (age at baseline 3-18 years), baseline insulin level was a significant predictor of SBP 3 and 6 years later [23]. However, insulin was not correlated with blood pressure changes once SBP continued to rise after completing puberty while insulin levels increased in parallel until puberty and then decreased progressively until the end of the follow-up (24 years of age).

In the present study the presentation of two separate models served to two purposes: 1) to test the hypothesis of distinct effects of total and central adiposity on the blood pressure, and 2) to avoid inaccurate estimates of coefficients and standard errors owing to the possible collinearity between FM and WHtR. However, the correlation between FM and MM was also not negligible in girls (r= 0.56) and boys (r= 0.35) and this might have affected the coefficients between the MM and the cluster of metabolic factors. This suspicion lays on the fact that the coefficient between MM and the cluster of metabolic factors was much smaller in the model with FM (Fig 3A and 3B) than with WHtR (Fig 3C and 3D).

**Limitations**

The cross-sectional design does not allow to determine cause and effect relationships. However, the hypothetical model was design based on several reports from longitudinal studies, but also based on clinical and physiological plausibility. The Sample was not randomly assigned and do not represent the population of children and adolescents. The accuracy of the estimates of fat mass and muscle mass by Multifrequency bioelectrical impedance has not been proved in children and adolescents. However, the reference methods themselves are limited for children since the algorithms for estimating body composition are based upon adult proportions.

In conclusion, our study highlighted to a sex-specific participation of UA and a cluster of metabolic factors as mediators in the association between body composition and SBP. These results indicate that the increase in the UA levels may affect blood pressure levels early in boys and even in the absence of obesity and other comorbidities. Further studies are necessary to address the independent effects of other metabolites, mainly of insulin, in the blood pressure regulation in girls because differently of uric acid during the accelerated growth spurt, insulin levels may be even higher in girls than boys [24].

**REFERENCES**

1. Gerber LM , Stern PM. Relationship of body size and body mass to blood pressure: sex-specific and developmental influences. *Hum Biol* 1999;71(4):505-528.
2. Zaniqueli D, Alvim RO, Baldo MP, Morra EA, Mill JG. Muscle mass is the main somatic growth indicator associated with increasing blood pressure with age in children and adolescents. *J Clin Hypertens* 2020; DOI: 10.1111/jch.14007.
3. Deeb A, Attia S, Mahmoud S, Elhaj G, Elfatih A. Dyslipidemia and fatty liver disease in overweight and obese children. *J Obes* 2018; DOI: 10.1155/2018/8626818.
4. Spreghini N, Cianfarani S, Spreghini MR, Brufani C, Morino GS, Inzaghi E, Convertino A et al. Oral glucose effectiveness and metabolic risk in obese children and adolescents. *Acta Diabetol* 2019; 56(8):955-962.
5. Modino SC, de Armas MGG, Mejías SM, Martínez JMM, Bolaños PI, Viveros MM, Quesada JML. Hyperuricemia and metabolic syndrome in children with overweight and obesity. *Endocrinol Nutr* 2012; 59(9):533-538.
6. Alvim RO, Siqueira JH, Zaniqueli D, Dutra DM, Oliosa PR, Mill JG. Influence of muscle mass on the serum uric acid levels in children and adolescents. *Nutr Metab Cardiovasc Dis* 2020; 30(2):300-305.
7. Teng F, Zhu R, Zou C, Xue Y, Yang M, Song H, Liang J. Interaction between serum uric acid and triglycerides in relation to blood pressure *J Hum Hypertens* 2011; 25(11):686-691.
8. Loeffler LF, Navas-Acien A, Brady TM, Miller ER 3rd, Fadrowski JJ. Uric Acid Level and Elevated Blood Pressure in U.S. Adolescents: National Health And Nutrition Examination Survey 1999-2006 *Hypertension* 2012; 59(4):811-817.
9. Park B, Lee HA, Lee SH, Park BM, Park EA, Kim HS, Cho SJ, Park H. Association between serum levels of uric acid and blood pressure tracking in childhood. *Am J Hypertens* 2017; 30(7):713-718.
10. Watanabe S, Kang DH, Feng L, Nakagawa T, Kanellis J, Lan H, Mazzali M, Johnson RJ. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. *Hypertension* 2002; 40(3):355-60.
11. Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension* 2001; 38(5):1101-1106.
12. Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, Kang DH et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am J Physiol Renal Physiol* 2002; 282(6):F991-997.
13. Sánchez-Lozada LG, Tapia E, Santamaría J, Avila-Casado C, Soto V, Nepomuceno T, Rodríguez-Iturbe B, et al. Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. *Kidney Int* 2005; 67(1):237-247.
14. Feig DI, Johson RJ. Hyperuricemia in childhood primary hypertension. *Hypertension* 2003; 42(3): 247-252.
15. Wang J, Qin T, Chen J, Li Y, Wang L, Huang H, Li J. Hyperuricemia and risk of incident hypertension: a systematic review and meta-analysis of observational studies. *PLoS One* 2014; 9(12):e114259.
16. Nichols A, Snaith ML, Scott JT. Effect of oestrogen therapy on plasma and urinary levels of uric acid. *Br Med J* 1973;1(5851):449-51.
17. Mumford SL, Dasharathy SS, Pollack AZ, Perkins NJ, Mattison DR, Cole SR, Wactawski-Wende J et al. Serum uric acid in relation to endogenous reproductive hormones during the menstrual cycle: findings from the BioCycle study. *Hum Reprod* 2013 Jul;28(7):1853-1862.
18. Alper AB Jr, Chen W, Yau L, Srinivasan SR, Berenson GS, Hamm LL. Childhood uric acid predicts adult blood pressure: The Bogalusa Heart Study. *Hypertension* 2005; 45(1):34-38.
19. Manolio TA, Savage PJ, Burke GL, Liu KA, Wagenknecht LE, Sidney S, Jacobs DR et al. Association of fasting insulin with blood pressure and lipids in young adults. The CARDIA study. *Arteriosclerosis* 1990;10:430-436.
20. Li N, Fu J, Koonen DP, Kuivenhoven JA, Snieder H, Hofker MH. Are hypertriglyceridemia and low HDL causal factors in the development of insulin resistance? *Atherosclerosis* 2014; 233(1):130-138.
21. Salvetti A, Brogi G, Di Legge V, Bernini GP. The inter-relationship between insulin resistance and hypertension. *Drugs* 1993; 46 Suppl 2:149-159.
22. Poirier P, Lemieux I, Mauriege P, Dewailly E, Blanchet C, Bergeron J, Despre´s JP. Impact of waist circumference on the relationship between blood pressure and insulin: the Quebec Health Survey. *Hypertension* 2005; 45:363-367.
23. Taittonen L, Uhari M, Nuutinen M, Turtinen J, Pokka T, Akerblom HK. Insulin and blood pressure among helthy children: Cardiovascular Risk in Young Finns. *Am J Hypertens* 1996;9(3):194-9.
24. Hirschler V, Maccallini G, Karam C, Gonzalez C, Aranda C. Are girls more insulin-resistant than boys? *Clin Biochem* 2009; 42(10-11):1051-1056.

**ACKNOWLEDGEMENTS**

We acknowledge the continuous support of "Estação Conhecimento" during the period of data collection of this work. This study received funding support from FAPES/PPSUS (No. 65854420/2014) and Fundação Vale

**Conflicts of interest**

There are no conflicts of interest.

**FIGURE LEGENDS**

**Figure 1.** Hypothetical model for the direct pathways between SBP and body composition (FM or WHtR and MM) and mediating pathways through the UA and a latent variable comprising TG/HDL, insulin, and glucose.

**Figure 2.** Systolic blood pressure values by quartiles of fat mass and muscle mass in girls (A and C) and boys (B and D). Adj(UA): adjusted for uric acid, Adj(UA+MF): adjusted for uric acid and the cluster of metabolic factors. \*Significant difference to 1st quartile, # Significant difference to 1st and 2nd quartiles, † Significant difference to 1st and 2nd, and 3rd quartiles. Significance set at P< 0.05 (ANCOVA).

**Figure 3.** Path analysis showing direct and mediating paths in the association of total fat mass (FM) and muscle mass (MM) with SBP (top panel) and of waist-to-height ratio (WHtR) and MM with SBP (bottom panel). Dotted arrows are nonsignificant direct effect (P> 0.05). Values are standardized β coefficient. Structural equation modelling.