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Associations of plasma uric acid and purine metabolites with blood pressure in children: the KOALA Birth Cohort Study

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Objective: Elevated serum uric acid concentration has been associated with high blood pressure (BP) and hypertension. A putative underlying mechanism is the accumulation of reactive oxygen species when uric acid is generated by an increased enzyme activity of xanthine oxidase (XO). The aims of the present study were to investigate the associations between plasma uric acid concentration, purine metabolite ratios, as proxies for increased XO activity, and SBP and DBP in school-age children.

Methods: Cross-sectional analyses were performed in 246 children (46% boys; mean age 7.1 years) from the Dutch KOALA Birth Cohort Study. Purine metabolites were determined with ultra-performance liquid chromatography–tandem mass spectrometry. During a home visit, a nurse collected a blood sample and measured BP three times; in addition, parents measured their child's BP on three consecutive days, in the morning and evening. Generalized estimating equations were used for analyses while controlling for variables such as sex, age, BMI, physical activity, and dietary intake.

Results: In multivariable analysis, uric acid (per SD of 38 $\mu\text{mol/l}$) was associated with DBP z-scores [β 0.07; confidence interval (CI), 0.01–1.14], but not with SBP z-scores. Higher ratios of uric acid/xanthine (per SD of 138) (β 0.09; CI, 0.01–0.17) and xanthine/hypoxanthine (per SD of 321) (β 0.08; CI, 0.02–0.17) were associated with higher DBP z-scores, but not with SBP z-scores.

Conclusion: In school-age children, uric acid and the ratios of uric acid/xanthine and xanthine/hypoxanthine were significantly associated with DBP z-scores. Suggesting that, both uric acid concentration and increased XO activity are associated with BP.

Keywords: blood pressure, blood pressure z-scores, children, hypertension, hypoxanthine, uric acid, xanthine, xanthine dehydrogenase, xanthine oxidase, xanthine oxidoreductase

Abbreviations: BP, blood pressure; CI, confidence interval; eNOS, endothelial nitric oxide synthase; FFQ, food frequency questionnaire; GEE, generalized estimating equations; HGPRT, hypoxanthine–guanine phosphoribosyltransferase; IMP, inosine monophosphate;

IQR, interquartile range; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg (A Dutch acronym for child, parents and health: lifestyle and genetic constitution); NAD(P)H, nicotinamide adenine dinucleotide (phosphate) (reduced form); NHBPEP, National High Blood Pressure Education Program; NO, nitric oxide; PPGP, pregnancy-related pelvic girdle pain; ROS, reactive oxygen species; UA, uric acid; XO, xanthine oxidase; XOR, xanthine oxidoreductase

INTRODUCTION

Recent meta-analyses show that elevated serum uric acid concentration increase the risk of incident hypertension among adults, independent of traditional risk factors [1,2]. Associations were significantly stronger in younger adults and tended to be larger in women, but studies among individuals younger than 18 years of age were not included in these meta-analyses [2]. During the past several years, a number of studies assessed this association in older children and adolescents, and extended evidence for elevated blood pressure (BP) and hypertension among those with elevated serum uric acid concentration to this age group (summarized in Table 1).

Several mechanisms have been proposed that causally link uric acid with hypertension. For example, uric acid

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TABLE 1. Overview of studies on association between uric acid and blood pressure in children and adolescents

Author (publication), country	Study design & setting (collection period)	Study population, n (boys %), Age, in years	Uric acid measurement & definition of hyperuricaemia	Blood pressure measurement & definition of hypertension	Results	Adjustment confounders
Viazzi <i>et al.</i> (2016), Italy [3]	Longitudinal intervention study, lifestyle changes (increase of physical activity and dietary modifications). Unit for CV risk assessment in children, (2005–2014). Follow-up: 1.5 years	248 (55). Range: 8.6–13.2	12-h fasting. Median sUA ($\mu\text{mol/l}$) for NT: 244 (208–285); PH: 262 (226–309); HT: 274 (214–309); HT 99th percentile: 274 (202–333)	Mean of three replicates after a 5-min rest, using an aneroid sphygmomanometer. NT: SBP and DBP <90th ($n = 124$); PH: SBP and DBP ≥ 90 th, but both <95th ($n = 41$); HT: SBP and DBP ≥ 95 th, but both <99th ($n = 59$); HT 99th: SBP and DBP ≥ 99 th percentile ($n = 24$) Ref. population: NHBPEP [4]	Baseline sUA associated with SBP z-scores at follow-up (β 0.15; CI, 0.04–0.26; $P < 0.01$), but not with DBP. Baseline sUA associated with HT 99th percentile during follow-up (OR 2.55; CI, 1.22–5.34; $P = 0.01$), but not with HT (OR 1.26; CI, 0.74–2.17; $P = 0.39$), after lifestyle changes	Age, sex, pubertal status, HOMA-index, and BMI z-scores (at baseline and follow-up)
Sun <i>et al.</i> (2015), Taiwan [5]	Longitudinal, MJ Health Screening Centre, (1999–2008). Median follow-up: 7.2 years	5303 (57). Range: 10–15	10-h fasting. Mean sUA (mg/dl) 6.7 ± 1.6 for boys and 5.4 ± 1.2 for girls. Hyperuricaemia: sUA > 7.6	Sitting position, by a nurse using a mercury sphygmomanometer. HT: SBP ≥ 130 and DBP ≥ 85 mmHg (number unspecified)	Baseline hyperuricaemia higher HR for HT at follow-up for boys (HR: 2.34; CI, 1.41–3.75; $P = 0.001$); but not for girls (HR: 2.76; CI, 0.45–15.26; $P = 0.26$)	Confounders unspecified
Goncalves <i>et al.</i> (2015), Portugal [6]	Cross-sectional, EPiteen Study, (2006–2007)	1286 (47). Mean: 17	12-h overnight fasting. Mean sUA (mg/l) for boys 52.6 ± 9.9 and 38.2 ± 8.2 for girls. Categorized in sex-specific quartiles	Mean of three replicates, separate by at least 10-min of rest, using a mercury sphygmomanometer using an auscultatory device	No sig. difference in SBP (boys: $P = 0.79$; girls: $P = 0.61$) and DBP (boys: $P = 0.36$; girls: $P = 0.28$) among sUA quartiles	–
Sun <i>et al.</i> (2014), USA [7]	Cross-sectional, Bogalusa Heart Study, (1987–1988). Including 1577 white and 1037 black children	Total 2614 (51). Range: 4–18. Mean: 10.8 ± 3.5 for white and 11.2 ± 3.6 for black children	12-h overnight fasting. Mean sUA (mg/dl) for white 4.5 ± 1.4 and black 4.1 ± 1.1 boys; for white 4.1 ± 1.1 and black 3.6 ± 1.0 girls	Mean of six replicates, in a relaxed sitting position, by two trained observers using a mercury sphygmomanometer. Outcome MAP: DBP + 1/3 pulse pressure	sUA associated with MAP in black girls (β 0.11; $P < 0.05$), but not in the total study population, black boys or in white boys and girls	Age and BMI
Pan <i>et al.</i> (2014), China [8]	Cross-sectional, Xinjiang Congenital Heart Disease survey, (2009–2010)	3778 (47). Range: 10–15	At least 8-h fasting. Mean sUA ($\mu\text{mol/l}$) 205.6 ± 65.2 . Categorized in quartiles	Mean of two replicates measured in 10-min, by doctor/nurse. HT: SBP or DBP ≥ 95 th percentile ($n = 352$). Ref. population: Chinese [9]	Increased sUA associated with increased DBP (sig. trend); not for SBP. Participants with sUA in Q3 (OR 1.60; CI, 1.10–2.32; $P < 0.01$) or Q4 (OR 1.81; CI, 1.18–2.78; $P < 0.01$) higher odds for HT versus Q1	Age, sex, ethnicity total cholesterol, HDL, LDL, triglycerides, eGFR, fasting glucose, and BMI
Viazzi <i>et al.</i> (2013), Italy [10]	Cross-sectional, San Gerardo Hospital, Unit for CV Risk Assessment in Children, (2004–2012)	501 (55.9). Range: 6–18; mean: 10.8 ± 2.4	12-h fasting period. Mean sUA (mg/dl) boys 4.38 ± 1.14 and girls 4.15 ± 0.95 . Categorized in quartiles	Mean of three replicates after 5-min rest, by a nurse using a calibrated aneroid sphygmomanometer; NT: SBP and DBP <90th ($n = 156$); PH: SBP and/or DBP between 90th and 95th ($n = 87$); HT: SBP and/or DBP ≥ 95 th percentile ($n = 136$); TH: family paediatrician diagnosed elevated BP but measured as NT ($n = 122$). Ref. population: NHBPEP [4]	sUA associated with SBP z-score ($P = 0.03$) and DBP z-score ($P = 0.02$), in adj. regression (no effect sizes). 1 mg/dl higher sUA (OR 1.40; $P < 0.01$) or sUA in Q4 (OR 1.67; $P = 0.05$) higher odds for HT versus the entire population. 1 mg/dl higher sUA (OR 1.60; $P < 0.01$; and OR 2.25; $P = 0.01$) or sUA in Q4; (OR 1.54; $P = 0.01$; and OR 2.04; $P = 0.02$) higher odds for HT or PH versus NT, respectively	Age, sex, pubertal status, BMI z-scores, HOMA index
Noone <i>et al.</i> (2013), UK [11]	Cross-sectional, Patients with ≥ 2 nephrology visits, (2004–2010)	117 (61). Range: 0.04–17; median: 6.5	Median sUA ($\mu\text{mol/l}$): 306 (range 127–780). Categories of hyperuricaemia differed among age ($n = 71$)	BP measured ≥ 2 occasions. HT: SBP > 95 th percentile for age, sex, and height adj. or taking antihypertensive drug ($n = 57$)	sUA related with elevated SBP ($r = 0.24$, $P = 0.004$). Participants with hyperuricaemia higher odds for HT (OR 2.1; $P = 0.03$) versus those who are nonhyperuricaemia	–

TABLE 1 (Continued)

Author (publication), country	Study design & setting (collection period)	Study population, n (boys %), Age, in years	Uric acid measurement & definition of hyperuricaemia	Blood pressure measurement & definition of hypertension	Results	Adjustment confounders
Loeffler et al. (2012), USA [12]	Cross-sectional, NHANES, (1999–2006)	6036 (51). Range: 12–17; mean: 14.5	Mean sUA (mg/dl): 5.0. Elevated sUA ≥ 5.5 mg/dl ($n = 83$)	Mean of three replicates after a 5-min rest, by a physician using a manual auscultation with a mercury manometer. NT: SBP and DBP < 95 th ($n = 5827$); HT: SBP and/or DBP ≥ 95 th percentile ($n = 209$). Continuous BP z-scores. Ref. population: NHBPEP [4]	For 0.1 mg/dl increase in sUA a higher odds for HT (OR 1.38; CI, 1.16–1.65) in total population, in boys (OR 1.45; CI, 1.17–1.80), but not in girls (OR 1.17; CI, 0.84–1.61). Participants with elevated sUA higher odds for HT in total population (OR 2.03; CI, 1.38–3.00), in boys (OR 2.23; CI, 1.16–4.31), but not in girls (OR 1.73; CI, 0.95–3.13)	Age, sex, race, BMI
Wang et al. (2012), Taiwan [13]	Longitudinal, MJ Health Screening Centre (1999–2008)	613 (100). Baseline: range 10–15; mean 12.9 $\pm$ 1.6. Follow-up: 15.6 $\pm$ 1.4	After 10-h fasting. Mean sUA (mg/dl) baseline: 6.9 $\pm$ 1.6; follow-up: 7.0 $\pm$ 1.4, categorized in quartiles	Sitting position, by nursing staff using standard mercury sphygmomanometers. HT: SBP ≥ 130 and DBP ≥ 85 mmHg	Baseline sUA correlated with SBP ($r = 0.30$) and DBP ($r = 0.21$) ($P < 0.01$) at follow-up. Participants in Q4 at baseline higher odds HT at follow-up (OR 3.72; CI, 1.07–13.73) versus those in Q1	Age
Hongo et al. (2010), Japan [14]	Cross-sectional, Junior high school students in various regions, (2006–2008)	958 (54). Range: 12.1–15.0	Overnight fasting sUA (mg/dl). sUA classified in four groups	After 10-min rest in sitting position, using a mercury-gravity sphygmomanometer. HT: SBP ≥ 140 or DBP ≥ 85 mmHg for boys and SBP ≥ 135 or DBP ≥ 80 mmHg for girls	Boys in group 4 versus higher SBP and DBP (DBP, 118 $\pm$ 12 versus 108 $\pm$ 11; SBP 64 $\pm$ 10 versus 60 $\pm$ 10) versus those in group 1. Boys in group 3 (OR, 2.04, CI, 1.16–3.59; $P < 0.05$) and group 4 (OR, 2.95; CI, 1.71–5.08; $P < 0.01$) higher odds for HT versus those in group 1. For girls, no sig. association between sUA and BP or HT	Age, POW and HbA _{1c}
Pacifico et al. (2009), Italy [15]	Case-control, Obese children ($n = 120$) enrolled at Department of Pediatrics, La Sapienza University of Rome (2007–2008)	170 (52). Mean: 10.6 for obese and 10.2 for controls	Overnight fast sUA (mmol/l). Mean sUA: obese 0.28 (range 0.27–0.30); controls 0.19 (range 0.17–0.20)	Sitting position, mean of three replicates after 10-min rest, during physical examination, using automated oscillatory device. Elevated BP SBP or DBP > 95 th. Ref. population: NHBPEP [4]	sUA associated with SBP (β 0.002; CI, 0.0001–0.003; $P < 0.05$)	Age, sex, Tanner-stage, and creatinine
Jones et al. (2008), USA [16]	Cross-sectional, American of African ($n = 74$), European ($n = 29$), and Asian ($n = 1$) descent	104 (64). Range: 7–18; mean: 13.7 $\pm$ 2.6	sUA in mg/dl. Elevated sUA > 4.9 .	ABPM every 20-min in 24-h period, using the auscultatory device (with oscillometric 'back-up'). Ref. population: German and Hungarian children [17]; HT: BPI > 1 ; for systolic (54%) and diastolic (16%)	sUA associated with 24-h SBP and 24-h DBP (β 1.77; $P = 0.048$; and β 1.55; $P = 0.02$) Participants with elevated sUA a higher odds for increased 24-h diastolic BPI (OR: 2.09; CI, 1.07–4.07; $P = 0.02$)	Race, sex, age, and BMI z-score
Gilardini et al. (2008), Italy [18]	Cross-sectional, Institution of Auxologico (national centre for the study of obesity)	89 (43). Range: 7–18	Plasma UA ($\mu\text{mol/l}$); mean: boys: 410.4 $\pm$ 107.1; girls and 344.9 $\pm$ 83.3	ABPM every 15-min during day and every 20-min during night-time, using oscillometric device. Daytime sustained HT: daytime SBP and/or DBP ≥ 95 th ($n = 59$). Ref. population: German and Hungarian children [17]	Plasma UA higher in those with night-time HT (6.9 $\pm$ 1.5) versus those with normal night-time BP (5.9 $\pm$ 1.7) ($P < 0.05$). Plasma UA not correlated with any BP parameters (adj. analyses)	BMI and waist circumference

TABLE 1 (Continued)

Author (publication), country	Study design & setting (collection period)	Study population, n (boys %), Age, in years	Uric acid measurement & definition of hyperuricaemia	Blood pressure measurement & definition of hypertension	Results	Adjustment confounders
Ford et al. (2007), USA [19]	Cross-sectional, NHANES (1992–2002)	1370. Range: 12–17	sUA ($\mu\text{mol/l}$): mean 301.9 ± 2.0 (range 113.0–719.7). Group sUA ≤ 291.5 (1: REF), (2) >291.5 – ≤ 339 ; and (3) >339	Mean of four replicates in a sitting position at the mobile examination centre, using a mercury-gravity manometer. HT: SBP and/or DBP ≥ 90 th percentile ($n = 59$). Ref. population: NHBPEP [4]	Elevated sUA was associated with a higher odds for HT. Group 2 (OR: 2.07; CI, 0.90–4.76) and group 3 (OR 3.74; CI, 1.86–7.52) versus group 1 ($P < 0.01$ for trend). In addition adj. Association attenuated (OR: 1.48; and 2.05; $P = 0.07$ for trend)	Age, sex, race, and concentration of C-reactive protein, MetS. In addition adj. for waist-circumference and BMI z-scores
Alper et al. (2005), USA [20]	Longitudinal, Bogalusa Heart Study, including 334 white and 243 (42) black children. Baseline (1973–1974); follow-up (1976–1977) mean follow-up: 11.4 years (range 7.4–15.0)	577 (42); mean boys: white 13.6 ± 3.2 and black 12.6 ± 3.8 ; mean girls: white 13.4 ± 3.1 and black 12.9 ± 3.7	sUA ($\mu\text{mol/l}$): mean sUA at baseline: boys: white 303.3 ± 83.2 and black 261.7 ± 89.2 ; girls: white 9 ± 65.4 and black 220.1 ± 71.4 ; mean sUA at follow-up: boys: white 362.8 ± 77.3 and black 350.9 ± 77.3 ; girls: white 261.7 ± 65.4 and black 255.8 ± 136.8	Mean of six replicates taken by each of two randomly assigned trained observers, using a mercury sphygmomanometers	Childhood sUA associated with SBP ($r = 0.31$; $P < 0.01$) and DBP ($r = 0.20$; $P < 0.01$) in childhood and adulthood SBP ($r = 0.29$; $P < 0.01$) and DBP ($r = 0.28$; $P < 0.01$). sUA at childhood correlated with childhood and adulthood SBP and DBP ($P < 0.01$) in girls, but not in black boys. Change in sUA was associated with SBP ($P = 0.03$) and DBP ($P = 0.01$)	Childhood analyses adj. for age and sex; adulthood analyses in addition adj. for childhood BMI, SBP and UA, and change in age and UA
Feig et al. (2003), USA [21]	Case-control, Paediatric Renal Clinic for hypertension ($n = 125$)	165 (61). Range: 6–18. Mean 13.4 ± 3.3	sUA (mg/dl). Mean 3.6 ± 0.8 ; for controls; 7 ± 1.3 for primary HT; 4.3 ± 1.4 for secondary HT; and 6 ± 0.7 for white-coat HT	Mean of three replicates in relaxed seated position, using a Dinamap device. HT: SBP or DBP > 95 th percentile. Secondary HT: identifiable cause for elevated BP. White-coat HT identified with 24-h ABPM. Ref. population: NHBPEP [4]	sUA higher in those with primary ($P < 0.01$) and secondary HT ($P < 0.01$) versus controls. sUA related with SBP ($r = 0.73$; $P < 0.01$) and DBP ($r = 0.61$; $P < 0.01$) (adj. BMI)	BMI
Goldstein and Manowitz (1993), USA [22]	Cross-sectional, Cycle III of NHANES	6268 (52). Range: 1–17	sUA (mg/dl).	BP levels reported as BP _{avg} BP _{end} of the physical examination, measured by nurse-members of the survey team, using a stethoscope mercury sphygmomanometer	sUA has a predictive effect on SBP _{avg} and DBP _{avg} (F_1 , 64.67, $P < 0.01$; F_1 , 7.62, $P = 0.006$) and SBP _{end} (F_1 54.23, $P < 0.01$). For white boys similar results, but for white girls only DBP remained sig. Black, association attenuated but trend remained	Sex, race, age, height, weight, and sexual maturity controlled

adj, adjusted; AMPB, ambulatory blood pressure; BP, blood pressure; CI, confidence interval; CV, cardiovascular risk; eGFR, estimated glomerular filtration rate; HbA1c, haemoglobin A1c; HOMA, homeostatic model assessment; HR, hazard ratio; HT, hypertension; MAP, mean arterial pressure; MetS, metabolic syndrome; NHANES, National Health and Nutrition Examination Survey; NHBPEP, National High Blood Pressure Education Program; NT, normotensive; OR, odds ratio; PH, pre hypertension; POW, percentage of overweight; RR, relative risk; sig, significant; sUA, serum uric acid; TH, transiently elevated blood pressure. When UA is classified in group or quartiles, group 1 refers to those with the lowest uric acid concentrations.

FIGURE 1 Purine metabolism. During the final stage of purine metabolism, xanthine oxidase breaks down hypoxanthine to xanthine, and xanthine to uric acid, meanwhile generating reactive oxygen species. These reactive oxygen species may cause oxidative stress and inactivate the vasodilator nitric oxide. GMP, guanosine monophosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; IMP, inosine monophosphate; NT, nucleotidase; PNP, purine nucleoside phosphorylase; XMP, xanthosine monophosphate.

stimulates the renin–angiotensin system, leading to increased production of the vasoconstrictor angiotensin II [23]. Another mechanism not directly related to uric acid concentration, but rather to its production, is the generation of reactive oxygen species (ROS) during the production of uric acid. In the terminal steps of the purine catabolism, the rate-limiting enzyme xanthine oxidase (XO) catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid (Fig. 1). The main sources of XO are the liver [24–26] and intestine [27]; but XO can also be released into plasma and circulate to remote sites in which it binds to the surface of endothelial cells [28]. When molecular oxygen acts as the electron acceptor, superoxide radical anion ($O_2^{\bullet-}$) [29], and hydrogen peroxide (H_2O_2) are generated as by-products of the oxidation step. These ROS inhibit endothelial nitric oxide (NO) production and consequently impair the vasodilatory reaction [28,30,31]. In addition, the ROS produced by XO activity induce the adherence of leukocytes to endothelial cells, which in turn leads to more ROS generation and the initiation of proinflammatory events [28].

We hypothesize that uric acid production, independent of its concentration, contributes to elevated BP and hypertension. As uric acid concentration is not an adequate marker for the production, proxies for uric acid production need to be investigated (Fig. 2). As is known that XO is a rate-limiting enzyme, an increased XO activity may lead to a relative decrease in the more upstream metabolites, thus, lower hypoxanthine compared with xanthine and lower xanthine compared with uric acid concentration, resulting in higher ratios of xanthine/hypoxanthine, uric acid/xanthine, and uric acid/hypoxanthine. The latter is based on animal studies and studies examining patients with xanthine oxidoreductase deficiency, a genetic disorder called xanthinuria, who have decreased concentrations of the more upstream metabolites [32–35].

In view of the above, the aims of the present study were to extend existing evidence on the relation between plasma

uric acid concentration and BP to school-age children, and to investigate associations between purine metabolite ratios, considered as proxies for an increased XO activity, and BP. Younger individuals are a desirable population in which to investigate this relationship, as common hypertension and metabolic risk factors are less likely to be present. Furthermore, uric acid may be involved in the development of hypertension before vascular damage has occurred but may have less influence on BP levels once vascular damage is permanent [36].

METHODS

Participants and study design

The current analyses were conducted in the context of the KOALA Birth Cohort Study [Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg (A Dutch acronym for child, parents and health: lifestyle and genetic constitution)] in the Netherlands [37]. In the years 2000–2002, pregnant women were recruited at 34 weeks of gestation and followed since. The cohort originates from two recruitment groups: healthy pregnant women with a conventional lifestyle ($n = 2343$) and pregnant women recruited through alternative channels ($n = 491$) (Fig. 2). The women with a conventional lifestyle have been retrieved from an ongoing prospective cohort study on pregnancy-related pelvic girdle pain study in the Netherlands. The second recruitment group consists of pregnant women acquired through anthroposophic doctors and midwives, anthroposophic under-five clinics, posters in organic food shops, magazines for special interest groups, and Steiner schools. The latter group of women was considered to have an alternative lifestyle that could involve dietary habits (vegetarian, organic), child rearing practices, vaccination schemes, and/or use of antibiotics. All parents had signed the informed consent and the study was approved by the Medical Ethics Committee of Maastricht University Medical Centre+ (Maastricht UMC+).

FIGURE 2 Flow chart of the study population. The KOALA Birth Cohort study included pregnant women with a conventional lifestyle (recruited from the on-going Pregnancy-related Pelvic Girdle Pain study) or those recruited through 'alternative' channels. Reasons for exclusion with numbers are presented.

A subgroup of 1204 parents was asked for consent for a home visit for anthropometric measurements, collection of venous blood, and a BP measurement from the child at the age of 6–7 years. This subgroup comprised participants who had home visits for blood collection from the mother during pregnancy and/or the child at age 2 years, and who were still active participants. Venous blood samples were obtained from 600 children and BP measurements from 712 children. Exclusion criteria for the current study were children with congenital heart disease, severe mental disability and/or severe autism, twins, and improbable values in purine concentrations. A total of 246 children that met the criteria were included in the present study (Fig. 2).

Information on potential confounders was prospectively collected by parent-completed questionnaires during pregnancy (weeks 14 and 34) and at ages 3, 7, 12, and

24 months, 4–5 years, and 6–7 years of the child. Dietary information was collected using a food frequency questionnaire (FFQ) covering a period of 4 weeks at the age of 4 years. The FFQ was specifically developed to assess childhood energy intake and validated by the doubly labelled water method [38]. Details on collection of dietary information are described elsewhere [39]. During the home visit at 6–7 years, the child's body weight and height were measured. BMI (weight/height^2 , kg/m^2) was standardized by recoding it into age-specific and sex-specific BMI z-scores using data from the Dutch reference population [40]. For descriptive purposes, children were classified into normal weight (BMI z-score <1.04 , corresponding to the 85th percentile), overweight (BMI z-score between ≥ 1.04 and <1.65 , corresponding to the 85th and 95th percentile), and obesity (BMI z-score between ≥ 1.65 , corresponding to the 95th percentile) [41].

Purine metabolite determination

Venous blood samples were collected in EDTA tubes during the home visit to assess uric acid, xanthine, and hypoxanthine concentrations in plasma. For ethical reasons children were not asked to fast overnight; however, 1.5 h prior to and during the home visit, the children were not allowed to eat or drink, resulting in a mean fast time of 2.2 h (SD 1.2 h). After collection, the samples were stored at -20°C and thawed in a water bath at 37°C prior to analysis and homogenated by vortex mixing. A volume of 30 μl plasma was mixed with 30 μl of internal standards solution, and while vortex mixing 500 μl of acetonitrile was added and vortex mixed for another 10 s. After centrifugation, the supernatant was transferred to a glass sample vial and evaporated to dryness under nitrogen gas at 45°C for ± 15 min. The sample was then taken up in 150 μl 50 mmol/l ammoniumformate pH 4.00. The purine metabolites were determined with ultra-performance liquid chromatography–tandem mass spectrometry [42]. The ratios of uric acid to xanthine [uric acid ($\mu\text{mol/l}$)/xanthine ($\mu\text{mol/l}$)], uric acid to hypoxanthine [uric acid ($\mu\text{mol/l}$)/hypoxanthine ($\mu\text{mol/l}$)], and xanthine to hypoxanthine [xanthine ($\mu\text{mol/l}$)/hypoxanthine ($\mu\text{mol/l}$)] were calculated.

Blood pressure measurement

Mean arterial BP was measured during the home visit, using a validated Omron 705IT oscillometric automated BP monitor (Omron Healthcare Europe BV, Hoofddorp, the Netherlands) [43]. The device automatically provided an estimated SBP and DBP. Up to three measurements were performed by a trained nurse after a 5-min rest, whereas the child was sitting and not allowed to talk or move. BP was measured up to three times on the upper left arm while it was resting on a table. Afterwards, parents were asked to measure their child's BP on three consecutive days, in the morning and in the evening. The parents were instructed by the trained nurse and the same protocol was used. SBP and DBP percentiles and z-scores were calculated according to the nomograms recommended by the National High Blood Pressure Education Program on High Blood Pressure in Children and Adolescents (in the Fourth Report) [4]. As BP was measured with an oscillometric device during a single study visit, a formal diagnosis of hypertension is not possible. Therefore, participants were characterized as having 'normal blood pressure' if the mean (measurement performed by the nurse) of SBP and DBP were both less than 95th percentile and 'elevated blood pressure' if the mean SBP or DBP percentile was at least 95th percentile.

Statistical analysis

The characteristics of the participants are given as numbers and proportions for categorical variables and as mean (SD) or median (interquartile range) values for continuous variables with a normal or nonnormal distribution, respectively. Missing values were classified as unknown for categorical variables (maternal smoking during pregnancy $n = 3$; passive smoking during pregnancy $n = 6$). Participants with missing values on the FFQ ($n = 11$) were excluded for the analyses in the third model. Data analysis was

performed using SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA).

Uric acid concentration differs between men and women; therefore, purine values and their ratios were compared between boys and girls with the independent *t* test [44] or Mann–Whitney test for values with a normal or nonnormal distribution data, respectively. Standardized SBP and DBP z-scores were compared across the three different conditions (home visit measurements versus self-measurement and self-measurements in the morning versus self-measurements in the evening), using the dependent *t* test, respectively.

Generalized estimating equation (GEE) models with unstructured correlation structure were used for analysis of the repeated BP measurements [studied as standardized z-scores (adj. for age, sex, and height z-scores)]. Up to nine measurements were available, taken under three different conditions. The BP measurement condition (as the index variable) and recruitment group (conventional; alternative) as confounder were included in the first model. The analyses included only one single purine metabolite or ratio at a time. In the second model, the following confounders were additionally included: age, infant sex (male, female), and BMI z-scores. In the third model, potential confounders that changed the regression coefficient of uric acid and SBP or DBP by more than 10% were added to the GEE model. The following variables met this criterion: place and mode of delivery (vaginal delivery at home, vaginal delivery in hospital, artificial delivery in hospital, and caesarean section in hospital), maternal smoking during pregnancy (no, yes, and unknown), passive smoking during pregnancy (<1 h a week, >1 h a week, and unknown), total physical activity at 4–5 years of age (h/week), energy intake (kJ/day), protein intake (%), carbohydrate intake (%), and fibre intake (g/day). By adding dietary variables as confounder in the analyses, we attempt to control for dietary factors that potentially affect BP. The following variables did not meet the criterion and were therefore not included in the third model: prematurity (born before 37 weeks of gestation), maternal prepregnancy BMI (kg/m^2), alcohol consumption during late pregnancy (number of consumptions), education level of the mother (lower education, middle education, and high education), birth weight (in grams), duration of breastfeeding (months), and fasting time before blood collection (h).

Finally, we tested whether the association between uric acid concentrations with BP z-scores was modified by sex or BP measurement condition by including an interaction term in the fully adjusted GEE model. The interaction terms were nonsignificant (P value > 0.05); therefore, the analyses were not stratified by sex or BP measurement condition.

RESULTS

The characteristics of the KOALA cohort ($n = 2834$) and the study population are shown in Table 2. The study population consisted of 246 children, who were on average ages 7.1 ± 0.4 years and of whom 114 (46.3%) were boys. In general, the two populations were similar, except that the study population consisted of less mothers who smoked

TABLE 2. Baseline characteristics of the KOALA Birth Cohort Study and the study population

		KOALA birth cohort study, <i>n</i> = 2834	Study population, <i>n</i> = 246
Sex, boy [<i>n</i> (%)]		1543 (50.9)	114 (46.3)
Recruitment group, conventional [<i>n</i> (%)]		2343 (82.7)	207 (84.1)
Age during home visit (years) (mean ± SD)		–	7.05 ± 0.41
BMI z-score during home visit (mean ± SD)		–	–0.24 ± 0.87
Normal weight [<i>n</i> (%)]			227 (92.3)
Overweight [<i>n</i> (%)]			16 (6.5)
Obese [<i>n</i> (%)]			3 (1.2)
Height z-scored (mean ± SD)			0.50 ± 0.88
Birth weight (g) (mean ± SD)		3504 ± 512	3551 ± 438
Maternal education ^a [<i>n</i> (%)]	Low	289 (9.5)	20 (8.1)
	Middle	1060 (34.9)	89 (36.2)
	High	1341 (44.2)	123 (50.0)
	Unknown	343 (11.3)	14 (5.7)
Maternal prepregnancy BMI (mean ± SD)		23.5 ± 4.5	23.5 ± 4.1
Maternal smoking during pregnancy [<i>n</i> (%)]	Yes	222 (7.3)	11 (4.5)
	Unknown	209 (6.9)	3 (1.2)
Maternal passive smoking during pregnancy (>1 h week) [<i>n</i> (%)]	Yes	448 (14.8)	23 (9.3)
	Unknown	255 (8.4)	6 (2.4)
Alcohol consumption during pregnancy (number of glasses) (mean ± SD)		0.3 ± 0.9	0.2 ± 0.7
Duration of pregnancy [<i>n</i> (%)]	Mean ± SD	39.8 ± 5.0	39.5 ± 1.1
	<37 weeks	93 (3.1)	0 (0)
	Unknown	202 (6.7)	1 (0.4)
Place and mode of delivery ^b [<i>n</i> (%)]	Natural delivery at home	1187 (39.1)	113 (45.9)
	Natural delivery in hospital	924 (30.5)	81 (32.9)
	Artificial delivery at home	1 (0.0)	0 (0.0)
	Artificial delivery in hospital	223 (7.4)	23 (9.3)
	Caesarean section in hospital	311 (10.3)	28 (11.4)
	Unknown	381 (12.6)	1 (0.4)
Duration of breastfeeding (months) (mean ± SD)		4.91 ± 4.55	5.5 ± 4.2
Total physical activity (h/week) (mean ± SD)		9.4 ± 4.5	9.6 ± 4.7
Dietary factors of the child at age four (mean ± SD) ^c	Total energy intake (kJ)	6172 ± 1286	6086 ± 1331
	Energy percentage from fat	29.6 ± 4.2	29.6 ± 3.9
	Energy percentage from protein	14.6 ± 2.1	14.8 ± 2.1
	Energy percentage from carbohydrate	55.8 ± 5.0	55.5 ± 4.7
	Fibre (g)	2.5 ± 0.5	2.5 ± 0.5

Numbers do not always add up to the total because of missing data.

^aLow: primary school, preparatory vocational or lower general secondary school, middle: vocational, higher general secondary or preuniversity education, high: higher vocational or academic education.

^bArtificial delivery: induced labour, as when started with drugs or medical devices.

^cMissing data for 13 participants on variables total physical activity and dietary factors.

during pregnancy (active and passive) and more children were delivered naturally at home than in the entire KOALA cohort.

Data on purine metabolite concentrations and their ratios are shown in Table 3. The mean plasma uric acid

concentration was slightly higher for boys [203.9 µmol/l (SD 39.6)] than for girls [201.8 µmol/l (SD 36.2)] but did not differ significantly ($P=0.67$). Median hypoxanthine [boys: 2.4 µmol/l (IQR 2.7); girls 2.5 µmol/l (IQR 3.5)] and mean xanthine concentrations [boys: 0.5 µmol/l (SD 0.20); girls:

TABLE 3. Plasma concentrations of uric acid, xanthine, and hypoxanthine and their ratios

		Total population, <i>n</i> = 246	Boys, <i>n</i> = 114	Girls, <i>n</i> = 132
Purine metabolite				
Uric acid (µmol/l)	Mean ± SD	202.8 ± 37.7	203.9 ± 39.6	201.8 ± 36.2
Xanthine (µmol/l)	Mean ± SD	0.53 ± 0.17	0.53 ± 0.20	0.53 ± 0.15
Hypoxanthine (µmol/l)	Median (IQR)	2.5 (3.2)	2.4 (2.7)	2.5 (3.5)
Ratio				
Uric acid/xanthine	Mean ± SD	417.2 ± 138.4	425.0 ± 154.1	410.5 ± 123.4
Uric acid/hypoxanthine	Median (IQR)	83.9 (94.5)	86.7 (102.5)	81.6 (86.5)
Xanthine/hypoxanthine	Median (IQR)	4.7 (4.8)	4.7 (4.6)	4.6 (4.8)

IQR, interquartile range.

TABLE 4. Blood pressure levels measured by a trained nurse during the home visit and by the parents in the morning and evening

		SBP z-scores	DBP z-scores
Home visit ^a			
	Mean ± SD	0.84 ± 0.69	0.49 ± 0.86
Self-measurement			
Morning ^b	Mean ± SD	0.78 ± 0.94	0.49 ± 0.88
Evening ^c	Mean ± SD	0.88 ± 0.94	0.49 ± 0.85

BP, blood pressure. ^aHome visit: BP is measured by a trained nurse, the mean of three replicates. Self-measurement: BP is measured by the parents on three consecutive days, in the ^bmorning and ^cevening. Missing for each condition: home visit: *n* = 5 (boys *n* = 1; girls *n* = 4); self-measurement: morning *n* = 16 (boys *n* = 5; girls *n* = 11) and evening *n* = 23 (boys *n* = 8; girls *n* = 15).

0.5 μmol/l (SD 0.15)] and the different ratios did not differ substantially by sex.

In Table 4, BP data are presented. Elevated BP was found in 64 (26%) of the children. The correlation among the self-measurement ranged from 0.20 up to 0.50. BP z-scores were higher when measurements were performed by the parents than these performed by the nurse (*P* value <0.001). Furthermore, measurements performed in the evening were higher than these performed in the morning for SBP z-score (*P* = 0.001), but not for DBP z-score (*P* = 0.96).

Purine metabolite concentrations and blood pressure

Multivariable analysis showed that a 1 SD (38 μmol/l) higher plasma uric acid concentration was associated with a higher DBP z-scores, although statistical significance was only reached in the fully adjusted model [adj. sβ 0.07; 95% confidence interval (CI), 0.01–0.14 mmHg]. No association was found with SBP z-scores (Table 5). Plasma xanthine and hypoxanthine concentrations were not associated with SBP z-scores or DBP z-scores, regardless of adjustment for potential confounders. Although the association between plasma hypoxanthine with DBP z-scores was close to

significance (adj. sβ 0.06; CI, −0.01 to 0.13; *P* = 0.10). An increase of 0.10 DBP z-score represents a difference of 0.93 mmHg for a 6-year-old boy with a height z-score of 0.00.

Purine metabolite ratios and blood pressure

The ratios uric acid/xanthine and xanthine/hypoxanthine were associated with DBP z-scores, although statistical significance was only reached in the fully adjusted model (adj. sβ 0.09; CI, 0.01–0.17) and (adj. sβ 0.08; CI, 0.02–0.14), respectively. None of the ratios was significantly associated with SBP z-scores. The ratio uric acid/hypoxanthine was associated with DBP z-scores in the crude model but lost significance after adjustment for potential confounders.

DISCUSSION

The present study showed that, in school-age children, elevated plasma uric acid concentration was associated with higher DBP z-scores. Furthermore, elevated ratios of uric acid/xanthine and xanthine/hypoxanthine, considered as proxies for increased XO activity, were associated with higher DBP z-scores. Our findings support the hypothesis that next to an elevated uric acid concentration, an increased XO activity is associated with elevated BP.

Although an association between serum uric acid concentration and BP has been described before in older children and adolescents (summarized in Table 1), we were the first to show an association in school-age children at a mean age of 7 years. The studies performed so far mainly studied older children (with a mean age of 10 years or higher) and adolescents [7,10,16,20,21]. Moreover, we found an effect in a relatively healthy group of children, with respect to both the participants' uric acid concentration [hyperuricaemia (plasma uric acid concentration >5.0 mg/dl) *n* = 0], whereas other studies often investigate

TABLE 5. Association between purine metabolite and their ratios with blood pressure z-scores in children

		Model 1 sβ (95% CI) ^a	<i>P</i>	Model 2 sβ (95% CI) ^b	<i>P</i>	Model 3 sβ (95% CI) ^c	<i>P</i>
Purine metabolite							
Uric acid	SBP	0.000 (−0.08 to 0.07)	0.99	0.01 (−0.07 to 0.08)	0.90	−0.02 (−0.10 to 0.06)	0.70
	DBP	0.04 (−0.02 to 0.11)	0.17	0.05 (−0.01 to 0.12)	0.08	0.07 (0.01 to 0.14)	0.03
Xanthine	SBP	−0.001 (−0.08 to 0.08)	0.99	0.002 (−0.08 to 0.08)	0.97	−0.03 (−0.10 to 0.05)	0.49
	DBP	−0.03 (−0.10 to 0.05)	0.61	−0.02 (−0.10 to 0.05)	0.54	−0.04 (−0.11 to 0.04)	0.35
Hypoxanthine	SBP	0.01 (−0.01 to 0.10)	0.76	0.01 (−0.07 to 0.09)	0.78	0.01 (−0.08 to 0.10)	0.82
	DBP	0.05 (−0.02 to 0.12)	0.13	0.06 (−0.01 to 0.12)	0.11	0.06 (−0.01 to 0.13)	0.10
Ratio							
Uric acid/xanthine	SBP	−0.01 (−0.10 to 0.08)	0.81	−0.01 (−0.09 to 0.05)	0.83	0.003 (−0.08 to 0.08)	0.93
	DBP	0.06 (−0.02 to 0.14)	0.15	0.07 (−0.01 to 0.15)	0.10	0.09 (0.01 to 0.17)	0.03
Uric acid/hypoxanthine	SBP	−0.002 (−0.04 to 0.04)	0.93	−0.01 (−0.05 to 0.04)	0.28	−0.003 (−0.06 to 0.06)	0.91
	DBP	−0.03 (−0.05 to −0.00)	0.04	−0.03 (−0.06 to 0.01)	0.11	−0.03 (−0.08 to 0.01)	0.17
Xanthine/hypoxanthine	SBP	0.01 (−0.07 to 0.10)	0.74	0.01 (−0.07 to 0.09)	0.79	0.03 (−0.06 to 0.12)	0.50
	DBP	0.06 (−0.00 to 0.12)	0.06	0.06 (−0.00 to 0.12)	0.06	0.08 (0.02 to 0.14)	0.01

CI, confidence interval. The purine metabolites and ratios expressed as SDs (uric acid 37.7 μmol/l; xanthine 0.17 μmol/l; hypoxanthine 6.9 μmol/l and the ratios uric acid/xanthine 138.4; uric acid/hypoxanthine 321.4, and xanthine / hypoxanthine 8.8). Outcome presented as standardized blood pressure z-scores (adj. for age, sex, and height z-scores; ref. population: NHBPEP [24]).

^aAdjusted for recruitment group (conventional; alternative).

^bIn addition, adjusted for age, sex, and BMI z-scores.

^cIn addition, adjusted for place and mode of delivery, maternal smoking during pregnancy (active and passive), total physical activity, and nutrition intake at 4 years of age (total energy intake (kcal), energy from carbohydrates (%) and energy from protein (%) and fibre intake (g). Analyses included 232 children, due to missing values on the FFQ (*n* = 11) and for total physical activity (*n* = 13).

children from clinical cohorts, who have a relatively high risk of cardiovascular diseases [3,11,15,16,18,21].

To the best of our knowledge, we were the first to study the association between BP and several ratios of purine metabolites as proxies for an increased XO activity in the vasculature. We showed that higher uric acid/xanthine and xanthine/hypoxanthine ratios were associated with higher DBP *z*-scores. Our hypothesis is based on studies examining the effect of a deficiency in XO on purine metabolite concentrations [33–35]; however, whether an opposite effect in purine metabolite concentrations occurs by an increased XO activity is unknown. Further investigation is required to examine whether increased XO activity leads to a relative increase in the more downstream metabolites and that this will not be compensated by alterations in urinary excretion of uric acid or xanthine, or the degradation of hypoxanthine to inosine monophosphate by hypoxanthine-guanine phosphoribosyltransferase [35].

In the present study, we found an association with DBP *z*-scores but not with SBP *z*-scores. Diastolic pressure and mean arterial pressure, the steady components of BP, reflect peripheral vascular resistance. Particularly in younger individuals (<50 years of age), DBP is the main predictor of cardiovascular risk. Later in life SBP and pulse pressure, the pulsatile components of BP become the main predictors [45–47]. As previously described, there are several plausible mechanisms linking uric acid to vascular resistance [23]. For example, uric acid in the circulation activates angiotensin II, thereby stimulating the nicotinamide adenine dinucleotide (phosphate) (reduced form) (NAD(P)H) oxidase that is the main source of ROS in the vasculature. ROS reduces the bioavailability of vasodilator NO, leading to vascular resistance. In turn, ROS increases the formation of peroxynitrite, which can increase endothelial NO synthase uncoupling resulting in even more ROS formation [48,49]. Moreover, Landmesser *et al.* [50] found that angiotensin II substantially increases endothelial XO activation in cultured endothelial cells, subsequent to NAD(P)H oxidase activation, leading to even more ROS formation. Moreover, another mechanism not directly related to uric acid concentration, but rather to its production, is the accumulation of ROS during the degradation of purines by increased XO activity.

The magnitude of the BP change found in our study is relatively small; an increase of 1 SD in uric acid concentration or uric acid/xanthine and xanthine/hypoxanthine ratios was associated with an increase of approximately 0.08 higher DBP *z*-score. This represents an increase in DBP by 2 percentiles (or 1 mg/dl increase in plasma uric acid leads to an increase in DBP by 3 percentiles). This is probably not clinically relevant during childhood, nevertheless when maintained during adolescence and adulthood, it can have clinical implications [3,20,21,51,52]. Prolonged exposure to increased peripheral vascular resistance and high BP leads to embedding of the vessel structure in a remodelled extracellular matrix [53,54]. Initially, the remodelling of arteries will be adaptive, but it eventually becomes maladaptive and increases stiffness, contributing to cardiovascular complications of hypertension [53]. Furthermore, an increase in uric acid concentration or XO activity may not have clinical implication at the time, but it increases the risk of hypertension later in life

[3,5,13,20]. For example, Viazzi *et al.* [3] showed, among children at high cardiovascular risk, that even a moderately increased serum uric acid concentration at baseline was associated with elevated BP after a mean follow-up of 1.5 years, regardless of appropriate lifestyle changes such as weight loss and diet. Accordingly, among participants in the Bogalusa Heart Study higher childhood serum uric acid concentration, even within the normal range, was associated with childhood and adult elevated BP (mean follow-up 11.4-year) [20].

In the current study, BP *z*-scores were higher than these from the reference population (Table 4). We made use of an oscillometric device to measure BP, whereas the available reference values have been obtained by an auscultatory device. Values obtained by an oscillometric device are considerably higher than these obtained by an auscultatory device. In addition, the average BP measured by the parents was higher than that measured by the trained nurse during the home visits, regardless of time of measurement (morning or evening). This same phenomenon has been observed previously in young adolescents and adults (<50 years) [55], but in young children it has not been studied extensively [56,57]. Our results show that these differences already occur in early childhood.

Our study has both strengths and weaknesses that deserve to be commented on. The adjustment for potential confounders, quantification of several purine metabolites, and the repeated BP measurements are major strengths of our study. However, the present study must be interpreted within the context of its potential limitations. First, we would like to emphasize the need for studies examining whether an increased XO activity causes shifts in several purine metabolite concentrations and their ratios. Second, we used a cross-sectional design, so no conclusions concerning causality can be drawn. Third, an oscillometric device was used to measure mean arterial BP, and SBP and DBP were subsequently estimated. Reference data on oscillometric BP measurements would have been more appropriate to create SBP and DBP *z*-scores. Unfortunately, referential data on oscillometric BP measurements are limited and insufficiently validated. Further studies are required before a complete substitution can be made from using conventional auscultation method to oscillometry. Last, blood samples to determine the purine metabolites were only collected once. As purine concentrations can vary during the day, multiple measures would have been more accurate.

In conclusion, this study showed that plasma uric acid concentration and the ratios uric acid/xanthine and xanthine/hypoxanthine are associated with DBP *z*-scores in school-age children. These findings support the role of uric acid as a determinant of BP, already in early childhood, and confirm our hypothesis that an increased XO activity, reflected by increased purine ratios, is associated with BP. These findings highlight the need for further investigation on increased XO activity and BP.

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Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

Strengths of the article:

This study shows that in a general population tendency to higher uric acid levels is associated with diastolic blood pressure. It is the first study showing that this phenomenon is related to elevated activity of xanthine oxidase.

Weaknesses of the article: the weak point is lack of analysis of potential associations between xanthine oxidase activity and subclinical arterial injury or oxidative stress.

Reviewer 2

The study is the first one in which, using repeated blood pressure measurements, the association between blood pressure and several ratios of purine metabolites, as proxies for an increased XO activity in the vasculature, was assessed. The results showed that higher uric acid/xanthine and xanthine/hypoxanthine ratios were associated with higher DBP z-scores in children at a mean age of 7 years. The weaknesses are related to the cross-sectional design, to the fact that blood samples to determine the purine metabolites were only collected once, and to the relatively small magnitude of blood pressure change that is clinically not relevant during childhood.