1 **The impact of *in utero* heat stress and nutrient restriction on progeny body**
2 **composition**

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13 **Running head:** Intrauterine stress and future body composition

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Abstract

We recently demonstrated that *in utero* heat stress (IUHS) alters future tissue accretion in pigs, but whether this is a conserved response among species, is due to the direct effects of heat stress (HS) or mediated by reduced maternal feed intake (FI) is not clear. Study objectives were to compare the quantity and rate of tissue accretion in rats exposed to differing *in utero* thermal environments while eliminating the confounding effect of dissimilar maternal FI. On d3 of gestation, pregnant Sprague Dawley rats (189.0 ± 5.9 g BW) were exposed to thermoneutral (TN; $22.2 \pm 0.1^\circ\text{C}$; $n=8$), or HS conditions (cyclical 30 to 34°C ; $n=8$) until d18 of gestation. A third group was pair-fed to HS dams in TN conditions (PFTN; $22.2 \pm 0.1^\circ\text{C}$; $n=8$) from d4 to d19 of gestation. HS increased dam rectal temperature ($p=0.01$; 1.3°C) compared to TN and PFTN mothers, and reduced FI ($p=0.01$; 33%) compared to TN *ad libitum* fed controls. Although litter size was similar ($p=0.97$; 10.9 pups/litter), pup birth weight was reduced ($p = 0.03$; 15.4%) in HS compared to PFTN and TN dams. Two male pups per dam [$n=8$ *in utero* TN (IUTN); $n=8$ IUHS; $n=8$ *in utero* PFTN (IUPFTN)] were selected from four dams per treatment based on similar gestation length, and body composition was determined using dual-energy x-ray absorptiometry (DXA) on d26, d46, and d66 of postnatal life. Whole-body fat content increased ($p=0.01$; 11.2%), and whole-body lean tissue decreased ($p=0.01$; 2.6%) in IUPFTN versus IUTN and IUHS offspring. Whole-body composition was similar between IUHS and IUTN offspring. Epididymal fat pad weight increased ($p=0.03$; 21.6%) in IUPFTN versus IUHS offspring. In summary and in contrast to pigs, IUHS did not impact rodent body composition during this stage of growth; however, IUPFTN altered the future hierarchy of tissue accretion.

1. Introduction

Suboptimal productivity due to heat stress (HS) jeopardizes efficient animal production and threatens global food security (Baumgard et al., 2012; Johnson et al., 2015a). This is primarily explained by decreased and inconsistent growth, poor reproductive performance, altered carcass composition, and increased morbidity and mortality (Brown-Brandl et al., 2004, Baumgard and Rhoads, 2013; Johnson et al., 2015a). The negative effects of HS will likely become more evident as climate models predict an increase in extreme summer conditions for most agricultural areas (Luber and McGeehin, 2008). While HS negatively impacts animals during postnatal life, exposure to HS during fetal development may also have lifelong consequences that undermine nutritional, management, and genetic advances made by the animal agriculture industries. Understanding the biology and mechanisms by which HS compromises animal performance both pre- and postnatally is a prerequisite to developing future climate change mitigation strategies.

Multiple insults experienced prenatally can have lasting effects on offspring performance (Desai et al., 2005; Roseboom et al., 2006; Limesand et al., 2007). One extensively researched experimental model is maternal undernutrition that increases offspring lipid accretion (Ravelli et al., 1976; Barker et al., 1993; Desai et al., 2005; Roseboom et al., 2006). In addition, we recently reported (Boddicker et al., 2014; Johnson et al., 2015b) that *in utero* HS increases future adipose deposition at the expense of skeletal muscle mass in pigs. Similarities between the *in utero* undernutrition and HS models, coupled with the fact that HS causes a well-documented reduction in feed intake (FI) in almost all species (Baumgard et al., 2012; Baumgard and Rhoads, 2013; Johnson

et al., 2015a), lends itself to the hypothesis that the two *in utero* insults may be mechanistically linked. Our study objectives were to investigate the direct and indirect effects (mediated by reduced FI) of *in utero* HS on the postnatal accretion of adipose and lean tissue in offspring derived from dams exposed to HS, feed restricted (to eliminate the confounding effect of dissimilar FI), or fed *ad libitum* in TN conditions throughout most of gestation. We hypothesized that postnatal adipose and lean tissue accretion would be similarly altered by both maternal feed restriction and *in utero* HS compared to *in utero* thermoneutral controls.

2. Materials and methods

2.1. Maternal environment

All animal work was approved by the Iowa State University Animal Care and Use Committee. A total of 24, first parity timed pregnant OB Sprague Dawley rats (189.0 ± 5.9 g BW) were obtained from Charles River Laboratories (Wilmington, MA) on d2 of gestation. Dams were fed a finely ground standard commercial chow diet (Harlan 2018; Harlan; Woodland, CA; 18% CP) and housed in individual cages (0.2 m x 0.4 m) in the Zumwalt Environmental Chambers at Iowa State University. Pregnant rats were exposed to thermoneutral (TN; constant $22.2 \pm 0.1^\circ\text{C}$; $34.6 \pm 0.1\%$ relative humidity (RH); $n = 8$), HS (cyclical 30.0°C daytime and 34.0°C nighttime; $20.1 \pm 0.1\%$ RH; $n = 8$), or pair-fed as a percent of BW to HS mothers in TN conditions (PFTN; constant $22.2 \pm 0.1^\circ\text{C}$; $34.6 \pm 0.1\%$ RH; $n = 8$) to eliminate the confounding effects of dissimilar FI. Thermoneutral temperature was selected based on recommendations that rats be maintained at 20 to 24°C (Hau and Shapiro, 2011), and pregnant dams were provided nesting material

(NestletsTM Nesting Material; Ancare; Radnor, PA) to reduce the potential for cold stress (Gaskill et al., 2013). Because rats are nocturnal species (Southern et al., 1946), peak HS was during the nighttime (dark from 0800-2000 h) when dams were active and baseline HS was during the daytime (light from 2000-0800 h) when dams were sleeping in order to match their circadian rhythm with the temperature cycles used in previous experiments with diurnal pregnant sows (Johnson et al., 2015b,c). Maternal environmental treatments began and ended on d3 and d18 of gestation, respectively, for TN and HS dams and on d4 and d19 of gestation, respectively, for PFTN mothers (Fig. 1), since by experimental design PFTN mothers were limit-fed to HS dams. To minimize the risk of post-parturition maternal cannibalism of offspring, and to ensure all progeny were exposed to similar lengths of *in utero* stress, all measurements ended and all dams were fed *ad libitum* and exposed to TN conditions (constant $22.2 \pm 0.1^{\circ}\text{C}$; $34.6 \pm 0.1\%\text{RH}$) from d19 (TN, HS) or d20 (PFTN) of gestation until parturition.

Rectal temperatures (T_{re}) were obtained on all dams at 1600 h (peak heat) on alternate days of the *in utero* treatment period using a calibrated and lubricated digital thermometer (Safety 1st; Model #TH050) inserted approximately 1 cm into the rectum of restrained dams. Tail skin temperature (T_{skin}) was measured twice daily (0800, 1600 h) using a calibrated infrared thermometer (Model 42505, Extech Instruments, Waltham, MA). Body weight, FI, and water intake (WI) were determined daily (0800 h) for all dams. At parturition, pup number and average individual pup birth weight (total weight of litter/pup number) were recorded for each dam. To eliminate the confounding effects of dissimilar gestation length and litter size on offspring phenotypes, pups were selected from only 12 dams ($n = 4/\text{treatment}$) with similar gestation lengths (22 d) and litter sizes

(10.9 ± 0.9 pups) at parturition. All analyses of maternal data were performed using only the 12 selected dams.

2.2. Postnatal environment

The Iowa State University Animal Care and Use Committee approved all procedures involving rat offspring. Between parturition and weaning (d 23 postnatal life), all pups were exposed to TN conditions (20 to 24°C) as recommended for neonatal rats (Hau and Schapiro, 2011). Female offspring were removed from the dams and euthanized on d4 of postnatal life to collect tissue for a separate experiment. The remaining male pups were balanced (n = 5/dam) to prevent postnatal milk consumption differences and excess male offspring were euthanized. At weaning, two male offspring per pregnant dam were randomly selected from *in utero* TN (IUTN; n = 8), *in utero* HS (IUHS; n = 8), and *in utero* PFTN (IUPFTN; n = 8) mothers and housed in individual cages (0.2 m x 0.4 m) in TN (21.8 ± 0.1°C; 28.3 ± 0.2% RH) conditions in the Kildee Hall small animal holding rooms at Iowa State University. By experimental design, gestation length was similar (22 d) in all selected offspring to eliminate the confounding effects of dissimilar gestation length on the direct effects of *in utero* environment. Feed was provided *ad libitum* at weaning in jars as a finely ground standard commercial chow diet (Harlan 2018; Harlan; Woodland, CA; 18% CP). Body weight was recorded weekly and FI was measured every third day to determine average daily FI and BW gain. Average daily FI was similar ($p = 0.16$; 4.2 g) between *in utero* treatments on the first 3 d (d 23 – 26 postnatal life) after weaning. An initial body scan was performed on anesthetized offspring (100 mg/mL ketamine and 10 mg/mL xylazine; administered at a dose of 0.2 mL/100 g BW IP) using a Hologic Discovery A dual-energy X-ray

absorptiometer (DXA; Hologic, Inc.; Bedford, MA) in small animal mode with V8.26a:3 software. Offspring were scanned after an overnight fast on d26 (68.4 ± 0.7 g BW), d46 (190.5 ± 4.6 g BW), and d66 of postnatal life (302.9 ± 7.5 g BW) to determine percent fat, lean and bone mineral content (BMC) in individual animals. Based on tissue content, total tissue quantity and accretion rates were calculated as we have previously described (Johnson et al., 2015b,c) with the following formula: (Final content, g of tissue – initial content, g of tissue) / days between DXA scans.

2.3. Tissue collection

Immediately following the final DXA scan (d66 of postnatal life), offspring were euthanized by anesthesia injection (100 mg/mL ketamine and 10 mg/mL xylazine; administered at a dose of 0.2 mL/100 g BW IP) followed by cervical vertebrae dislocation. The epididymal fat pad, head, heart, liver, spleen, testis, gastrocnemius, and soleus muscles were removed and weighed, and the right leg tibia length was measured. Subcutaneous flank fat and visceral fat were collected, fixed in OCT compound (Tissue-Tek[®] O.C.T. Compound; Sakura Finetek USA, Inc.; Torrance, CA), and stored at -80°C for histology.

2.4. Histology

Fixed samples of subcutaneous flank fat and visceral fat were sent to the Iowa State University Veterinary Diagnostic Laboratory for sectioning (10 μ m) and hematoxylin and eosin staining. Adipocytes from each sample (1 slide/rat; 3 images/slide) were imaged using Q-capture Pro 6.0 software (Qimaging[®], Surrey, BC, Canada), and adipocyte size (μ m²) was determined using ImageJ 1.47v software (National

Institutes of Health; Bethesda, MD, USA). All adipocytes in each image were measured and average adipocyte size for each image was used for the final analysis.

2.5. Statistics

All data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). For analysis of all maternal environment data, statistical model components included maternal environment (TN, HS, PFTN), day of gestation (d2 – d19), and their interactions. Maternal environment variables included, BW, FI, T_{re} , T_{skin} , WI, BW gain, feed efficiency, pup number, and average pup birth weight. The statistical model for maternal environmental data analysis was: $Y_{ijk} = \mu + ME_i + D_j + ME*D_{ij} + e_{ijk}$. Where, Y = parameter of interest, μ = mean, ME = maternal environment, D = day of gestation, and e = residual. Statistical model components for *in utero* environment data included *in utero* environment (IUTN, IUHS, IUPFTN), day of postnatal age (d26, d46, d66), and their interactions. *In utero* environment variables included, BW, FI, BW gain, feed efficiency organ weights, tissue content, tissue accretion and adipocyte size. The statistical model for *in utero* environment data analysis was: $Y_{ijk} = \mu + IE_i + D_j + IE*D_{ij} + e_{ijk}$. Where, Y = parameter of interest, μ = mean, IE = *in utero* environment, D = day of postnatal age, and e = residual. Dam and offspring(dam) were used as random effects for all analyses of *in utero* environment variables. For repeated analyses, each rat's respective parameter was analyzed using repeated measures with an auto-regressive covariance structure using day as the repeated /effect. Data are presented as LSmeans, statistical significance was defined as $p \leq 0.05$, and a tendency was defined as $0.05 < p \leq 0.10$.

3. Results

3.1. Maternal environment

3.1.1. Thermal indices

Over the entire maternal treatment period, T_{re} was increased ($p = 0.01$; 1.3°C) in dams exposed to HS compared to TN and PFTN conditions (Table 1). A considerable overall increase ($p = 0.01$; 36%) in T_{skin} was observed in HS-exposed compared to TN and PFTN dams (Table 1).

3.1.2. Growth variables

Overall, FI was reduced ($p = 0.01$; 32.9%) in HS compared to TN controls (Table 1; Fig. 2), and by design the magnitude of reduction and temporal FI pattern was similar (13.9 g/d) between HS and PFTN dams (Table 1; Fig. 2). Although initial maternal BW was similar ($p = 0.68$; 189.0 g; Table 1) between maternal environmental treatments, final maternal BW was reduced in HS and PFTN dams ($p = 0.01$; 15.5 and 38.7 g, respectively) compared to TN controls, and PFTN dams had decreased final BW ($p = 0.01$; 23.2 g) compared to HS dams (Table 1). A maternal environment by day of gestation interaction was detected ($p = 0.01$) as HS dams had reduced BW (22.6 g) from d10 to d19 of gestation compared to TN controls (Fig. 3), and PFTN dams had decreased BW compared to TN and HS-exposed dams (41.4 and 18.8 g, respectively; Fig. 3). Overall, BW gain per gram of FI was increased ($p = 0.05$; 15.4%) in HS dams compared to PFTN mothers (Table 1); however, no differences were detected when comparing HS and PFTN dams to TN controls (Table 1). Throughout the entire maternal environmental treatment period, WI was increased ($p = 0.02$; 20.5%) in HS dams compared to PFTN and TN-exposed mothers (Table 1). Although litter size was similar amongst treatments

($p = 0.97$; 10.9 pups/litter), average individual pup birth weight was reduced ($p = 0.05$; 15.4%) in HS-exposed dams compared to PFTN and TN controls (Table 1).

3.2. Offspring variables

3.2.1. Growth

In utero PFTN offspring had increased BW ($p = 0.04$; 6.7%) compared to IUTN rats on d26 of postnatal life; however, no *in utero* treatment BW differences ($p = 0.85$) were detected on d46 (189.7 g) or d66 of postnatal life (302.9 g; Table 2). Daily BW gain, FI, and feed efficiency were similar ($p > 0.28$) in offspring from all *in utero* treatments from d26 to d46, d46 to d66, and d26 to d66 of postnatal life (Table 2).

3.2.2. Tissue composition and accretion

Overall, IUPFTN offspring had reduced whole-body percent muscle mass ($p = 0.01$) compared to IUTN and IUHS progeny by 2.4 and 2.7%, respectively (Fig. 4A). When considering the entire postnatal growth period (d26 to d66 of postnatal life), whole-body percent adipose tissue was increased ($p = 0.01$) in IUPFTN compared to IUTN and IUHS offspring (10.6 and 12.5%, respectively; Fig. 4B). A postnatal day of life effect was observed, regardless of *in utero* treatment, where whole-body percent lean decreased ($p = 0.01$; Fig. 4A) and whole-body percent adipose tissue increased ($p = 0.01$) as progeny matured (Fig. 4B). No differences in whole-body percent BMC were detected (Fig. 4C). No other body composition differences were detected comparing *in utero* environment, postnatal day of life, or their interaction.

No postnatal tissue accretion differences amongst *in utero* treatments were detected from d26 to d46 of postnatal life ($p = 0.17$; Table 3), or d46 to d66 of postnatal life ($p = 0.55$; Table 3). From d26 to d66 of postnatal life, grams of whole-body lean

tissue gain per gram of BW gain were reduced ($p = 0.04$; 3.8%), and whole-body adipose tissue gain per gram of BW gain and the ratio of adipose to lean accretion from d26 to d66 of postnatal life increased ($p = 0.03$; 15.0 and 19.2%, respectively) in IUPFTN compared to IUHS rats; however, no differences were detected when compared to IUTN controls (Table 3). No other *in utero* treatment differences in tissue accretion were observed (Table 3).

3.2.3. Organ and tissue weights

Epididymal fat pad weight was increased ($p = 0.05$; 0.77 g) in IUPFTN compared to IUHS rats (Table 4), but no differences were detected when comparing IUHS and IUPFTN offspring to IUTN controls (Table 4). *In utero* PFTN rats had increased ($p = 0.03$) epididymal fat pad weight as a percent of BW compared to IUTN and IUHS rats (17.1 and 21.8%, respectively; Table 4). Testis weight was increased ($p = 0.04$) and testis weight as a percent of BW tended to be increased ($p = 0.10$) in IUPFTN compared to IUTN and IUHS rats by 10.6 and 13.4%, and 7.4 and 11.5%, respectively (Table 4). Gastrocnemius and soleus muscle weight, and gastrocnemius and soleus muscle weight as a percent of BW were similar ($p > 0.72$; 1.77 g and 0.13 g, respectively, and 0.58%, and 0.04%, respectively) amongst *in utero* treatments (Table 4). No other organ and tissue weight differences were detected between *in utero* treatments (Table 4).

3.2.4. Histology

Subcutaneous adipose tissue adipocyte size was increased ($p = 0.01$) in IUPFTN compared to IUTN and IUHS offspring by 83 and 92%, respectively (Table 4; Fig. 5). No adipocyte size differences in visceral adipose tissue ($p = 0.64$; $3895 \pm 506 \mu\text{m}^2$; Table 4) were detected amongst *in utero* treatments.

4. Discussion

Heat stress jeopardizes efforts by animal agriculture industries to efficiently produce animal products for human consumption (Baumgard and Rhoads, 2013). The negative effects of HS will likely become more apparent as climate models predict an increase in extreme summer conditions for most U.S. animal producing areas (Luber and McGeehin, 2008). Although the effects of HS on animal production during postnatal life are well-documented (St-Pierre et al., 2003; Johnson et al., 2015a), the direct and indirect effects of *in utero* HS on postnatal animal performance and metabolism are not well defined.

Intrauterine stress alters postnatal phenotypes and can negatively impact future metabolism and nutrient partitioning (Foxcroft et al., 2009; Limesand et al., 2007; Johnson et al., 2015a,b). In particular, maternal undernutrition alters postnatal nutrient partitioning priorities in offspring resulting in increased postnatal adiposity (Ravelli et al., 1976; Barker et al., 1993; Roseboom et al., 2006). Likewise, *in utero* HS increases postnatal whole-body adipose accretion at the expense of skeletal muscle mass in pigs (Johnson et al., 2015b). Considering the well-documented energy intake reduction of most species in response to HS (St-Pierre et al., 2003; Johnson et al., 2015a), the negative intrauterine programming effects of prenatal HS may be an indirect result of reduced maternal feed intake. This confounding effect of dissimilar dam FI was accounted for in the current study; however, IUPFTN offspring had increased whole-body adipose tissue compared to IUTN progeny (Fig. 4B), and contrary to our observations in pigs (Johnson et al., 2015b), body composition in IUHS offspring did not differ from IUTN controls. Increased adiposity in IUPFTN compared to IUHS offspring was accompanied by a

reduction in total lean tissue content (Fig. 4A), likely due to modified postnatal nutrient partitioning resulting from either reduced protein synthesizing capacity caused by *in utero* stress as previously reported (Gokulakrishnan et al., 2012; Johnson et al., 2015b), decreased glucose uptake by skeletal muscle as described in intrauterine growth restricted rodents (Ozanne et al., 2003).

The stark differences in postnatal phenotypes described above may shed light on the mechanism by which HS affects bioenergetics. A previous study by our group (Pearce et al., 2013) reported increased BW gain in heat-stressed pigs compared to pair-fed counterparts in TN conditions. Similarly, heat-stressed dams in the present study gained more BW (29 g), and these gains cannot be explained by increased water intake alone (7.3 mL) assuming that this water was retained. Furthermore, HS dams had increased BW gain per day (19%) and BW gain per gram of FI (15%) compared to PFTN mothers (Table 1), and this BW differential may have actually been greater (in terms of total maternal tissue mass) when considering that total litter weight of IUHS mothers was 11.9 g less compared to PFTN dams (Table 1). The latter point is supported by the fact that approximately 70% of the body weight gain of IUHS mothers was accounted for by fetal growth whereas fetal growth accounted for 98% of the body weight change in PFTN dams. Because their litter weights were smaller, IUHS mothers were either unable to enlist homeorhetic mechanisms to fully support the pregnancy and/or intrinsic defects within the gravid uterus prevented its growth potential. While specific mechanisms are unknown, increased BW gain and efficiency of converting dietary nutrients into tissue by the dams may be the result of HS-induced reductions in maintenance costs (Johnson et al., 2015b,c), thus sparing energy for additional growth. This could imply that although

311 HS and PFTN mothers consumed similar amounts of gross energy, nutrient availability
312 for maternal growth may have been greater in HS dams (especially considering that they
313 apparently did not partition additional nutrients into fetal development).

314 Subcutaneous adipocyte hypertrophy is closely linked with obesity (Shankar et
315 al., 2008; O'Connell et al., 2010) and is a postnatal phenotypic consequence of *in utero*
316 undernutrition (Nguyen et al., 2010; García et al., 2011). In the present study,
317 subcutaneous adipocyte size was increased (87%) in IUPFTN compared to IUHS and
318 IUTN progeny (Table 4; Fig. 5). A report by O'Connell and colleagues (2010) indicates
319 that the degree to which adipocytes can expand to accommodate excess lipid may be
320 determined by intrauterine influences (i.e. maternal undernutrition, *in utero* stress). As a
321 result, hypertrophic adipocytes may influence systemic metabolism because larger
322 adipocytes are less sensitive to insulin action (Salans et al., 1968; Hirsch and Knittle,
323 1970), and may be an independent predictor of type II diabetes (Weyer et al., 2000; Lonn
324 et al., 2010).

325 Increased epididymal fat pad weight is frequently reported in obese rodent models
326 (Knittle and Hirsch, 1968), and may be a postnatal consequence of *in utero*
327 undernutrition (Ford et al., 2007). In accordance with the aforementioned reports,
328 epididymal fat pad weight and epididymal fat pad weight as a percent of BW was
329 increased (25 and 22%, respectively) in IUPFTN compared to IUHS offspring (Table 3).
330 The increased epididymal fat pad weight corroborates the overall body composition data
331 (Fig. 4B) and because offspring FI and BW gain were similar, it is likely a consequence
332 of preprogrammed nutrient partitioning that favors adipose deposition in IUPFTN
333 progeny. In addition, testis weight and testis weight as a percent of BW increased (12

and 9%, respectively) in IUPFTN versus IUTN and IUHS progeny (Table 3). These results are in agreement with those reported by Rae and colleagues (2002) where sheep fetuses exposed to prenatal malnutrition had numerically increased testis weight. Causes for how or why prenatal nutrient restriction increases testis size is unknown, but may have practical implications for reproductive success in agriculturally important animals. Although whole-body lean tissue content was reduced (2.6%) in IUPFTN compared to IUHS and IUTN offspring, no *in utero* treatment differences in gastrocnemius and soleus muscle weights were detected. Reasons for the inconsistency between whole-body lean tissue and specific muscle mass measurements are unknown, but a potential explanation is that the decrease in whole-body lean content of IUPFTN offspring was not large enough to be detected by gross measurements of individual muscle mass in a small sample size.

Reduced birth weight is a well-documented effect of *in utero* undernutrition (Ravelli et al., 1976; Barker et al., 1993; Roseboom et al., 2006; Spencer, 2012) and hyperthermia (Shelton and Huston, 1968; Reynolds et al., 1985; Tao et al., 2012), and is likely a consequence of intrauterine growth retardation and shortened gestation length. In the present study, IUHS offspring had reduced birth weight (16%) compared to IUTN controls, but IUPFTN treatment did not impact pup weight (Table 1). Although birth weight was reduced in IUHS rats, litter size and gestation length (by experimental design) were similar in all *in utero* treatments. Additionally, since no pup birth weight differences were observed in IUPFTN offspring, reduced birth weight in IUHS rats was likely not a function of maternal restricted FI or reduced gestation length. Instead, reduced birth weight may be a direct impact of prenatal hyperthermia, possibly mediated

by reduced placental blood flow as has been previously suggested (Reynolds et al., 1985; Nakamura et al., 2000). Since fetal development was retarded despite the overall apparent increase in available nutrients in heat-stressed dams (as indicated by enhanced dam growth compared to IUPFTN), it may imply that the previously reported (Johnson et al., 2015b) negative postnatal consequences of *in utero* HS in pigs are mediated by intrauterine growth retardation (possibly due to reduced blood flow and fetal nutrient availability) as opposed to maternal undernutrition. Further, as low birth weight is a significant risk factor for the development of obesity in later life (Spencer, 2012), it is unknown if IUHS offspring would have accumulated more adipose tissue if the postnatal aspect of the experiment had lasted longer.

Although IUHS treatments reduced offspring birth weight, pup BW at weaning was similar to IUTN controls. This likely indicates that lactogenic capacity of heat-stressed mothers was not compromised and was copious enough to allow for “catch-up” or compensatory growth in IUHS offspring; although, differences in milk composition from parturition to weaning may have partially contributed the postnatal body composition differences (as described in rat models of maternal undernutrition; Watzet et al., 2014) between IUHS and IUPFTN offspring. While IUPFTN conditions altered offspring body composition compared to IUHS treatment during postnatal growth to d66, no BW or FI differences were detected during postnatal growth. These results likely represent pre-programmed differences in the hierarchy of tissue accretion between IUPFTN and IUHS offspring, independent of energy intake and growth rate. However, a lack of FI differences from d26 to d66 of postnatal life in IUPFTN offspring compared to IUTN controls was surprising, since hyperphagia is a commonly reported postnatal

consequence of prenatal undernutrition (Vickers, et al., 2000; Bellinger and Langley-Evans, 2005), possibly mediated by altered expression of the appetite controlling system (Ikenasio-Thorpe et al., 2007; Coupé et al., 2009). Reasons for the lack of postnatal hyperphagia in IUPFTN offspring may be due to less severe maternal nutrient restriction in the current experiment compared to previous reports (Vickers et al., 2000; Bellinger and Langley-Evans, 2005), since the primary objective was to mirror the reduced FI in heat-stressed dams (33% reduction) and not to induce marked malnutrition.

A previous report by our group (Johnson et al., 2015b) indicated that *in utero* HS alters postnatal nutrient partitioning in growing pigs; however, no differences were observed when comparing IUHS versus IUTN rats in the present study. Reasons for the difference between models are unclear but may be due to differences in maternal environment (rat = cycling temperature 30 to 34°C; pig = cycling temperature 27 to 37°C), timing of the gestational insult (rat = 73% of gestation length; pig = 95% of gestation length), differences in thermoregulation, species differences in tissue development, or because FI reductions did not occur in heat-stressed compared to TN sows due to the experimental inclusion of the standard swine industry practice of limit-feeding dams during gestation (Johnson et al., 2015b). Additionally, there may be species differences in how maternal HS affects fetal and postnatal development. Regardless, determining the effects of HS during *in utero* development is of interest in other agriculturally important species.

5. Conclusions

Since our previous work indicated that *in utero* HS alters postnatal nutrient partitioning in pigs we hypothesized that this prenatal programming may be mediated by reduced maternal feed intake. However, the current rodent model of *in utero* HS challenges our hypothesis. Specifically, offspring from pair-fed dams in TN conditions had altered body composition compared to progeny from heat-stressed dams, despite similar levels of maternal nutrient restriction. These results may have implications for HS bioenergetics and could represent reductions in maternal maintenance costs (as indicated by enhanced dam growth). Although an apparent increase in available nutrients occurred in heat-stressed dams, IUHS birth weight was reduced, potentially indicative of intrauterine growth retardation and predictive of enhanced adiposity later in life. Although body composition differences were not observed comparing IUHS and IUTN progeny during this particular stage of life, it may simply be a result of study design or species differences. The mechanisms by which *in utero* HS alters postnatal nutrient partitioning remain unclear, and deciphering which species are sensitive to *in utero* heat stress is of interest.

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Table 1: Effects of thermoneutral environment, heat stress environment, and pair-feeding in a thermoneutral environment on thermal indices, growth, and litter characteristics in pregnant Sprague Dawley rats (n = 4/treatment) from d2 to d19 of gestation.

Parameter	Environment			P	
	TN ¹	HS ²	PFTN ³	SEM ⁴	ME ⁵
T _{re} (°C) ⁶	37.2 ^a	38.5 ^b	37.2 ^a	0.1	0.01
T _{skin} (°C) ⁷	27.0 ^a	36.8 ^b	27.2 ^a	0.2	0.01
Initial BW (g) ⁸	186.3	187.4	193.3	5.9	0.68
Final BW (g) ⁹	298.8 ^c	283.3 ^b	260.1 ^a	6.7	0.01
Body weight gain (g/d)	5.96 ^c	4.39 ^b	3.70 ^a	0.18	0.01
Feed intake (g/d)	21.0 ^c	14.1 ^b	13.7 ^b	0.6	0.01
FI as % of BW (g/g) ¹⁰	9.3 ^b	6.3 ^a	6.3 ^a	0.1	0.01
BW gain (g) : FI (g) ¹¹	0.28 ^{ab}	0.30 ^b	0.26 ^a	<0.01	0.05
Water intake (mL/d)	41.7 ^a	52.1 ^b	44.8 ^a	2.2	0.02
Birth weight ¹² (g)	7.5 ^b	6.3 ^a	7.4 ^b	0.3	0.05
Pup number	11.0	10.8	10.8	0.9	0.97

¹Thermoneutral
²Heat-stressed
³Pair-fed in thermoneutral conditions
⁴Standard error of the mean
⁵Maternal environment
⁶Rectal temperature
⁷Tail skin temperature
⁸Initial body weight on d 2 of gestation
⁹Final body weight on d 19 of gestation
¹⁰Feed intake % of body weight
¹¹Feed efficiency
¹²Mean birth weight of individual pups
Letters (a < b) within a row indicate maternal environment differences (*p* ≤ 0.05)

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Table 2: Effect of *in utero* thermoneutral environment, heat stress environment, and maternal pair-feeding in a thermoneutral environment on male Sprague Dawley rat offspring (n = 8/treatment) growth parameters

Parameter	Environment			SEM ⁴	<i>P</i> IE ⁵
	IUTN ¹	IUHS ²	IUPFTN ³		
Body weight					
Day 26 of postnatal life (g)	67.4 ^a	68.9 ^{ab}	71.9 ^b	0.9	0.04
Day 46 of postnatal life (g)	189.7	187.8	191.7	4.9	0.85
Day 66 of postnatal life (g)	302.8	300.4	305.6	7.5	0.88
26 to 46 d ⁶					
Feed intake (g/d)	16.6	15.9	16.4	0.4	0.47
Body weight gain (g/d)	6.1	5.9	5.9	0.2	0.81
BW gain : FI (g/g) ⁷	0.37	0.38	0.36	0.01	0.81
46 to 66 d					
Feed intake (g/d)	24.3	21.1	20.6	3.8	0.60
Body weight gain (g/d)	5.4	4.7	4.1	0.6	0.29
BW gain : FI (g/g)	0.23	0.23	0.23	0.01	0.88
26 to 66 d					
Feed intake (g/d)	18.7	18.4	18.8	0.7	0.93
Body weight gain (g/d)	5.9	5.7	5.8	0.2	0.82
BW gain : FI (g/g)	0.32	0.32	0.31	0.01	0.91

¹*In utero* thermoneutral

²*In utero* heat-stressed

³*In utero* pair-fed thermoneutral

⁴Standard error of the mean

⁵*In utero* environment

⁶Day of postnatal life

⁷Ratio of body weight gain to feed intake

Letters (a < b) within a row indicate *in utero* environment differences ($p \leq 0.05$)

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Table 3: Effect of *in utero* thermoneutral environment, heat stress environment, and maternal pair-feeding in a thermoneutral environment on postnatal tissue accretion in male Sprague Dawley rat offspring (n = 8/treatment).

Parameter	Environment			SEM ⁴	<i>P</i>
	IUTN ¹	IUHS ²	IUPFTN ³		IE ⁵
26 to 46 d ⁶					
Adipose (g/d)	1.33	1.15	1.34	0.11	0.40
Lean (g/d)	4.75	4.75	4.57	0.19	0.77
BMC ⁷ (g/d)	0.15	0.14	0.14	0.01	0.34
Adipose : Lean (g/g)	0.29	0.24	0.28	0.02	0.17
Adipose (g/g BW gain) ⁸	0.21	0.19	0.22	< 0.01	0.17
Lean (g/g BW gain)	0.77	0.79	0.77	< 0.01	0.23
BMC (g/g BW gain)	0.02	0.02	0.02	< 0.01	0.24
46 to 66 d					
Adipose (g/d)	1.28	1.22	1.38	0.11	0.62
Lean (g/d)	4.37	4.41	4.32	0.15	0.92
BMC (g/d)	0.13	0.14	0.14	< 0.01	0.46
Adipose : Lean (g/g)	0.29	0.28	0.32	0.03	0.56
Adipose (g/g BW gain)	0.22	0.22	0.24	0.02	0.55
Lean (g/g BW gain)	0.77	0.78	0.76	0.02	0.59
BMC (g/g BW gain)	0.02	0.02	0.02	< 0.01	0.55
26 to 66 d					
Adipose (g/d)	1.30	1.18	1.36	0.06	0.18
Lean (g/d)	4.56	4.58	4.45	0.15	0.80
BMC (g/d)	0.14	0.14	0.14	< 0.01	0.95
Adipose : Lean (g/g)	0.29 ^{ab}	0.26 ^a	0.31 ^b	< 0.01	0.03
Adipose (g/g BW gain)	0.22 ^{ab}	0.20 ^a	0.23 ^b	< 0.01	0.03
Lean (g/g BW gain)	0.77 ^{ab}	0.79 ^b	0.76 ^a	< 0.01	0.04
BMC (g/g BW gain)	0.02	0.02	0.02	< 0.01	0.66

¹*In utero* thermoneutral

²*In utero* heat-stressed

³*In utero* pair-fed thermoneutral

⁴Standard error of the mean

⁵*In utero* environment

⁶Day of postnatal life

⁷Bone mineral content

⁸Grams of tissue gain per gram of body weight gain

Letters (a < b) within a row indicate *in utero* environment differences ($p \leq 0.05$)

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Table 4: Effect of *in utero* thermoneutral environment, heat stress environment, and maternal pair-feeding in a thermoneutral environment on carcass components in male Sprague Dawley rat offspring (n = 8/treatment) at d 66 of postnatal life.

Parameter	Environment			SEM ⁴	<i>P</i>
	IUTN ¹	IUHS ²	IUPFTN ³		IE ⁵
Weight					
Epididymal fat pad (g)	3.23 ^{ab}	3.05 ^a	3.82 ^b	0.19	0.05
Gastrocnemius (g)	1.81	1.76	1.75	0.07	0.80
Head (g)	23.34	22.50	22.92	0.33	0.27
Heart (g)	1.03	1.06	0.99	0.04	0.49
Liver (g)	11.84	10.66	11.54	0.45	0.19
Soleus (g)	0.13	0.13	0.13	<0.01	0.97
Testis (g)	1.61 ^a	1.57 ^a	1.78 ^b	0.05	0.04
Percent of final body weight					
Epididymal fat pad % BW ⁶	1.05 ^a	1.01 ^a	1.23 ^b	0.05	0.03
Gastrocnemius % BW	0.59	0.58	0.57	0.02	0.73
Head % BW	7.57	7.49	7.51	0.13	0.92
Heart % BW	0.34	0.35	0.32	<0.01	0.25
Liver % BW	3.91	3.55	3.77	0.12	0.16
Soleus % BW	0.04	0.04	0.04	<0.01	0.92
Testis % BW	0.54	0.52	0.58	0.02	0.10
Tibia length (cm)	5.14	5.10	5.31	0.06	0.08
Subcutaneous adipocyte size (μm ²)	1803 ^a	1719 ^a	3293 ^b	370	0.01
Visceral adipocyte size (μm ²)	4158	4013	3513	506	0.64

¹*In utero* thermoneutral

²*In utero* heat-stressed

³*In utero* pair-fed thermoneutral

⁴Standard error of the mean

⁵*In utero* environment

⁶Body weight

Letters (a < b) within a row indicate *in utero* environment differences ($p \leq 0.05$)

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Fig. 1. Ambient temperature (T_a ; °C) by day of gestation in pregnant Sprague Dawley rats. Abbreviations are: maternal thermoneutral environment (TN; constant $22.2 \pm 0.1^\circ\text{C}$), and maternal heat stress environment (HS; cyclical 30.0 daytime to 34.0°C nighttime).

Fig. 2. Effects of maternal environment on the temporal changes in feed intake (FI) in pregnant Sprague Dawley rats (n = 4/treatment) averaged by day of treatment. Abbreviations are: maternal thermoneutral environment (TN; constant $22.2 \pm 0.1^{\circ}\text{C}$), maternal heat stress environment (HS; cycling 30.0°C daytime to 34.0°C nighttime), mothers pair-fed to HS-exposed dams in a TN environment (PFTN; constant $22.2 \pm 0.1^{\circ}\text{C}$). Error bars on d 1 and d 15 of treatment indicate standard error of the mean in each maternal environment.

Fig. 3. Effects of maternal environment on the temporal changes in body weight (BW) by day of gestation in pregnant Sprague Dawley rats ($n = 4/\text{treatment}$). Abbreviations are: maternal thermoneutral environment (TN; constant $22.2 \pm 0.1^\circ\text{C}$), maternal heat stress environment (HS; 30.0 daytime to 34.0°C nighttime), mothers pair-fed to HS-exposed dams in a TN environment (PFTN; constant $22.2 \pm 0.1^\circ\text{C}$). Error bars on d 2 and d 19 of gestation indicate standard error of the mean in each maternal environment. Letters ($a < b < c$) indicate daily differences between maternal treatments ($p = 0.01$). Vertical dashed lines indicate the beginning (d 3) and end (d 18) of the environmental treatment period.

Fig. 4. Effect of *in utero* environment on (A) percent lean tissue, (B) percent adipose tissue, and (C) percent bone mineral as determined by DXA scans on d26, d46, and d66 of postnatal life in male Sprague Dawley rat offspring (n = 8/treatment). Abbreviations are: *in utero* thermoneutral environment (IUTN), *in utero* heat-stressed environment (IUHS), and *in utero* pair-fed to HS-exposed rats in TN conditions (IUPFTN). Numbers above error bars indicate tissue percent at each postnatal day of life. Letters (a < b) indicate overall differences ($p = 0.01$) between *in utero* treatments.

Fig. 5. Estimation of adipocyte size in the subcutaneous adipose tissue of male Sprague Dawley rat offspring (n = 8/treatment). Abbreviations are: *in utero* thermoneutral environment (IUTN), *in utero* heat-stressed environment (IUHS), and *in utero* pair-fed to HS-exposed rats in TN conditions (IUPFTN). Letters (a < b) in the graph indicate significance ($p = 0.01$) comparing *in utero* treatments.