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DISSERTATION

Salt intake as a risk factor for hypertensive disorders of pregnancy and importance of gestational aldosterone availability

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Anna Birukov

aus Bolschenarymskoje, Kasachstan

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Abbreviations

24h u-aldo	24-hour urinary aldosterone excretion
24h u-K^+	24-hour urinary potassium excretion
24h u-Na^+	24-hour urinary sodium excretion
95% CI	95% confidence interval
95% KI	95% Konfidenzintervall
ACTH	adrenocorticotrophic hormone
AT_1R	angiotensin II type 1 receptor
AT_2R	angiotensin II type 2 receptor
BMI	body mass index
BP	blood pressure
BW	birth weight
BW sds	birth weight standard deviation score
CVD	cardiovascular diseases
DBP	diastolic blood pressure
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ENaC	epithelial sodium channel
g	gram
GA	gestational age
g/day	gram per day
GDM	gestational diabetes mellitus
GH	gestational hypertension
g/mmol	gram per millimole
h	hour
HR	hazard ratio
HRP	horseradish peroxidase
IQR	interquartile range
IUGR	intrauterine growth restriction
K^+	potassium
kg/m^2	kilogram per square meter

LGA	large for gestational age
mg/day	milligram per day
µl	microliter
mmHg	millimeter of mercury
mmol	millimole
mmol/day	millimole per day
mmol/l	millimole per liter
Na^+	sodium
NaCl	sodium chloride, common salt
NO	nitric oxide
NP	normotensive pregnancy
OGTT	oral glucose tolerance test
p-aldo	plasma aldosterone
PCOS	polycystic ovary syndrome
PE	preeclampsia
pg/ml	pictogram per milliliter
PIH	pregnancy-induced hypertension
PlGF	placental growth factor
PW	placental weight
RAAS	renin-angiotensin-aldosterone system
SD	standard deviation
sFlt-1	soluble fms-like tyrosine kinase-1
sFlt-1/PlGF	soluble fms-like tyrosine kinase-1 to placental growth factor ratio
SGA	small for gestational age
u-	urinary
VEGF	vascular endothelial growth factor

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Abstract

Background: Aldosterone plays a pivotal role in sodium reabsorption, plasma volume expansion and blood pressure regulation. The effects of aldosterone in pregnancy beyond maternal plasma volume expansion are not fully understood. In preeclampsia, both plasma volume and aldosterone availability are reduced. In vitro studies have demonstrated that aldosterone is implicated in the trophoblast cell proliferation. The activity of the renin-angiotensin-aldosterone system is influenced by salt intake. High salt and low potassium intakes are established risk factors for hypertension and cardiovascular disease outside of pregnancy but less is known about their role in pregnancy. We hypothesized that aldosterone acts as a direct feto-placental trophic factor, and that high-salt in combination with low potassium intakes decrease maternal aldosterone secretion, resulting in attenuated placental and birth weights and a higher incidence of hypertensive disorders of pregnancy. We further evaluated the utility of aldosterone in predicting preeclampsia.

Methods: We analyzed data from the Odense Child Cohort, a Danish prospective population-based cohort study. 24-hour urine collections and plasma samples from gestational week 29 in a subsample of 569 pregnant women were available for the analyses. Plasma and urinary aldosterone were determined by ELISA, urinary sodium and potassium excretions by flame photometry. Sodium and potassium intakes were estimated by 24-hour urinary sodium and potassium excretions. Relationships between aldosterone levels, sodium and potassium intakes on the one hand and preeclampsia, placental and fetal weights on the other hand were assessed independent of maternal clinical and demographic characteristics and offspring covariates.

Results: In the adjusted models, urinary aldosterone excretion was associated with birth and placental weights (adjusted β coefficients [95% CI]: 24.50 [9.66; 39.35] and 9.59 [4.57; 14.61], respectively) independent of maternal and offspring covariates. Aldosterone availability did not associate with preeclampsia or pregnancy-induced hypertension. Salt intake >6 gram/day increased the hazard for development of preeclampsia by 5.7 times.

Conclusions: Aldosterone levels in early 3rd trimester contributed to placental and birth weights. Our data suggest that aldosterone has pregnancy-specific functions beyond plasma volume expansion, with maternal aldosterone being a marker for placental and fetal growth. Suppression of aldosterone in pregnancy may have adverse trophic effects. In perspective,

therapeutic interventions increasing aldosterone availability might be considered for pregnancies at high risk for intrauterine growth restriction and should be tested in rodent and human studies. Additionally, we identified high salt intake as an important modifiable risk factor for preeclampsia and pregnancy-induced hypertension. Further studies and analyses are needed to evaluate sodium and potassium intakes for dietary recommendations in pregnancy.

Zusammenfassung

Hintergrund: Aldosteron spielt eine entscheidende Rolle bei der Rückresorption von Natrium und Wasser, Expansion des Plasmavolumens und Blutdruckregulation. Die Wirkungsmechanismen des Aldosterons während der Schwangerschaft - abgesehen von der Expansion des mütterlichen Plasmavolumens - sind nicht vollständig verstanden. In präeklamptischen Schwangerschaften sind Plasmavolumen und Aldosteronspiegel reduziert. In-vitro-Studien haben gezeigt, dass Aldosteron bei der Proliferation fetaler Trophoblastenzellen eine Rolle spielt. Die Aktivität des Renin-Angiotensin-Aldosteron-Systems wird durch die Natriumzufuhr beeinflusst. Salzreiche, kaliumarme Diät ist ein etablierter Risikofaktor für Hypertonie und kardiovaskuläre Erkrankungen außerhalb der Schwangerschaft, jedoch ist wenig bekannt über ihren Einfluss während der Schwangerschaft. Wir stellten die Hypothese auf, dass Aldosteron direkt als ein feto-plazentarer trophischer Faktor agiert, und dass eine salzreiche und kaliumarme Diät die Aldosteronsynthese hemmt, und somit zu reduziertem Wachstum der Plazenta und des Fetus und zu höherer Inzidenz von hypertensiven Schwangerschaftserkrankungen führt. Außerdem bewerteten wir die Nutzbarkeit des Aldosterons in Bezug auf die Präeklampsie-Vorhersage.

Methoden: Wir analysierten die Daten aus einer dänischen prospektiven populationsbezogenen Kohorte (Odense Child Cohort). Für die Analysen wurden 24-Stunden-Urin und Plasma aus der 29. Schwangerschaftswoche von 569 Schwangeren verwendet. Plasma- und Urinaldosteron wurden mit ELISA, Natrium- und Kalium-Urinausscheidungen mit einem Flammenphotometer gemessen. Natrium- und Kaliumeinnahmen wurden auf der Basis von Natrium- und Kalium-Urinausscheidungen geschätzt. Wir untersuchten die Beziehungen zwischen Aldosteronspiegel, Natrium- und Kaliumeinnahme einerseits und Geburts- und Plazentagewicht, Inzidenz von Präeklampsie und schwangerschaftsinduzierter Hypertonie

andererseits unabhängig von den mütterlichen klinischen und demografischen und fetalen Charakteristika.

Ergebnisse: Urinaldosteron assoziierte mit Geburt- und Plazentagewicht unabhängig von maternalen und fetalen Parametern (adjustierte β-Koeffiziente [95% KI]: 24.50 [9.66; 39.35] und 9.59 [4.57; 14.61]). Aldosteronspiegel war nicht mit Präeklampsie oder schwangerschaftsinduzierter Hypertonie assoziiert. Salzeinnahme >6 Gramm/Tag war mit einem 5.7-fach höheren Hazard für Präeklampsie-Entwicklung assoziiert.

Schlussfolgerungen: Aldosteronspiegel zu Beginn des 3. Trimesters trug zum Geburts- und Plazentagewicht bei. Unsere Ergebnisse deuten auf eine schwangerschaftsspezifische Funktion Aldosterons über Plasmaexpansion hinaus hin, wobei Aldosteron als ein Marker des feto-plazentaren Wachstums fungiert. Hemmung der Aldosteronsynthase in der Schwangerschaft kann negative trophische Effekte haben. Perspektivisch könnte man therapeutische Maßnahmen für die Erhöhung des Aldosteronspiegels bei den Schwangerschaften mit hohem Risiko für fetale Wachstumsretardierung in Erwägung ziehen, die in Tier- und humanen Studien getestet werden sollten. Des Weiteren haben wir Hochsalzdiät als einen wichtigen modifizierbaren Risikofaktor für Präeklampsie und schwangerschaftsinduzierte Hypertonie identifiziert. Weitere Studien und Analysen sind notwendig, um Natrium- und Kaliumeinnahmen für diätetische Empfehlungen in der Schwangerschaft zu evaluieren.

1. State of the art

Pregnancy constitutes nine months of dramatic physiological changes in the female body. It can be viewed as a direct cardio-metabolic stressor, resulting either in an appropriate adaptation to the changing environmental conditions, or maladaptation – as some women will develop signs of impaired cardiovascular adaptation (gestational hypertension [GH] or preeclampsia [PE]) or impaired glucose tolerance. The present work is focusing predominantly on PE. PE is a disease which affects 2-8% of pregnancies[1, 2] and is characterized by the new-onset hypertension after 20 weeks of gestation accompanied by proteinuria, or intrauterine growth restriction (IUGR), or maternal end-organ damage, such as acute kidney or liver dysfunction or neurological complications[3]. Patients with a previous PE express signs of maternal cardiac dysfunction[4, 5] and are more susceptible to cardiovascular disease (CVD) later in life[6, 7]. The syndrome is thought to be related to a defective deep placentation, which

can also be seen in IUGR, preterm birth, preterm rupture of membranes, late spontaneous abortion and placental abruption[8]. Risk factors for PE include prior PE, renal disease (most conditions with albuminuria), chronic hypertension, diabetes mellitus, primiparity, systemic lupus erythematosus, antiphospholipid antibody syndrome, multiple gestation, family history of CVD or PE, obesity, excessive gestational weight gain and advanced (≥40 years) maternal age[9].

In non-pregnant physiology, the mineralocorticoid aldosterone increases the reabsorption of salt and water by the kidney tubules, thereby reducing their loss via the urine while at the same time causing an expansion of blood and extracellular fluid volumes, and by extension the long-term elevation of the arterial pressure. In pregnancy, aldosterone plays an important role in the physiological expansion of maternal plasma volume, which is essential for maintaining circulating blood volume, blood pressure, and optimal uteroplacental perfusion. Circulating levels of aldosterone in plasma are increased during healthy pregnancy[10-13], supported by the augmented release of active renin from the kidney in response to decreased vascular resistance and the need for elevated blood volume, and by the increased angiotensinogen secretion by the liver driven by placental production of estrogens, starting as early as in the 1st trimester of pregnancy[14]. Levels of all components of the renin-angiotensin-aldosterone system (RAAS) in the maternal circulation increase throughout the pregnancy and reach 3- to 7-fold higher levels at the end of gestation compared to pre-pregnancy levels[14]. Furthermore, despite this increase in angiotensin II and aldosterone, maternal blood pressure (BP) is unchanged or even lower with higher aldosterone levels at birth[15, 16]. Further studies have shown an elevated aldosterone-to-renin ratio in healthy pregnancy, suggesting that additional factors – such as vascular endothelial growth factor (VEGF), adrenocorticotrophic hormone (ACTH) or potassium (K^+) – might stimulate or augment aldosterone secretion both directly and indirectly[17, 18]. As has already been demonstrated in animal and *in vitro* studies, aldosterone availability is necessary for placental development[19], contributing to a normal fetal development by inducing placental growth factor (PlGF) expression and trophoblast cell proliferation[20]. Although RAAS is usually considered to increase blood pressure, healthy pregnant women do not usually present with hypertension, despite relatively high angiotensin II and aldosterone levels. Several counterbalancing mechanisms might be in play: The mineralocorticoid receptor antagonist actions of progesterone and the increased glomerular filtration rate facilitate natriuresis despite the sodium retaining properties of aldosterone. Moreover, physiological pregnancy is a state of relative vascular insensitivity to the pressor

effect of angiotensin II, and the vasodilator angiotensin II type 2 receptor (AT_2R) is induced under the influence of estrogens[21]. Newly discovered factors of the RAAS may contribute to vasodilation as well, such as the heptapeptide angiotensin 1-7 with its own Mas-receptor, which exerts antiangiogenic, anti-inflammatory, antiproliferative and vasodilatory properties[22, 23]. In addition, dilatory effects are exerted by nitric oxide (NO), kallikrein-kinin system, prostacyclin and relaxin[21].

In contrast to normal pregnancy, maternal plasma volume is reduced in manifest PE, paralleled by a suppression of aldosterone production, vasoconstriction, reduced extracellular volume expansion and abundant Na^+ retention[10-13, 24-30]. Notably, PE is characterized by attenuated adrenal aldosterone sensitivity to the stimulatory effects of ACTH[18], high levels of soluble fms-like tyrosine kinase-1 (sFlt-1, an endogenous VEGF inhibitor) and low VEGF and PlGF levels, which might all contribute to the decreased bioavailability of aldosterone in PE[17]. Another possible explanation for the reduced levels of aldosterone in PE could be a deficiency in enzymatic pathways. Mutations with reduced methyl oxidase activity in the aldosterone synthase gene CYP11B2 have been described in PE[25]. In addition, vascular responsiveness to angiotensin II is enhanced in PE despite reduced circulating levels of RAAS compared to physiological pregnancy[30, 31]. One potential mechanism for the increased angiotensin II sensitivity is the presence of circulating autoantibodies against angiotensin II type 1 receptor (AT_1R) in the sera of preeclamptic women[32-34]. Studies suggest that RAAS activity during pregnancy is influenced by dietary salt (NaCl) intake[35, 36]. We thus hypothesized that chronic high NaCl intake paralleled by low K^+ intake would suppress aldosterone availability with adverse implications for placental development and fetal growth and would result in higher incidence of PE and pregnancy-induced hypertension (PIH)[37].

There is currently a paucity of insight into the underlying pathophysiological mechanisms of PE. The impact of dietary NaCl intake, NaCl sensitivity and renal function on health is receiving much attention internationally, with ground-breaking publications from our group[38] and others[39-41], demonstrating hitherto unknown regulatory mechanisms of NaCl on immune functions in non-pregnant setting. Outside of pregnancy, the role of excessive dietary NaCl and insufficient K^+ intakes in causing hypertension, CVD and stroke is well documented in a number of animal studies, clinical and epidemiological trials both within and across populations[42-55]. Yet, sodium (Na^+) is also an essential nutrient necessary for healthy physiological function, as it is required for maintenance of plasma volume, acid-base balance,

transmission of nerve impulses and normal cell function. In its Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013–2020, World Health Organization (WHO) identified nine key targets for the reduction of chronic diseases, including "a 30% relative reduction in mean population intake of salt/sodium"[56, 57]. The recommended level of dietary NaCl intake is <6 g per day (g/day), which equates to 2300 mg of Na^+, however, dietary NaCl intake is above this recommended daily amount in the majority of countries[58]. Salt sensitivity of BP is increased in conditions with albuminuria (even if urinary albumin is merely within high-normal range) in the non-pregnant setting[59, 60], but as of yet, the effect of NaCl on PE and other hypertensive disorders of pregnancy is understudied.

The objective of the current study was to assess the associations of NaCl intake >6 g/day – the recommended upper limit of daily NaCl consumption as defined by the WHO[58] – and aldosterone availability with maternal outcomes PE and PIH incidence, and fetal outcomes birth weight (BW) and placental weight (PW)[37].

Circulating angiogenic markers sFlt-1 and PlGF are released from the placenta and endothelium, and their imbalance plays a pivotal role in the pathogenesis of PE[61, 62]. Since they are widely accepted biomarkers for PE and utilized in the clinical routine[61-71], we investigated whether aldosterone levels and high NaCl intake associated with PE development independent of placental angiogenic marker concentrations.

2. Methods

2.1 Odense Child Cohort

The project was based on the data from the Odense Child Cohort study. The Odense Child Cohort is a prospective population-based cohort from the Municipality of Odense, Southern Denmark, comprising approximately 2500 active mother-infant dyads as of now[72]. Between January 1st 2010 and December 31st 2012 all pregnant women in the Municipality of Odense (n = 6707) were approached for participation in the study. In total, 2874 women (42.9%) accepted the enrolment material and were included into the Odense Child Cohort[72]. The children will be followed up until 18 years of age, with currently 7th year of examination in progress. The study was approved by the local Ethics Committee under the protocol number S-20090130 and by the Danish Data Protection Agency under the number j.no. 2008-58-

0035[72]. It was conducted according to the 2nd Helsinki Declaration and all participating women gave written informed consent.

2.2 Sub-sample with available 24h urine specimens from the Odense Child Cohort

Inclusion criteria for the present retrospective nested sub-study were: available 24h urine collections, available data on maternal diagnoses PE and GH, gestational length, offspring sex, PW and BW[37]. Exclusion criteria encompassed confirmed cases of gestational diabetes mellitus (GDM) defined by the Danish diagnostic criteria, i.e. one-step 75 g oral glucose tolerance test (OGTT) with 2h venous plasma glucose level ≥9.0 mmol/l[73, 74], twin pregnancies, preexisting hypertension, manifest PE or GH at the time of sampling, or incomplete 24h urine specimens, **Figure 1**. Urine specimen was considered as complete, if urine volume was ≥500 ml/day[75] paralleled by 24h u-creatinine excretion ≥600 mg/day. Maternal BP from 1st trimester and at the time of sampling was extracted from the patients' medical charts. PIH encompassed both PE and GH. The diagnoses of PE and GH were retrospectively validated through evaluation of patients' medical charts[76]. PE was diagnosed according to the Danish criteria from 2007–2012, i.e. as *de novo* hypertension after 22 weeks of gestation with proteinuria (>0.3 g/day or at least +1 on sterile urine dipstick), GH as *de novo* hypertension without proteiuria[76]. Severe PE was characterized as PE with either BP >160/110 mmHg, elevated urate or transaminases, low platelets and/or symptoms including pulmonary edema, visual disturbances, abdominal pain or persistent headache[77]. Of total 2874 recruited women, 607 (21%) provided 24h urine specimens and of these, 569 fulfilled the inclusion criteria for the present sub-study, **Figure 1**. 57% of women who provided 24h urine collections (n = 347) had risk factors for GDM, as specified by the principal investigators: BMI >27 kg/m^2; previous GDM, previous infant birthweight >4500 g; family history of diabetes; PCOS; or glycosuria detected during pregnancy[73]. This patient collective underwent an OGTT at gestational age (GA) 28–30 weeks. Additionally, women without known GDM risk factors from the Odense Child Cohort were matched to the women with GDM risk factors based on GA; they likewise underwent an OGTT[37].

Fetal outcomes comprised BW, PW and BW standard deviation score (BW sds). BW sds is adjusted for gestational length and sex of the infant and was based on the formula developed for Scandinavian population by Marsal and colleagues[78]:

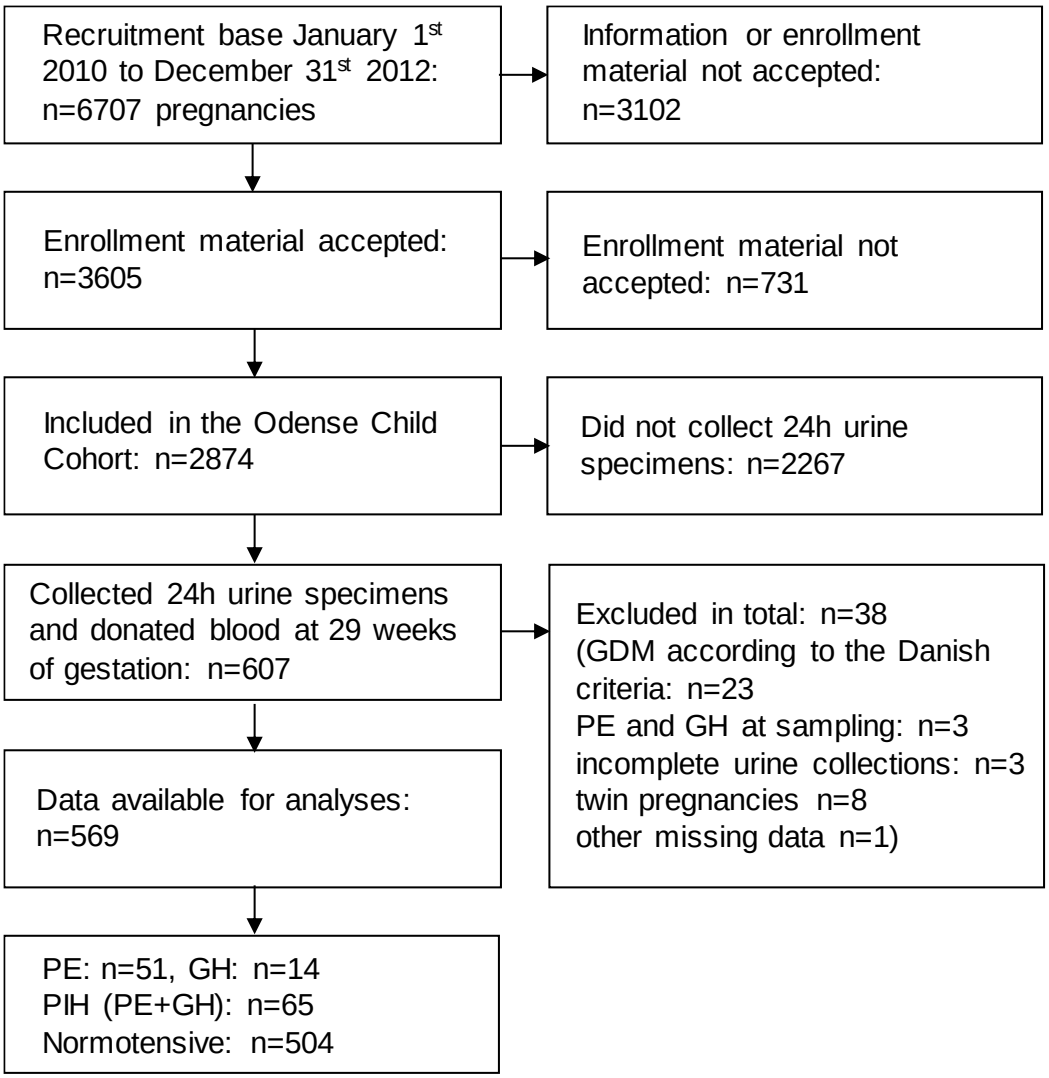

Figure 1. Flowchart of inclusion and participation in the Odense Child Cohort and the present sub-study.

GA adjusted BW for boys (reference) = $-1.907345e^{-6} * x^4 + 1.140644e^{-3} * x^3 + (-1.336265e^{-1}) * x^2 + 1.976961 * x + 2.410053e^2$ and

GA adjusted BW for girls (reference) = $-2.761948e^{-6} * x^4 + 1.744841e^{-3} * x^3 + (-2.893626e^{-1}) * x^2 + 1.891197e^1 * x + (-4.135122e^2)$,

where x = GA at delivery (in days)[78].

BW sds < -2 was characterized as small for gestational age (SGA), BW sds >2 as large for gestational age (LGA).

Maternal outcome was specified as PE. We further conducted sensitivity analyses in predicting PIH and severe PE[37].

2.3. Laboratory measurements

All urine was collected for a 24h period and 100 ml aliquots were immediately frozen and stored at -80° for later analyses. The urine volume was determined gravimetrically. Na^+ and K^+ concentrations in 24h urine samples were analysed with a clinical flame photometer (EFUX 5057, Eppendorf, Hamburg, Germany). Creatinine was measured by an automated technique. Aldosterone in urine and plasma was determined by commercially available ELISA (MS E-5200, Labor Diagnostika Nord GmbH & Co. KG, Germany). According to the manufacturer's instructions for use, we diluted the urine aliquots 1:50 with urine diluent (Labor Diagnostika) and 50 µl incubated them with aldosterone HRP conjugate for 1h. Accuracy was confirmed for urine by running a dilution series. Intra-assay variation was tested 2 times with n = 10 repetitive determinations each time and was 5.6% and 7.1%, respectively. We used the same human EDTA plasma aliquots as internal standard in all plasma and urine aldosterone assays (72.2 ± 11.4 pg/ml for plasma aldosterone assay and 68.2 ± 17.3 pg/ml for urinary aldosterone). Between-assay coefficient of variation was 8.1% for plasma aldosterone analyses and 11.19% for urinary aldosterone analyses. According to the manufacturer, the ELISA had no cross-reactivity with progesterone and cortisol[37].

We calculated daily renal Na^+, K^+ and aldosterone excretions as 24h u-Na^+, 24h u-K^+ and 24h u-aldo, respectively, by multiplication of urine Na^+, K^+ and aldosterone concentrations and urine volume. Daily NaCl and K^+ intakes were estimated from the 24h u-Na^+ and 24h u-K^+ excretions[37]:

NaCl intake (g/day) = 24h u-Na^+ (mmol/day) / 17.1 (mmol)[79],

K^+ intake (g/day) = 24h u-K^+ (mmol/day) * 0.039 (g/mmol).

Measurements of sFlt-1 and PlGF concentrations in serum were performed on the fully automated KRYPTOR compact Plus system (KRYPTOR PlGF and KRYPTOR sFlt-1; Thermo Fisher Scientific) according to the manufacturer's instructions for use[80]. According to the manufacturer, the sFlt-1 assay covered a measuring range of 22-90000 pg/ml. The limit of detection was 22 pg/ml, and the limit of quantitation (functional sensitivity) was 29 pg/ml. The PlGF assay covered a measuring range of 3.6-7000 pg/ml. The limit of detection was 3.6

pg/ml, and the limit of quantitation was 6.9 pg/ml[80]. Angiogenic marker concentrations from gestational week 29 were available in 535 (94.0%) corresponding serum samples.

2.4. Statistical analyses

First, the distribution of all quantitative variables was checked. Normally distributed data were reported as means ± standard deviation (SD), and the differences between the normally distributed data were compared by the independent samples t-test. Levene's test was used to compare variability between groups. Non-normally distributed data were reported as medians ± interquartile range (IQR), and differences in these distributions were compared by the Mann-Whitney U test. The distribution of the angiogenic markers sFlt-1, PlGF, sFlt-1/PlGF was positively skewed. Therefore, we used log-transformed variants of these variables in all statistical analyses. Binary (categorical) data were shown as absolute values and percentages. Likelihood-ratio chi-squared test and Fisher's test were used to test for differences in distribution of the categorical characteristics as appropriate[37].

24h u-aldo was used as proxy of integrated secretion over 24h in all statistical analyses because it is less susceptible to short-term fluctuations due to posture, time of the day, physical activity and stress than plasma aldosterone (p-aldo)[81, 82].

To explore the relationships between aldosterone, Na^+ and K^+ intakes and fetal outcomes PW and BW, we first plotted the data and calculated the Person correlation coefficients between 24h u-aldo, u-Na^+ and u-K^+ (as proxy for Na^+ and K^+ intakes) and PW and BW. Upon confirming a statistically significant relationship between the aforementioned biochemical parameters and PW and BW in the simple correlation analyses, we performed multiple regression analyses adjusted for confounders (maternal: BMI, age, smoking status, BP, gestational length, placental angiogenic markers; fetal: sex of the infant) to assess the associations of Na^+ and K^+ intakes, 24h u- and p-aldo with BW, PW and BW sds[37].

To take into account the left truncation (enrollment in the study at urine and blood sampling, i.e. at gestational week 29) and time-to-event data, we constructed Cox proportional hazards regression models to analyze the effects of NaCl intake >6 g/day, K^+ intake, 24h u- and p-aldo levels relative to the risk of PE, severe PE and PIH[37]. These models were adjusted for maternal covariates BMI, age, smoking status, 1st trimester BP and BP at sampling, and placental angiogenic markers PlGF and sFlt-1. A pregnancy was considered to be at risk of

PE, PIH or severe PE from enrollment in the study until 1) PE, including severe PE, occurred, 2) GH occurred, or 3) survival until delivery.

IBM SPSS version 25 was used for all statistical analyses and GraphPad Prism version 6 was used to create graphs. A two-sided p-value <0.05 was considered significant, p-values 0.05 - 0.10 were considered as trends.

3. Results

3.1. Study participants

We included 569 women in this study. Participants' characteristics are given in **Table 1** in the publication[37]. Women who collected 24h urine specimens differed in several regards from the entire Odense Child Cohort: they had significantly higher BMI and BP already at 1st trimester, were predominantly of Caucasian ethnicity, more prone to the development of PE (9.3% vs. 5.6%, p<0.01) and PIH (12.0% vs. 8.0%, p<0.01), and their infants had higher birth length and BW sds, **supplemental Table S1** in the publication[37].

Women who developed PE did not differ in age, smoking status or prevalence of preterm delivery, but had higher BMI and elevated BP in the 1st trimester and at sampling, as compared to the rest of the women with available urine collections, **Table 1** in the publication[37]. PE group further presented with higher sodium-to-potassium ratio, though there were no significant differences in urinary Na^+ or K^+ excretions, or aldosterone levels as compared to the normotensive pregnancies (NP) with 24h urine specimens[37]. There were significantly more women with NaCl intake>6 g/day in the future PE group, while the fraction of K^+ intake <3.5 g/day (recommended lower limit of daily K^+ consumption as defined by the WHO) was similar, **Table 1** in the publication[37]. Placental angiogenic balance was shifted towards anti-angiogenesis in PE-prone pregnancies, with lower PlGF concentrations and higher sFlt-1/PlGF[37]. In the sensitivity analysis, PIH-prone women showed a trend to a larger fraction of high NaCl intake compared to NP group, **Table 1** in the publication[37], while the fraction of high NaCl consumption among women who developed severe features of PE was not significantly different from the NP group (data not shown).

3.2. Relationships of birth and placental weights with aldosterone levels, Na^+ and K^+ intakes at gestational week 29

Aldosterone levels, Na^+ and K^+ intakes (by proxy 24h u-Na^+ and u-K^+) and sodium-to-potassium ratio all positively correlated to the fetal outcomes BW, PW and BW sds in the crude analyses, **Figures 1-2** and **supplemental Figure S1** in the publication[37]. After the adjustment for other maternal and offspring covariates (as specified in **Methods**), the associations between Na^+ and K^+ intakes and offspring outcomes lost their statistical significance, **Table 2** and **supplemental Table S2** in the publication[37]. 24h u-aldo remained a significant predictor of BW, PW and BW sds, even when adjusted for the above confounders, **Table 2** and **supplemental Table S2** in the publication[37]. An increase of 1 µg/day in urinary aldosterone excretion at gestational week 29 contributed to 25 g increase in fetal body weight and to 10 g increase in PW[37]. Further significant predictors of BW and PW were maternal BMI, gestational length and PlGF concentrations. Parity and sex of the infant also significantly contributed to birth weight.

3.3. Risk of PE, severe PE and PIH incidence based on aldosterone levels, Na^+ and K^+ intakes and other risk factors at gestational week 29

Aldosterone availability did not predict PE or PIH. Based on the urinary Na^+ excretion at gestational week 29, women with daily NaCl consumption above WHO recommended limit (6 g/day) had 5.7 higher hazard for developing PE and 3.6 higher hazard for developing PIH in the later course of pregnancy, **Figure 3** in the publication[37]. This effect was independent of BMI, BP and age, since the model was adjusted for maternal pre-pregnancy BMI, age, smoking status, BP, parity, placental angiogenic markers sFlt-1 and PlGF[37]. Additionally, pre-pregnancy BMI and elevated BP at sampling were associated with the development of PE and PIH independent of other maternal characteristics. Higher serum PIGF concentrations were protective against PE and PIH development[37]. NaCl intake >6 g/day was not associated with severe PE (data not shown), probably because of the lack of statistical power due to the low number of severe PE cases in our sub-study (n = 23).

3.4. Associations of urinary aldosterone with maternal and fetal outcomes by NaCl intake at gestational week 29

In an additional analysis we further evaluated whether the relationships of aldosterone availability with fetal (birth and placental weights) and maternal (blood pressure at gestational week 29) are modified by salt intake. To this end, we stratified the cohort according the median NaCl intake in this cohort (8 g/day).

As demonstrated in the PE prediction model, aldosterone did not associate with later PE or PIH incidence. This was corroborated by the modification analysis with NaCl intake: No association of urinary aldosterone excretion with maternal blood pressure at gestational week 29 could be detected independent of NaCl intake, **Table 3**.

Aldosterone correlated positively to birth and placental weights, independent of NaCl intake, **Table 4**. However, in women with NaCl intake above the median, a slightly larger contribution of aldosterone to birth weight and BW sds could be seen compared to women with NaCl intake below the median (adjusted β coefficients (95% CI): 27.38 (4.82 to 49.94) g vs 25.34 (4.38 to 46.30) g birth weight and 0.07 (0.02 to 0.12) vs 0.06 (0.008 to 0.11) BW sds per 1 µg/d increase in 24h urine aldosterone excretion at gestational week 29), **Table 4**. This contribution was independent of maternal BMI and other factors associated with larger birth weight.

Table 3. Adjusted associations between urinary aldosterone excretion and maternal blood pressure at sampling, gestational week 29, stratified by maternal NaCl intake.

	Adjusted β coefficients (95% CI) for SBP, mmHg	**Adjusted β coefficients (95% CI) for DBP, mmHg**
NaCl intake ≥8 g/d at GA 29 wk		
At inclusion		
Maternal BMI, kg/m^2	0.10 (-0.18 to 0.38)	0.23 (0.02 to 0.43)
Maternal age, y	-0.17 (-0.62 to 0.17)	-0.02 (-0.26 to 0.23)
Maternal smoking (1=yes, 0=no)	2.68 (-3.86 to 9.22)	0.55 (-4.14 to 5.25)
1st trimester SBP, mmHg	0.38 (0.26 to 0.49)	--
1st trimester DBP, mmHg	--	0.37 (0.26 to 0.49)
Parity, n	-0.58 (-2.82 to 1.66)	-1.36 (-2.97 to 0.26)
At sampling GA 29 wk		
24h u-aldo, µg/d	0.18 (-0.30 to 0.66)	0.18 (-0.17 to 0.52)
24h u-Na^+, mmol/d	-0.03 (-0.06 to 0.01)	0.01 (-0.02 to 0.04)
24h u-K^+, mmol/d	0.03 (-0.04 to 0.11)	-0.02 (-0.07 to 0.03)
Log10 (PlGF, pg/ml)	-0.60 (-6.15 to 4.96)	-1.37 (-5.38 to 2.63)
Log10 (sFlt-1, pg/ml)	2.62 (-3.90 to 9.13)	0.07 (-4.69 to 0.78)
NaCl intake <8 g/d at GA 29 wk		
At inclusion		
Maternal BMI, kg/m^2	0.08 (-0.22 to 0.37)	0.26 (0.04 to 0.48)
Maternal age, y	0.23 (-0.11 to 0.57)	0.09 (-0.16 to 0.34)
Maternal smoking (1=yes, 0=no)	4.33 (-3.30 to 11.96)	-2.46 (-7.99 to 3.07)
1st trimester SBP, mmHg	0.47 (0.34 to 0.61)	--
1st trimester DBP, mmHg	--	0.32 (0.19 to 0.44)
Parity, n	-3.08 (-5.25 to 0.91)	-0.76 (-2.34 to 0.83)
At sampling GA 29 wk		
24h u-aldo, µg/d	0.39 (-0.15 to 0.92)	0.12 (-0.27 to 0.51)
24h u-Na^+, mmol/d	0.04 (-0.03 to 0.11)	0.02 (-0.03 to 0.07)
24h u-K^+, mmol/d	-0.09 (-0.18 to 0.006)	-0.05 (-0.11 to 0.02)
Log10 (PlGF, pg/ml)	-2.79 (-8.60 to 3.02)	-4.09 (-8.29 to 0.11)
Log10 (sFlt-1, pg/ml)	2.71 (-4.11 to 9.53)	-2.14 (-7.09 to 2.81)

Table 4. Adjusted associations between urinary aldosterone excretion at gestational week 29 and birth and placental weights, BW sds, stratified by maternal NaCl intake.

	Adjusted β coefficients (95% CI) for BW, g	**Adjusted β coefficients (95% CI) for BW SDS**	**Adjusted β coefficients (95% CI) for PW, g**
NaCl intake ≥8 g/d at GA 29 wk			
At inclusion			
Maternal BMI, kg/m^2	9.42 (-4.60 to 23.43)	0.02 (-0.01 to 0.05)	3.55 (-1.02 to 8.12)
Maternal age, y	4.51 (-12.14 to 21.16)	0.01 (-0.03 to 0.05)	3.57 (-1.85 to 9.00)
Maternal smoking (1=yes, 0=no)	-189.08 (-501.06 to 122.90)	-0.42 (-1.13 to 0.29)	-33.80 (-135.50 to 67.90)
1st trimester SBP, mmHg	3.43 (-3.39 to 10.25)	0.009 (-0.007 to 0.02)	0.61 (-1.62 to 2.83)
1st trimester DBP, mmHg	2.27 (-7.00 to 11.54)	0.004 (-0.02 to 0.03)	0.64 (-2.38 to 3.67)
At sampling GA 29 wk			
24h u-aldo, µg/d	27.38 (4.82 to 49.94)	0.07 (0.02 to 0.12)	7.43 (0.007 to 14.86)
24h u-Na^+, mmol/d	0.11 (-1.65 to 1.88)	0.0004 (-0.004 to 0.004)	-0.07 (-0.65 to 0.51)
24h u-K^+, mmol/d	2.91 (-0.61 to 6.42)	0.007 (-0.001 to 0.02)	0.67 (-0.48 to 1.82)
Log10 (PlGF, pg/ml)	599.46 (338.12 to 860.79)	1.36 (0.78 to 1.94)	109.57 (24.39 to 194.75)
Log10 (sFlt-1, pg/ml)	162.07 (-149.25 to 473.40)	0.43 (-0.27 to 1.14)	84.71 (-17.09 to 186.52)
SBP at sampling, mmHg	-0.19 (-7.83 to 7.45)	0.0001 (-0.02 to 0.02)	0.34 (-2.15 to 2.83)
DBP at sampling, mmHg	-1.46 (-11.81 to 8.89)	-0.005 (-0.03 to 0.02)	-1.87 (-5.25 to 1.50)
At delivery			
Gestational length, wks	164.71 (120.94 to 208.49)	--	30.99 (16.70 to 45.29)
Parity, n	150.20 (48.11 to 252.29)	0.34 (0.11 to 0.57)	11.45 (-21.90 to 44.80)
Offspring sex (1=female, 0=male)	-123.13 (-244.90 to -1.36)	--	-7.59 (-47.32 to 32.14)
NaCl intake <8 g/d at GA 29 wk			

At inclusion			
Maternal BMI, kg/m^2	16.80 (5.08 to 28.51)	0.04 (0.01 to 0.07)	5.50 (1.51 to 9.49)
Maternal age, y	-7.66 (-22.23 to 6.92)	-0.02 (-0.05 to 0.02)	-0.87 (-5.85 to 4.11)
Maternal smoking (1=yes, 0=no)	-38.46 (-347.36 to 270.44)	-0.12 (-0.84 to 0.60)	29.81 (-75.29 to 134.91)
1st trimester SBP, mmHg	-1.67 (-8.44 to 5.10)	-0.004 (-0.02 to 0.01)	1.13 (-1.18 to 0.59)
1st trimester DBP, mmHg	4.92 (-3.51 to 13.35)	0.01 (-0.009 to 0.03)	0.68 (-2.19 to 3.54)
At sampling GA 29 wk			
24h u-aldo, µg/d	25.34 (4.38 to 46.30)	0.06 (0.008 to 0.11)	13.32 (6.17 to 20.47)
24h u-Na^+, mmol/d	0.17 (-2.56 to 2.90)	0.00002 (-0.006 to 0.006)	-0.11 (-1.04 to 0.82)
24h u-K^+, mmol/d	0.46 (-3.48 to 4.40)	0.001 (-0.008 to 0.01)	-1.45 (-2.82 to -0.09)
Log10 (PlGF, pg/ml)	549.65 (310.40 to 788.90)	1.30 (0.75 to 1.84)	161.89 (80.33 to 243.45)
Log10 (sFlt-1, pg/ml)	113.17 (-185.73 to 412.06)	0.31 (-0.36 to 0.98)	-19.03 (-120.85 to 82.79)
SBP at sampling, mmHg	2.07 (-4.33 to 8.47)	0.005 (-0.009 to 0.02)	1.59 (-0.59 to 3.77)
DBP at sampling, mmHg	-3.01 (-12.65 to 6.63)	-0.007 (-0.03 to 0.02)	-2.78 (-6.06 to 0.511)
At delivery			
Gestational length, wks	183.08 (145.00 to 221.17)	--	20.66 (7.67 to 33.64)
Parity, n	130.70 (38.32 to 223.08)	0.30 (0.09 to 0.51)	16.68 (-15.31 to 48.67)
Offspring sex (1=female, 0=male)	-93.27 (-217.70 to 31.16)	--	-15.34 (-57.83 (27.15)

Discussion

4.1. Main findings in light of other evidence

The main findings of the present study were that 24h urinary aldosterone excretion as integrated measure of daily aldosterone secretion was a predictor of placental and birth weights independent of maternal and fetal characteristics, and high NaCl intake was associated with hypertensive pregnancy disorders PE and PIH[37].

4.1.1. Aldosterone's contribution to placental and birth weights

Adverse effects of deleted aldosterone synthase on fetal outcome were shown in a rodent study, where deletion of CYP11B2 gene resulted in increased number of necrotic placentas, reduced litter size and diminished fetal weight[19]. A human case-control study[15] has already shown that higher urinary tetrahydro-aldosterone excretion and higher BW were found in normal pregnancies as compared to lower tetrahydro-aldosterone excretion and reduced BW in manifest PE. However, no adjustments for maternal and fetal characteristics were performed, and particularly no adjustment for the differences in BMI and blood pressure was made in that study, which might at least partially explain the finding. We now demonstrate that the positive relationship between neonatal size and maternal aldosterone availability is present even after correction for maternal (including BMI and blood pressure) and offspring confounders on a population-based level. We further evaluated the utility of aldosterone in predicting PE and PIH, however, aldosterone levels were not significantly different between normotensive pregnancies and pregnancies, which went on to develop PE or PIH in our study[37]. This is in line with other studies[29, 83, 84], where aldosterone levels were also not different before the onset of the clinical PE condition. RAAS suppression does not commonly occur prior to the clinical symptoms of PE[13, 29, 83, 84], but is probably a secondary response to vasoconstriction and augmented Na^+ reabsorption in PE[29, 84]. Thus, aldosterone is not a likely biomarker for PE, and at the beginning of 3rd trimester it did not associate with later PE or PIH incidence[37]. Interestingly, aldosterone response to exogenous low dose of ACTH is dampened in PE patients, possibly because of the suppressed angiotensin II and impaired synthetic capacity[18].

4.1.2. High salt intake as a risk factor for hypertensive disorders of pregnancy

Brown and colleagues[36, 83] suggested that women with established PE retain more of an acutely given Na^+ load than those with normotensive pregnancies. In another study by Brown et al., the ability to increase renin and aldosterone upon furosemide stimulation was lost in several women with PE[85] and Nielsen showed that women with PE had impaired reactivity of renin to low salt[35]. Both findings are compatible with excess distal Na^+ retention in PE as shown also in the Na^+ infusion experiments by Brown[36]. It could be speculated that this impaired Na^+ excretion is due to aldosterone-independent mechanisms[37]. One such mechanism may be that proteases such as plasmin(ogen), abundantly present in PE[86], result in an increased activation of epithelial Na^+ channel (ENaC) currents with subsequent Na^+ retention[86-89].

Scientific evidence regarding the effects of salt restriction or salt supplementation in preventing the incidence of hypertensive disorders of pregnancy is still limited and profoundly controversial. To date, there is only one Cochrane systematic review from 1999[90] which tried to examine the effects of low-salt diet on major obstetric outcomes including PE. The authors concluded that there was not enough reliable evidence about the effects of NaCl intake restriction during normal pregnancy, since only two trials were eligible for the analysis[90]. The trials were insufficient in size and did not enroll women with PE, so it was not possible to provide information about the effects of dietary NaCl restriction for treatment of PE. On the other hand, an interventional study which was published in Lancet in 1958 concluded that salt supplementation in pregnancy reduced the occurrence of PE ("toxæmia"), edema, perinatal death, antepartum hemorrhage and bleeding during pregnancy and improved the disease course in women with early-onset PE[91]. The study however had several limitations: The intervention was not randomized, the actual salt intake was not measured (the intervention consisted of advice to either increase or reduce salt intake during cooking, at meal times, or eating salty/less salty food, respectively), and no adjustments for potential confounders were performed in that early study. Further studies followed from the group of Dr. Mohaupt, which demonstrated that Na^+ supplementation of 3-6 g/day was paralleled by lowered blood pressure in a case-report on a pregnant woman with chronic hypertension and hypoaldosteronism[92]. In another study, the authors showed that high salt intake was rather inversely related to blood pressure in healthy normotensive pregnant women in contrast to the non-pregnant state[16]. However, also in this study no adjustments for potential confounders

were performed, and the dietary intervention was not randomized. Therefore, the observed drop in blood pressure with higher salt intake in the first trimester could in fact be caused by other factors that were not considered in the analyses. As a matter of fact, blood pressure follows a U-shaped trajectory in physiological pregnancy due to the decrease in perfusion pressure, the marked reduction in total systemic vascular resistance and other hemodynamic and cardiovascular adaptations to the pregnancy state[93].

A recent Danish registry study[94] based on follow up of 66 651 singleton pregnancies with 1809 cases of hypertensive disorders of pregnancy (including 1300 PE cases) confirmed our results regarding high salt intake as a risk factor for PIH, including PE[37]. The authors reported that women in the highest quintile of Na^+ intake (median 3.70 g/day) in the 2nd trimester had 54% higher risk of GH and 20% higher risk of PE compared to women in the lowest quintile of Na^+ intake (median 2.60 g/day)[94]. Higher 3rd trimester 24h urinary Na/K ratio among women with PE was associated with higher systolic and diastolic blood pressure, higher creatinine, lower birth weight, shorter gestational length and increased incidence of severe features of PE in another study[95]. In a study conducted in Bangladesh, drinking saline water increased the odds of PE and GH in a dose-dependent manner[96]. In addition, women after a preeclamptic pregnancy appear to be more prone to salt-sensitivity of blood pressure, an important cardiovascular risk factor at any blood pressure level[97].

For the reasons discussed above, it is currently too early to propose recommendations for salt intake in pregnancy. In fact, WHO and other institutions do not currently recommend Na^+ restriction during pregnancy for prevention of PIH, including PE[98-100], but the quality of evidence is still low[99, 100]. Yet, the avoidance of an “excessive” dietary salt intake is considered as a healthy dietary practice in pregnancy[99]. Further studies are needed to identify the optimal dietary Na^+ intake in pregnancy.

4.2. Strengths and limitations

Our study benefits from an unprecedentedly high number of 24h urine collections in a pregnancy setting[37]. We used validated diagnoses of hypertensive disorders of pregnancy, which is considered a strength in population-based research. Further, we performed all sensitivity analyses on BW sds, which was adjusted for fetal sex and gestational length and calculated by the formula specifically developed for the Scandinavian population, it is a more precise parameter in the context of Odense Child Cohort and less prone to bias[37]. The

sensitivity analyses proved the robustness of our findings. Moreover, even though the observed associations between aldosterone and placental and neonatal size and between high NaCl intake and incidence of hypertensive disorders of pregnancy were limited to one time in pregnancy (gestational week 29) in our study, they were robustly present even after the adjustment for maternal age, pre-pregnancy BMI, smoking status, parity, BP, placental angiogenic factors, urinary Na^+, K^+ and aldosterone excretions, gestational length and sex of the child[37].

Our study naturally has some limitations. Limitations include the cross-sectional character of this nested sub-study, and specifically the absence of 24h urine specimens from the early gestation to evaluate the clinical utility of aldosterone and dietary Na^+ and K^+ at earlier stages of pregnancy[37]. As has been already shown by us[101] and others[102], an isolated 24h urine excretion might not be representative of chronic dietary Na^+ and K^+ intakes. Longitudinal 24h urine samples collected over the entire pregnancy might be necessary to correctly classify women as high or low NaCl or K^+ intakers. The cohort was ethnically very homogenous, with few of African descent, which are reported to be more prone to salt-sensitive hypertension[103-105] and PE[106-109]. Half of the participants who provided 24h urine specimens were enriched with risk for GDM and another half were controls based on gestational age and no risk factors for GDM. However, we excluded women who went on to develop GDM according to the Danish criteria from all analyses[37]. Another source of selection bias might be that only 21% of participants in the Odense Child Cohort provided urine samples[37]. The cohort itself differed in few regards from the background population: participating women were on average older, more often nulliparous and of Danish origin, smoked less, had longer gestational length and self-reported higher education status[72]. Moreover, this study had an observational character, thus, the observed associations cannot necessarily be deemed causal.

4.3. Perspectives and clinical implications

We unraveled a substantial beneficial effect of aldosterone on placental and birth weights, with no apparent adverse effects on the maternal outcome[37]. This is in contrast to the detrimental effects of aldosterone on cardiovascular outcomes in CVD patients outside of pregnancy, where it is beneficial to pharmacologically block the mineralocorticoid signaling. Future prospective studies should evaluate the utility of aldosterone and its interplay with cortisol in screening and monitoring programs of high-risk pregnancies. Our finding indicates

that the role of aldosterone in pregnancy goes beyond maternal plasma volume expansion and the suppression of aldosterone synthase in pregnancy may have direct adverse trophic effects on the fetus[37]. It might pave the way for possible pharmacologic strategies (such as mineralocorticoid supplementation) in pregnancies with high risk for IUGR in order to achieve normal or slightly elevated levels of aldosterone and thus benefit placental and fetal development. Most strikingly, we demonstrated an association between high salt intake and incidence of hypertensive disorders of pregnancy[37]. Currently there are no recommended guidelines for NaCl and K^+ intake during pregnancy. Since the evidence is still limited and controversial, it is currently too early to propose dietary recommendations for Na^+ or K^+ intake in pregnancy. Future longitudinal studies and randomized controlled trials should consider Na^+ and K^+ intakes for further analysis to determine appropriate dietary electrolyte intakes in pregnancy[37].

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Eidesstattliche Versicherung

„Ich, Anna Birukov, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema „Salt intake as a risk factor for hypertensive disorders of pregnancy and importance of gestational aldosterone availability“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

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Datum Unterschrift

Ausführliche Anteilserklärung an der erfolgten Publikation

Publikation: **Birukov A**, Andersen LB, Herse F, Rakova N, Kitlen G, Kyhl HB, Golic M, Haase N, Kräker K, Müller DN, Jørgensen JS, Andersen MS, Dechend R, Jensen BL. Aldosterone, salt and potassium intakes as predictors of pregnancy outcome, including preeclampsia. Hypertension. 2019 Jun 10:HYPERTENSIONAHA11912924. doi: 10.1161/HYPERTENSIONAHA.119.12924. [Epub ahead of print].

Beitrag im Einzelnen:

Konzept und Hypothesenaufstellung

Die ursprüngliche Hypothese von Prof. Jensen (hohe diätetische Salzeinnahme hemmt das Renin-Angiotensin-Aldosteron-System mit negativen Auswirkungen auf plazentare Entwicklung und fetales Wachstum) habe ich insofern ergänzt, dass die Hypothese als einen zweiten Endpunkt auch maternale Outcomes (Inzidenz von Präeklampsie- und schwangerschaftsinduzierter Hypertonie in der Kohorte) beinhaltete.

Laborbestimmungen

In Zusammenarbeit mit Dr. Natalia Rakova habe ich den Elektrolytgehalt von Natrium und Kalium in den 24-Stunden-Urinsammlungen (n=607) mithilfe der Flammenphotometrie im Labor von unserer Arbeitsgruppe am ECRC bestimmt. Ferner verbrachte ich drei Wochen im Labor von Prof. Boye Jensen in Odense, Dänemark, wo ich Aldosteronkonzentrationen im Plasma und Urin in den selbigen Proben (n=607) mithilfe von ELISA und mit Unterstützung der dortigen Laborassistentin Gitte Kitlen gemessen habe. Mütterliche Blutdruckwerte wurden von mir ebenfalls in Dänemark aus Patientenakten extrahiert.

Statistische Auswertung

Alle statistischen Analysen (Prädiktion von Geburts- und Plazentagewichten, sowie von Präeklampsie-Aufkommen) mit den *apriori* und im Einklang mit den Koautoren ausgewählten Variablen wurden von mir alleine und ohne Unterstützung Dritter ausgeführt. Die

angewandten Analysen beinhalteten diverse statistische Tests für den Vergleich der Variablenverteilungen in den Subgruppen (Tabelle 1 und S1 in der Publikation), einfache Korrelationen (Abbildungen 1-2 und S1), multiple Regressionen für die Prädiktion von Geburts- und Plazentagewichten (Tabellen 2 und S2), sowie die Cox-Regression für die Prädiktion von Präeklampsie-Inzidenz und Aufkommen schwangerschaftsinduzierter Hypertonie in der Kohorte (Abbildung 3). Für die statistischen Analysen wurde IBM SPSS Version 25 genutzt.

Tabellen und Abbildungen

Alle Tabellen und Abbildungen für die Publikation (Tabellen 1-2, Abbildungen 1-3, Graphical Abstract, alle Tabellen und Abbildungen im Supplement) wurden von mir alleine angefertigt. Für Abbildungen wurde die Software GraphPad Prism Version 6 benutzt.

Verfassen der Publikation

Die erste Version des Manuskripts habe ich in Zusammenarbeit mit dem korrespondierenden Autor Prof. Boye Jensen angefertigt, die durch andere Koautoren revidiert wurde.

Revision

Ich habe zusätzliche Analysen für die Reviewer durchgeführt und die erste Version der revidierten Fassung des Manuskripts vorbereitet, die von den anderen Koautoren überarbeitet wurde.

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Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

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Unterschrift der Doktorandin

Excerpt from the Journal Summary List (ISI Web

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI Selected Categories: **"PERIPHERAL VASCULAR DISEASE"** Selected Category Scheme: WoS

Gesamtanzahl: 65 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	CIRCULATION	167,719	18.880	0.223630
2	CIRCULATION RESEARCH	52,753	15.211	0.082820
3	HYPERTENSION	36,908	6.823	0.049510
4	STROKE	65,854	6.239	0.088520
5	ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY	34,074	6.086	0.044820
6	THROMBOSIS AND HAEMOSTASIS	16,701	4.952	0.025770
7	JOURNAL OF THROMBOSIS AND HAEMOSTASIS	17,663	4.899	0.034380
8	Journal of Stroke	694	4.750	0.002880
9	ATHEROSCLEROSIS	23,013	4.467	0.039120
10	ANGIOGENESIS	2,712	4.351	0.004860
11	JOURNAL OF HYPERTENSION	16,916	4.092	0.025250
12	EUROPEAN JOURNAL OF VASCULAR AND ENDOVASCULAR SURGERY	8,352	3.877	0.012910
13	International Journal of Stroke	3,825	3.859	0.014880
14	CURRENT OPINION IN LIPIDOLOGY	3,849	3.853	0.006100
15	AMERICAN JOURNAL OF PHYSIOLOGY-HEART AND CIRCULATORY PHYSIOLOGY	28,039	3.569	0.027570
16	HYPERTENSION RESEARCH	5,064	3.439	0.006250
17	CURRENT OPINION IN NEPHROLOGY AND HYPERTENSION	3,324	3.370	0.006500
18	SEMINARS IN THROMBOSIS AND HEMOSTASIS	3,876	3.345	0.006270
19	Diabetes & Vascular Disease Research	1,253	3.340	0.003320
20	JOURNAL OF VASCULAR SURGERY	24,792	3.294	0.030300
21	CURRENT HYPERTENSION REPORTS	2,564	3.234	0.006250

Aldosterone, salt and potassium intakes as predictors of pregnancy outcome, including preeclampsia

https://doi.org/10.1161/HYPERTENSIONAHA.119.12924

Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publication list

Original articles

Birukov A, Andersen LB, Herse F, Rakova N, Kitlen G, Kyhl HB, Golic M, Haase N, Kräker K, Müller DN, Jørgensen JS, Andersen MS, Dechend R, Jensen BL. Aldosterone, salt and potassium intakes as predictors of pregnancy outcome, including preeclampsia. Hypertension. 2019 Aug;74(2):391-398. doi: 10.1161/HYPERTENSIONAHA.119.12924. Epub 2019 Jun 10. Impact factor (2017): 6.82

Birukov A, Muijsers HEC, Heidecke H, Drost JT, Cunnigham Jr. MW, Kräker K, Haase N, Frolova A, Müller DN, Herse F, Maas AHEM, Dechend R. Regulatory antibodies against GPCR in women ten years after early-onset preeclampsia. Front Biosci (Landmark Ed). 2019 Jun 1;24:1462-1476. Impact factor (2017): 2.35

Bækgaard Thorsen LH, Bjørkholt Andersen L, **Birukov A**, Lykkedegn S, Dechend R, Stener Jørgensen J, Thybo Christesen H.Prediction of birth weight small for gestational age with and without preeclampsia by angiogenic markers: an Odense Child Cohort study. J Matern Fetal Neonatal Med. 2018 Sep 25:1-8. doi: 10.1080/14767058.2018.1519536. [Epub ahead of print]. Impact factor (2017): 1.49

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Birukov A, Rakova N, Lerchl K, Olde Engberink RHG, Johannes B, Wabel P, Moissl U, Rauh M, Luft FC, Titze JM. Ultra-long-term human salt balance studies reveal interrelations between sodium, potassium, and chloride intake and excretion. Am J Clin Nutr. 2016 Jul;104(1):49-57. doi: 10.3945/ajcn.116.132951. Epub 2016 Mai 25. Impact factor (2017): 6.55

Abstracts

Birukov A, Andersen LB, Herse F, Rakova N, Kitlen G, Kyhl HB, Müller DN, Jørgensen JS, Andersen MS, Dechend R, Jensen LB: Urinary aldosterone and electrolytes: Predictors of birth complications [abstract]. Presented at the Conference of the Nordic Federation of Obstetrics and Gynecology (NFOG), Odense, Denmark, June 10-13, 2018.

Birukov A, Andersen LB, Herse F, Nielsen JH, Kyhl H, Jensen LB, Andersen MS, Müller DN, Jørgensen JS, Dechend R. Blood pressure in pregnancy and offspring cardiometabolic profile [abstract]. Presented at the Conference of the Nordic Federation of Obstetrics and Gynecology (NFOG), Odense, Denmark, June 10-13, 2018.

Birukov A, Muijsers H, Heidecke H, Haase N, Kräker K, Müller DN, Herse F, Maas A, Dechend R. Impact of regulatory (auto-) antibodies against G-protein-coupled receptors on blood pressure in women ten years after early-onset preeclampsia [abstract]. In: Advances in RAB Research 2018 Sep 28;1(1). Article ID: 1809017. doi: 10.18416/RAB.2018.1809017. Presented at the 2nd Symposium on Regulatory Autoantibodies Targeting G-Protein-Coupled Receptors, Lübeck, Germany, September 28-30, 2018.

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