Maternal High-Salt Diet During Pregnancy in Sprague Dawley Rats Programs Exaggerated Stress-Induced Blood Pressure and Heart Rate Responses in Adult Female Offspring

Summer Hayes King
Brigham Young University - Provo

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MATERNAL HIGH-SALT DIET DURING PREGNANCY IN SPRAGUE-DAWLEY RATS PROGRAMS EXAGGERATED STRESS-INDUCED BLOOD PRESSURE AND HEART RATE RESPONSES IN ADULT FEMALE OFFSPRING

by

Summer H. King

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Physiology and Developmental Biology

Brigham Young University

December 2007
This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

Date

James P. Porter, Chair

Date

Allan M. Judd

Date

Brent L. Nielsen
As chair of the candidate’s graduate committee, I have read the thesis of Summer H. King in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

Date

James P. Porter
Chair, Graduate Committee

Accepted for the Department

Dixon J. Woodbury
Graduate Coordinator

Accepted for the College

Rodney J. Brown
Dean, College of Life Sciences
ABSTRACT

MATERNAL HIGH-SALT DIET DURING PREGNANCY IN SPRAGUE-DAWLEY RATS PROGRAMS EXAGGERATED STRESS-INDUCED BLOOD PRESSURE AND HEART RATE RESPONSES IN ADULT FEMALE OFFSPRING

Summer H. King

Department of Physiology and Developmental Biology

Master of Science

The prenatal environment has been shown to have lasting effects on cardiovascular health. In the present study, pregnant rats were fed a 0.7% NaCl normal salt (NS) diet or an 8% NaCl high salt (HS) diet throughout pregnancy. Adult offspring were fitted with radiotelemetry probes to continuously measure blood pressure and heart rate. Rats were placed in restraining cages to test for a programmed acute stress hyperresponsiveness. Offspring were challenged with HS diet for one week to determine if blood pressure salt sensitivity had been programmed by the prenatal HS diet. Animals were killed following resting and acute stress conditions, after which brains and blood were collected for in situ hybridization for corticotropin releasing hormone (CRH) and radioimmunoassay for corticosterone. In order to determine the contribution of gene
expression to differences seen, total brain RNA was analyzed with microarray. Rats were injected with a ganglionic blocker and an adrenergic receptor antagonist before restraint to examine the autonomic component of the stress response. High salt offspring of either sex did not have basal hypertension. Female HS offspring had an increased pressor and tachycardic response to acute stress compared with NS females. There were no differences between male NS and HS offspring during acute stress. Salt sensitivity was not induced during high salt challenge. According to the microarray, 11 genes were upregulated and 10 were downregulated in adult brains, while 17 were upregulated and 17 were downregulated in pup brains. These data indicate that there are long-term changes due to HS diet. CRH levels were higher in the paraventricular nucleus (PVN) of female HS offspring than in female NS offspring during both basal and stressed conditions, though no differences were seen in CRH expression levels of male offspring. Autonomic blockade completely abolished the enhanced tachycardic response seen in female HS offspring. However, a difference in NS and HS blood pressures remained. Thus, female offspring of mothers fed an 8% NaCl diet have alterations in cardiovascular control, indicated by an enhanced tachycardic response as adults, due to changes in the autonomic nervous system, and enhanced pressor response to stress mediated by unknown mechanisms.
ACKNOWLEDGMENTS

I would like to thank Dr. Porter for the guidance he has given me as my advisor and for his generosity with his patience, accessibility, and financial support. I would also like to thank Dr. Judd and Dr. Nielsen for their suggestions and viewpoints along the way. Special thanks to my parents for their encouragement and love. Finally, I would like to express my appreciation to my husband, Spencer King, for his unending support and his belief that I can do anything.
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Experimental Objectives:

In the present study, our experimental objectives were:

Chapter 1

1. To determine whether offspring of rats fed an 8% NaCl high salt diet during pregnancy are hypertensive as adults.
2. To determine whether offspring of rats fed a high salt diet during pregnancy are salt sensitive as adults.
3. To determine if there is a sex-dependent difference in the blood pressure and heart rate of male and female offspring of rats fed a high salt diet.
4. To determine whether the reaction to stress is the same in NS and HS rats.

Chapter 2

5. To determine the contribution of genetics to differences between NS and HS rats.

Chapter 3

6. To determine the contribution of the autonomic nervous system to the differences seen between NS and HS rats.
Chapter 1: Effects of High Salt Diet

Introduction:

High blood pressure is an important modifiable risk factor for stroke and other vascular disease, and affects more than 65 million Americans (52). However, the origins of hypertension are not well understood. The fetal origins hypothesis states that an adverse fetal environment may lead to permanent physiological and metabolic alterations, establishing pre-susceptibility to adult disease (4). This process is called fetal programming. Correlations between maternal malnutrition and poor offspring health have been noted for many years (5 and 76). Even today, studies find that undernutrition during pregnancy predisposes offspring to low birth weight and cardiovascular disease (61).

Several animal models of fetal programming of hypertension have been developed. The protein undernutrition model, in which a maternal diet that is low in protein causes low birth weight of offspring, has been studied often (39, 40, 41, 54, 79). This model produced offspring that were hypertensive as adults (42, 43, 57, 77). Epidemiological studies have shown an inverse relationship between cardiovascular disease and birth weight (15 and 44), although this idea has been challenged (31). Injection of dexamethasone during pregnancy also led to offspring that have low birth weight and were hypertensive as adults (64 and 67). Surgically decreased placental blood flow, often performed by putting clips over uterine arteries, caused low birth weight and altered renal activity in offspring, as well as hypertension (2, 60, 76). Prenatal exposure to interleukin-6 resulted in hypertension and increased hypothalamic-adrenal-pituitary
activity in adult offspring (59). Each model demonstrates that fetal environment can lead to permanent alterations in adult cardiovascular health.

A high salt diet has also been studied as a method to program hypertension in Sprague Dawley rats (13, 69). Grollman and Grollman showed that pregnant dams given 2% NaCl in drinking water gave birth to offspring who were hypertensive as adults (28). However, Myers et al. showed that the offspring of dams fed a high salt (2.3% NaCl) chow during pregnancy were not hypertensive (50). Contreras et al. found that giving a high-salt diet (3%) before and during pregnancy, as well as during lactation and for 10 days after weaning, produced hypertensive offspring (13). This model, however, is not necessarily one of fetal programming since the high salt diet is given during lactation and weaning in addition to prenatal exposure. However, giving a higher percentage of salt, such as an 8% NaCl diet, during pregnancy may be sufficient to produce lasting hypertension in adult offspring.

Occasionally, programmed blood pressure differences are transient, rather than permanent. For instance, an increase in blood pressure during and after stress may last longer in rats exposed to an adverse fetal environment (56). Igosheva et al. observed that prenatally stressed rats had higher and longer-lasting systolic arterial pressure elevations to restraint stress and during recovery compared to control (32). Malnourished rats responded similarly to ammonia stress, with enhanced blood pressures during stress and recovery (71). Other indicators of altered physiologic stress response can also be present, such as an increase or decrease compared to control in the activity of the HPA axis in rats prenatally exposed to dexamethasone responding as adults to cold or restraint stress (22 and 67). These enhanced responses to stress occurred even when the adverse fetal
environment did not cause permanent change in adult cardiovascular health. The consequences of repeated hyperresponsiveness to acute stress include vascular remodeling (24) and an increase in human cardiovascular mortality (11, 49, 62).

Additionally, stress-induced differences in blood pressure or HPA activity may be sex-specific. There is disagreement about whether males or females are most affected. Bhatnagar et al. found that prenatal stress prevented habituation to repeated restraint stress in male, but not female rats (8). Igosheva et al. observed a higher increase in blood pressure during restraint stress in prenatally stressed female rats than in prenatally stressed male rats (32). Human females showed a correlation between blood pressure during psychological stress and size at birth, while no relationship was seen in males (74). Thus, sex of the offspring may be an important factor in the blood pressure and heart rate changes seen in high salt offspring.

Sprague Dawley rats are not genetically susceptible to salt-induced hypertension; a high salt diet does not produce elevated arterial pressure in these rats (7). Some reports have indicated that exposure to high salt in utero or other models of fetal programming can lead to a permanent salt sensitivity (13, 77). Offspring of rats fed an 8% high salt diet during pregnancy may have an increased blood pressure when fed a high salt diet as adults.

In this study we determined the effects of fetal exposure to a high salt diet on basal and stress-related blood pressure and heart rate levels in male and female rats (58). Additionally, we examined whether such offspring were salt-sensitive as adults. Our results indicate HS rats do not have basal hypertension, but that female HS offspring have
a hyperresponsive tachycardic and pressor response to restraint stress. Prenatal HS diet does not confer salt sensitivity.
Materials and Methods:

Prenatal HS and NS protocol:

Female Sprague Dawley (Harlan Sprague Dawley) rats received 8% NaCl Harlan Teklad diet (product ID# 92012) as the HS treatment or 0.7% NaCl (product ID# 96329) as the NS control treatment. Proven breeder male Sprague Dawley rats were given standard lab chow (Harlan Teklad Rodent Diet (0.29% Na⁺, 0.49% Cl⁻)) during this time. Rats were allowed to adjust to the experimental diet (HS or NS) while caged separately for one week prior to breeding, after which they were placed in hanging wire cages in breeding pairs. Once vaginal mucus plugs were found (usually 1-5 days), the males were removed and females were placed in polycarbonate cages and allowed to eat experimental diet and drink water ad libitum. Corncob bedding was changed every 2-3 days. Pregnant dams were allowed to progress to delivery. Litter size, birth weights, and sex were determined within 24 hours of delivery and litters were culled to 10, keeping an equal ratio of males and females. After parturition, experimental diet was discontinued and dams were fed standard chow while lactating. Pups were weaned on PD 21 and given standard lab chow to eat. Hence, the only exposure of offspring to the high-salt environment was during the in utero period.

Implantation of the radiotelemetry probe:

Adult offspring were anesthetized with ketamine/acepromazine (140 mg/kg/1.4mg/kg, im) and the abdominal aorta was exposed through a ventral midline incision. Blood pressure probes (TA11PA-C40, DSI, Arden Hills, MN) were inserted into the aorta distal to the renal arteries and cemented in place with Vetbond (3M, St. Paul, MN) and secured with a cellulose (0.5 cm²) patch. After the incision was closed,
rats were injected with Rimadyl (5 mg/kg, *im*, Pfizer, Exton, PA) and allowed one week to recover in plastic cages placed on top of radio receiving units (RPC-1, DSI, 1 per rat). During this recovery week, children’s acetaminophen syrup was added to the drinking water to provide analgesia. Only animals without surgical complications were included in the study (2 NS female offspring were excluded). Blood pressure and HR were monitored using a scheduled sampling protocol which included 30 seconds of recording (500Hz) every 20 minutes throughout the light and dark cycles. Data were digitized and stored on a hard drive using Dataquest ART software (DSI).

**Expression of CRH mRNA:**

Animals were killed by decapitation and, in some rats, trunk blood was collected into chilled tubes containing 5 µl of heparin (2,000 U/ml). Brains were quickly removed and frozen in plastic molds containing O.C.T. compound. The blocks of tissue were stored at -93°C until processing for *in situ* hybridization.

**In situ hybridization:**

Coronal sections (20µm) through the PVN were cut (1 in 3 series) with a cryostat and thaw-mounted onto slides (Superfrost Plus, Fisher Scientific). One series of sections was subsequently fixed (4% buffered paraformaldehyde) and acetylated. Hybridization, *in situ*, was carried out overnight at 57°C using a ³²P-UTP-labeled (3 X 10⁶ cpm per slide) antisense riboprobe to CRH mRNA (Dr. Kelly Mayo, Northwestern University, Evanston, IL). Unincorporated probe was removed by incubating the slides in RNase (14 µg/ml, Sigma) for 30 min followed by washes in buffer without RNase, 1X SSC (Saline Sodium Citrate, room temperature) and 0.5X SSC (60°C). Visualization of the hybridized sections, together with ¹⁴C-standards (American Radiolabeled Chemicals,
Inc., St. Louis, MO) utilized 48-hr exposure to autoradiographic film (Hyperfilm MP, Amersham Biosciences). Autoradiographic films were then developed using a standard x-ray developer.

For data analysis, sections through the whole extent of the PVN were scanned from each brain. The region bounded by both wings of the PVN was outlined as the region of interest and mean optical density (O.D., calibrated to µCi/g using the $^{14}$C standards) and area (in square pixels) were determined (Scion Image, Scion Corporation, Frederick, MD). An identical area over the region just dorsal to the PVN was used to determine the background O.D. For each section, the O.D. (total minus background) was multiplied by the area and all values from a given brain were averaged. Since not all sections were processed in the same assay, the data required normalization. This was done by dividing the value for each individual rat by the average of the NS offspring for a given assay. Within each sex, the normalized data for HS and NS groups during basal and stressed conditions were compared using two-way ANOVA.

**Corticosterone assay:**

Blood was centrifuged for 15 min. at 4° C and plasma was stored at -20° C until the time of the assay. Radioimmunoassay for corticosterone was performed using a commercially available kit (MP Biomedical, Orangeburg, NY).

**Experimental protocols:**

**Basal recordings**

One week after surgery, resting MAP and HR were measured as outlined above for 7 days. During this time, the animals were left undisturbed except for periodic changing of cages and routine feeding by the animal care facility workers. For males,
adult offspring ranged in age from 2 to 6 months at the time of initial surgery and came from 10 different litters (5 HS and 5 NS). For females, adult offspring ranged in age from 2 to 4.5 months and came from 12 different litters (6 HS and 6 NS).

For each rat, the 36 data points recorded for each light or dark period for each day were averaged separately to give 7 light-period values and 7 dark-period values. Within male or female groups, 2-way ANOVA for repeated measures was used to compare resting MAP and HR between HS and NS groups over the 7 days (SigmaStat, SSI, Richmond, CA). Light-period values were compared separately from dark-period values.

Acute restraint stress

Two weeks after surgery, scheduled sampling was changed from a 30-second sample once every 20 minutes to a 30-second sample once every 5 minutes. The experimental procedure room was kept quiet the morning before the stress. Beginning at 13:00, several samples were taken to establish initial MAP and HR. At the end of the final five-minute baseline period, rats were placed into Plexiglass restraining tubes (5 cm x 7 cm x 14 cm for rats under 300 g and 5 cm x 8 cm x 15 cm for rats greater than 300 g) which were placed back into the home cages sitting on top of the radio receivers in time to record the next five-minute sample. After one hour, rats were removed from the restraining cages and allowed to recover in their home cages for an additional hour. After recovery, schedules sampling was changed back to one 30-second sample every 20 minutes. For each rat, pre-stress values for MAP and HR were averaged (2-10 values) to give a single baseline number. The change in MAP and HR from the initial was then determined for each 5-min sample during the 60 minutes of stress and the 60 minutes of recovery. Two-way ANOVA with repeated measures (time) was used to compare the
effects in NS and HS offspring across the 120 minutes. Where appropriate post hoc analysis was performed using the Student-Newman-Keuls test.

**High-salt challenge**

One day after completion of the acute restraint stress, all rats were fed the 8% NaCl diet for one week followed by one week of recovery on standard rat chow. Control MAP and HR values were obtained for each rat by averaging all data from the day (both light- and dark-values) prior to the high-salt challenge. All data points for the entire 7-day high-salt challenge were averaged to give the experimental value for each rat. The recovery values were obtained by averaging all data points obtained during the seventh day (24 hours) of recovery. Within male and female groups, control, experimental, and recovery data were compared using 2-way ANOVA for repeated measures. In addition, day-by-day values for MAP and HR during the week of high-salt challenge were compared for male and female groups during light or dark periods using 2-way ANOVA for repeated measures. Where appropriate post hoc analysis was performed using the Student-Newman-Keuls test. Rats were killed at the end of the final day of recovery from the high-salt challenge and brains were collected at this point for subsequent determination of basal CRH mRNA expression. In some rats, trunk blood was also collected for subsequent determination of plasma levels of corticosterone.

**Data analysis**

For all statistical analyses, a p-value less than 0.05 was considered statistically significant. In addition to the statistical analyses mentioned above, the following were also used. The female to male ratio of each litter, the ratio of live pups to total pups in each litter for HS and NS groups, and litter size between HS and NS groups were
compared using the Student t test. Within male and female groups, HS and NS pup birth
weight and plasma corticosterone levels during basal and stressed conditions were
compared using 2-way ANOVA. All values are reported as the mean ± SEM.
Results:

Pup birth weight:

Offspring of NS and HS rats were weighed one day after birth. Though female pups weighed less than male pups (6.25 ± 0.07 vs. 6.5 ± 0.07 g, respectively, p = 0.002), there were no significant differences in the birth weights of NS and HS pups (6.32 ± 0.07 vs 6.47 ± 0.07 g, respectively, p = 0.11). The individual group birth weights were NS: males – 6.49 ± 0.09 g, n=54; females – 6.17 ± 0.10 g, n=45; HS: males – 6.62 ± 0.09 g, n=50; females – 6.32 ± 0.09 g, n=44. Therefore, the prenatal HS diet did not cause low birth weight.

Basal recordings:

Resting blood pressure was the same (figure 1) for NS and HS male offspring during both light (F(1,24) = 0.153, p = 0.70) and dark (F(1,24) = 0.314, p = 0.58) periods. Heart rate of male offspring was also the same for HS and NS groups during both light (F(1,24) = 0.001, p = 0.98) and dark (F(1,24) = 0.92, p = 0.35) periods. For females, resting blood pressure was the same (figure 1) for NS and HS offspring during both light (F(1, 28) = 0.063, p = 0.80) and dark (F(1, 28) = 0.001, p = 0.99) periods. Heart rate in females was the same for both light (F(1,28) = 0.002, p = 0.96) and dark (F(1,28) = 0.011, p = 0.92). A prenatal high salt diet did not program adult hypertension or changes in basal heart rate in offspring.

Acute restraint stress:

Baseline data collected at five-minute intervals before restraint stress revealed no differences in blood pressure values between HS and NS rats regardless of sex (males: NS -121.1 ± 3.7, HS – 120.0 ± 2.7 mm Hg; females: NS -108.2 ± 2.7, HS -107.1 ± 2.0
Two-way ANOVA showed that baseline MAP for males was significantly greater than for females (p<.001). There were also no differences in pre-stress HR (males: NS - 348 ± 8, HS - 342 ± 10; females: NS - 353 ± 6, HS - 340 ± 6).

Female HS offspring had a significantly larger change in blood pressure from baseline during one hour of restraint stress and one hour of recovery than did female NS offspring (MAP: diet, F(1,28) = 6.15, p = 0.019) across the entire 60 minutes of restraint and 60 minutes of recovery (figure 2). HS females also had a significant increase in heart rate during restraint and recovery compared to NS female offspring (HR: diet, F(1,28) = 12.95, p = 0.001). There was no significant interaction (diet x time) in any of the analyses. Hence, the HS female offspring had difficulty accommodating the restraint stress. Normal salt females were able to recover to baseline values sooner than HS females.

During one hour of restraint stress and one hour of recovery, NS and HS male offspring had similar changes in blood pressure and heart rate (figure 3) (HR: diet, F(1,24) = 0.0005, p = 0.982; MAP: diet, F(1,24) = 0.0003, p = 0.986).

**High salt challenge:**

There was no difference in the HR or MAP response to the high-salt challenge between NS and HS offspring (figure 4) in either males (HR: F(1,22) = 0.10, p = 0.76; MAP: F(1,22) = 0.04, p = 0.85) or females (HR: F(1,28) = 0.42, p = 0.52; MAP: F(1,28) = 0.76, p = 0.39). Both males and females exhibited a significant (p< 0.001) increase in MAP during the week of high-salt diet and returned to control levels by the seventh day after returning to the standard chow. Heart rate was significantly increased by the high-
salt challenge in the male group, and fell significantly below control during the recovery period in all groups (p<0.001).

**CRH In Situ Hybridization:**

In situ hybridization was performed on brains of resting NS and HS rats. The paraventricular nucleus (PVN) was the only area where a hybridization signal was found. Significantly higher expression of CRH was found in the PVN of adult female HS offspring as compared to NS female brain sections in resting rats (figure 5) (t(25) = 3.28, p = 0.003). Male NS and HS offspring showed no difference (t(16) = 0.0125, p = 0.99) in CRH expression in the PVN. In one group of male rats, the hybridization failed to produce any detectable signal.

**Corticosterone:**

Radioimmunoassay was performed on blood from resting NS and HS rats. Results are shown in Table 1. ANOVA found no difference in plasma corticosterone levels under basal conditions between NS and HS females or between NS and HS males, (though levels were higher in female animals than males).
**Discussion:**

**Blood Pressure in Adults:**

Administering an 8% NaCl prenatal diet is not sufficient to produce a low birth weight model in rats. There was no permanent adult hypertension seen in resting rats using this model. However, the female HS rats in the study were hyperresponsive to restraint stress in that an enhanced pressor and tachycardic response was seen in the HS females as compared to NS females during stress. The absence of permanent adult hypertension indicates that the critical period for programming high blood pressure may not be during pregnancy only. Multiple critical periods have been identified using a low-protein model, though not all low-protein diets result in permanent hypertension (43, 75). Contreras et al. have shown that feeding high salt during pregnancy, lactation, and for one week after weaning leads to adult hypertension.

Our model may be a useful tool to study fetal programming without the complications of low birth weight. Several researchers have shown that low birth weight pups do not necessarily have high blood pressure (9 and 20). The increase in blood pressure observed during stress may, if repeated, lead to a permanent insult to the cardiovascular health of the animal. This high salt model may be useful in the study of the effects of repetitive stresses in everyday human life.

**Salt Sensitivity:**

Sprague-Dawley rats are not genetically salt-sensitive, meaning that a high-salt diet does not confer lasting hypertension (7). However, some models of fetal programming cause salt sensitivity. Contreras et al. found increased MAP during 3% NaCl feeding compared to normal salt feeding as adults in rats fed prenatal high salt (13).
A low protein diet during pregnancy also caused salt sensitivity as adults (77), though not all low protein models lead to salt sensitivity (42). During one week of a high salt challenge, there were no significant differences in NS vs. HS groups of animals. Thus, our rats were not salt sensitive.

**Male and Female Differences:**

Fetal programming has been shown to cause differing reactions in male rats versus female rats. Adult female NaCl-resistant spontaneously hypertensive rats fed 8% NaCl diet during pregnancy and lactation produced female offspring who, when HS feeding continued for one week after weaning, exhibited an increase in MAP compared to females exposed to 1% NaCl diet (12). Male offspring of the mothers fed the HS diet did not develop an increase in MAP (12). Normally fed female offspring of lard-fed mothers developed hypertension as adults, while male offspring were not adversely affected (37). Prenatal stress caused a greater increase in systolic blood pressure after restraint stress and longer recovery time in female offspring than in male offspring (33). Additionally, prenatally stressed female offspring had an increase in corticosterone levels compared to controls (33). In the present study, female HS offspring had an enhanced tachycardic and pressor response compared to female NS offspring, though the same trend did not apply to male groups. There is evidence that the locus coeruleus (LC) is more activated in females than in males during hypotensive stress (14). In addition, CRH has been shown to activate the LC (73). As estrogen has been shown to increase CRH expression during stress (72), estrogen-mediated increases in CRH expression could be the cause of the enhanced cardiovascular response to stress in female HS rats.
Chapter 2: Microarray

Introduction:

Microarray technology is widely used to measure gene expression. The Affymetrix GeneChip technique uses hybridization to small, high-density arrays containing tens of thousands of synthetic oligonucleotides to measure genome-level gene expression (45). The arrays are redundant, allowing for multiple measurements of individual RNA sequences to ensure accurate expression levels (45). Though questions have arisen as to the precision of microarray probe sequences, the most recent GeneChip technology has been shown to be quite accurate (1 and 48).

Analysis of microarray data can provide valuable insight into the changes that occur due to experimental treatment. Comparison of gene expression between control and treatment groups may reveal candidate genes causing differences in the two groups. For instance, Kinnunen et al. show that prenatally stressed offspring have differential expression of 35 genes in the frontal lobe as compared to non-stressed rats (38). Therefore, our hypothesis was that one or more genes may be differentially expressed in offspring of mothers fed a high salt diet during pregnancy. Because the experimental treatment is given during pregnancy, one-day-old pup brains were examined for evidence of early changes in gene expression. Adult brains were studied with microarray to give a picture of gene expression at the developmental stage when acute stress was administered and the hyperreactivity was seen. Understanding genetic differences may lead to development of preventative measures to eliminate the negative long-term effects associated with prenatal programming of adult disease.
In this study we examined possible genetic contributions to the differences in blood pressure and heart rate seen in NS and HS females during acute stress. We have found that there are several differentially expressed genes in adult and in pup brains that could be influencing the stress response.
Materials and Methods:

RNA isolation

Adult females: One to two naïve female offspring (NS, n=4; HS, n=4) were selected from each litter for analysis. Animals were killed by decapitation at 12-13 weeks of age and brains were collected into liquid nitrogen.

    Pup females: One to two female pups (NS, n=3; HS, n=3) were selected from each litter. One-day-old pups were killed by decapitation and brains were collected into liquid nitrogen.

    Total brain RNA was collected by homogenization in TRIzol (Invitrogen) reagent. Chloroform was added to the homogenate and the mixture was shaken and centrifuged. The aqueous phase was transferred to a fresh tube where the RNA was precipitated in isopropyl alcohol, centrifuged, and washed in 75% ethanol. The resultant RNA was redissolved into water and concentration was measured using spectrophotometry.

Microarray Analysis

Microarray analysis was performed according to the standard Affymetrix protocol. A 20 µg sample of RNA from each brain was sent to the Utah State University’s Center for Integrated Biosystems for analysis. A biotin-labeled cRNA mix was transcribed and hybridized to a GeneChip Rat Genome 230 2.0 Array (Affymetrix Inc., Santa Clara, CA, USA). Each GeneChip was washed and stained using a Fluidics Station (Affymetrix Inc.) and scanned with a GeneArray Scanner (Affymetrix Inc.). The microarray image data obtained were returned to us, and data were analyzed using
GeneSifter (VizX Labs). For statistical analyses, a fold change of ± 1.5 and a p value < 0.05 was considered significant.
Results:

The GeneChip analysis on the brains from NS and HS adult females was carried out, and the data were processed statistically against the NS group using GeneSifter. According to the results, there were a total of 21 genes found to be differentially expressed in the adult female HS rats as compared to NS female controls using a ± 1.5 cutoff with a p ≤ 0.05. Eleven of the 21 genes that were differentially expressed were genes that had been upregulated, while 10 of the genes had been downregulated. Table 2 indicates individual genes and expressed sequence tags (ESTs) represented on the array that showed differences between NS and HS groups. In addition to the genes that met our initial criteria for significance, there were several potential candidate genes that were altered less than 1.5 fold. These included Angiotensin Type II receptor (AT2), downregulated 1.16 fold, and angiotensin converting enzyme (ACE), downregulated 1.23 fold (p < 0.05).

In analysis of female one-day-old pup brains, a total of 34 genes were found to be differentially expressed in HS females as compared to NS females. The number of genes which showed significant changes included 17 that were upregulated genes and 17 that were downregulated genes. Table 3 lists the individual genes and ESTs from the array that showed differences between NS and HS pup groups. Catechol-o-methyltransferase (COMT), an enzyme that inactivates catecholamines, was downregulated 1.44 fold in HS pup brains, though this change did not meet our criterion of a 1.5 fold change.
**Discussion:**

Prenatal programming is understood to imprint changes in brain function (63). This study addresses for the first time the differences caused by a prenatal 8% high salt diet at the level of global gene expression in the brain using microarray. We have identified several differentially regulated genes that are potential candidates to explain the differences observed in female rats during acute stress.

There is a large variety of mechanisms that can contribute to the onset of hypertension, and many genes that would, if altered, lead to such development. Interestingly, the data obtained from our microarray showed that most of these genes were not significantly altered. However, there were expression changes in a few genes commonly associated with hypertension that did not meet our threshold requirements of a 1.5 fold change. For instance, AT2 was downregulated 1.16 fold in the adult female HS brain (p < 0.05), and ACE was downregulated 1.23 fold in the adult female HS brain (p < 0.05). Expression changes in AT2 and ACE in the kidney have been associated with renal remodeling and blood pressure changes in rats exposed to prenatal low-protein diet (26 and 47). In pup brains, COMT was downregulated 1.44 fold in HS as compared to NS females (p < 0.05). Rats that developed in a hypoxic environment had decreased levels of COMT and altered adrenal function at one day of birth compared to control, which could be associated with the hyperresponsive nature of HS females (25). Further study may reveal whether these genes play a role in the hyperactive stress response seen in HS females.

The microarray analysis revealed that soluble epoxide hydrolase (Epxh2) was significantly downregulated in adult female brains. Epoxide hydrolase is an enzyme that
catalyzes the conversion of epoxides to their respective diols (3, 51). In the periphery, soluble epoxide hydrolase deactivates epoxyeicosatrienoic acids (EETs), decreasing vasodilation and increasing blood pressure (34). Male soluble epoxide hydrolase-null mice had a lower systolic blood pressure than did wild-type mice, though female null mice did not exhibit the same trend (68). Epoxide hydrolase was found in higher levels in the kidneys (66) and brains (65) of spontaneously hypertensive rats (SHR) than in Wistar Kyoto (WKY) rats. Surprisingly, however, blocking the brain activity of soluble epoxide hydrolase raised the blood pressure and increased the heart rate of SHR rats (65). Thus, it has been hypothesized that decreased levels of epoxide hydrolase in the brain actually worsen hypertension. As such, the decreased levels of Epoxh2 in the brains of adult female HS rats observed in the present study could contribute to the increased tachycardic and pressor responses to acute stress.

Several differentially expressed genes, including Tenascin R (Tnr) and Sry-box containing gene 11 (Sox11) in pups and Neuron navigator 1 (Nav1-predicted) in adults, affect neuronal development. Though the specific mechanism by which alteration of these genes may lead to hyperresponsiveness to stress is unknown, it is possible that critical regulatory brain circuitry is altered in HS brains.

Exact location of the genes that were upregulated or downregulated in the brain is difficult to determine as the whole brain was studied in this case. Future studies may reveal more detailed changes if specific sections of the brain are studied individually. Changes that occurred over a smaller distance may be exposed by study specific brain areas.
Chapter 3: Role of Autonomic Nervous System

Introduction:

Cardiovascular responses to stress are mediated by more than one regulatory system. For instance, the adrenal medulla and the sympathetic nervous systems have been shown to play a role in the cardiovascular response to stress (6). Activation of the sympathetic nervous system is involved in the increased HR and MAP during stress (21, 35). Blocking sympathetic outflow has been shown to inhibit normal pressor responses during acute stress (6, 29). There is evidence that central corticotropin releasing hormone (CRH) also regulates sympathoadrenal responses during stress (35). CRH is involved in the regulation of sympathetic activity during stress, increasing sympathetic outflow and anxious behavior during stressful situations (29). Rats fed a high salt diet had increased blood pressure that was associated with an increase in lumbar sympathetic nerve activity and total peripheral resistance (10). The paraventricular nucleus (PVN) and brainstem show increased catecholamine neuronal activation during restraint stress (16). In murine models, administration of CRH i.c.v. produced an increase in mean arterial pressure (MAP) and heart rate (HR), as well as altered baroreflex function (19, 23, 53). Interestingly, CRH-stained neurons in the PVN of patients with primary hypertension have been found to be much more numerous than in those with normal blood pressure (27). Thus, prenatally programmed cardiovascular and autonomic responses to stress may be caused by changes in CRH in the PVN.

In this study we determined the contribution of the autonomic nervous system to the differences seen between NS and HS rats. We also examined the differences in CRH and corticosterone in male and female offspring of mothers fed a high salt diet to
determine whether other factors were involved in the hyperresponsiveness, or lack thereof, seen in NS and HS animals. Our results show that there is increased expression of CRH under resting and stressed conditions in female HS rats as compared to NS females. Additionally, the results indicate that there is likely a non-sympathetically mediated effect that is contributing to the enhanced pressor response in addition to the sympathetically mediated mechanisms that are in place.
Materials and Methods:

Stress-induced CRH expression and corticosterone levels

Naïve (no telemetry implant) NS and HS adult offspring of both sexes (NS:males—n=4; HS:males—n=4; NS:females—n=9; HS:females—n=9) were subjected to 60 minutes of restraint stress as outlined above. At the end of the stress period, the rats were removed from the restraining tube and 5 minutes later were killed by decapitation. Blood was collected for determination of plasma corticosterone and brains were prepared for subsequent *in situ* hybridization for CRH.

Contribution of autonomic nervous system

Adult male and female NS and HS rats were fitted with radiotelemetry probes and allowed one week of recovery. Scheduled sampling was set to record a 30-second sample once every 5 minutes. Rats were injected with chlorisondamine (5 mg/kg, *ip*), a nicotinic acetylcholine receptor antagonist, to cause ganglionic blockade. After a fifteen-minute waiting period to allow the drug to take effect, rats were placed in plastic restraining tubes for 30 min. The restraint was limited to 30 min rather than 60 min to ensure that the effect of the drug did not wear off during the experiment. Blood pressure recording continued during the restraint and for 30 min of recovery after removal from the restraining tubes. Two days later, the same protocol was repeated using a mixture of chlorisondamine (5 mg/kg, *ip*) and propranolol (6.5 mg/kg, *ip*), a β₁, β₂ adrenergic receptor antagonist, to block ganglionic transmission and beta-adrenergic receptors. For each rat, pre-stress values for MAP and HR (after drug injection) were averaged (2 values) to give a single baseline number. The change in MAP and HR from the initial was then determined for each 5-min sample during the 30 minutes of stress and the 30
minutes of recovery. Two-way ANOVA with repeated measures (time) was used to compare the effects in NS and HS offspring across the 60 minutes. The effect of ganglionic blockade and beta-adrenergic receptor blockade on the pressor and tachycardic effect of restraint was tested using three-way ANOVA. Where appropriate post hoc analysis was performed using the Student-Newman-Keuls test.
**Results:**

**CRH:**

Brain sections were taken through the hypothalamus of rats subjected to one hour of restraint stress. For female offspring, there was a significant main effect of diet (F(1,41) = 10.62, p = 0.002); that is, female HS offspring had significantly higher relative CRH mRNA levels in the PVN than did NS females averaged over basal and stressed conditions (figure 5A & 5B). Since the CRH data were normalized to the NS group in both the basal and stressed conditions there was no significant main effect of stress on CRH mRNA expression. However, when absolute expression levels were compared, the CRH mRNA expression of stressed females was approximately twice that of the basal group (10140 ± 523 vs 5152 ± 723 µCi/g X pixel^2, p < 0.001). Male HS and NS CRH levels did not differ (F(1,28) = 0.15, p = 0.70) (figure 5A & 5C). Absolute expression of CRH mRNA was also significantly increased by stress in the male rats (stress—6300 ± 215 vs basal—3407 ± 345 µCi/g X pixel^2, p < 0.001).

**Corticosterone:**

Radioimmunoassay for corticosterone was performed on blood from NS and HS rats after one hour of restraint stress. Results are shown in Table 1. In both the male and female offspring there was a significant main effect of stress (males—F(1,14) = 79.0, p < 0.001; females—F(1,33) = 430.7, p < 0.001). However, there was no difference between NS and HS offspring in either sex (males—F(1,14) = 0.96, p = 0.34; females—F(1,33) = 0.083, p = 0.77).

**Autonomic nervous system:**
Female NS and HS rats were injected $ip$ with chlorisondamine. Baseline MAP in NS females fell from $109.0 \pm 2.6$ mmHg to $69.8 \pm 2.6$ mmHg, and HR dropped from $397 \pm 10$ bpm to $326 \pm 8$ bpm after the injection. HS female MAP fell from $109.1 \pm 1.4$ mmHg to $73.4 \pm 3.7$ mmHg, and HR dropped from $393 \pm 13$ bpm to $326 \pm 21$ bpm after the injection. During the first five minutes of restraint, MAP increased significantly (figure 6). By ten minutes after the beginning of restraint, MAP had returned to pre-stress levels and remained there throughout the restraint and recovery. HR of both NS and HS rats increased significantly during the first five minutes of restraint (figure 7). There was a secondary significant rise in HR when animals were removed from the restraining cages. The stress-induced increase in HR after chlorisondamine was smaller than in untreated animals but remained still the same. This suggested that the ganglionic blocker may not have prevented all adrenal release of epinephrine.

Adult male NS and HS offspring were given an injection of chlorisondamine $ip$ for ganglionic blockade. The chlorisondamine produced a significant decrease in baseline MAP (NS: from $112.9 \pm 2.8$ mmHg to $74.0 \pm 2.5$ mmHg, HS: from $111.9 \pm 1.7$ mmHg to $64.5 \pm 3.5$ mmHg) and HR (NS: from $350 \pm 10$ bpm to $314 \pm 7$ bpm, HS: from $367 \pm 11$ bpm to $322 \pm 6$ bpm). MAP increased significantly during the first five minutes of restraint in both NS and HS (figure 8). By 10 min after the onset of acute stress, MAP levels had returned to pre-stress values and were constant during the remainder of stress and recovery. Heart rate in NS and HS males was significantly increased during the first five minutes of restraint and declined to pre-stress levels by 30 min (figure 9). A secondary increase in HR was observed when the animals were removed from restraint. There was no difference in the response of NS males as compared to HS males.
Combined blockade with chlorisondamine and propranolol was administered to females via *ip* injection before restraint. During combined blockade, there was a significant decrease in MAP (NS: 104.8 ± 4.3 mmHg, HS: 98.9 ± 1.0 mmHg) and HR (NS: 359 ± 10 bpm, HS: 344 ± 6 bpm) from baseline to decreased levels (MAP—NS: 77.1 ± 5.1 mmHg, HS: 72.1 ± 2.2 mmHg; HR—NS: 302 ± 5 bpm, HS: 298 ± 3 bpm) (figures 10 and 11). Restraint did not cause an increase in MAP or HR during combined blockade. There were no significant differences between HR reactions of NS and HS females. However, there was a significant effect of HS diet on MAP response during restraint (*p* < 0.001).

A combination of chlorisondamine and propranolol was injected *ip* and male rats were placed in restraining cages for 30 min. MAP and HR fell from baseline (MAP—NS: 110.4 ± 2.6 mmHg, HS: 117.6 ± 1.7 mmHg; HR—NS: 342 ± 12 bpm, HS: 385 ± 11 bpm) to decreased levels (MAP—NS: 77.2 ± 4.4 mmHg, HS: 79.6 ± 3.8 mmHg; HR—NS: 298 ± 10 bpm, HS: 309 ± 8 bpm) (figures 12 and 13). With combined blockade, restraint produced no increase in MAP or HR in either group. There was no difference between NS and HS male groups.
Discussion:

There was significantly higher expression of CRH mRNA in the PVN of adult HS female, but not male, animals during both resting and stressed conditions. There is evidence that CRH acts centrally to increase sympathetic nervous activity and blood pressure during stress (23, 35). CRH may be affecting central pathways that mediate baroreceptor-induced activation of cardiac vagal neurons (23), or may be acting through the locus coeruleus which results in norepinephrine release (73). The presence of both increased CRH mRNA expression and stress hyperresponsiveness in females, along with the absence of these effects in males, suggests the possibility of a cause-and-effect relationship that needs further investigation. The absence of increased resting blood pressure despite the increased basal expression of CRH mRNA was likely due to baroreflex buffering. The enhanced pressor and tachycardic response with stress likely occurred as the baroreflex reset to higher pressures (46). An augmented blood pressure and heart rate response to acute stress, if repeated each time a stressor is encountered, has the potential to gradually lead to permanent hypertension and/or heart disease due to structural adaptations of the heart and blood vessels in response to the added load (24).

Ganglionic blockade using chlorisondamine did not completely abolish the tachycardic effects of acute stress. It is possible that the adrenal medulla was still receiving stimulation to release epinephrine through neurotransmitters other than acetylcholine (55). Thus, we added a combination of propranolol and chlorisondamine to the rats in an attempt to fully block autonomic response to restraint stress. Upon administration of the propranolol and chlorisondamine mixture, there was no longer an increase in heart rate during stress. The heart rate hyperresponsiveness in HS females was
clearly eliminated with combined blockade, indicating that the greater response during restraint in untreated rats was likely due to autonomic overactivation. The difference in MAP, but not HR (HR was totally blocked) suggests blood vessel involvement since cardiac output was the same in both groups after combined blockade.

Though the pressor response to restraint was eliminated by combined blockade, the difference between HS and NS females was not clearly eliminated. This raises the possibility that some non-sympathetic effect is contributing. For example, arginine vasopressin (AVP) is a peptide that is not affected by peripheral ganglionic blockade. AVP has been associated with an increase in MAP after acute restraint (36, 70). Additionally, injecting AVP increased systolic blood pressure without raising heart rate (78). Pretreatment with an AVP antagonist attenuated the increase in MAP seen during exposure to cigarette smoke (30). Furthermore, maternal treatment during pregnancy in ewes led to programmed alteration in AVP secretion in adult offspring (17 and 18). Taken together, these findings indicate that AVP is a potential mediator of the enhanced stress-induced pressor response seen in HS females even after autonomic blockade. This notion can easily be tested in future studies using a combination of pharmacological blockade of V1 receptors and autonomic receptors. In addition, there may be other potential peptides that could be contributing to the pressor response, such as adrenocorticotropic hormone or neuropeptide Y.
Summary

In summary, this study provides evidence that a high salt diet during pregnancy leads to enhanced cardiovascular responsiveness to stress in female offspring. Limiting exposure of the high salt diet to pregnancy only is not sufficient to cause lasting hypertension or salt sensitivity in offspring. There is, however, an adult tachycardic hyperresponsiveness to stress in female animals that can be eliminated by administration of ganglionic blockade along with beta-receptor antagonist. The enhanced pressor response during acute stress in females may be regulated by mechanisms in addition to the autonomic nervous system. Female HS offspring also have increased expression of CRH in the PVN during basal and stressed conditions. Several genes, including soluble epoxide hydrolase, are differentially expressed in the female animals that exhibit hyperresponsiveness to stress. These genes may contribute to the lasting changes seen in adult females. Over time, repeated stress may lead to permanent cardiovascular dysfunction in animals that have a hyperactive response. A more complete understanding of how a prenatal high salt diet affects mechanisms that cause cardiovascular insult may aid in the prevention of adult disease.
References:


15. **Davies AA, Smith GD, May MT, and Ben-Shlomo Y.** Association between birth weight and blood pressure is robust, amplifies with age, and may be underestimated. *Hypertension* 48:431-436, 2006.


42. **Langley-Evans SC and Jackson AA.** Rats with hypertension induced by in utero exposure to maternal low-protein diets fail to increase blood pressure in response to a high salt intake. *Ann Nutr Metab* 40:1-9, 1996.


45. **Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, and Brown EL.** Expression


50. **Myers JB, Smidt VJ, Doig S, Di Nicolantonio R, and Morgan TO.** Blood pressure, salt taste and sodium excretion in rats exposed prenatally to high salt


Figure Legends:

Figure 1: Basal recordings of mean arterial pressure (MAP) and heart rate (HR).

MAP and HR recordings using radiotelemetry during day (D) and night (N) periods in male and female offspring of mothers fed a high salt diet during pregnancy.

Figure 2: Female acute stress recordings. The change in HR (top panel) and MAP (bottom panel) from baseline during one hour of restraint stress and one hour of recovery in female offspring of mothers fed a high salt diet during pregnancy. The p values are for the diet factor (NS vs HS) in the two-way ANOVA for repeated measures.

Figure 3: Male acute stress recordings. The change in HR (top panel) and MAP (bottom panel) from baseline during one hour of restraint stress and one hour of recovery in male offspring of mothers fed a high salt diet during pregnancy. The p values are for the diet factor (NS vs HS) in the two-way ANOVA for repeated measures.

Figure 4: High salt challenge. Effect of a high-salt challenge (HS) on HR and MAP in adult male and female offspring of mothers eating high salt or normal salt during pregnancy. The initial values represent 24 hours of HR and MAP while eating standard (ST) rat chow. The HS values represent the 7-day average HR and MAP while eating 8% NaCl. The final values represent 24 hours of HR and MAP on the 7th day after returning (R) to the standard rat chow. *p<0.001 compared to ST
and HS, *p<0.001 compared to ST and R. The numbers in parentheses depict the group sizes.

Figure 5: CRH expression. Effect of maternal diets on relative CRH mRNA basal expression in PVN of adult female and male offspring. The inset on top depicts representative images scanned from the autoradiographic film. *p=0.003 compared to NS offspring.

Figure 6: Effect of chlorisondamine on MAP in females. The change in MAP from baseline with chlorisondamine during 30 min of restraint stress and 30 min of recovery. In figures 6 through 13, gray lines represent data previously collected during acute stress with no ganglionic blockade. The dotted line reflects the difference of the duration of restraint during treated and untreated animals (30 min vs. 60 min, as explained in the text).

Figure 7: Effect of chlorisondamine on HR in females. The change in HR baseline with chlorisondamine during 30 min of restraint stress and 30 min of recovery. See explanation in figure legend 6 for information about gray lines and dotted lines.

Figure 8: Effect of chlorisondamine on MAP in males. The change in MAP from baseline with chlorisondamine during 30 min of restraint stress and 30 min of recovery. See explanation in figure legend 6 for information about gray lines and dotted lines.
Figure 9: Effect of chlorisondamine on HR in males. The change in HR baseline with chlorisondamine during 30 min of restraint stress and 30 min of recovery. See explanation in figure legend 6 for information about gray lines and dotted lines.

Figure 10: Effect of chlorisondamine and propranolol on MAP in females. The change in MAP from baseline with chlorisondamine and propranolol during 30 min of restraint stress and 30 min of recovery. Three-way ANOVA showed a significant difference between NS and HS adult offspring (F(1, 533) = 51.0, p < 0.001). See explanation in figure legend 6 for information about gray lines and dotted lines.

Figure 11: Effect of chlorisondamine and propranolol on HR in females. The change in HR from baseline with chlorisondamine and propranolol during 30 min of restraint stress and 30 min of recovery. See explanation in figure legend 6 for information about gray lines and dotted lines.

Figure 12: Effect of chlorisondamine and propranolol on MAP in males. The change in MAP from baseline with chlorisondamine and propranolol during 30 min of restraint stress and 30 min of recovery. Three-way ANOVA showed no difference between NS and HS adult offspring. See explanation in figure legend 6 for information about gray lines and dotted lines.
Figure 13: Effect of chlorisondamine and propranolol on HR in males. The change in HR from baseline with chlorisondamine and propranolol during 30 min of restraint stress and 30 min of recovery. Three-way ANOVA showed no difference between NS and HS adult offspring. See explanation in figure legend 6 for information about gray lines and dotted lines.
**Table Legends:**

Table 1: Plasma corticosterone levels (ng/ml) in NS and HS male and female offspring under basal and stressed conditions.

Table 2: Gene expression in adult offspring. Differential gene expression in the brains of adult HS female offspring as compared to NS female offspring. EST is an expressed sequence tag, meaning little information is available for the gene beyond the tag.

Table 3: Gene expression in pups. Differential gene expression in the brains of one-day-old female HS offspring as compared to NS female offspring.
Figures:

Figure 1

Males

![Graph showing HR and MAP for males with Normal Salt and High Salt conditions.]

Females

![Graph showing HR and MAP for females with Normal Salt and High Salt conditions.]

Figure 1
Figure 2

Delta HR (bpm)

- HS Offspring, n=16
- NS Offspring, n=14

P = 0.001

Time (min)

Delta MAP (mm Hg)

P = 0.019

Restrain

Figure 2
Figure 3
Figure 4

MAP (mm Hg)                HR (bpm)
100
110
120
130
325
350
375
400
425

Normal Salt Offspring
High Salt Offspring

Diet: ST HS R ST HS R
(11)  (13)  (16)  (14)
*  +  *

Males        Females
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9
Figure 10
Figure 12
Figure 13
Table 1. Plasma corticosterone levels (ng/ml) in NS and HS male and female offspring under basal and stressed conditions.

<table>
<thead>
<tr>
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<th>Basal</th>
<th>Stressed</th>
<th>Basal</th>
<th>Stressed</th>
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</thead>
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<td>Male</td>
<td>44.1 ± 18.7 (5)</td>
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<td>127.0 ± 29.3 (9)</td>
<td>692.7 ± 30.0 (9)</td>
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<td>Female</td>
<td>49.8 ± 10.5 (5)</td>
<td>317.8 ± 57.6 (4)</td>
<td>140.5 ± 21.0 (10)</td>
<td>695.2 ± 27.8 (9)</td>
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</table>

NS = normal salt offspring, HS = high salt offspring. The n of each group is indicated in the parentheses. For both male and female groups there was a significant main effect of stress (p < 0.001 for both), but no significant main effect of diet.
**Table 2. Genes that increase or decrease expression in response to treatment with prenatal HS diet in adults**

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<th>GenBank Accession No.</th>
<th>Probe Set ID</th>
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Table 3. Genes that increase or decrease expression in response to treatment with prenatal HS diet in pups

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<td>Tenascin R (Tnr)</td>
<td>0.007</td>
<td>1.78</td>
<td>Cell adhesion; axonal guidance</td>
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<tr>
<td>NM_019341</td>
<td>1369957_at</td>
<td>Regulator of G-protein signaling 5 (Rgs5)</td>
<td>0.043</td>
<td>1.74</td>
<td>Signal transduction</td>
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<td>BE104595</td>
<td>1381208_at</td>
<td>Histone 2A (H2a)</td>
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<td>Nucleosome assembly, DNA binding</td>
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<td>AA850895</td>
<td>1371765_at</td>
<td>Histone 2A (H2a)</td>
<td>0.025</td>
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<td>Nucleosome assembly, DNA binding</td>
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<td>AA891661</td>
<td>1369625_at</td>
<td>Aquaporin 1 (Aqp1)</td>
<td>0.001</td>
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<td>Water transport</td>
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<tr>
<td>NM_053349</td>
<td>1387275_at</td>
<td>SRY-box containing gene 11 (Sox 11)</td>
<td>0.035</td>
<td>1.55</td>
<td>Transcription factor; nervous system development</td>
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<td>BG377412</td>
<td>1384519_at</td>
<td>SEC63-like (S. cerevisiae) (Sec63-predicted)</td>
<td>0.040</td>
<td>1.54</td>
<td>Protein folding</td>
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<tr>
<td>BF396210</td>
<td>1378592_at</td>
<td>Tripartite motif-containing 59 (Trim59-predicted)</td>
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<tr>
<td>AA799328</td>
<td>1379170_at</td>
<td>Similar to expressed sequence AW413625</td>
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<td>(RGD1560913-predicted)</td>
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<td>BG380482</td>
<td>1376774_at</td>
<td>Exocyst complex component 4 (Exoc4)</td>
<td>0.035</td>
<td>1.52</td>
<td>Protein targeting to membrane</td>
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<tr>
<td>BF396209</td>
<td>1378492_at</td>
<td>FERM, RhoGEF (Arhgef) and pleckstrin domain protein 1 (chondrocyte-derived) (Farp1-predicted)</td>
<td>0.017</td>
<td>1.51</td>
<td>Rho guanyl-nucleotide exchange factor activity</td>
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<tr>
<td>BM388525</td>
<td>1374779_at</td>
<td>Coagulation factor XIIIa (F13a1)</td>
<td>0.017</td>
<td>1.5</td>
<td>Blood coagulation</td>
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<tr>
<td>BF261153</td>
<td>1388340_at</td>
<td>Protocadherin beta 21; Aldolase A; Similar to hypothetical protein FLJ90652</td>
<td>0.019</td>
<td>1.5</td>
<td>Cell adhesion</td>
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</table>
### Genes that Decrease Expression

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Accession</th>
<th>Description</th>
<th>Fold Change</th>
<th>P-Value</th>
<th>Function</th>
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<tbody>
<tr>
<td>BF404113</td>
<td>1396884_at</td>
<td>ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1</td>
<td>0.043</td>
<td>-4.15</td>
<td>Aminophospholipid transport</td>
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<tr>
<td>BF403099</td>
<td>1396392_at</td>
<td>Dynactin 6 (Dctn6-predicted)</td>
<td>0.010</td>
<td>-2.15</td>
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<tr>
<td>BM389467</td>
<td>1376279_at</td>
<td>Processing of precursor 1, ribonuclease P/MRP family, (S. cerevisiae)</td>
<td>0.007</td>
<td>-1.82</td>
<td>RNA processing</td>
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<tr>
<td>NM_031010</td>
<td>1387796_at</td>
<td>Arachidonate 12-lipoxygenase (Alox15)</td>
<td>0.008</td>
<td>-1.79</td>
<td>Carbohydrate metabolism</td>
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<tr>
<td>AW527942</td>
<td>1396395_at</td>
<td>EST</td>
<td>0.011</td>
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<td>AW530163</td>
<td>1394913_at</td>
<td>Similar to OL-protocadherin isoform</td>
<td>0.037</td>
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<tr>
<td>BE105500</td>
<td>1382020_at</td>
<td>Vesicle transport through interaction with t-SNAREs homolog 1A (yeast) (Vti1a)</td>
<td>0.031</td>
<td>-1.66</td>
<td>Membrane fusion</td>
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<tr>
<td>AW527798</td>
<td>1381940_at</td>
<td>Hypothetical protein LOC619558 (LOC619558)</td>
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<td>AI70544</td>
<td>1390262_a_at</td>
<td>DnaJ (Hsp40) homolog, subfamily C, member 9 (DnaJC9-predicted)</td>
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<td>1398727_at</td>
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<td>BE113272</td>
<td>1374908_at</td>
<td>Similar to hypothetical protein B230399E16</td>
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<tr>
<td>AI059075</td>
<td>1393523_at</td>
<td>Coilin (Coll)</td>
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<td>-1.51</td>
<td>Protein binding; metal ion binding</td>
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<tr>
<td>AI501282</td>
<td>1378124_at</td>
<td>Protein phosphatase 1B, magnesium dependent, beta isoform (Ppm1b)</td>
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<td>-1.51</td>
<td>Magnesium ion binding; dephosphorylation</td>
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</tbody>
</table>
Summer Hayes King

1. Education

<table>
<thead>
<tr>
<th>Degree</th>
<th>Institution</th>
<th>Year</th>
<th>Major</th>
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<tr>
<td>MS</td>
<td>Brigham Young University</td>
<td>2007</td>
<td>Physiology and Developmental Biology</td>
</tr>
<tr>
<td>BS</td>
<td>Brigham Young University</td>
<td>2005</td>
<td>Physiology and Developmental Biology</td>
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</table>

2. Honors and Awards

- University Scholarship Recipient: 2002-2007
- Graduation *Cum Laude*: 2005
- Dean’s List: Winter 2005
- Dean’s List: Spring 2003

3. Work Experience

- **Apr. 2005-Present**: Research Assistant
  Brigham Young University, Provo, UT
  Mentored undergraduate students. Performed various lab techniques, including PCR; RNA isolation; in situ hybridization; tissue sectioning on a cryostat; fixing, washing, and staining slides; growth and isolation of plasmids; spectrophotometry; and gel electrophoresis. Created lab protocol book. Presented research papers in lab meetings. Writer for scientific papers.

- **Jan. 2007-Apr. 2007**: Teaching Assistant, PDBio 305 (Physiology)
  Brigham Young University, Provo, UT
  Held weekly office hours and review sessions. Assisted in writing a weekly quiz. Taught two review sessions to 90-student class. Graded weekly quizzes.

- **Nov. 2004-Dec. 2005**: Research Assistant
  Brigham Young University, Provo, UT
  Located relevant scientific articles and summarized. Writer for scientific paper on effective methods for teaching science. Performed various lab techniques including fluorescence spectroscopy to study membrane mechanics.

- **Aug. 2004-Apr. 2005**: Writing Fellow
  Brigham Young University, Provo, UT
  Classes tutored: Religion C 341 and Honors Physical Science 100. Edited student papers. Wrote letters in response to show students how to improve writing. Held two conferences each with 15 students from each course. Developed personal writing skills. Completed three-credit course to improve writing and tutoring skills.
Aug.-Dec. 2004 Teaching Assistant, Biology 120 (Biology) Brigham Young University, Provo, UT
Taught three lectures to class of 220 students. Held weekly office hours and help sessions for one-on-one tutoring. Created and presented reviews before exams. Contributed to course outline, lecture plans, and exams. Answered student questions during lecture. Attended weekly meetings with the professor. Proctored and graded exams. Graded various homework assignments.

4. Peer-Reviewed Publications


5. Meetings Attended

2006 Experimental Biology Author on abstract and presented poster

2006 Neuroscience Meeting Author on abstract and presented poster

2007 Experimental Biology Author on abstract and presented poster