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**Title:** KIDNEY VOLUME AND ALBUMINURIA AS MARKERS OF BIRTH WEIGHT – BLOOD PRESSURE RELATIONSHIP IN ESSENTIAL HYPERTENSION

**Running title:** Birth weight - blood pressure relationship in essential hypertension

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## Abstract

Our aim was to analyze whether birth weight contributes to future hypertension through reduced kidney volume, and could albuminuria be a marker of this pathway.

We included 103 patients with newly-diagnosed essential hypertension and 92 normotensive controls. Blood pressure (BP) was measured using mercury sphygmomanometer and ABP monitor. Kidney volume was determined by ultrasound. Data on birth weight were obtained from mothers. Albuminuria was determined in 24-hour urine samples.

Hypertensive patients had lower birth weight and higher albuminuria than normotensives. There was no difference in kidney volume between the two groups. We found negative correlation between birth weight and systolic BP in hypertensive group. BP was significantly correlated with BMI and albuminuria in the hypertensive group. Multiple regression analysis had shown the greatest impact of BMI on BP and had also demonstrated that 24h systolic BP showed the greatest risk for developing albuminuria in hypertensive patients.

In conclusion, birth weight influences BP values in adult age, but it is not mediated by a reduced kidney volume. Strong correlation, independent of birth weight, was observed between albuminuria and BP values. Increased BMI is the most important independent risk factor responsible for BP increase, even in an early phase of essential hypertension.

Keywords: hypertension, birth weight, kidney volume, albuminuria

## Introduction

A broad range of epidemiological data supports the hypothesis that risk for developing essential hypertension is, in part, determined before birth. Negative correlation between the size at birth and blood pressure (BP) in later life has been observed [1-3]. This phenomenon, termed "the programming" has, since recently, been the subject of an intensive investigation, undertaken in order to determine possible underlying mechanisms [4]. Numerous mechanisms have been proposed to underlie the observed relationship, the reduced number of nephrons being one of them [5,6]. Several studies were conducted to the end of exploring the correlation between the kidney size and the number of nephrons, and most of them agree that the number of nephrons in a kidney correlates with the physical dimensions and size of the organ [7]. Ultrasonographic measurement of renal dimensions and volume represents a simple and reliable method applicable in clinical settings [8-11]. Given the facts discussed above, within the frame of this study we had investigated the justifiability of the proposed pathophysiologic pathway, i.e lower birth weight - lower kidney volume (reflecting a lower nephron number) – higher blood pressure in later life. Albuminuria has been recognized as an important predictor of renal and generalized vascular disease in both diabetic and non-diabetic patients [12,13]. Recent data support the role of albuminuria as a marker of endothelial damage and also as an integrated marker of cardiovascular risk in patients with essential hypertension [14]. Albuminuria has also been linked to poor fetal growth, arising as a consequence of hyper-filtration taking place in remnant nephrons [15,16]. The role of albuminuria as the marker of early renal damage in patients with essential hypertension is less clear [17]. Our aim was to analyze whether birth weight contributes to future development of hypertension through lower kidney volume (reflecting a lower nephron number) and whether albuminuria could be a marker that points towards these developments.

## Patients

The study embraces patients treated in the Outpatient Clinic of the Division of Nephrology and Arterial Hypertension, Zagreb University Hospital Center during the 1999-2003 time-frame. Anthropometric characteristics were determined in 103 patients (60 M/ 43 F, age  $37.3 \pm 9.1$  years) with newly-diagnosed essential hypertension, lacking any target organ damage whatsoever and being antihypertensive medications-naive, as well as in 92 normotensive controls (47 M/ 45 F, age  $35.6 \pm 7.5$

years) (Table 1). Patients with positive parental history of hypertension and those with “white-coat” hypertension were excluded. “White-coat” hypertension was defined as the blood pressure values  $\geq$  140/90 mm Hg measured with mercury sphygmomanometer and  $<$  120/80 mmHg daytime BP values measured using an ambulatory blood pressure device. Subjects constituting the control group were recruited from the hospital staff and the pool of patients visiting our hospital for other reasons, who managed to fulfil the following inclusion criteria: negative personal history of kidney and cardiovascular disease, absence of any actual kidney disease or malformation which could influence kidney morphology or function, normal serum creatinine levels, and normotension, i.e. BP  $<$  140/90 mm Hg. All participants signed an informed consent.

## Methods

Blood pressure was measured in clinical settings after a five-minute lag period in a sitting position, using a mercury sphygmomanometer equipped with standard-sized cuff for adult persons (three consecutive measurements were made, based on which the average value was calculated), and using the ambulatory blood pressure device Spacelabs 90207. BP  $\geq$ 140/90 mm Hg, measured using a mercury sphygmomanometer, and daytime BP  $\geq$  140/90 mm Hg, measured using the ambulatory blood pressure device, were considered as hypertensive values. Birth weight data were obtained from mothers. Although this method could be prone to recall bias, it was used in other studies exploring birth weight-blood pressure relationship as well (18,19). The method itself was also tested by O’Sullivan et al; in their study, parental recall data on their children’s birth weight were compared to the data entered into birth registries. The study showed the parental recall data to be highly accurate (20). Low birth weight was defined as less than, or equal to 2500 g. Both kidneys were examined by ultrasound (Siemens Sonoline SI 250) on a single occasion, by one observer who was unaware of patient’s characteristics. Three consecutive measurements of kidney length, width and depth were made, and the mean value was calculated for each measured parameter used for the calculation of kidney volume by virtue of the ellipsoid formula (Volume=length x width x depth x 0,523) after Solvig and Dinkel [21,22] . Kidney volume was corrected for BSA and the combined kidney volume was calculated:  $1.73 \times 1/2 (KV_{\text{right}} + KV_{\text{left}})/BSA$  [23] . BMI was calculated in the following manner: body weight (kg)/height (m<sup>2</sup>), while the BSA (m<sup>2</sup>) was obtained as follows:  $71.84 \times \text{body weight}^{0.425} \times \text{body height}^{0.725} \times 10^{-4}$ . Obesity was defined as BMI  $>$ 30 kg/m<sup>2</sup>.

Albuminuria (30-300 mg/dU) was determined in a 24-h urine sample using immune-nephelometric method (Behring, Nephelometer Analyzer II), while 24-h urinary sodium was measured by photometric method (Efox photometer 5053). Increased urinary sodium was defined as values > 220 mmol/24h.

## Statistics

Statistical analysis was performed with WinStat ver. 4.0 Statsoft, Inc. Normality of data was tested using  $\chi^2$  test and Kolmogorov-Smirnov test. Albuminuria showed a skewed distribution; for the purposes of correlation and regression analyses, albuminuria logarithmic values were used. The distribution of continuous variables was described using means and SDs, except for albuminuria where median and range were employed to that goal. Differences in means established between the groups were tested using either Student t-test or Mann-Whitney U-test. Inter-group comparisons of prevalence rates were attained by  $\chi^2$  test. Relationships between birth weight, kidney volume, albuminuria and blood pressure were examined by Pearson correlation coefficient ( $r$ ). The strength of the association between blood pressure, albuminuria and several other variables (i.e. birth weight, reduced kidney volume, BMI, smoking) was quantified by virtue of multiple regression analysis. The statistical evaluation outcome was considered statistically significant in case of the  $P$  value < 0.05.

## Results

Characteristics of patients with essential hypertension and those of normotensive controls are shown in Table 1. Among patients with hypertension an increased prevalence of lower birth weight (30% vs 17%,  $\chi^2 = 3.63$ ,  $P=0.047$ ), microalbuminuria (21% vs 5%  $\chi^2 = 6.05$ ,  $P=0.013$ ) and increased urinary sodium (79% vs 32%  $\chi^2 = 6.82$ ,  $P=0.009$ ) was established as compared to the normotensive group. Maternal smoking was not significantly more frequent in the hypertensive group (19% vs 15%  $\chi^2 = 0.60$ ,  $P=0.440$ ). Hypertensive subjects were more often obese (36% vs 9.6%,  $\chi^2 = 10.13$ ,  $P=0.001$ ) and smokers (49% vs 31%  $\chi^2 = 6.50$ ,  $P=0.017$ ) than those constituting the normotensive group. Both male and female hypertensive patients had shorter and wider kidneys and a greater kidney volume than the normotensive ones. However, when corrected for BSA, the observed correlation was not statistically significant ( $P=0.898$ ) (Table 2). Kidney dimensions correlated best with the body proportions. We found negative correlation between birth weight and office blood pressure in

the hypertensive ( $r=-0.25$ ;  $P=0.038$ ), but not in the normotensive group ( $r=-0.14$ ;  $P=0.234$ ). Birth weight did not correlate with the combined corrected kidney volume in either group (Table 3). Blood pressure showed significant correlation with BMI ( $r=0.30$   $P=0.002$ ), but not with the combined corrected kidney volume ( $r=0.11$   $P=0.786$ ). Multiple regression analysis of the correlation between BMI, birth weight, the combined corrected kidney volume and blood pressure in the hypertensive group yielded a significant regression coefficient ( $\beta=0.35$ ,  $P=0.005$ ) only when it comes to BMI (Table 4).

When gender-based differences in the group of hypertensive patients were analyzed, it was observed that male patients exhibited larger body proportions (BSA  $2.11 \pm 0.1$  vs  $1.78 \pm 0.1$  m<sup>2</sup>,  $P < 0.001$ ) and slightly increased kidney volume ( $113.1 \pm 19.2$  vs  $109.2 \pm 25.5$  cm<sup>3</sup>/1.73m<sup>2</sup>,  $P=0.621$ ). Women were proven to have higher office systolic blood pressure values ( $157.7 \pm 15.2$  vs  $150.9 \pm 14.3$  mm Hg,  $P=0.022$ ) and albuminuria ( $23.2 \pm 16.7$  vs  $17.9 \pm 13.5$  mg/24h,  $P=0.384$ ). There were no differences in birth weight (men  $3154 \pm 734$  vs women  $3187 \pm 529$  g,  $P=0.847$ ). The observed characteristics were not found in the normotensive group.

There existed a significant positive correlation between blood pressure and albuminuria in the hypertensive group. This goes particularly for the 24-h systolic blood pressure ( $r=0.40$   $P < 0.001$ ). Birth weight and the combined corrected kidney volume failed to show any correlation with albuminuria in either group (birth weight:  $r=0.13$   $P=0.892$ ; corrected.kidney volume  $r=0.18$   $P=0.729$ ). Multiple regression analysis pointed towards 24-h systolic BP as the major risk factor for developing albuminuria ( $\beta= 0.31$ ,  $P=0.049$ ) in the hypertensive group (Table 5).

## Discussion

The correlation between birth weight and blood pressure was found to be negative in both study groups; however, in the hypertensive arm that correlation was proven statistically significant. This observation is in agreement with the outcome of several epidemiological studies [1-3]. Following the adjustment for body proportions, either the differences in kidney volume between the two groups, or the correlation between the kidney volume and blood pressure failed to be found. Nyengaard and Bendtsen proved that glomerular adaptation to increased metabolic demands occurs due to an increased glomerular volume and due to the fact that BSA correlates best with the glomerular volume [7]. Raman et al. failed to find significant correlation between the kidney volume and blood pressure. Blood pressure

correlated best with BMI [24]. The absence of difference in kidney volume may be explained by the fact that the study was performed on an adult population in which the exact duration of blood pressure elevation remained unknown, as well as by possible compensatory glomerular hypertrophy, as proposed by Keller et al. [25]. In that study, the nephron number in hypertensive patients was reduced by 46%, while the glomerular volume was increased by 133% as compared to the control group, resulting in an increased total glomerular volume ( $4.56 \times 10^3$  vs  $3.98 \times 10^3$ ).

We also found that hypertensive women exhibit a tendency towards a lower kidney volume and a significantly higher ambulatory systolic blood pressure. Given that women were shown to have smaller kidneys with a lower nephron mass (~ 10%) [5], the underlying mechanism could be a lower nephron number, since other factors contributing to blood pressure elevation (i.e. an increased BMI, smoking, 24h urinary sodium) were more often found in men.

Our results do not support the hypothesis that the influence of birth weight on blood pressure levels is mediated by a lower kidney volume. However, a significant correlation between blood pressure and BMI was observed. This is in agreement with the results of Falkner and Seidman, who explained the increase in blood pressure in lower birth weight persons by obesity and increased BMI [26,27]. In our study, the hypertensive group members had higher body weights, higher BMIs and were more often smokers. All these characteristics may independently contribute to an increased BP values. Nevertheless, birth weight could also contribute to blood pressure values by several other mechanisms, for instance increased glucocorticoid levels, impaired vascular development, enhanced sympathetic activity, or an enhanced postnatal catch-up growth and obesity [28-31].

In our study, hypertensive patients were more often diagnosed with microalbuminuria. It correlated best with 24-h systolic blood pressure, which is in agreement with the results of other authors [32]. Although other authors reported a negative correlation between microalbuminuria and birth weight [33,34], we failed to find such correlation in our group. There was also no correlation between albuminuria and kidney volume. Other authors reported similar results. In the study of 545 middle-aged Danes, Johansen et al. did not confirm the hypothetical association between fetal growth and adult albumin-creatinine ratio [35]. Yudkin reports a weak association between microalbuminuria and low ponderal index at birth, but not lower birth weight in non-diabetic subjects [16]. This could be explained by the fact that birth weight is a poor indicator of growth retardation during the third trimester, when an



increase in nephron number takes place. According to our results, albuminuria is not a reliable marker when it comes to the corroboration of the proposed pathophysiological sequence: lower birth weight -> reduced kidney volume -> higher blood pressure at adult age.

In conclusion, birth weight influences blood pressure values at adult age, but this influence is not mediated by a lower kidney volume. Our study did not support an association between birth weight, kidney volume, higher blood pressure and the onset of albuminuria at adult age. Strong correlation, independent of birth weight, was observed between albuminuria and blood pressure. The mechanisms other than kidney volume (i.e. increased BMI) could be more important for the development of hypertension.

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Table 1 Characteristics of patients with essential hypertension and normotensive controls

|   | Hypertensive group, n=103 | Normotensive group, n=92 | P                  |
|---|---------------------------|--------------------------|--------------------|
| Age (y)   | 37.3 (9.1)                | 35.6 (7.5)               | 0.151              |
| Men (n/%)   | 60 (58)                   | 47 (52)                  | 0.314              |
| Height (m)  | 1.73 (0.1)                | 1.74 (0.1)               | 0.735              |
| Weight (kg)   | 83.5 (16.3)               | 73.3 (15.1)              | <0.001             |
| Body mass index (kg/m <sup>2</sup> )                              | 27.6 (4.2)                | 24.1 (3.9)               | <0,001             |
| Body surface area (m <sup>2</sup> )                               | 1.97 (0.2)                | 1.87 (0.2)               | 0.001              |
| Birth weight (g)  | 3,168 (648.9)             | 3,453 (622.7)            | 0.013              |
| Low birth weight $\leq$ 2500 g (n/%)                              | 31 (30)                   | 16 (17)                  | 0,047              |
| Smoking (n/%)   | 51 (49)                   | 29 (31)                  | 0.017              |
| Blood pressure (mmHg)   |                           |                          |                    |
| Systolic  | 153.7 (15.0)              | 123.8 (9.1)              | <0.001             |
| Diastolic   | 102.1 (8.5)               | 80.8 (6.7)               | <0.001             |
| 24-h blood pressure (mmHg)  |                           |                          |                    |
| Systolic  | 145.5 (12.9)              | 115.8 (8.7)              | <0.001             |
| Diastolic   | 94.9 (10.4)               | 70.6 (6.2)               | <0.001.            |
| Albuminuria (mg/24h) (median/range)                               | 10.75(2.33-257.0)         | 7.98(1.17-67.52)         | 0.016 <sup>†</sup> |
| Microalbuminuria (n/%)  | 22 (21)                   | 5 (5)                    | 0,013              |
| 24h urinary Na (mmol/24h)   | 220.5 (94.1)              | 182.4 (70.0)             | 0.004              |
| Comb.correct.kidney volume (cm <sup>3</sup> /1,73m <sup>2</sup> ) | 109.9 (21.9)              | 110.3 (14.3)             | 0.898              |

Unless stated otherwise, all values are expressed as means ( $\pm$ SDs).

Continuous variables were compared using Student-t test and Mann-Whitney U test where indicated (<sup>†</sup>).

Categorical variables were analyzed using  $\chi^2$  test

Table 2 Kidney dimensions measured in patients with essential hypertension and in normotensive controls

|   | Hypertensive group<br>n=103 | Normotensive<br>group n=92 | <i>P</i> |
|---|-----------------------------|----------------------------|----------|
| Kidney length (mm)                                  |                             |                            |          |
| Right   | 110.4 (13.6)                | 113.7 (6.6)                | 0.032    |
| Left  | 113.3 (7.6)                 | 115.8 (6.4)                | 0.016    |
| Kidney width (mm)                                   |                             |                            |          |
| Right   | 46.7 (6.7)                  | 44.0 (4.9)                 | 0.002    |
| Left  | 51.0 (6.0)                  | 48.9 (4.9)                 | 0.009    |
| Kidney depth (mm)                                   |                             |                            |          |
| Right   | 40.1 (5.4)                  | 39.3 (4.9)                 | 0.315    |
| Left  | 43.1 (4.9)                  | 41.6 (3.8)                 | 0.022    |
| Right KV (cm <sup>3</sup> )                         | 115.1 (32.7)                | 110.8 (22.5)               | 0.235    |
| Left KV (cm <sup>3</sup> )                          | 135.8 (31.9)                | 127.5 (22.2)               | 0.042    |
| Comb.corr KV (cm <sup>3</sup> /1,73m <sup>2</sup> ) | 109.9 (21.9)                | 110.3 (14.3)               | 0.898    |

All values are mean ( $\pm$ SD)

Continuous variables were compared using Student-t test

Table 3 Pearson correlation coefficients established between birth weight and clinical variables

|                                  | Birth weight                |                            |
|----------------------------------|-----------------------------|----------------------------|
|                                  | Hypertensive<br>group n=103 | Normotensive<br>group n=92 |
| Office systolic blood pressure   | -0.25*                      | -0.14                      |
| Office diastolic blood pressure  | -0.23**                     | -0.12                      |
| Albuminuria logarithmic values   | 0.13                        | 0.06                       |
| Combined corrected kidney volume | 0.10                        | 0.06                       |

\**P*=0.038; \*\* *P*=0.045

Table 4. Multiple regression analysis between office systolic blood pressure and clinical variables in the hypertensive group of patients

|                                  | $\beta$ | Standard error | <i>P</i> |
|----------------------------------|---------|----------------|----------|
| Body mass index                  | 0.35    | 0.1239         | 0.005    |
| Birth weight                     | -0.20   | 0.1240         | 0.11     |
| Combined corrected kidney volume | 0.04    | 0.1202         | 0.71     |

Table 5. Multiple regression analysis between log albuminuria and selected exposure variables in the hypertensive group of patients

|                                    | $\beta$ | Standard error | <i>P</i> |
|------------------------------------|---------|----------------|----------|
| Age                                | 0.08    | 0.156          | 0.586    |
| Sex (female vs male)               | 0.05    | 0.145          | 0.689    |
| Smoking                            | 0.01    | 0.143          | 0.934    |
| Body mass index                    | 0.20    | 0.142          | 0.841    |
| Birth weight                       | 0.13    | 0.143          | 0.341    |
| Combined corr.kidney volume        | 0.24    | 0.151          | 0.136    |
| Ambulatory systolic blood pressure | 0.31    | 0.153          | 0.049    |