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ORIGINAL ARTICLE

Normative data on the Bonn Risk Index for calcium oxalate crystallization in healthy children

Tadeusz Porowski · Walentyna Zoch-Zwierz · Anna Wasilewska · Anthony Spotyk · Jerzy Konstantynowicz

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Abstract Bonn Risk Index (BRI) is being used for the assessment of urinary calcium oxalate (CaOx) crystallization. There are no published data regarding BRI during growth. The objective of this study was to establish age- and sex-dependent BRI values in healthy children and adolescents. A total of 1,050 Caucasian subjects aged 3–18 years (525 males, 525 females) without a history of kidney stone disease were enrolled in the cross-sectional study. The study group was divided into 15 ranges according to age, each comprising 70 subjects. Urinary ionized calcium [Ca^{2+}] was measured using a selective electrode while the onset of spontaneous crystallization was determined using a photometer and titrating with 40 mmol/L ammonium oxalate (Ox^{2-}). The calculation of BRI value was based on the ratio of [Ca^{2+}] to the required amount of ammonium oxalate added to 200 ml of urine to induce crystallization. The median BRI was 0.26 1/L and the values of the 5th and 95th percentiles were 0.06 1/L and 1.93 1/L, respectively. BRI correlated positively with body-area-related BRI ($1/L \times 1.73\ m^2$) (R=0.18; P<0.05), whereas a negative correlation was found between BRI and body weight ($1/L \times kg$) (R=−0.85; P<0.05). Neither sex nor age differences were detected in BRI across studied children and adolescents. The values of Bonn Risk Index were constant during growth and there was a limited influence of age and sex on BRI in children over 3 years of age. The BRI may be valuable in the evaluation of pediatric patients at risk for kidney stones, particularly if the BRI from stone formers is demonstrated to be higher than in normal children.

Keywords Bonn Risk Index (BRI) · Children · Normative values · Oxalate crystallization

Abbreviations

AP	activity product
BMI	Body Mass Index
BRI	Bonn Risk Index
[Ca^{2+}]	ionized calcium concentration
CaOx	calcium oxalate
(Ox^{2-})	amount of ammonium oxalate added to 200 ml of urine to induce crystallization

T. Porowski (✉) · W. Zoch-Zwierz · A. Wasilewska
First Department of Pediatrics,
Medical University of Bialystok, Children's Hospital,
17 Waszyngtona St.,
15-274 Bialystok, Poland
e-mail: tporowski@biaman.pl

A. Spotyk
Department of Nuclear Medicine,
Medical University of Bialystok,
Bialystok, Poland

J. Konstantynowicz
Department of Pediatrics and Auxology,
Medical University of Bialystok,
Bialystok, Poland

Introduction

Urolithiasis is a frequently reported condition in children and is diagnosed even in neonates and infants [1, 2]. The disease may be the first sign of congenital and acquired metabolic disturbances, or the consequence of anatomic or genetic abnormalities [2]. The majority of kidney stones are composed of calcium oxalate and calcium phosphate [1]. Insight into this pathology is increasing and is focused on the pathogenesis of deposit formation in the urinary tract [3–5]. Investigation is being conducted into more effective

methods which would enable the detection of risk factors for urolithiasis. One of the risk factors is an increase in the crystallization of calcium oxalate (CaOx) in urine. Kavanagh and Laube recently published a review of methods used to assess the crystallization of CaOx in urine [6]. During the last few years, activity products (AP) of crystallization have usually been assessed by means of the AP_{CaOx} index or as the relative supersaturation (RS) of urinary calcium oxalate (RS_{CaOx}) using the computer program EQUIL [7, 8].

Laube et al. showed that the ratio of calcium ions [Ca^{2+}] to the amount of ammonium oxalate added to 200 ml of urine to induce crystallization [designated here as (Ox^{2-})] may be, at the moment of spontaneous crystallization of CaOx, an indicator of the risk of CaOx crystal formation [9]. The authors made this determination with a direct urine collection, without an initial processing. This ratio is known as the Bonn Risk Index (BRI): BRI=[Ca^{2+}]/(Ox^{2-}) 1/L [9, 10]. Due to its characteristics, this index is an accurate indicator of the individual state of balance between the quantities of the most important promoters and inhibitors of the crystallization process within urine [9, 10]. Measuring BRI is simple, cost-effective, and the results are repeatable. In patients with calculi formation, CaOx assessed using BRI is significantly higher when compared to healthy subjects [9]. A lack of published studies concerning BRI in pediatric patients led us to conduct the study in children and adolescents. The purpose of this study was to define the BRI value in healthy subjects aged 3–18 years, in relation to age and sex.

Materials and methods

The study was performed on a group of 1,050 healthy Caucasian children and adolescents (group I) aged 3–18 years (mean±SD: 10.51±4.33), comprised of 525 boys and 525 girls. The study population was divided into 15 age groups, consisting of 70 children in each 1-year group (35 boys and 35 girls). The children reported no history of dietary restrictions. All participants met the criteria of the standard dietary energy and nutrient intakes recommended in Poland [11]. These children were free of infection at the time of the examination (serum C-reactive protein CRP <0.4 mg/dL, blood leukocyte count $<10\times10^9$/L). Prior to inclusion, all participants were screened regarding serum levels of protein, albumin, calcium, phosphate, potassium, uric acid, creatinine and alkaline phosphatase as well as urine concentrations of citrate, oxalate, potassium, calcium and phosphate. Urinary dipstick testing (Bayer Diagnostics, Bridgend, UK) detecting nine parameters, including leukocytes, protein and blood, did not reveal any abnormalities. Children with a family history of kidney stones were excluded from the study. Subjects with diseases known to affect oxalate, calcium and phosphate metabolism and children treated with antibiotics were excluded. All children were screened using renal ultrasound examination to exclude urolithiasis (Toshiba SSH-140A apparatus; probe Convex 3.75 MHz). Participants and their legal guardians gave informed consent, and the study was approved by the Ethical Committee of the Medical University of Bialystok.

Bonn Risk Index

The Bonn Risk Index was assessed using the method of Laube [12]. Each child had a 24-h urine collection into sterile containers, without additional preserving substances, which was stored at 4°C. The testing was always performed twice using the same urine collection from each subject. Two consecutive urine samples (each 200 mL) were incubated immediately after collection, at a temperature of 37°C and the calcium ion concentration was measured using calcium ion-selective electrodes of type Rapilab 855 (Bayer, Germany) and titrated with ammonium oxalate solution (40 mmol/L) at a rate of 0.75 mL/min. The onset of spontaneous crystallization was detected using an Eppendorff photometer (filter 585 nm) with a decrease in light transmission to 98% of the initial value. Each analysis was repeated twice. The BRI is presented as [Ca^{2+}] mmol/L / (Ox^{2-}) mmol = 1/L. Calibration and quality assurance procedures, based on the calibration curves, were conducted every day.

Statistical analysis was performed using the program Statistica 6.0 PL. Mann-Whitney test was used for the analysis of two non-parametric independent variables, with $P<0.05$ considered statistically significant. Assessment of the rank of two independent variables was conducted using Spearman correlation, with $P<0.05$ considered statistically significant. For the purpose of plotting the curve of spontaneous crystallization (an association between the number of calcium ions and the amount of added ammonium oxalate leading to the spontaneous crystallization), we used the computer program with the range of values as a scatterplot with the option of adding curves.

Results

The characteristics of the study group are presented in Fig. 1. The anthropometric traits of participants, based on weight and height measurements and body mass index (BMI), were within the normal range in each subgroup, relative to the Polish references described by other authors [13].

Figure 2 shows the detailed results for the whole study group for BRI, defined as the ratio of [Ca^{2+}] concentration

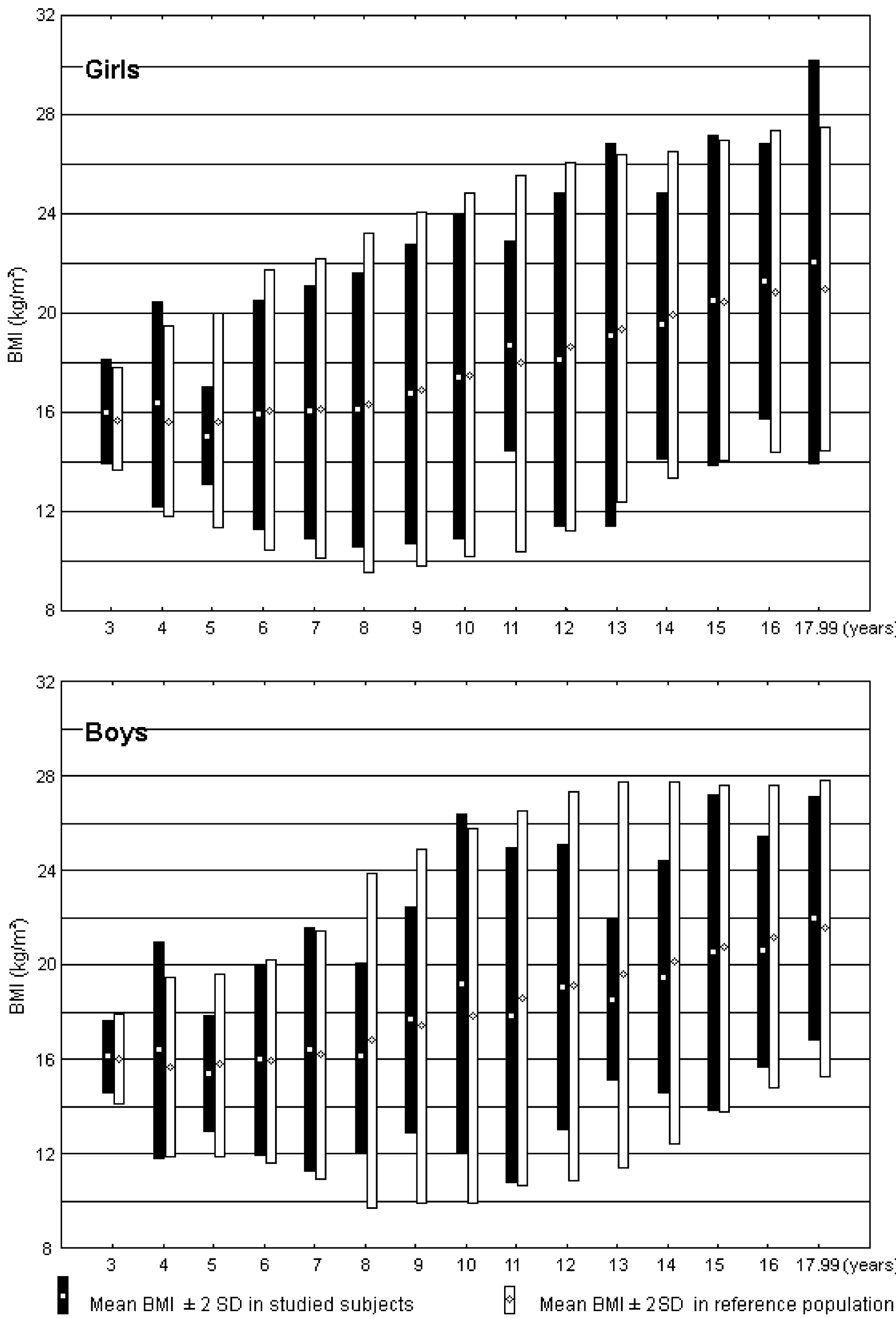

Fig. 1 Body mass index (BMI) in studied girls and boys aged 3–18 years compared to age and sex-matched reference range

in urine to the amount of added ammonium oxalate (Ox^{2-}) necessary for the spontaneous crystallization of CaOx. BRI in healthy children ranged between 0.06 and 1.93 1/L. The values between the 5th and 95th percentiles are found between the two borderline arrows. This diagram also presents the minimum and maximum concentrations of [Ca^{2+}] and the required amount of added oxalate (Ox^{2-}). The concentration of [Ca^{2+}] in urine ranged from 0.25 mmol/L (5th percentile) to 0.89 mmol/L (95th percentile) with a median of 0.42 mmol/L, and an amount of added (Ox^{2-}) ranging from 0.46 mmol (5th percentile) to 3.53 mmol (95th percentile) with a median of 1.65 mmol.

Figure 3 presents the BRI values in children representing various age groups. We considering the subjects in two main subgroups, younger children aged 3–9 years, and older children and adolescents aged 10–18 years. In

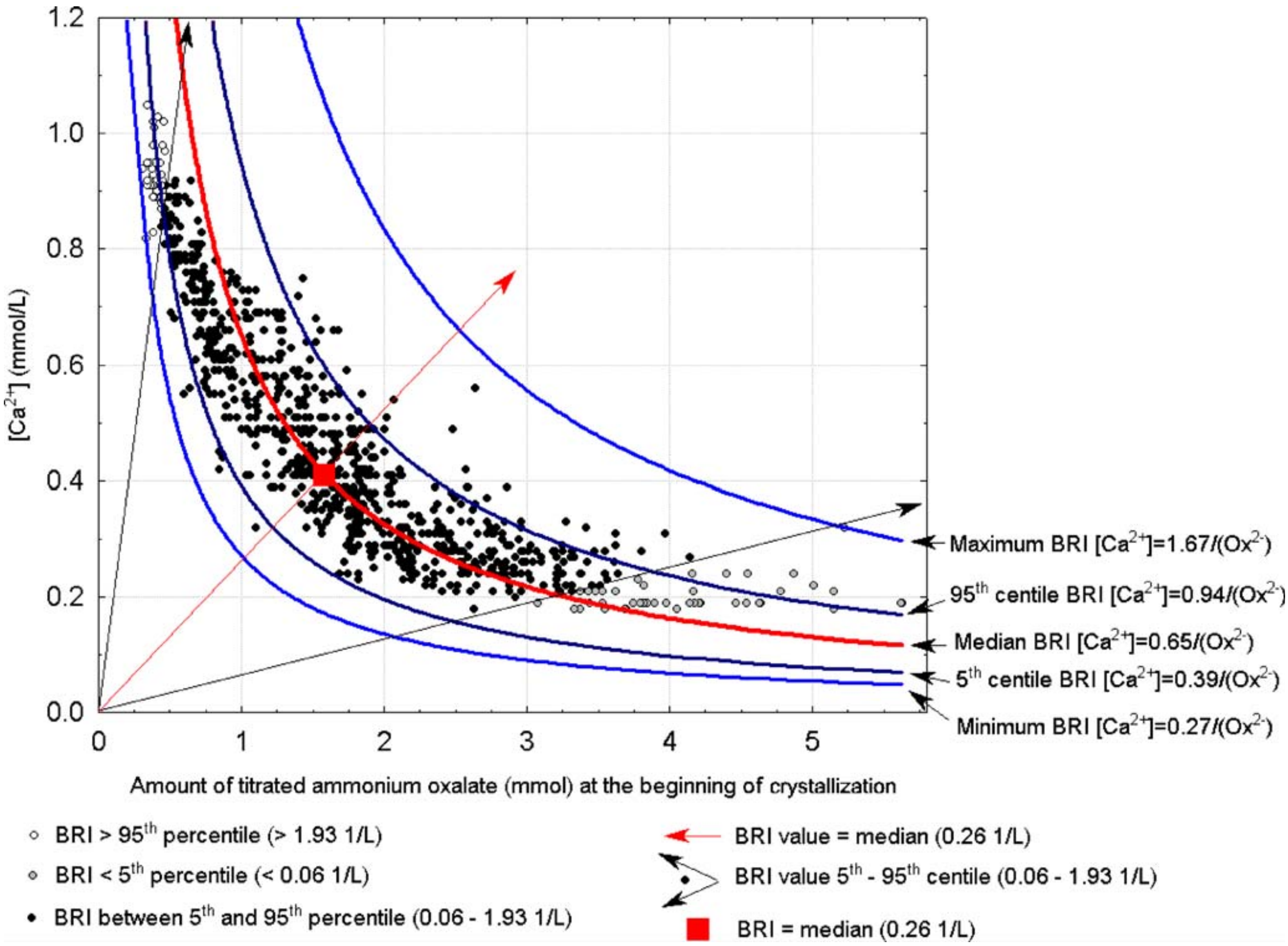

Fig. 2 The spontaneous crystallization of CaOx in urine of 1,050 children aged 3.00–17.99 years. The *x-axis* shows amount of ammonium oxalate (Ox^{2-}) in mmol necessary for the onset of spontaneous crystallization. The *y-axis* shows the concentration of calcium ions [Ca^{2+}] before adding Ox^{2-}. The extremes of the hyperbola represent minimum and maximum values. The minimum hyperbola is defined by the equation [Ca^{2+}]=0.27/(Ox^{2-}), and the maximum is [Ca^{2+}]=1.67/(Ox^{2-}). The BRI values for the 5th to the 95th percentile are found between the hyperbolas [Ca^{2+}]=0.39/(Ox^{2-}) and [Ca^{2+}]=0.94/(Ox^{2-}), median [Ca^{2+}]=0.65/(Ox^{2-})

children 3–9 years of age, the lowest values of BRI were found in the youngest children, i.e. those 3 years old (median 0.18 1/L), whilst the highest values were found in 9-year-old children (median 0.34 1/L). However, statistical analyses did not show a difference between the values across the various age groups. The lowest median BRI value was 0.03 1/L in 9-year old children, whilst the highest median value was 2.48 1/L in 4-year-old children. The BRI values for younger children corresponding to the 5th percentile ranged from 0.03–0.08 1/L, and the 95th percentile from 1.38–2.27 1/L.

In older children and adolescents aged 10–18 years, the lowest BRI values were found in the participants who were 12 years old (median 0.17 1/L), whilst the highest were in 17-year-old adolescents (median 0.31 1/L) (Fig. 3). However, statistical analysis did not show significant differences between the age subgroups. The minimum value of BRI was 0.02 1/L, and was found in 11-year-old children, whereas the maximum value was 3.1 1/L in children aged 10 and 17 years. The BRI values for older children corresponding to the 5th percentile ranged between 0.05 and 0.13 1/L, whilst the 95th percentile was from 1.69–2.66 1/L.

The crystallization values of CaOx, based on BRI in healthy children aged 3–18, did not exceed 2.66 1/L. No significant differences were found in the BRI values between boys and girls in either age group.

Table 1 presents the BRI and BRI related to the 1.73 m^2 body surface area and body mass (kg). The median BRI in relation to body surface area ($1/L \times 1.73\ m^2$) was 0.39 $1/L \times 1.73\ m^2$, with 5th and 95th percentile medians of 0.09 and 3.01 $1/L \times 1.73\ m^2$, respectively. However, the median BRI in relation to 1 kg of weight was 0.008 $1/L \times kg$ with corresponding 5th and 95th percentiles of 0.0015 and 0.06 $1/L \times kg$, respectively. A weak positive correlation was found between BRI and BRI/1.73 m^2 (R=0.18, P<0.05), and a negative correlation was found between BRI/1.73 m^2 and BRI/kg (R=−0.86, P<0.05). No differences were found

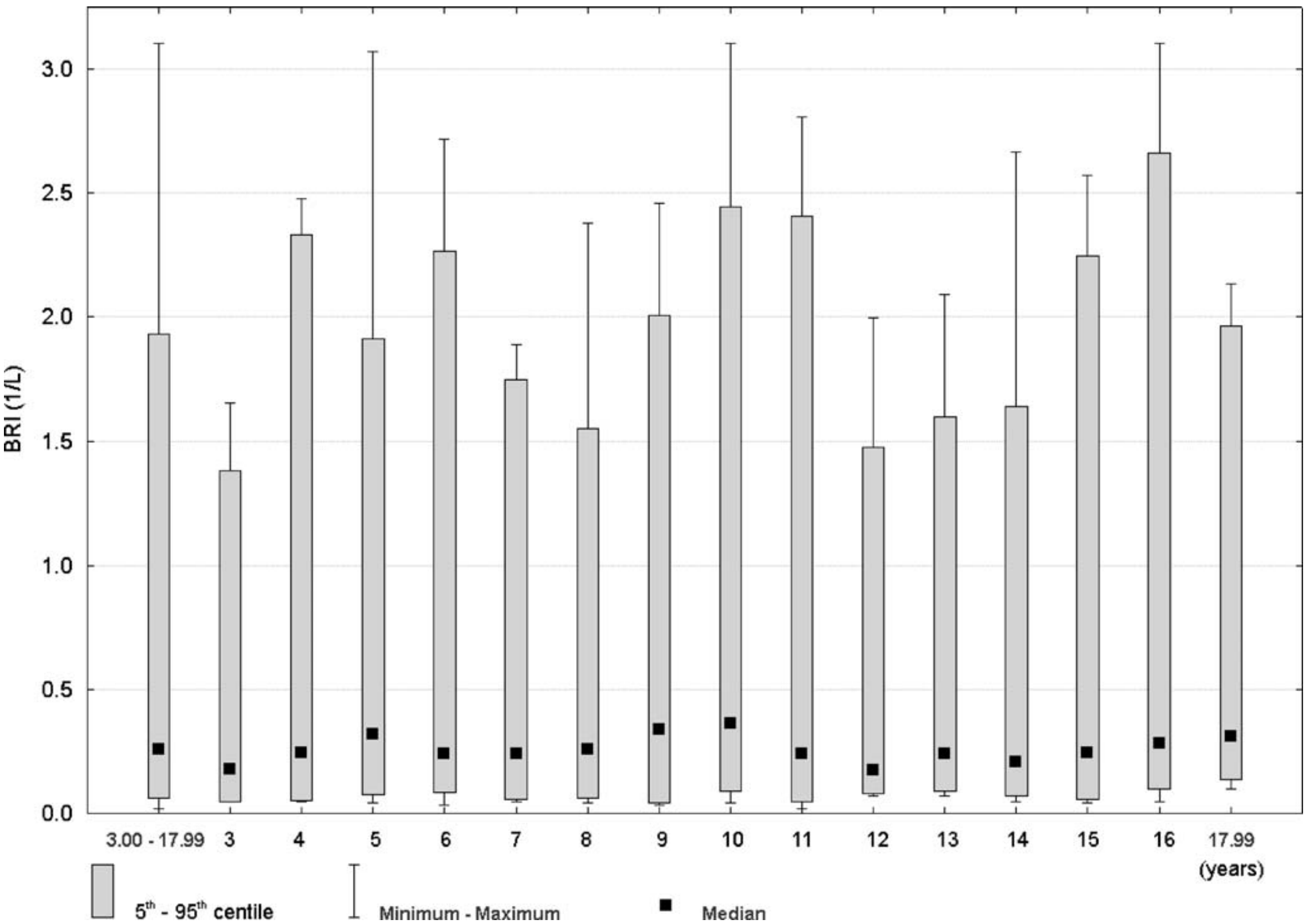

Fig. 3 Bonn Risk Index (BRI) in the whole studied group (*left box*) and in separate 1-year age groups for children aged 3–18 years

between the values of BRI/1.73 m^2 and BRI/kg in the various age groups or in relation to sex.

Discussion

Urinary stones are considered a major health problem in society, both in adults and in children, due to their recurrent nature and the cost of treatment [14]. The pathogenic pathways leading to stone formation in kidneys have not been fully explained. During processes of calculus formation, a number of phenomena have been reported such as excess of crystallizing substances, nucleation, crystallization, aggregation and stone formation [15, 16]. However, it has not been explained why calculi do not form in all subjects, despite a large amount of urinary crystallization products. Over the past few years, there have been many attempts to define the risk factors leading to urinary stone formation on the basis of the ability to form oxalate crystals, a main component of calculi [7, 8, 17]. Tiselius et al. described the practical importance of assessing the activity of calcium oxalate ions in urine using the AP_{CaOx} index [7].

Stone formation has also been evaluated in terms of excess urinary calcium oxalate, using the computer program EQUIL [18, 19]. Laube et al. showed a strong correlation between the concentration of free calcium ions [Ca^{2+}] in urine and the quantity of ammonium oxalate (Ox^{2-}) added to invoke spontaneous urinary crystallization of CaOx [9]. The authors suggested that the BRI index in healthy subjects was significantly lower than in those forming urinary calculi. The study, based on a group of 72 healthy subjects, provided mean BRI values of 1.05±

Table 1 Bonn Risk Index values in children aged 3–18 years in relation to body surface area and body weight

	Median	Minimum	Maximum	5th percentile	95th percentile
BRI (1/L)	0.26	0.02	3.10	0.06	1.93
BRI/1.73 m^2 (1/L×1.73 m^2)	0.39	0.03	6.65	0.09	3.01
BRI/kg (1/L×kg)	0.008	0.0006	0.15	0.0015	0.06

1.038 1/L with a range of 0.06–4.89 1/L. In other studies involving 85 healthy adult subjects, the mean BRI values were similar, 0.89±0.91 1/L. Higher BRI values have been described by Lewandowski et al. whose study group of 15 Caucasian adults demonstrated a mean BRI of 4.90 1/L, and 15 African adults had a mean BRI of 2.04 1/L [10]. The literature review did not reveal any larger or more comprehensive studies regarding BRI in children.

Our findings in the large group of healthy children showed that the normal BRI value ranged from 0.06 1/L (5th percentile) to 1.93 1/L (95th percentile). The maximum values obtained in children were lower than those described by other authors in adult populations [6, 9, 20]. This may be due to the fact that the mechanism which inhibits crystal formation in children's urine is more effective when compared to adults. Teller et al. observed that urinary glycosaminoglycans in children reduced the aggregation of crystals more effectively than in adults [21]. A similar age-related effect regarding stone formation has been observed by Bergsland et al., who showed that the urine of subjects aged less than 20 years produced a strong inhibitory effect on crystal formation [22]. Ricchiuti et al. investigated the process in boys aged less than 10 years and found increased activity of plasma protease inhibitor (inter-alpha inhibitor), blocking urinary crystallization during various stages [23].

Our results did not show a significant difference in BRI values between boys and girls. A minor difference was found in relation to age, but this was not significant. The BRI/1.73 m^2 and BRI/kg behaved similarly. High correlations between BRI, BRI and body weight, and BRI and body surface area of studied subjects enable practical, more objective use of BRI and allow for comparisons between individuals. For this reason, these results are presented as median values with ranges for the 5th to 95th percentiles for the whole study group.

The study has several limitations. So far, the use of our normative data appears to be limited to the Polish population. It is difficult to relate the results to other geographical regions or to different ethnic groups as there are no published data regarding BRI in children. Further comparative and prospective investigations are needed because a cross-sectional study is not able to reveal whether some children with a normal BRI value will be at risk for stone disease in the future. Moreover, some studied children may have had urolithiasis despite normal results of renal ultrasound. However, this study provided consistent normative data on BRI, due to both the large age representation and the stringent selection criteria.

In summary, the Bonn Risk Index, expressed as the ratio of ionized calcium to the amount of ammonium oxalate necessary to initiate spontaneous CaOx urinary crystal formation, is lower in healthy children and adolescents aged 3–18 years than in studies of adults by other authors. The BRI during growth appears to be independent of age and sex. Thus, our results may contribute to the effective screening of kidney stone disease in pediatric subjects. We conclude that the BRI may be valuable in the evaluation of pediatric patients at risk for kidney stones, particularly if the BRI from stone formers is demonstrated to be higher than in normal children.

References

1. Kraus SJ, Lebowitz RL, Royal SA (1999) Renal calculi in children: imaging features that lead to diagnoses: a pictorial essay. Pediatr Radiol 29:624–630
2. Cameron MA, Sakhaee K, Moe OW (2005) Nephrolithiasis in children. Pediatr Nephrol 20:1587–1592
3. Asplin JR (2002) Hyperoxaluric calcium nephrolithiasis. Endocrinol Metab Clin North Am 31:927–949
4. Sayer JA, Carr G, Simmons NL (2004) Nephrocalcinosis: molecular insights into calcium precipitation within the kidney. Clin Sci 106:549–561
5. Hoppe B, Leumann E, von Unruh G, Laube N, Hesse A (2003) Diagnostic and therapeutic approaches in patients with secondary hyperoxaluria. Front Biosci 8:437–443
6. Kavanagh JP, Laube N (2006) Why does the Bonn Risk Index discriminate between calcium oxalate stone formers and healthy controls? J Urol 175:766–770
7. Tiselius HG (1997) Risk formulas in calcium oxalate urolithiasis. World J Urol 15:176–185
8. Werness PG, Brown CM, Smith LH, Finlayson B (1985) EQUIL 2: a basic computer program for the calculation of urinary saturation. J Urol 134:1242–1244
9. Laube N, Schneider A, Hesse A (2000) A new approach to calculate the risk of calcium oxalate crystallization from unprepared native urine. Urol Res 28:274–280
10. Lewandowski S, Rodgers AL, Laube N, von Unruh G, Zimmermann D, Hesse A (2005) Oxalate and its handling in a low stone risk vs a stone-prone population group. World J Urol 6:1–4
11. Kunachowicz H, Nadolna I, Przygoda B, Iwanow K (1998) Food composition tables. National Food and Nutrition Institute, Warsaw
12. Laube N, Hergarten S, Hesse A (2002) Comparison of a laser-probe and photometric determination of the urinary crystallization risk of calcium oxalate. Clin Chem Lab Med 40:595–599
13. Palczewska I, Szilágyi-Pągowska I (2002) Ocena rozwoju somatycznego dzieci i młodzieży. Med Prakt Ped 3200:140–148
14. Chandhoke PS (2002) When is medical prophylaxis cost-effective for recurrent calcium stones? J Urol 168:937–940
15. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC (2003) Time trends in reported prevalence of kidney stones in the United States: 1976–1994. Kidney Int 63:1817–1823
16. Kok DJ (2002) Clinical implications of physicochemistry of stone formation. Endocrinol Metab Clin North Am 31:855–867
17. Kavanagh JP (2006) In vitro calcium oxalate crystallisation methods. Urol Res 14:1–7
18. Hoppe B, Jahnen A, Bach D, Hesse A (1997) Urinary calcium oxalate saturation in healthy infants and children. J Urol 158:557–559
19. Lande MB, Varade W, Erkan E, Niederbracht Y, Schwartz GJ (2005) Role of urinary supersaturation in the evaluation of children with urolithiasis. Pediatr Nephrol 20:491–494
20. Laube N, Hergarten S (2005) Can the Bonn Risk Index be replaced by a simple measurement of the urinary concentration of free calcium ions? J Urol 175:2175–2177

21. Teller WM, Burke EC, Rosevaer JW, McKenzie BF (1962) Urinary excretion of acid mucopolysaccharides in normal children and patients with gargoylism. J Lab Clin Med 59:95–101
22. Bergsland KJ, Kinder JM, Asplin JR, Coe BJ, Coe FL (2002) Influence of gender and age on calcium oxalate crystal growth inhibition by urine from relatives of stone forming patients. J Urol 167:2372–2376
23. Ricchiuti V, Hartke DM, Yang LZ, Goldman HB, Elder JS, Resnick MI, Marengo SR (2002) Levels of urinary inter-alpha-trypsin inhibitor trimer as a function of age and sex-hormone status in males and females not forming stones. BJU Int 99:513–517