

Physiological and Molecular Responses to Altered Sodium Intake in Rat Pregnancy

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Background—In pregnancy, a high plasma volume maintains uteroplacental perfusion and prevents placental ischemia, a condition linked to elevated maternal blood pressure (BP). Reducing BP by increasing Na^+ intake via plasma volume expansion appears contra-intuitive. We hypothesize that an appropriate Na^+ intake in pregnancy reduces maternal BP and adapts the renin-angiotensin system in a pregnancy-specific manner.

Methods and Results—BP was measured by implanted telemetry in Sprague-Dawley rats before and throughout pregnancy. Pregnant and nonpregnant animals received either a normal-salt (0.4%; NS), high-salt (8%; HS), or low-salt (0.01%; LS) diet, or HS (days 1–14) followed by LS (days 14–20) diet (HS/LS). Before delivery (day 20), animals were euthanized and organs collected. Food, water, and Na^+ intake were monitored in metabolic cages, and urinary creatinine and Na^+ were analyzed. Na^+ intake and retention increased in pregnancy (NS, LS), leading to a positive Na^+ balance (NS, LS). BP was stable during LS, but reduced in HS conditions in pregnancy. The renin-angiotensin system was adapted as expected. Activating cleavage of α - and γ -subunits of the renal epithelial Na^+ channel and expression of full length medullary β -subunits, accentuated further in all LS conditions, were upregulated in pregnancy.

Conclusions—Pregnancy led to Na^+ retention adapted to dietary changes. HS exposure paradoxically reduced BP. Na^+ uptake while only modestly linked to the renin-angiotensin system is enhanced in the presence of posttranslational renal epithelial Na^+ channel modifications. This suggests (1) storage of Na^+ in pregnancy upon HS exposure, bridging periods of LS availability; and (2) that potentially non-renin-angiotensin-related mechanisms participate in ENaC activation and consecutive Na^+ retention. (*J Am Heart Assoc.* 2018;7:e008363. DOI: 10.1161/JAHA.117.008363.)

Key Words: ion transport • kidney • physiology • pregnancy • renin angiotensin system

Volume expansion prevails in pregnancy, while tight control by the renin-angiotensin system (RAS) and consecutive aldosterone production via tubular Na^+ sensing¹ is uncoupled in pregnancy.^{2–4} In pregnancy, aldosterone levels are high despite plasma volume expansion and also less responsive to increased Na^+ intake. This volume expansion does not translate into the expected rise in blood pressure

(BP),⁵ only partially explained by the aldosterone-induced release of vasodilatory placental growth factor.⁶

As both high aldosterone levels and enhanced Na^+ intake have been linked to improved pregnancy outcome and reduced BP, it is unclear whether Na^+ or aldosterone is primarily responsible.^{7–11} A beneficial role of an increased Na^+ uptake during pregnancy was postulated,¹⁰ yet to what

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Accompanying Data S1, Tables S1 through S4 and Figures S1, S2 are available at <http://jahajournals.org/content/7/15/e008363/DC1/embed/inline-supplementary-material-1.pdf>

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Clinical Perspective

What Is New?

- These animal data suggest that Na⁺ intake is an important environmental factor in pregnancy with a high salt intake paradoxically associated with lower blood pressure, that Na⁺ is retained by posttranslational activation of Na⁺ transport via the epithelial Na⁺ channel and stored, and that prior high salt intake was able to bridge a consecutive period of low salt intake by maintaining a lower blood pressure.

What Are the Clinical Implications?

- Strict Na⁺ restriction in pregnancy must be reconsidered; yet as periods of lower salt intake during later stages of pregnancy might be compensated by a higher salt intake beforehand, the optimal Na⁺ intake during different stages of gestation should be derived from observational studies.

extent of Na⁺ intake remains to be elucidated. This information is relevant to advise national health authorities as to an appropriate Na⁺ intake in pregnancy,¹² while lowering Na⁺ intake in pregnancy may even present a risk of adverse outcomes in healthy pregnancies to mothers and to their offspring.^{13,14} On the contrary, given the benefits of aldosterone in pregnancy, its suppression by excessive Na⁺ intake might be disadvantageous.¹⁵

In preeclampsia, a frequent hypertensive disease in pregnancy with systemic endothelial dysfunction,^{16,17} plasma volume reduction precedes the clinical onset of the disease (summarized in Luft et al¹⁸), and preventing the disease by reducing salt intake was unsuccessful.^{16,19} Supporting an increased salt intake were findings of a reduced incidence of preeclampsia and perinatal complications as well as lower numbers of neonatal fetal deaths in women receiving a high Na⁺ diet.²⁰ In conditions of a genetically reduced aldosterone availability of both human pregnancy with a homozygous loss-of-function mutant of the aldosterone synthase gene *CYP11B2* and in a knockout mouse model, increasing daily Na⁺ intake led to a persistent reduction in BP and, in the latter, to an improvement of the intrauterine growth-restricted phenotype and placental hypoxia.^{8,9,11} Pharmacological inhibition of aldosterone reduced umbilical perfusion in rat fetuses.⁸

Effects of aldosterone on Na⁺ uptake are mainly mediated via the epithelial Na⁺ channels (ENaCs), which are controlled by transcriptional regulation and posttranslational modifications via cleavage of inhibitory segments.²¹ For the latter, a role of pregnancy independent of aldosterone is conceivable.

To elucidate the relevance of gestational Na⁺ intake, we hypothesized that Na⁺ intake during pregnancy is linked to a

maternal BP response and that elements of the RAS are specifically adapted to transport high amounts of Na⁺ in pregnancy. We investigated the BP responses and changes of elements of the RAS and ENaCs in pregnant and nonpregnant rats in conditions of stable salt intake throughout pregnancy and upon challenge with acute changes in salt availability.

Materials and Methods

The data and analytic methods, but not study materials, will be available to other researchers for purposes of reproducing the results or replicating the procedure. Material is not freely available because of the limited amounts and potential degradation. Extended methods and materials can be found in Data S1.

Animals

The experiments were approved by the Ethical Committee for Animal Experiments of the Veterinary Department of the University of Bern.

Eight-week-old female Sprague-Dawley rats (Janvier Labs, Saint-Berthevin Cedex, France) received an implanted telemetry device. Following 3 weeks of recovery, the estrus cycle was verified. Day 1 of pregnancy was determined as described earlier.²² Rats remaining nonpregnant were used as controls.

Stable housing conditions consisted of controlled 12-hour light cycles and temperature, with free access to chow and water in individual cages. Before mating, rats were fed a standard chow containing 0.4% NaCl (Kliba Nafag AG, Kaiseraugst, Switzerland). On day 1 of pregnancy, rats were randomly fed with either a normal-salt (NS; 0.4% NaCl), high-salt (HS; 8% NaCl), or low-salt (LS; 0.01% NaCl) diet, respectively. An additional group of animals (HS/LS) received a HS diet for the first 14 days, followed by a LS diet for the next 6 days of pregnancy. Each group consisted of 4 rats. Nonpregnant rats were divided in similar groups, with each group consisting of 3 rats in all but the HS group (n=5). On day 20, animals were euthanized; blood was collected and kidneys were weighted and stored at -70°C for further analyses (Figure S1).

Statistical Analysis

All graphs are presented as mean±standard deviation (SD) except for Figure S2. Daily averages of absolute BP data obtained during either the active (nighttime) or the inactive (daytime) phase were calculated for each individual animal and presented following correction for baseline BP levels of each animal individually. The weekly BP load is presented

as area under the curve normalized for each animal's individual baseline except days in the metabolic cage. In addition, daily means for each time point were tested. The means of water and food intake is a summary of measurements during the metabolic assessments (Data S1). All statistical analyses for multiple comparisons were performed using ANOVA, followed by Tukey post hoc tests to compare all pairs of groups. With small samples sizes in molecular analyses, the Kruskal-Wallis test was applied. In all other analyses (nonpregnant versus pregnant) the Student *t* test for unpaired comparisons was used. Blinding was done for group allocation during analysis and for molecular analysis except Western blotting for gel arrangement. Power analysis was performed based on an earlier paper of our group using tail-cuff measurements¹¹ indicating a sample size per group of 3 for 90% power, which we could slightly increase to account for an overestimated effect size on this manual technique after discussion with the animal ethics committee. Statistical significance was assumed at $P<0.05$ and analyzed using GraphPad Prism (version 6; GraphPad Software, USA).

Results

Effect of Na⁺ Intake on Weight and Water and Food Intake

In nonpregnant animals, HS feeding led to increased water intake ($P=0.020$ versus the NS group), a finding also observed in the HS/LS group under HS (Table S1). After switching from an HS to LS diet, water intake dropped accordingly ($P=0.040$ for the change of HS to LS). Weight gain did not differ between the groups.

In pregnant rats, a similar association could be observed with animals on an HS diet, showing higher water intake than the other groups. The weight gain was reduced with an altered dietary salt intake, which was most obvious with the LS diet ($P<0.001$). The short exposure to the LS diet in the HS/LS group did not affect weight gain as compared with the HS group. If corrected for pub number, the weight gain was highest with NS diet similar to the absolute weight increase. The smallest weight rise was in the LS group ($P<0.05$ versus NS). The HS diet did not show the highest weight gain, and the HS/LS diet tended toward a higher weight increase than an LS diet alone. Food intake was stable in both nonpregnant and pregnant rats, irrespective of salt consumption, except for a reduction upon the abrupt switch from an HS to LS diet ($P=0.03$) in pregnant rats.

Effect of Diet on Na⁺ Balance

The Na⁺ intake in pregnant rats fed an NS diet was higher as compared with their nonpregnant counterparts if the whole

observation period was considered (0.197 ± 0.01 versus 0.147 ± 0.01 g/day; $P=0.02$) (Figure 1). No such difference was observed for the remaining diets, while the switches to the extremes of Na⁺ intake were accompanied by the expected changes.

In nonpregnant rats, the urinary Na⁺ excretion remained stable with a NS diet throughout the observational period, whereas it initially changed as expected in the extreme diets and remained only stable at the respective levels thereafter. During pregnancy, urinary Na⁺ excretion dropped with a NS diet from baseline (13.1 ± 1.2 versus 24.1 ± 3.5 mmol/mmol creatinine versus nonpregnant rats; $P=0.049$). In pregnancy, Na⁺ excretion did not alter with HS and HS/LS, but dropped with an LS diet (first versus third week; $P=0.031$).

NS and HS diets in pregnancy led to Na⁺ retention ($P=0.015$ and 0.014, respectively) throughout pregnancy. With a LS diet, following an initial drop in both nonpregnant and pregnant rats ($P=0.004$ and 0.0003, respectively, baseline versus dietary week 1), they later progressively retained salt with advancing gestational age ($P=0.027$ and 0.008 during weeks 2 and 3, respectively). The retention during week 3 was completely abolished in an HS/LS diet ($P<0.0001$ LS versus HS/LS diet).

Effect of Na⁺ on BP in Nonpregnant and Pregnant Animals

Systolic and diastolic BP was measured by telemetry, clearly indicating a circadian rhythm (Figure 2, Figure S2). We focused on nighttime BP measurements during active periods of the animals, as the daytime patterns were similar yet attenuated. As diastolic BP did not add further information while showing a similar pattern as compared to the systolic records, these data are not presented. After normalization to baseline BP levels, no difference in relative BP behavior was observed between nonpregnant and pregnant rats fed a NS diet.

In contrast to NS, a HS diet in nonpregnant rats led to a rise in BP within 1 week, which remained high throughout the experiment. The BP of pregnant versus nonpregnant rats dropped with a HS diet (combined overall $P<0.0001$) starting on days 5 to 9 ($P=0.004$ –0.044) and further reduced toward term ($P=0.013$ –0.031).

A LS diet did not affect the systolic BP except for a reduction during the last 2 days of the observation in pregnant rats (statistically significant only at day 19). Though not reaching statistical significance, the BP tended to remain slightly higher in pregnant as compared with nonpregnant rats between days 5 and 9.

Applying the HS/LS diet in nonpregnant rats led to the expected rise in systolic BP with HS followed by a drop upon

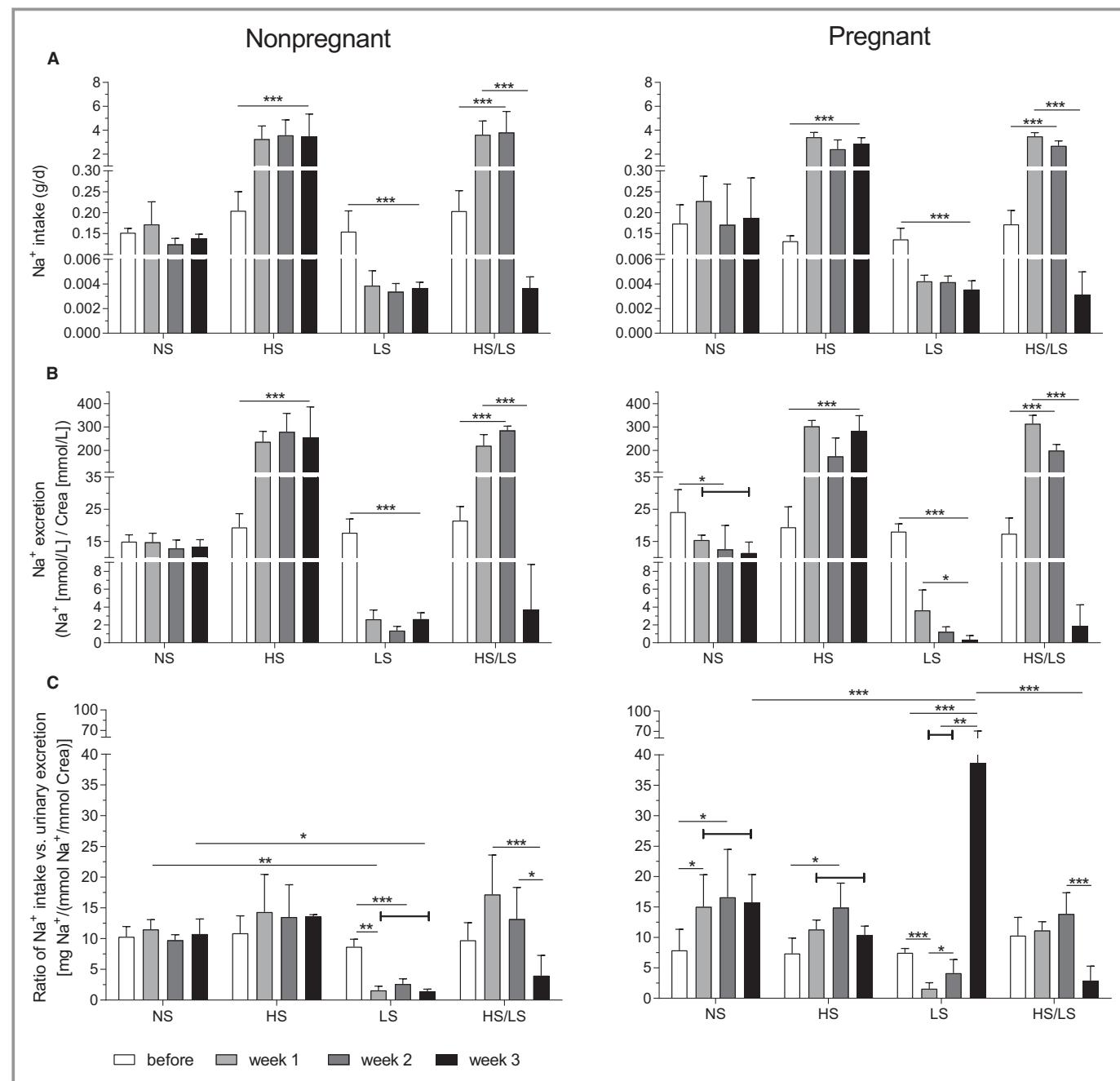


Figure 1. Metabolic Na⁺ steady state of nonpregnant and pregnant rats. In both groups, high-salt (HS) and low-salt (LS) diet led to increased and decreased Na⁺ intake (A) and excretion (B), respectively. In the group being switched from HS to LS diet, patterns similar to the individual dietary changes were observed. In addition, in normal salt (NS) conditions, pregnant rats decreased their Na⁺ excretion along pregnancy. C, Na⁺ balance as ratio of Na⁺ intake over urinary excretion rose in pregnant animals provided with either an NS or an HS diet rose, indicating Na⁺ retention. While nonpregnant rats undergoing an LS diet did not retain Na⁺, pregnant animals progressively retained it, reaching a maximum at week 3. This response to LS was absent in the HS/LS group. The time periods are indicated as before mating (baseline [white]), weeks 1, 2, and 3 (light gray, dark gray, and black bar, respectively) (n=4 for pregnant rats; n=3–5 for nonpregnant rats). Data are presented as mean±SD and tabulated in Table S4. *P<0.05, **P<0.01, ***P<0.001.

switching to a LS diet. In pregnant animals, again a BP reduction occurred in the HS diet, which remained stable upon switching to a LS diet, similar to nonpregnant rats. In pregnant animals, the BP dropped further below the

nonpregnant range only on the last experimental day. Comparing the overall BP changes, the cumulative BP was similar in NS, LS, and HS/LS diets irrespective of pregnancy. In contrast, a HS diet led to lower BP in pregnancy ($P=0.001$).

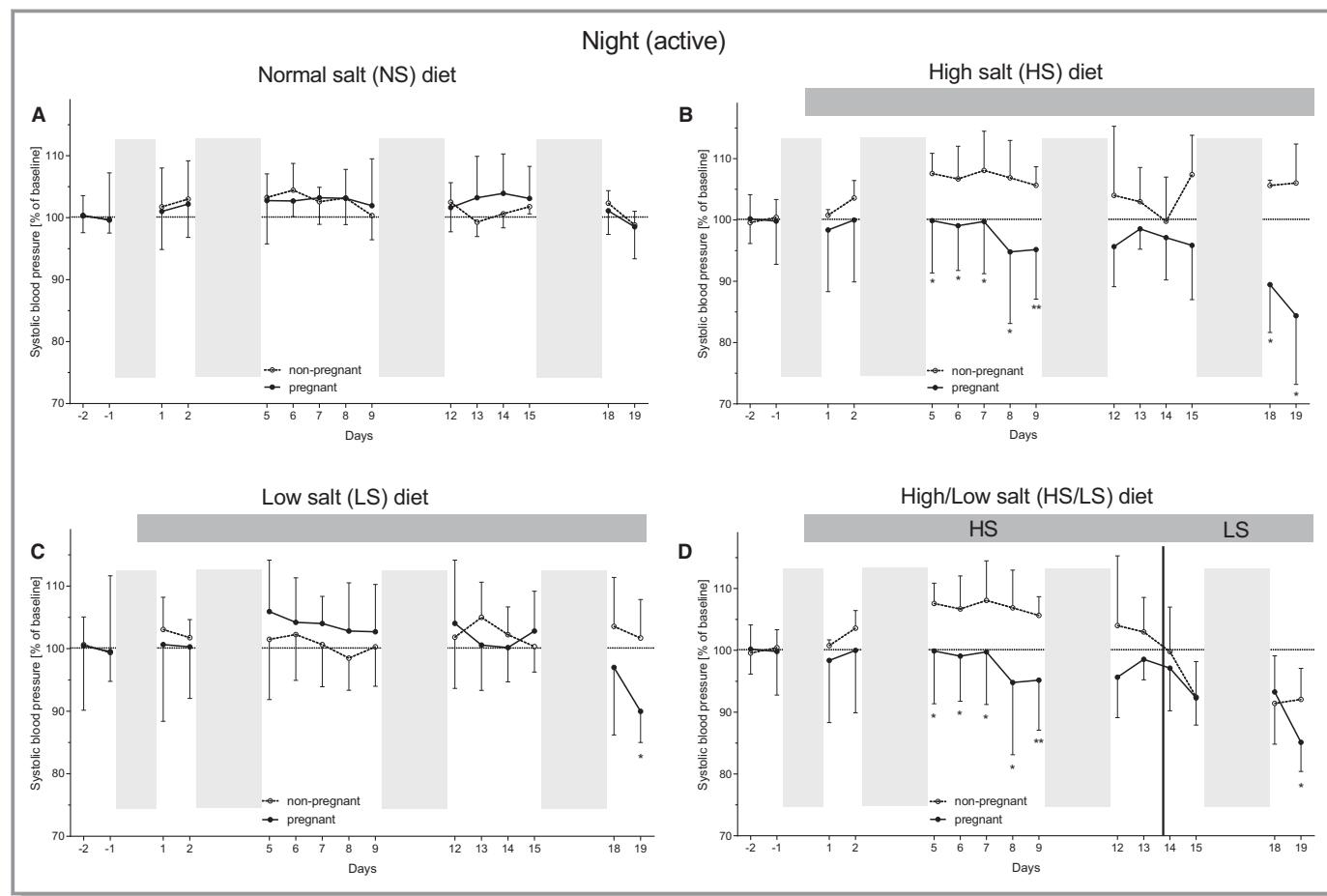


Figure 2. Normalized systolic blood pressure (BP) (%) during night activity is given in nonpregnant (white symbol, dashed line) and pregnant (black symbols, continuous line) during normal-salt (NS; A), high-salt (HS; B), low-salt (LS; C) and HS/LS diet (D; dietary change indicated by the vertical line). Days with metabolic assessment without telemetry are indicated by gray bars. While HS diet increased BP in nonpregnant rats, it was associated with a BP drop in pregnant rats. This drop was present during only the last 2 days of observation in pregnant animals on an LS diet. The group with the dietary change demonstrated a drop for both nonpregnant and pregnant rats upon introduction of the LS diet, but the effect was more intense in pregnant rats ($n=4$ for pregnant rats; $n=3-5$ for non-pregnant rats). Data are presented as mean \pm SD and tabulated in Table S4. * $P<0.05$, ** $P<0.01$.

Effect of Na⁺ on the BP Area Under the Curve Between Nonpregnant and Pregnant Animals Within Individual Dietary Interventions

Weekly changes in systolic BP indicated as area under the curve individually normalized for each animal showed in nonpregnant rats on an HS diet an increased BP ($P<0.05$ versus LS) (Figure 3). The BP in the LS and NS groups were similar. In the HS/LS group, the BP dropped with LS ($P<0.01$).

In pregnant rats, BP tended to rise with LS and drop with HS ($P=0.0108$ versus NS) but fell in both of these diets and also with HS/LS toward the third gestational week. The latter resembled the HS pattern and is different from those animals continuously having been exposed to the LS diet.

Expression of Elements of the Renal RAS System in Pregnant and Nonpregnant Rats

Angiotensinogen transcripts were more prominently expressed in the renal medulla as compared with the cortex with a tendency to similar or slightly higher levels in pregnant rats (Figure 4, Table S2). Cortical angiotensin-converting enzyme mRNA expression was lower in pregnant rats with NS intake. A comparable relationship was seen in the medulla with a HS intake. In contrast, a HS followed by a LS intake led to a distinct upregulation of angiotensin-converting enzyme. As expected, renin expression was low with HS and high with LS intake. The angiotensin receptor subtypes 1a and 1b had lower expression in pregnancy except for the medullary expression of the 1b subtype.

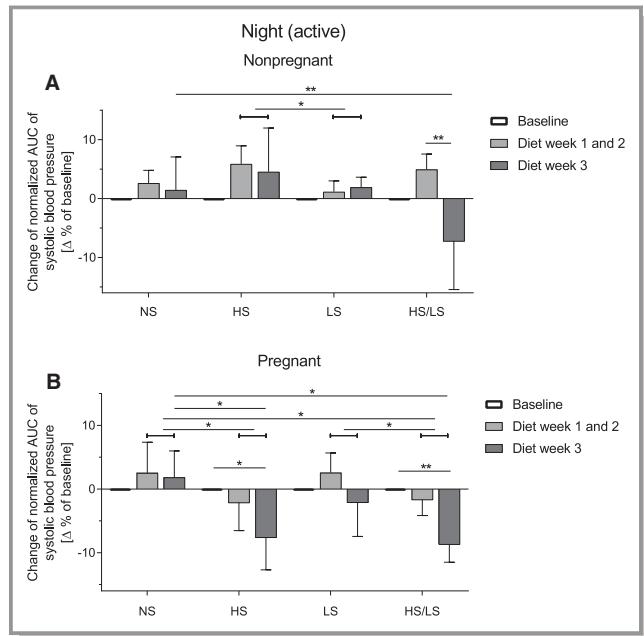


Figure 3. The change of the normalized area under the curve (AUC) of systolic blood pressure (BP) to baseline (%) during night activity is given in nonpregnant (A) and pregnant rats (B) at baseline (white), during weeks 1 and 2 (light gray bar), and on week 3 (dark gray bar). The respective diet is indicated on the x axis. While rats on a normal-salt (NS) diet behaved similarly, those on a high-salt (HS) diet showed a divergent response, with nonpregnant rats increasing their BP load and pregnant rats reducing their BP load ($P<0.001$). The pregnant animals on low salt (LS) first suffered an increase, yet later a slight but consistent drop in BP load. Of interest, nonpregnant rats with the dietary change HS/LS reduced their BP load strikingly, whereas pregnant HS/LS rats behaved as HS animals irrespective of the switch to LS in the third week of pregnancy (n=4 for pregnant rats; n=3–5 for nonpregnant rats). Data are presented as mean \pm SD and tabulated in Table S4. * $P<0.05$, ** $P<0.01$.

Adjustments of Elements of Aldosterone-Dependent Na⁺ Transport in Pregnant Rats

While ENaC- α subunit mRNA levels were not remarkably regulated, - β and - γ subunits showed a high expression in HS conditions in the nonpregnant rat (Figures 5 and 6, Table S3). In pregnant animals, this change was absent and the overall transcript amount was lower.

As ENaC- α and - γ subunits are activated by cleavage, both full-length and cleaved subunits were determined. The highest expression of full-length proteins was in HS/LS conditions in both groups in the renal cortex, but not in medullary tissue. In nonpregnant, in contrast to pregnant rats, ENaC- α abundantly appeared in its cleaved form in the renal cortex in LS and even stronger in HS/LS conditions. Medullary samples showed no gestational specificity, yet a high expression in pregnancy. Cortical ENaC- β protein expression only mildly increased in pregnant rats exposed to the HS/LS challenge. In contrast,

the ENaC- β subunit protein strongly increased ($P=0.0062$) in the medulla in pregnancy independent of diet. This was paralleled for the full-length ENaC- γ subunit with an increase in medullary tissue only with pregnancy. As expected in the nonpregnant setting, the cleaved γ -subunit was more abundant in cortical tissue in LS conditions, yet reversed in pregnancy and unchanged in the medulla.

Discussion

We investigated Na⁺ handling in nonpregnant and pregnant rats exposed to extremes of Na⁺ intake, or rapid changes of Na⁺ availability. The Na⁺ intake was higher in pregnant animals in NS conditions resembling human behavior.¹⁰ No such differences regarding the Na⁺ intake were present for the other diets. During pregnancy, Na⁺ appears to be retained progressively, most obviously in LS conditions, and more rigorously than in nonpregnant rats, as shown by Na⁺ excretion. The Na⁺ balance revealed Na⁺ retention in all conditions, with striking differences during the last week of LS intake in pregnant as compared with nonpregnant animals. Surprisingly, a prior HS converted pregnant animals with the switch to LS to behave as LS animals in nonpregnant conditions, suggesting a profound Na⁺ storage earlier during the preceding HS period. While nonpregnant HS rats tended to increase their BP, pregnant ones demonstrated a reduced BP. These findings in a rat model confirm our recent observations in pregnant women¹⁰ in a model with accurate Na⁺ balance.

A prior HS diet in those pregnant animals chosen to switch to a LS diet after 2 weeks of pregnancy abolished the BP drop seen with HS during the third gestational week, whereas a LS diet alone had no such effect. These observations strongly suggest a mechanism permitting BP lowering with a HS diet in pregnancy, which occurs in association with the pronounced Na⁺ retention. This Na⁺ retention may even outlast periods of low Na⁺ availability such as is simulated in our HS/LS switch group. With respect to the expected changes of the renal RAS system, including the angiotensin receptor subtype 1, only mild responses occurred because of pregnancy. Of interest, the high renin state of pregnancy was not reflected by the intrarenal transcript levels. Likewise, expression of ENaC subunits as Na⁺ transporters was unchanged or rather low. The medullary expression of the active cleaved ENaC- α and β subunits was very strong in pregnancy, suggesting a facilitated Na⁺ transport. Although the medullary full-length ENaC- γ subunit was also highly expressed, the cleaved form was low.

Changes in Na⁺ intake during pregnancy have been studied in many models. As suggested by findings in humans, a diet reduced in Na⁺ content does not improve the clinical course of pathological pregnancies.^{13,19} This was supported by observations in animal experiments, where either a low-Na⁺ diet or low-aldosterone conditions caused by chronic

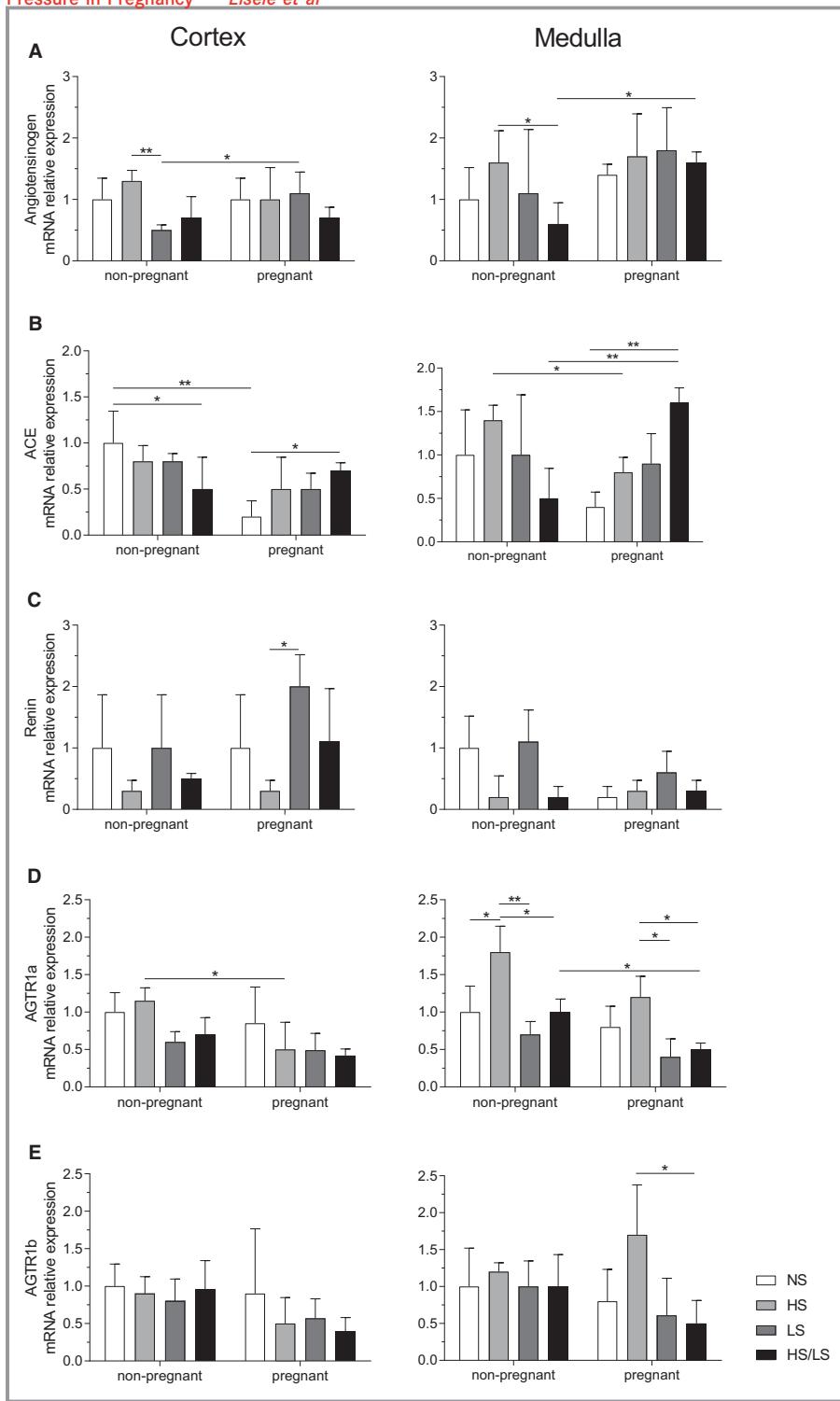


Figure 4. Expression of components of the renin-angiotensin system (RAS) as assessed by TaqMan polymerase chain reaction. The expression of angiotensinogen (A, upper panel), angiotensin converting enzyme (ACE; B, middle panel), renin (C, lower panel) and angiotensin II receptor subtype 1a (D, upper panel) and 1b (E, lower panel) is given in nonpregnant (left-hand columns) and pregnant rats (right-hand columns) at baseline (white), during weeks 1 and 2 (light gray bar), and on week 3 (dark gray bar) in renal cortex (left panel) and renal medulla (right panel) separately. A low-salt (LS) diet rather increased renal angiotensinogen expression in pregnancy. In pregnancy, a high-salt (HS)/LS diet led to the highest renin expression in pregnancy, while the response was reverse in nonpregnant animals. Renin expression was regulated as physiologically expected (lower panel) ($n=4$ for pregnant rats; $n=3-5$ for nonpregnant rats). Data are presented as mean \pm SD and tabulated in Table S4. * $P<0.05$, ** $P<0.01$.

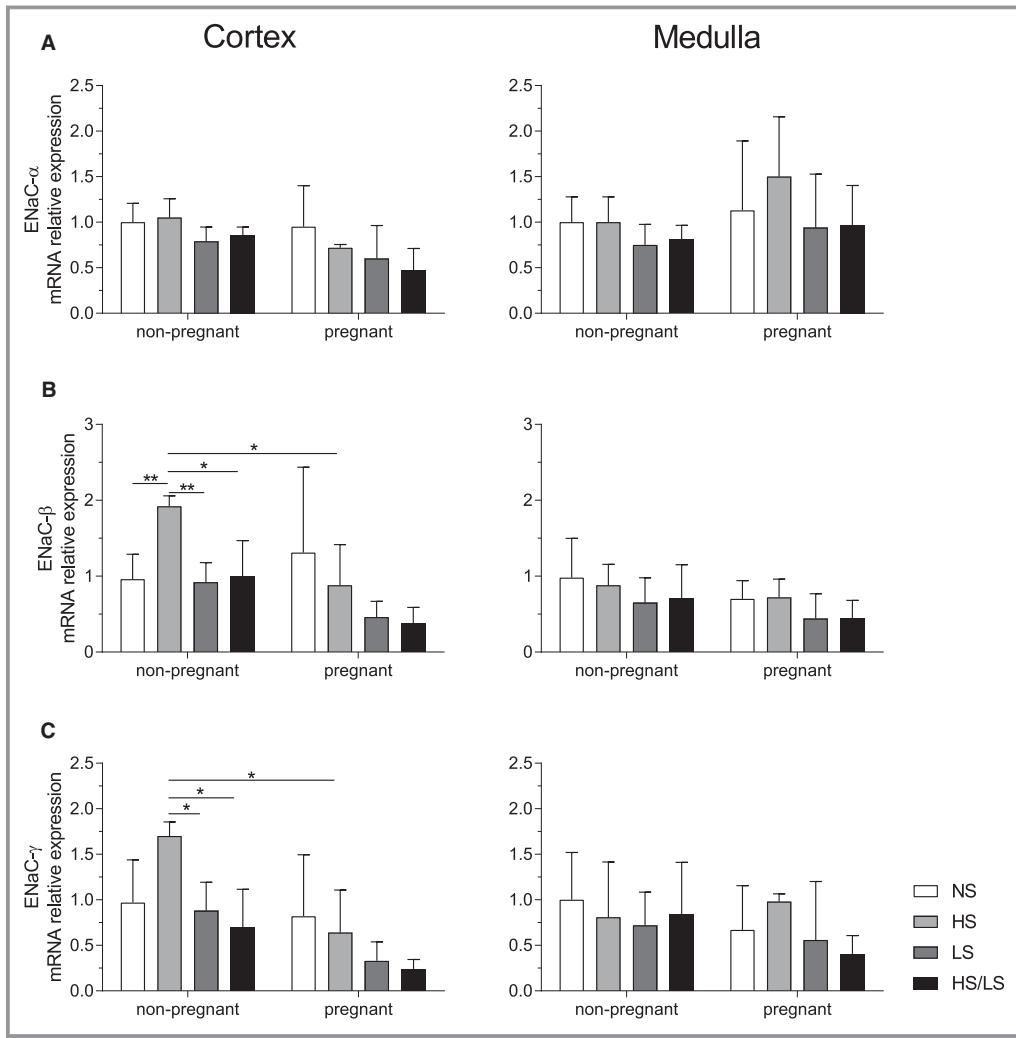


Figure 5. Expression of epithelial Na⁺ channel (ENaC) subunit transcripts as assessed by TaqMan polymerase chain reaction. The expression of the ENaC α - (A, upper panel), β - (B, middle panel), and γ -subunit (C, lower panel) is given in nonpregnant (left-hand columns) and pregnant rats (right-hand columns) at baseline (white), during weeks 1 and 2 (light gray bar), and on week 3 (dark gray bar) in renal cortex (left panel) and renal medulla (right panel) separately. ENaC- α subunit mRNA levels were not remarkably regulated. In contrast, ENaC- β and - γ subunits show a high expression in high-salt (HS) conditions in the nonpregnant rat, while this change was absent in pregnant animals with the overall transcript amount being also lower ($n=4$ for pregnant rats; $n=3-5$ for nonpregnant rats). Data are presented as mean \pm SD and tabulated in Table S4. * $P<0.05$, ** $P<0.01$. HS indicates high-salt; HS/LS, HS followed by LS; LS, low-salt; NS, normal-salt.

spironolactone treatment as well as aldosterone synthase knockout led to reduced umbilical perfusion and/or intrauterine growth restriction.^{8,11,23}

Pregnant NS rats increased Na⁺ intake while excretion dropped as pregnancy progressed, suggesting an absolute Na⁺ retention, a finding that has recently been attributed to the renal ENaC in the late normal pregnant rat.²⁴ The enhanced spontaneous Na⁺ intake is again in line with findings by our group in human pregnancy.¹⁰ Although exposed to an excessive Na⁺ load, even pregnant animals of the HS group overall retained Na⁺. Such a paradox in salt handling in pregnancy underlines the

assumption that aldosterone and Na⁺ regulation are (at least partially) uncoupled in pregnancy. Stimulation of aldosterone production by pregnancy-derived angiogenic signals is well conceivable as a contributor of aldosterone stimulation independent of the RAS.⁴ The salt retention is extreme during LS conditions in pregnancy, indicating a highly capable mechanism.

As the RAS, in part, even in pregnancy contributes to aldosterone production and volume regulation, we aimed to challenge this system by first providing large amounts of Na⁺ and, upon perfusion of the rat placenta beyond day 14 of gestation, to abruptly switch to a LS diet. This challenge did not

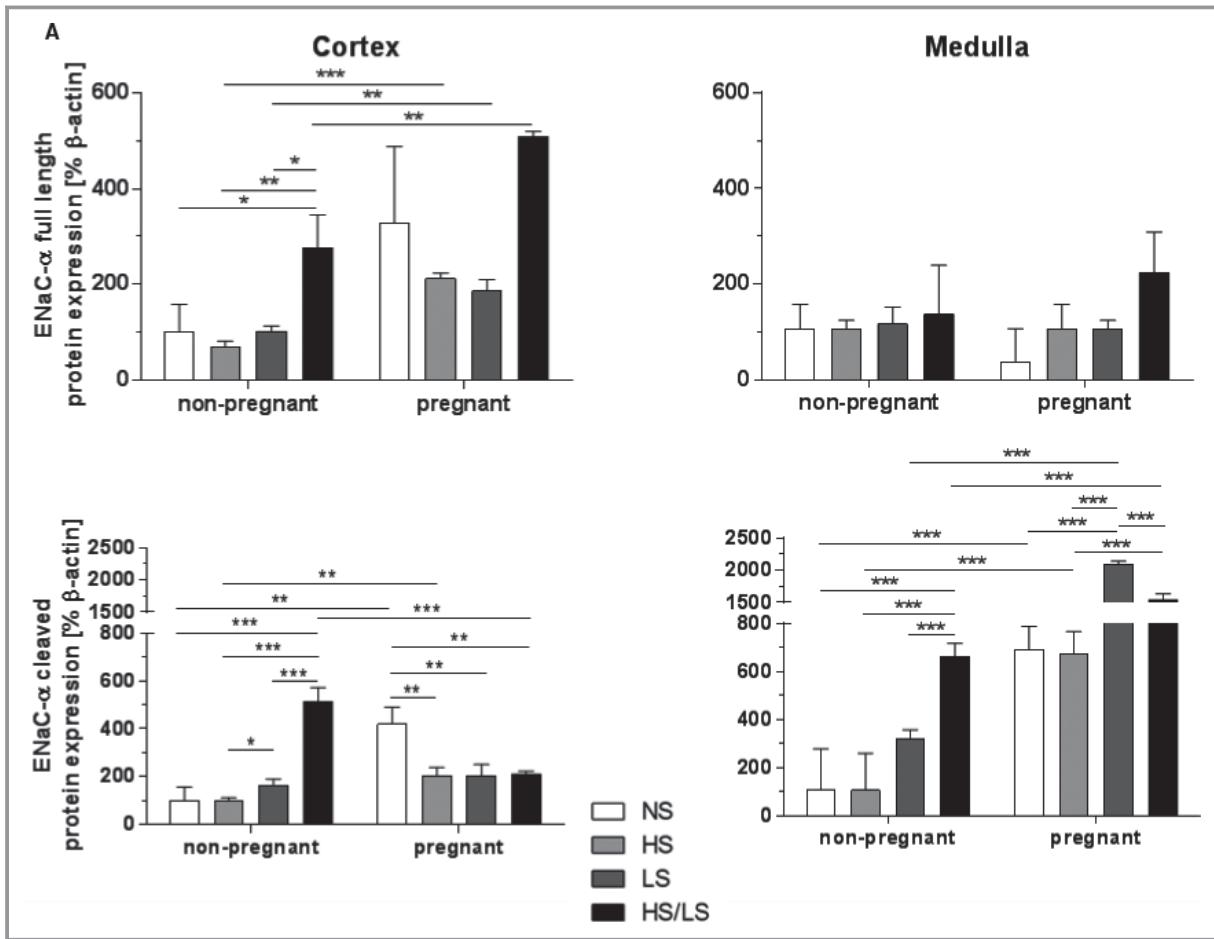
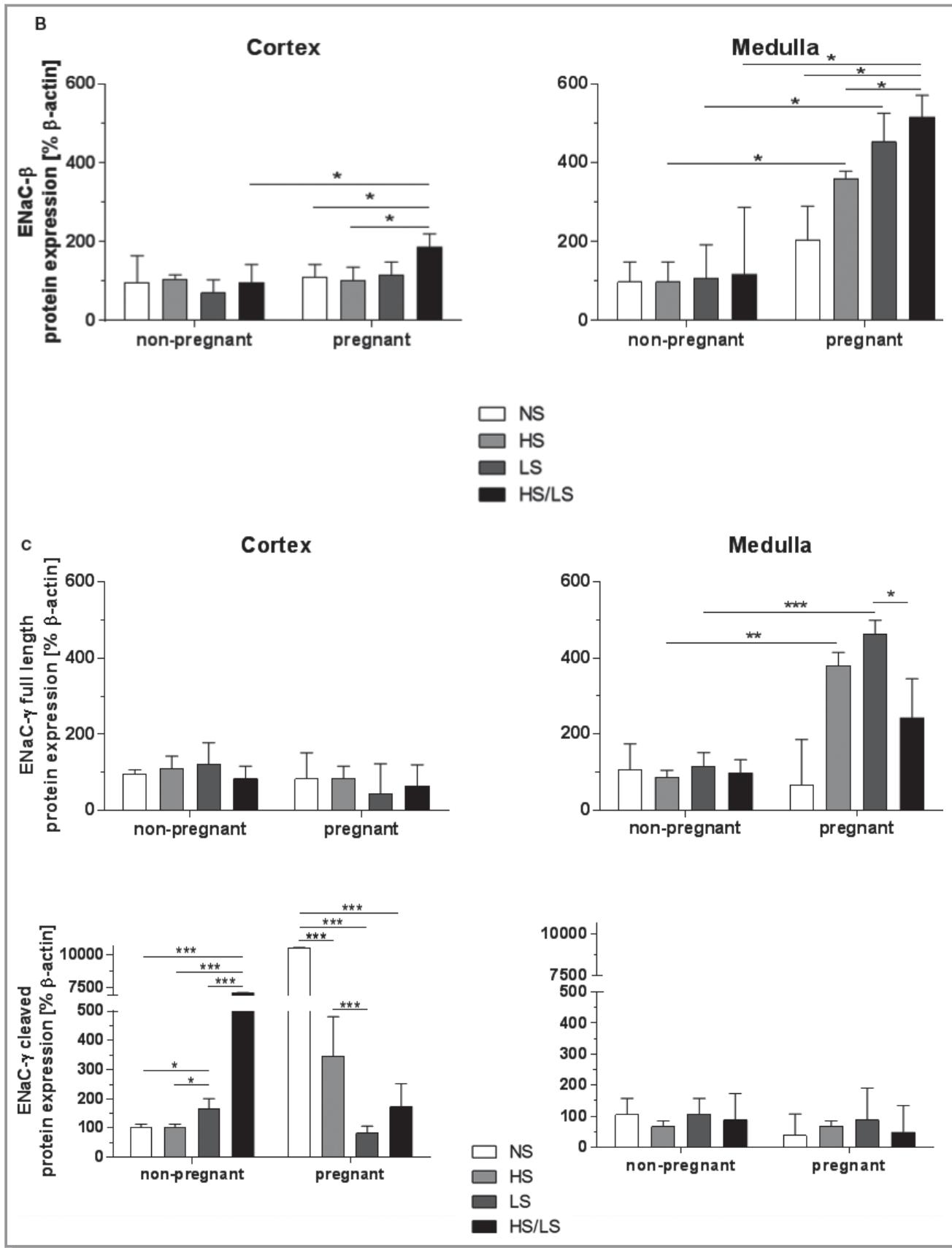


Figure 6. Expression of ENaC subunit protein as assessed by Western blot analysis. The expression of the ENaC α -subunit (A, upper panel, full-length protein; lower panel, cleaved protein), β -subunit (B, full-length protein), and γ -subunit (C, upper panel, full-length protein; lower panel, cleaved protein) is given in nonpregnant (left-hand columns) and pregnant rats (right-hand columns) at baseline, during weeks 1 and 2, and on week 3 (white, light gray, and dark gray bars, respectively) in renal cortex (left panel) and renal medulla (right panel) separately. Full-length transcripts were highest expressed in low-salt (LS) conditions following a high-salt (HS) exposure in both nonpregnant and pregnant rats in the renal cortex, yet not in medullary tissue. In contrast to pregnant rats, in nonpregnant rats, in LS and even stronger in HS/LS conditions, ENaC- α abundantly appeared in its cleaved form in the renal cortex. Both nonpregnant and pregnant animals showed a similar pattern in medullary samples, though the expression levels in pregnancy were hugely elevated (A). Cortical ENaC- β protein expression only mildly increased in pregnant rats exposed to the HS/LS challenge. A huge increase was observed in the medulla with pregnancy for the ENaC- β subunit protein for all diets (B). A similar picture was experienced for the full-length ENaC- γ subunit with an increase in medullary tissue only with pregnancy (C). As expected in the nonpregnant setting, the cleaved γ -subunit was more abundant in cortical tissue in LS conditions and reversed with pregnancy, yet unchanged in the medulla (n=3 for each group). Data are presented as mean \pm SD and tabulated in Table S4. * P <0.05, ** P <0.01, *** P <0.001. HS indicates high-salt; HS/LS, HS followed by LS; LS, low-salt; NS, normal-salt.

produce the expected LS Na⁺ retention phenotype in pregnant rats but paralleled the pattern seen in nonpregnant rats. This suggests that prior Na⁺ retention during the HS diet supplied sufficient residual Na⁺ or an inability of the animals to rapidly adapt to the environmental change. This is interesting because the HS/LS group was considered to be extremely challenged by first a suppression of aldosterone with the HS diet, followed by a marginal salt availability only at times of placental perfusion and required plasma volume expansion. Our observation of the importance of an HS diet is highlighted in contrast to earlier

findings, in which a limited exposure to a LS diet during the last week of gestation not preceded by a HS phase resulted in similar changes as compared with a LS diet throughout pregnancy.^{23,25} Given the highest and lowest weight gain with NS and LS diets, respectively, and not with the HS and HS/LS challenge, indicates a more complex issue of time and optimal dosing of salt intake during pregnancy.

Contrary to LS, the extreme HS diet led to decreased BP in pregnant rats. It was accentuated in late pregnancy after gestational day 14, when the plasma volume usually increases

**Figure 6.** Continued

in rat pregnancies.²⁶ This observation is in line with findings in mice with a less stringent HS diet, in which fetal growth and placental hypoxia were improved without increasing maternal BP.¹¹ Furthermore, our results indicate an inverse relationship between BP and Na⁺ intake, in line with our previous results in first-trimester pregnant women, where HS intake was related to lower BP levels.¹⁰

The changes toward a higher expression of ENaC subunits and a more pronounced cleavage with enhanced activity resemble ENaC expressional changes observed in nephrotic syndrome, where plasmin appears to have a causative role in activating the ENaC.²⁷ Given the high levels of plasminogen activator inhibitor-1 and -2 levels in pregnancy, other serine proteases might be involved. In humans, urinary exosomes show an enhanced ENaC expression and also processing by cleavage.²⁸ Interfering with the RAS toward a higher aldosterone level by a LS diet or direct aldosterone infusion also enhanced ENaC- α and - γ subunit exosomal excretion in humans.²⁹ Although ENaC mRNA levels were unable to exactly predict active ENaC protein abundance, blocking ENaC expression could not be compensated for in late pregnant rats.²⁴ Thus, ENaC appears to be a central element in Na⁺ regulation in pregnancy. Although its transcript regulation is mediated via aldosterone,³⁰ further activating modifications are due to protein-cleaving conditions.

The strength of our study was the use of a telemetry device as well as detailed and controlled metabolic analysis of the Na⁺ balance. In addition, the dietary changes were major, in contrast to other studies in pregnancy, and not limited to the final phase of the pregnancy. Given the dynamic adaptation throughout pregnancy, such a study might be difficult to interpret.³¹ In that study, a late dietary adjustment was made limited to the third trimester of rat pregnancy. As BP reduction was present only after some latency in our model, which we started at gestation, a delayed intermediary response is likely involved. This could indicate that sensing and adaptation to a higher-than-NS intake might require exposure during earlier phases of pregnancy or may simply require time. In line with these considerations are studies by Machnik et al,³² who describe the skin as a relevant storage and regulatory compartment in response to Na⁺.

A limitation of this study is the small number of animals attributable to the extended experimental setup. Furthermore, a broader dosage range should be investigated in further studies to identify an optimal Na⁺ intake as well as the optimal times to eventually alter the salt regimen in a less extreme condition, combined with an analysis of Na⁺ storage such as has been otherwise identified in the skin.³²

In conclusion, to date, only limited information is available in human pregnancy, suggesting clear positive effects of increasing salt intake, such as in genetic aldosterone

synthase deficiency and in the first trimester of healthy women.^{9,10} The current study in rats provides further insight with respect to increasing salt intake in pregnancy. Although the sensitive balance between Na⁺ and aldosterone as well as placental development requires further attention and studies to optimize the dosing and perhaps timing of salt delivery, this study contributes to better comprehension the impact of Na⁺ availability in pregnancy beyond its impact on the RAS. Coexisting conditions leading to an activation of ENaC might contribute or even abolish functional activating pathways such as with increased plasminogen activator inhibitor-1 levels.³³

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Disclosures

None.

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