

**Heat stress alters animal physiology and post-absorptive metabolism during
pre- and postnatal development**

by

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LIST OF ABBREVIATIONS

%RH = PERCENT RELATIVE HUMIDITY
ADG = AVERAGE DAILY GAIN
BPM = BREATHS PER MINUTE
BW = BODY WEIGHT
cAMP = CYCLIC AMP
CK = CREATINE KINASE
CM = CHYLOMICRON
DAG = DIACYLGLYCEROL
DHAP = DIHYDROXYACETONEPHOSPHATE
DM = DRY MATTER
EBW = EMPTY BODY WEIGHT
ER = ENDOPLASMIC RETICULUM
FE = FEED EFFICIENCY
FFA = FREE FATTY ACIDS
FI = FEED INTAKE
FSG = FINAL SLAUGHTER GROUP
GA3P = GLYCERALDEHYDE -3 – PHOSPHATE
GE = GROSS ENERGY
GHS = GESTATIONAL HEAT STRESS
GLUT = GLUCOSE TRANSPORTER
GTN = GESTATIONAL THERMAL NEUTRAL
HS = HEAT STRESS
HSHS = HEAT STRESS ENTIRE GESTATION
HSL = HORMONE-SENSITIVE LIPASE
HSP70 = HEAT SHOCK PROTEIN 70
HSTN = HEAT STRESS FIRST HALF OF GESTATION
ISG = INITIAL SLAUGHTER GROUP
IUGR = INTRAUTERINE GROWTH RETARDATION
IUHS = *IN UTERO* HEAT STRESS
IUPFTN = *IN UTERO* PAIR-FED IN THERMAL NEUTRAL CONDITIONS
IUTN = *IN UTERO* THERMAL NEUTRAL
MAG = MONOACYLGLYCEROL
NEFA = NON-ESTERIFIED FATTY ACIDS
NI = NUTRIENT INTAKE
PFTN = PAIR-FED IN THERMAL NEUTRAL CONDITIONS
PUN = PLASMA UREA NITROGEN
RR = RESPIRATION RATE
TAG = TRIACYLGLYCEROL
TCI = THERMAL CIRCULATION INDEX
T_{CORE} = CORE BODY TEMPERATURE
T_{EAR} = EAR SKIN TEMPERATURE
TN = THERMAL NEUTRAL
TNHS = HEAT STRESS SECOND HALF OF GESTATION
TNTN = THERMAL NEUTRAL ENTIRE GESTATION

T_{RE} = RECTAL TEMPERATURE

T_{RUMP} = RUMP SKIN TEMPERATURE

T_{SHOULDER} = SHOULDER SKIN TEMPERATURE

T_{SKIN} = SKIN TEMPERATURE

T_{TAIL} = TAIL SKIN TEMPERATURE

VLDL = VERY LOW DENSITY LIPOPROTEIN

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ABSTRACT

Heat stress (HS) is a key limiting factor to efficient animal production and negatively impacts health and development during postnatal life. In addition, hyperthermia during *in utero* development can permanently alter postnatal phenotypes and negatively impact future animal performance. While the teratogenic effects of prenatal HS have been extensively evaluated, the impact of *in utero* HS exposure on future mammalian thermoregulation, nutrient partitioning, and bioenergetics is undefined. To determine the postnatal consequences of *in utero* HS, pregnant first parity sows and rats were exposed to either thermal neutral (TN) or HS conditions for the entire gestation, the first half, or second half of gestation. To account for differences in maternal nutrient intake, we utilized an *ad libitum* TN control group and a pair-fed TN control group of rats. Progeny were evaluated for differences in production performance, nutrient partitioning, thermoregulation, and post-absorptive metabolism. In a series of experiments, it was determined that prenatal HS exposure increased postnatal adipose deposition at the expense of skeletal muscle mass and permanently increased core body temperature during future development. When compared with *in utero* HS-exposed rats, pair-fed TN exposed progeny had increased adipose tissue and reduced lean tissue mass. In opposition to some previously published reports, postnatal HS exposure seems to reduce maintenance costs, which may have implications toward energy efficiency during times of thermal stress. In summary, HS modifies animal metabolism and physiology during both pre- and postnatal development and reduces livestock production efficiency.

CHAPTER I: LITERATURE REVIEW

Introduction

Environmentally induced heat stress (HS) results from the imbalance between thermal energy flowing into and out of an animal (Kleiber, 1961), and negatively impacts health and development. Typical responses to HS include slower and inconsistent growth, reduced milk synthesis, poor fertility, altered metabolism, morbidity and mortality, and altered body composition characterized by increased adiposity and reduced skeletal muscle mass (Collin et al., 2001; Brown-Brandl et al., 2004; Baumgard and Rhoads, 2013). As climate models predict an increase in extreme summer conditions for most U.S. animal producing areas, the negative effects of HS will likely become more significant in the future (Luber and McGeehin, 2008). Furthermore, because increased basal heat production is an unintended consequence of most genetic selection programs (Brown-Brandl et al., 2004), some suggest faster growing animals are more sensitive to HS (Nienaber and Hahn, 2007). In addition to its aforementioned postnatal effects, HS during gestation can impact a variety of fetal development parameters (Graham et al., 1998), and has the potential to adversely affect animals lifetime productivity. Although primarily an animal welfare and economic issue in developed countries, most third-world nations and small stakeholders lack the resources to afford HS abatement strategies. Therefore, in these developing countries, climate change and specifically HS is a food security and humanitarian concern (Baumgard and Rhoads, 2013). Consequently, there is an urgent need to better understand the mechanisms by which HS compromises efficient production of high quality animal protein.

Global Impact of Heat Stress

Climate Change

Heat stress-induced suboptimal animal performance is already a considerable economic problem and food security issue. However, if climate change continues as expected (Bernabucci et al., 2010), the negative consequences of HS could be a serious threat to global animal agriculture. Climate change affects ambient temperature, weather patterns, and sea levels, and it is thought that deforestation and green house gas emissions are a significant contributor to the changing climate (U.S. EPA, 2013). According to the U.S. Environmental Protection Agency (2013), average global temperatures are expected to increase by 1.1 to 6.4°C by 2100 and will warm at least twice as much as it has in the last 100 years. Further, an increase in the average temperature worldwide would likely cause more frequent and intense extreme heat events with days over 32.2°C expected to increase from 60 to 150 days annually in the U.S. alone (U.S. EPA, 2013). With increased frequency of heat waves and periods of extreme high temperature expected, incidences of heat-related maladies for humans and animals are likely to increase. Therefore, there is an urgent need to better understand how hyperthermia affects physiology and ultimately productivity in agriculturally important species.

Economic and Food Security Impacts of Heat Stress

The economic impact of HS-related maladies are estimated to account for billions of dollars in lost revenue due to reduced production in almost every aspect of animal agriculture. In the United States alone, estimated annual losses resulting from HS are greater than \$1.6 billion/year for dairy, beef, swine and poultry species (St-Pierre et al., 2003; Pollman, 2010). Despite improved management practices and cooling technology (shade,

sprinklers, fans), animal productivity remains suboptimal during the summer months (St-Pierre et al., 2003).

Economic losses continue to occur because animals are raised in regions and during seasons where the ambient temperature ventures outside the zone of thermal comfort (St-Pierre et al., 2003). During HS, efficiency is compromised because nutrients are diverted to maintain euthermy as preserving a safe core temperature is of highest priority and product synthesis is de-emphasized (Baumgard and Rhoads, 2013). The effects of HS are especially evident in tropical and sub-tropical regions where many developing countries are located (Battisti and Naylor, 2009; Muller et al., 2010). As a result, these regions may experience extended periods of HS compared with temperate climates, making HS a significant economic, food security, and humanitarian concern (Battisti and Naylor, 2009). Further, as many developing countries (i.e., African nations, China, India) populations continue to rapidly grow (Godfray et al., 2010), so will the need for increased food supply, thus amplifying the negative consequences of economic and production losses due to HS.

Heat Related Illnesses in Humans and Animals

Elevated core temperature can negatively impact human and animal health, and in extreme cases may result in mortality depending on the severity of the heat load (Jackson and Rosenberg, 2010). In the U.S. from 1999 through 2003, 3,442 heat-related human deaths were recorded where heat exposure was indicated as the cause (Jackson and Rosenberg, 2010). In addition, a California heat wave purportedly resulted in the death of more than 30,000 dairy cows (CDFA, 2006), and a recent heat wave in Iowa killed at least 4,000 head of beef cattle (Drovers Cattle Network, 2011), illustrating that most geographical locales in the U.S. are susceptible to extreme and lethal heat. Symptoms of heat-related illnesses

include heat edema, heat cramps, heat syncope, heat exhaustion, and eventually heat stroke (Table 1.1).

Table 1.1: Symptoms of heat-related illnesses¹ from mild (1) to severe (5).

<p>1. Heat edema</p> <ul style="list-style-type: none"> - Mildest form of heat-illness caused by transient peripheral vasodilation from the heat and orthostatic pooling during prolonged sitting or standing 	<p>4. Heat exhaustion</p> <ul style="list-style-type: none"> - Excess sweating - Heat cramps - Nausea/vomiting - Headache - Hypotension
<p>2. Heat cramps</p> <ul style="list-style-type: none"> - Painful spasms of skeletal muscle - Increased body temperature - Thirst - Sweating - Tachycardia 	<p>5. Heat stroke</p> <ul style="list-style-type: none"> - Heat exhaustion - Dehydration - Endotoxemia - Hyperglycemia - Lactic acidosis - Cardiovascular abnormalities - Increased glucocorticoids - Renal failure - Hyperventilation - Arrhythmia
<p>3. Heat syncope</p> <ul style="list-style-type: none"> - Dizziness - Inadequate cardiac output - Fainting 	

¹Adapted from Leon, 2007; Aggarwal et al., 2008, and Pearce, 2011

Management Strategies to Reduce Heat Load

Despite increased efforts to combat HS through nutritional strategies, management and employing heat abatement programs still represent the main approach to relieve HS (Baumgard and Rhoads, 2013). Providing shade, ventilation, and evaporative cooling can be strategies implemented to reduce the harmful effects of HS. Stressors of any kind (i.e. vaccinations, restraint) should be avoided during hotter parts of the day, as the combination of HS and handling stress is unfavorable (Collier et al., 2011). Additionally, social stressors such as regrouping should be avoided as it has been demonstrated to reduce growth and feed

intake during HS (McGlone et al., 1987). Administration of aspirin and other non-steroidal anti-inflammatory drugs should be avoided as they may exacerbate gastrointestinal integrity issues (Stoakes et al., 2013). Further, although extensive engineering and animal expertise must be considered, building design can have a significant impact on the health and well-being of pigs and poultry, and must take into account animal heat and moisture production responses to changing genetics, nutrition, and thermal environment (Brown-Brandl et al., 2004).

Nutritional Strategies to Reduce Heat Load

Proper management strategies can be complemented by nutritional interventions during periods of prolonged HS. Previous studies have demonstrated that insulin action (Wheelock et al., 2010; Rhoads et al., 2013; Pearce et al., 2013a), heat of nutrient processing (Curtis, 1983), and gut integrity (Lambert, 2002; Pearce et al., 2013b) can be significantly impacted during HS, and can benefit from changes in diet composition or the addition of nutritional supplements. Since proper insulin action is thought to be one of the key components of successfully adapting to and surviving a heat load, enhancing insulin sensitivity may be an effective tactic to improve animal performance during HS (Rhoads et al., 2013). Tactics to improve sensitivity can include the addition of chromium (Mertz, 1993), lipoic acid (Diesel et al., 2007), or thiazolidinediones (Ranganathan et al., 2006) to the diet, which are known to improve insulin sensitivity (Rhoads et al., 2013).

Dietary composition is another easily manipulated nutritional strategy that may benefit animal health. During HS, growing pigs will reduce fasting heat production by 18%, daily heat production by 22%, and the thermic effect of feed by 35% (Collin et al., 2001). The reduced thermic effect of feeding is likely due to a reduction in feed intake, which while

immediately beneficial to the animal, can be detrimental to growth potential and animal production. A good strategy to alleviate losses due to inadequate nutrient intake is to replace dietary fiber with energy dense fat sources that can reduce the impact of HS on growing animals and increase the amount of energy received per kg of feed intake (Schoenherr et al., 1989).

The gastrointestinal (GI) tract is highly susceptible to the effects of HS and is a target that may be manipulated by nutritional interventions. Animals that are heat-stressed divert blood flow to the periphery in an attempt to dissipate heat, leading to hypoxia and eventual GI tract damage (Hall et al., 1999; Lambert, 2002). To alleviate this damage, nutritional strategies like including glutamine (Lima et al., 2005), zinc (Alam et al., 1994; Zhang and Guo, 2009), or betaine (Kettunen et al., 2001; Hassan et al., 2011) may be considered. Glutamine is a conditionally non-essential amino acid, which serves as the primary energy source for enterocytes (Singleton and Wischmeyer, 2006). Supplemental glutamine has been demonstrated to improve intestinal barrier function, and its effect is potentially mediated through activation of heat-shock protein 70 (Lima et al., 2005). Further, glutamine improves milk production in dairy cattle during periods of HS (Caroprese et al., 2013). Zinc ions are essential for normal intestinal barrier function and supplementation can improve intestinal integrity in both acute and chronically heat-stressed pigs (Pearce et al., 2013b; Sanz-Fernandez et al., 2014). Although its mode of action is currently unknown, it may include the up-regulation of tight junction proteins (Zhang and Guo, 2009), or its role as an antioxidant via induction of metallothioneins (Wang et al., 2013). Finally, betaine (trimethylglycine) is an osmotic regulator and methyl donor, which may protect against intestinal osmotic stress by reducing sodium potassium pump activity (Cronje, 2007).

Additionally, betaine ameliorates the effects of HS on weight gain, immunity and body temperature indices in rabbits (Hassan et al., 2011), and improves milk production parameters in dairy cattle (Peterson et al., 2012; Dunshea et al., 2013). Despite some documentation of the positive effects of betaine supplementation, lack of sufficient evidence in support of or against its role in HS alleviation warrants the need for further investigation (Stoakes et al., 2013).

Direct and Indirect Effects of Heat Stress

Reduced feed intake during HS is a highly conserved response among livestock species (Reneau et al., 2008; Baumgard et al., 2012), and presumably represents an attempt to decrease metabolic heat production (Whittow, 1971). It has traditionally been assumed that inadequate feed intake caused by an excessive thermal load was responsible for decreased animal production (Fuquay, 1981; Beede and Collier, 1986; Collin et al., 2001). However, recent results challenge this dogma, as disparate slopes in feed intake and milk yield exist in response to cyclical heat load (Shwartz et al., 2009), and growth rate of heat-stressed pigs is increased compared to pair-fed animals despite similar reductions in nutrient intake (Pearce et al., 2013a). These studies employ the use of a thermal neutral pair-fed group that allows for evaluating thermal stress while eliminating the confounding effects of dissimilar nutrient intake. Using this model, previous experiments have demonstrated that reduced feed intake only explains 35-50% of decreased milk yield in dairy cattle during environmentally induced hyperthermia (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2012). Further, heat-stressed gilts do not lose as much body weight and condition as their pair-fed counterparts (Pearce et al., 2013a), and heat-stressed lambs lose more body weight compared to pair-fed counterparts (Mahjoubi et al., 2014). Although the

direct impact of HS appears to differ between monogastrics and ruminants (i.e., pigs grow faster while lambs lose weight), these data indicate that HS imposes direct effects independently of reduced feed intake. Furthermore, the composition of body weight gain in HS-exposed animals is presumably skewed towards adipose production at the expense of lean muscle mass synthesis, while pair-fed animals gain lean tissue at the expense of adipose production (Heath, 1983; Sano et al., 1983; Geraert et al., 1996; Ronchi et al., 1999).

Although the pair-fed thermal neutral (PFTN) model attempts to separate the direct and indirect effects of HS, some limitations should be considered. The negative consequences of HS on productivity may be mediated by reduced intestinal integrity (Pearce et al., 2013b; Sanz Fernandez et al., 2014), likely due to decreased intestinal blood flow (Lambert et al., 2002). Reduced blood flow leads to hypoxia at the intestinal epithelium, which can alter intestinal morphology, and may increase the permeability of tight junctions (Yan et al., 2006; Pearce et al., 2013b). Enhanced intestinal permeability can increase the risk of bacterial translocation (Baumgart and Dignass, 2002), and may decrease nutrient digestibility and absorption in heat-stressed animals as suggested by Pearce and colleagues (2013b), likely putting HS pigs at an even lower plane of nutrition than pair feeding can account for. However, it is important to note that nutrient restriction may also increase intestinal permeability (Ferraris and Carey, 2000; Pearce et al., 2013b), but it is unknown to what extent this occurs when compared to heat-stressed animals. In addition, voluntarily reduced FI during HS is likely a strategy to reduce basal heat production (i.e., the thermic effect of feeding) in a concerted effort to acclimate to hyperthermia (Curtis, 1983). Whereas, limit-feeding pigs in TN conditions may initiate a stress response resulting in increased activity levels, abnormal behavior, and enhanced stress hormone production since nutrient

restriction in PFTN pigs is involuntary. Therefore, it is likely that PFTN animals are not only nutrient restricted, but may also be under greater or differing levels of psychological stress compared to HS counterparts. A report by our lab (Pearce et al., 2013a) indicated that pair feeding reduces core body temperature when compared to pigs fed *ad libitum* and raised in thermal neutral (TN) conditions. This core temperature decrease could result from reduced heat production from nutrient processing (i.e., the thermic effect of feeding), and in an attempt to maintain homeothermy, PFTN pigs may increase fasting heat production compared to HS pigs. Increased fasting heat production would imply greater maintenance costs, thus reducing energy efficiency in PFTN pigs compared to HS counterparts. Finally, because PFTN pigs lose more BW when compared to heat-stressed counterparts (Pearce et al., 2013a), nutrient restriction in HS-exposed pigs may not be as metabolically stressful compared to PFTN pigs. Pair feeding as a percent of BW can help mitigate nutrient intake differences due to altered rates of BW gain. However, despite its limitations the PFTN model is currently the best method to minimize the confounding effects of dissimilar FI during HS experiments.

Thermoregulatory Response to Heat Stress

Thermoregulation

In the short-term, an animal's HS response is to increase heat loss by the body in an attempt to remain euthermic. As a result, animals can maintain normal production within a wide range of ambient temperatures and environments (DeShazer, 2009). Temperature regulation can be achieved through alterations in animal physiology, behavior, and morphology (Angilletta, 2009), and these changes serve to preserve thermal homeostasis so

that thermal input equals heat loss to the environment (DeShazer, 2009). Control of thermal exchange starts at the level of the neuron and external temperature is sensed by thermoreceptors located throughout the body (Curtis, 1983). These receptors react to changes in ambient temperature by increasing their firing rate in reaction to either warm (warm receptors) or cold (cold receptors) external temperatures. The rate of firing is not in response to absolute temperature, but instead to rapid changes in temperature (Curtis, 1983). This ability to collect temperature information from many parts of the body initiates a physiological or behavioral response allowing for an appropriate feedback to a changing environment. Table 1.2 demonstrates euthermic rectal temperature of various species.

Table 1.2: Euthermic rectal temperature (T_{re}) of multiple species.

Species	T_{re} (°F)	T_{re} (°C)
Avian	107.6	42.0
Bovine	101.0	38.3
Canine	102.0	38.9
Caprine	103.1	39.5
Equine	100.0	37.8
Feline	101.5	38.6
Human	98.6	37.0
Leporine	101.5	38.6
Ovine	102.3	39.1
Porcine	102.5	39.2
Rodents	99.1	37.3

Adapted from Campbell et al., 2003; O'Brien, 2008; and Pearce, 2011

Conductive Exchange

Conduction is a method of heat exchange defined as moving heat from the core to the periphery, and then transferring it to surrounding objects in contact with the skin surface (Curtis, 1983). Conductive heat transfer depends on several variables including thermal

conductivity, temperature gradient, and area of contact (Curtis, 1983; Meat and Livestock Australia, 2006). Heat exchange by conduction at the skin (expressed in Watts) is defined by the following equation, which demonstrates its dependence on thermal conductance, surface area, and temperature gradients:

$$Q_k = (A_k) (k) [(T_1 - T_2) \div (s)]$$

Where; Q_k is the conductive heat flux (cal sec^{-1}), A_k is the animal's effective conductive surface area (cm^2), k is the thermal conductivity of environmental substance ($\text{cal cm}^{-2} \text{sec}^{-1} \text{C}^{-1} \text{cm}$), T_1 is the temperature of environmental substance at a point some distance from the animal-contact point ($^{\circ}\text{C}$), T_2 is the environmental surface temperature at point of contact with animal ($^{\circ}\text{C}$), and s is the distance between points where T_1 and T_2 were measured (cm); (Curtis, 1983).

Pigs raised in conventional housing facilities do not have the opportunity for behavioral wetting (i.e. wallowing), so they rely on methods such as conduction for enhanced heat dissipation (Curtis, 1983). Conductive heat exchange in pigs is achieved primarily through skin to ground contact (20% of pigs surface area; Bruce, 1977) and is influenced by thermal resistance of the material (Bruce and Clark, 1979). However, as the ambient temperature approaches core temperature, conduction is of little importance as the thermal gradient is reduced (McDowell, 1972).

Convective Exchange

Convection is a method of heat loss animals employ to facilitate heat loss to their environment using the air or water as a mobile medium for heat exchange. It is defined as the net rate of heat transfer in a moving gas or fluid between different parts of an organism,

or between an organism and its external environment (IUPS Thermal Commission, 2001).

Convective heat transfer is described as:

$$Q_h = (h) (A_h) (T_a - T_s)$$

Where; Q_h is the convective heat flux (kcal min^{-1}), h is the convection coefficient ($\text{kcal min}^{-1} \text{m}^{-2} \text{C}^{-1}$), A_h is the animals surface area that is affected by convection, T_a is air temperature, and T_s is the skin or surface temperature for the exchange site (Curtis, 1983).

Heat exchange by convection is achieved by increasing blood flow to the skin through vasodilation, allowing for heat loss to the surrounding environment by use of a medium such as increased air flow (DeShazer, 2009). In the pig, convective heat loss occurs primarily in peripheral regions (extremities) due to the close proximity of blood vessels to the skin surface and lack of subcutaneous adipose tissue preventing efficient heat loss to the environment (McDowell, 1972). Further, convective heat loss can occur in the respiratory tract since airflow through the nasal passages and upper respiratory tract can carry a large amount of deep, internal body heat to the outside environment (Robertshaw, 1985).

Radiative Exchange

Radiant heat exchange is the only means by which heat flows without the aid of a contact medium. It occurs by the transfer of electromagnetic energy between an organism and its environment. Radiative exchange is described as:

$$Q_r = A_r \sigma [(eT_s^4) - (aT_e^4)]$$

Where; Q_r is the radiative heat flux (cal sec^{-1}), A_r is the effective radiant-surface area of the animal (m^2), a is the absorptivity of animal surface for thermal radiation, σ is Stefan-Boltzmann constant, T_e is the average absolute temperature of animal's radiant environment

(°K), e is the average emissivity of environmental surfaces for thermal radiation, and T_s is the average absolute temperature of animals radiant surface (°K).

Heat exchange by radiation involves electromagnetic waves and consists of heat transfer within the visible and infrared portions of the electromagnetic spectrum. The amount of heat transfer is determined by the color of the surface within the visible spectrum 0.38-0.78 μm wavelengths (Cena and Monteith, 1975). Heat transfer within the infrared spectrum involves the emission of electromagnetic waves. These waves will transmit energy either away or towards an object, with the net direction dependent on the surface-temperature gradient (Curtis, 1983). Further, heat energy is gained in the visible spectrum and lost in the form of thermal radiation by the animal.

Evaporation

Evaporation is a powerful means of dissipating heat using vapor pressure gradients independent of temperature. This is a useful method of heat loss when temperature gradients become too narrow (or negative) for radiative, conductive or convective heat losses (Curtis, 1983). The latent heat of vaporization for water is $596 \text{ cal} - .56 \text{ kcal/T cal g}^{-1}$, where T is water temperature (°C). For every gram of water evaporated at 25°C, 582 cal or .58 kcal of heat is lost (Curtis, 1983). In other words, evaporation can effectively reduce the temperature gradient and allow animals to dissipate body heat even as ambient temperature approaches or exceeds core body temperature. Unlike humans and some livestock species (i.e. cattle, horses, sheep), pigs do not possess functional sweat glands and thus cannot effectively utilize evaporation without behavioral wetting of the skin (Ingram, 1965; Brown-Brandl et al., 2004). Further, as the opportunity for behavioral wetting is virtually impossible in commercial practice (without the use of sprinklers), pigs must instead rely solely on

increasing respiration rate (RR) as their primary route of latent heat loss (Baumgard et al., 2012).

Respiration

Increasing RR is employed by nearly all heat-stressed animals and effectively decreases a heat load through the evaporation of water from the respiratory tract (Robertshaw, 1985). This is particularly important for pigs as they do not possess functional sweat glands and must rely solely on heat dissipation through the skin or respiratory tract evaporation during times of HS (Ingram, 1965). Elevated respiratory ventilation (panting) due to heat exposure involves increased ventilation of dead space without a subsequent change in alveolar ventilation. This is because the frequency of RR is increasing while the tidal volume decreases (Robertshaw, 1985).

Countercurrent heat exchange occurs in the mucosa of the upper respiratory tract, where deep body heat and moisture are provided by the blood to the nasal mucosa, while cooled blood drains into the venous sinuses at the base of the skull. There, it joins blood draining from the head and encircles the rete mirabile (a network of veins and arteries) to utilize countercurrent blood flow and maintain a temperature gradient (Robertshaw, 1985). As deep body temperature increases, RR will increase until it reaches a maximal value (Findlay and Whittow, 1966). In mild HS, an increase in RR and minute volume (volume of gas inhaled or exhaled) occurs with a corresponding decrease in tidal volume; however, a rise in core body temperature will cause the RR to decrease while the tidal and minute volume increases (Findlay and Whittow, 1966; Whittow, 1971). The transition from elevated RR with low tidal volume to reduced RR and increased tidal volume represents a breakdown in

thermal polypnea as a physiological mechanism permitting maximum respiratory cooling without disturbing blood gas homeostasis (Whittow, 1965, 1971).

Feed Intake

The primary consequence of HS on whole-body energy balance is the reductive effect it has on feed intake (FI), and this has been well-documented in livestock species (Reneau, 2008, 2010; Baumgard and Rhoads, 2013). In general, consuming feed increases basal heat production (i.e. the thermic effect of feeding; Curtis, 1983), which is a disadvantage during HS and requires enhanced heat loss to maintain homeothermy (Whittow, 1971; Speakman and Krol, 2010). The thermic effect of feeding is defined as the difference between total heat production minus fasting heat production and heat production due to physical activity (Van Milgen and Noblet, 2000). Processes such as nutrient fermentation and metabolism are thought to be the primary contributors to FI-induced heat production (Curtis, 1983; Van Milgen and Noblet, 2003). Heat production by fermentation occurs due to heat produced anaerobically by microbes in the digestive tract (Curtis, 1983). In ruminants for example, the amount of heat produced by this process can range from about 0.8 kcal hr⁻¹ per kg of concentrates, to about 0.4 kcal hr⁻¹ per kg of roughages and accounts for approximately 5% of a ruminant animals total heat production under normal conditions (Curtis, 1983). In addition, nutrient metabolism also contributes to the thermic effect of feeding since the bioconversion of nutrients is an inefficient process resulting in the loss of consumed energy as heat (Van Milgen and Noblet, 2003). By reducing FI during times of HS, pigs can help balance their heat production with their heat loss. Table 1.3 demonstrates the impact of HS on dry matter intake of various species under constant or diurnal patterns of HS.

Table 1.3: The impact of heat stress (HS) on dry matter intake (DMI) of various species.

Species	HS Pattern	Max (°C)	Min (°C)	↓ DMI	Reference
Pigs	Constant	34.1	34.1	27.5%	3, 4, 9, 10
Pigs	Diurnal	35.0	24.8	17.0%	1, 5
Cows	Constant	29.0	29.0	35.8%	11, 13
Steers	Constant	35.0	35.0	35.4%	2, 8
Steers	Diurnal	40.0	29.4	9.5%	7
Chickens	Constant	35.0	35.0	52.0%	6
Chickens	Diurnal	35.0	23.9	24.0%	6
Rodents	Constant	31.0	31.0	9.9%	12

1. Becker et al., 1992

2. Brown-Brandl et al., 2003

3. Collin et al., 2001

4. Kim et al., 2009

5. Lopez et al., 1991

6. Mashaly et al., 2004

7. O'Brien et al., 2010

8. Pereira et al., 2008

9. Renaudeau et al., 2008

10. Spencer et al., 2005

11. Spiers et al., 2004

12. Spiers et al., 2005

13. Wilson et al., 1998

Van't Hoff Arrhenius Equation and the Q₁₀ Effect

Although seemingly counterintuitive, it is thought that HS increases metabolic rate and maintenance costs, and this is primarily attributed to the Van't Hoff Arrhenius equation and the Q₁₀ effect. The Van't Hoff Arrhenius equation is a formula stating that chemical reactions are temperature dependent and that the rate of a reaction will increase as temperature increases (Van't Hoff, 1898). The Van't Hoff Arrhenius equation is as follows:

$$k = Ae^{-Ea/(RT)}$$

Where; k is the rate constant of a chemical reaction, A is the pre-exponential factor, Ea is the activation energy, R is the universal gas constant, and T is the absolute temperature (in Kelvin). With this equation, the rate of a chemical reaction can be determined based on the temperature it occurs in (Van't Hoff, 1898), and it is thought to partially explain the enhanced energy expenditure during HS due to greater chemical reaction rates and ultimately increased maintenance costs (Kleiber, 1961; Fuquay, 1981).

Alternatively, the Q_{10} effect states that the rate of the reaction will increase for every 10-degree rise in temperature (T; Whittow, 1971). The Q_{10} equation is as follows:

$$Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)}$$

Where; R_2 and R_1 are the final and initial rates of the reaction, and T_2 and T_1 are the final and initial temperatures at which the reaction takes place. Using this equation, it can be determined if the rate of a reaction is temperature independent ($Q_{10} > 1$) or dependent ($Q_{10} < 1$). The more temperature dependent a physiological process is the greater its Q_{10} value becomes, thus increasing the need to reduce heat production (i.e. reduced FI) and decrease the Q_{10} value (Whittow, 1971).

Adaptation and Acclimation

In the long-term, the normal animal response to HS is adaptation, and this can be achieved through both physical and physiological changes over time (Yousef, 1985). Adaptation is defined as a change that reduces the physiological strain produced by a stressful component of the total environment over an animal's lifetime (Yousef, 1985). It involves either genotypic adaptations that can occur across generations by influencing changes in a species to favor survival in a particular environment, or phenotypic alterations that reduce physiological strain placed on an animal within its lifetime (Yousef, 1985). Physical adaptations often occur due to genetic selection or heritable characteristics transferred across generations. For example, pigs (Renaudeau et al., 2008) and cattle (Robertshaw et al., 1985) from subtropical regions are better adapted to HS than those from temperate areas due to physical adaptations (i.e. hair coat color and consistency, limb size, and skin thickness; Brody 1956; Robertshaw, 1985; Renaudeau et al., 2008). Physiological adaptations can be metabolic or physiologic adjustments within the cell or tissues resulting

from a long-term stress exposure that improves the ability of the animal to cope with a subsequent challenge (Young et al., 1989). Unlike physical adaptations, physiological adaptations do not involve the passage of genetic material from one generation to the next to allow an individual to survive (Young et al., 1989). Instead, they involve changes in metabolism, blood flow and sweating that allow for long-term animal survival (Young et al., 1989).

Sometimes confused with adaptation, acclimation is defined as the functional compensation over a period of days to weeks in response to a single environmental factor as in controlled experiments used as models to predict animal response to climate change (Gaughan, 2012). Typically these studies are short term and do not truly reflect long-term adaptations. Acclimation is illustrated by gradual reductions in core temperature, improved feed intake, or reduced respiration rates in response to HS (Gaughan, 2012). Further, the acclimation response is highly variable between individuals and may occur at different rates or stages throughout the period of the insult (Gaughan, 2012).

Nutrient Metabolism and Partitioning

Nutrient metabolism is defined simply as the utilization of foods by living organisms for normal growth, reproduction and maintenance of health (Slavin, 2013). Nutrients in the organic or carbon-containing group make up the bulk of diet and provide energy and essential organic compounds required for life. Organic macronutrients include carbohydrates (sugar and starches), protein, and fats, which are obtained through the digestion and metabolism of various plant and animal foods. Although many energy sources can be

considered nutrients, the aforementioned are generally considered the primary sources of energy in living systems (Slavin, 2013).

Carbohydrates

Carbohydrates are the most abundant organic components in most fruits, vegetables, legumes and cereal grains, and typically provide all the dietary glucose used by omnivore monogastrics (Leturque and Brot-Laroche, 2013). Glucose is an essential energy source in tissues that may be derived from the diet (sugars and starches), glycogen stores, or by synthesis *in vivo* from gluconeogenic precursors such as amino acid carbon skeletons (Leturque and Brot-Laroche, 2013). Additionally, glucose serves as a precursor for lactose in milk production, ribose in nucleic acid synthesis, and the sugar residues found in covalently bound constituents of glycoproteins, glycolipids, and proteoglycans in the body (Leturque and Brot-Laroche, 2013). Although carbohydrates can be classified into available (sugar and starch) or nonavailable (fiber) sources, most in the modern commercial pig diet fall into the available category.

Digestion and Absorption

Digestible carbohydrates include: monosaccharides, disaccharides, oligosaccharides, and polysaccharides (Leturque and Brot-Laroche, 2013). Digestion begins at the mouth by action of the enzyme α -amylase (Leturque and Brot-Laroche, 2013). Production and secretion of α -amylase is restricted to the salivary and pancreatic exocrine glands located in the mouth and small intestines, respectively. As food is chewed and mixed with saliva, α -amylase starts the process of carbohydrate breakdown, but its action is halted once the food mixes with the acidic secretions of the stomach since α -amylase activity requires neutral pH (Rosenblum et al., 1988). Once in the stomach, food is mechanically and chemically

digested and it enters the duodenum where pancreatic α -amylase breaks down poly- and oligosaccharides into monosaccharides (glucose and fructose) for absorption (Leturque and Brot-Laroche, 2013). Glucose is transported into the enterocyte by SGLT transporters that function using a sodium-potassium pump, while fructose passes through by facilitated diffusion using the GLUT5 transporter (Leturque and Brot-Laroche, 2013). The absorbed glucose and fructose is then transported into portal circulation by GLUT2 and travels to the liver and peripheral tissues using specific GLUT transporters (Table 1.4; Leturque and Brot-Laroche, 2013).

Table 1.4: Glucose transporters and their function¹.

Transporter	Function
1. GLUT1	- Glucose to brain
2. GLUT2	- Glucose to portal circulation
3. GLUT3	- Glucose into neurons and placenta
4. GLUT4	- Insulin dependent glucose transporter present in muscle and adipose
5. GLUT5	- Fructose transport into enterocyte
6. GLUT6	- Unknown
7. GLUT7	- Uptake of residual glucose/fructose
8. GLUT8	- Glucose transport to gametes
9. GLUT9	- Unknown
10. GLUT10	- Unknown
11. GLUT11	- Muscle specific fructose transporter
12. GLUT12	- Unknown

¹Adapted from Zhao and Keating, 2007

Synthesis and Oxidation

Following glucose absorption into portal circulation, it moves through the liver and to peripheral tissues as an energy source. Since some tissues are entirely dependent on glucose as an energy source (red blood cells, brain), it is critical for the body to maintain its glucose supply (McGrane, 2013). To maintain blood glucose within its strictly regulated

concentration range (4 to 6 mmol/L in humans), the body is able to produce glucose by breaking down hepatic glycogen stores and by endogenous biosynthesis (McGrane, 2013). The balance among the glucose oxidation, biosynthesis, and glucose storage is dependent upon the hormonal and nutritional state of the cell, tissue and organism.

Glycolysis begins the oxidation process of glucose. It involves the catabolism of glucose (6 carbons) to two pyruvates (3 carbon/pyruvate) in a series of reactions (Berg et al., 2007), and acts as an intermediate for other pathways such as lipid metabolism, the pentose phosphate pathway, and the TCA cycle. In addition to oxidizing pyruvate, the TCA cycle is also involved in some pathways of glucose synthesis (gluconeogenesis). Gluconeogenesis results in the generation of glucose from non-carbohydrate carbon substrates such as propionate, pyruvate, lactate, glycerol, and gluconeogenic amino acids (Berg et al., 2007). Primarily, gluconeogenesis occurs in the liver during periods of fasting, starvation, low dietary carbohydrates, and intense exercise, and is highly endergonic until ATP or GTP are utilized, making the process exergonic (Berg et al., 2013).

Heat Stress and Carbohydrate Metabolism

During HS, carbohydrate utilization is amplified (Streffer, 1988), and this has been demonstrated in pigs (Pearce et al., 2013a) and cattle (Wheelock et al., 2010). Acute HS was first reported to cause hypoglycemia in cats (Lee and Scott, 1916) and originally thought to be responsible for reduced worker productivity in summer months (Baumgard and Rhoads, 2013). Additionally, athletes exercising at high ambient temperatures have consistently elevated hepatic glucose production and whole-body enhanced carbohydrate oxidation at the expense of lipids (Fink et al., 1975; Febbraio, 2001). Moreover, exogenous glucose is unable to blunt hepatic glucose output (Angus et al., 2001), likely due to increased glycogenolysis

(Febbraio, 2001), and gluconeogenesis (Collins et al., 1980). A proposed mechanism for the enhanced hepatic glucose output is increased pyruvate carboxylase expression (a rate limiting enzyme that controls lactate and alanine entry into the gluconeogenic pathway) during times of HS (O'Brien et al., 2008; White et al., 2009), likely resulting from increased plasma lactate (presumably due to an increase in muscle lactate production; Hall et al., 1980; Elsasser et al., 2009).

Despite the well-documented reduction in nutrient intake and increase in body weight loss (Baumgard and Rhoads, 2013), heat-stressed animals are often hyperinsulinemic (Wheelock et al., 2010; O'Brien et al., 2010; Pearce et al., 2013a). This increase in insulin (a potent anabolic hormone) during catabolic conditions (i.e. HS) is a biological paradox, but an explanation may include insulin's key role in activating and up-regulating heat shock proteins (Li et al., 2006). Regardless of the reason, HS is one of the very few non-diabetic models where nutrient intake is markedly reduced but basal and stimulated insulin levels are increased. Table 1.5 shows the impact of heat stress on biomarkers of post-absorptive carbohydrate metabolism.

Table 1.5: The impact of heat stress on parameters of carbohydrate metabolism.

Parameter	Species	Response	Reference
<u>Blood</u>			
Alanine	Pigs	Increase	9
	Rodents	Increase	26
	Humans	Increase	21
Glucose	Humans	Increase	1, 4, 6
	Rats	Decrease	17, 18
	Rats	Increase	25
	Pigs	Increase	20
	Chickens	Decrease	7, 32
	Cows	Decrease	22, 23, 24, 29
	Lambs	Decrease	16
	Cats	Decrease	14
Insulin	Cattle	Increase	19, 29
	Pigs	Increase	20
	Rats	Increase	27
	Humans	None	11
	Lambs	Increased	16
	Buffalo	Reduced	10
	Lactate	Pigs	Increase
	Cattle	Increase	3
	Humans	Increase	6, 13, 15, 21
	Dogs	Increase	13
Pyruvate	Pigs	Increase	9
	Rodents	Increase	26
	Dogs	Increase	13
<u>Tissue</u>			
Pyruvate Carboxylase	Cattle	Increase	19, 28, 30, 31
	Coral	Increase	12
Gluconeogenesis	Humans	Increase	1, 2, 4, 5, 8
	Rats	Increase	17
	Coral	Increase	12
1. Angus et al., 2001	9. Hall et al., 1980	17. Miova et al., 2013	25. Simon, 1953
2. Collins et al., 1980	10. Haque et al., 2012	18. Mitev et al., 2005	26. Teague et al., 2007
3. Elsasser et al., 2009	11. Kappel et al., 1997	19. O'Brien et al., 2010	27. Torlinska et al., 1987
4. Febbraio et al., 1994	12. Kenkel et al., 2013	20. Pearce et al., 2013a	28. Wheelock et al., 2008
5. Febbraio et al., 2001	13. Kozlowski et al., 1985	21. Pettigrew et al., 1974	29. Wheelock et al., 2010
6. Fink et al., 1975	14. Lee and Scott, 1916	22. Rhoads et al., 2007	30. White et al., 2009
7. Frascella et al., 1977	15. Lorenzo et al., 2010	23. Rhoads et al., 2009	31. White et al., 2012
8. Gonzalez-Alonso et al., 1999	16. Mahjoubi et al., 2014	24. Shwartz et al., 2009	32. Yalcin et al., 2009

Protein

Proteins are a distinct class of biological molecules made of amino acid residues linked by peptide bonds. They are involved in virtually every intracellular process and have a wide range of functions as enzymes, transcription factors, binding proteins, transmembrane transporters, and hormones (Stipanuk, 2013). Animals must consume proteins to meet their essential amino acid requirements (Stipanuk, 2013). As such, protein is considered an essential macronutrient that is vital to all life processes.

Digestion and Absorption

Enzymatic digestion of protein begins in the stomach by pepsin. Pepsin is a digestive enzyme that begins as the zymogen pepsinogen (secreted by chief cells) and is eventually transformed to pepsin by HCl (secreted by parietal cells; Moughan and Stevens, 2013). It acts to hydrolyze proteins and break peptide linkages that hold together amino acid residues (Moughan and Stevens, 2013). After transport to the duodenum, amino acids are broken into di- and tri- peptides and free amino acids by pancreatic peptidases, which are released as inactive zymogens from the pancreas using secretin (Moughan and Stevens, 2013). After the release of trypsinogen, it is transformed by enterokinase to its active form trypsin. Trypsin activates chymotrypsinogen to chymotrypsin, proelastase to elastase, and procarboxypeptidase A and B to carboxypeptidase A and B (Moughan and Stevens, 2013). The resulting products of protein breakdown include oligopeptides, di- and tripeptides, and some free amino acids.

Most absorption of free amino acids occurs at the brush border through multiple energy dependent systems with overlapping specificity for amino acids (Moughan and Stevens, 2013). For oligopeptide absorption, Pept1 is used while Pept2 is used for the

absorption of di- and tri- peptides in a Na⁺ independent, proton (H⁺) electrochemical potential manner (Liebach and Ganapathy, 1996). Alternatively, free amino acids (L-stereoisomers) are absorbed through Na⁺ dependent pathways. Once in the enterocyte, glutamine is extensively metabolized as an energy source to provide ATP, thus sparing dietary glucose and fatty acids for use by other tissues (Moughan and Stevens, 2013). Amino peptidases within the cytosol of the enterocytes hydrolyze peptides into constituent free AA that are then transported across the basolateral membrane to provide energy and building blocks for proteins (Moughan and Stevens, 2013).

Synthesis and Degradation

Protein synthesis and degradation encompass the interchange between body protein and the pool of free amino acids (Figure 1.1; Anthony and McNurlan, 2013). Synthesis of protein occurs on ribosomes located in the cytosol (either free or bound to the endoplasmic reticulum), and is required for generation of new tissue during times of growth and pregnancy, as well as maintenance of existing tissues (Anthony and McNurlan, 2013). The maintenance function of synthesis encompasses the replacement of proteins that are degraded as well as the synthesis of regulatory proteins when needed. This occurs in response to the degradation of body proteins by intracellular enzymes, particularly by the proteolytic subunits of protein-digesting bodies (proteosomes), and by acid hydrolyses in the lysosomes of cells (Anthony and McNurlan, 2013). This process is known as protein turnover (Figure 1.1; Anthony and McNurlan, 2013).

Protein synthesis is initialized by the mammalian target of rapamycin (mTOR) pathway; a protein that regulates cell growth, proliferation, motility, and protein synthesis by integrating input from insulin, insulin like growth factors (IGF-1 and IGF-2), and amino

acids (particularly leucine; Anthony and McNurlan, 2013). Once activated, mTOR phosphorylates S6K1 and 4E-BP1 to stimulate the activation of S6 ribosomal protein (component of the ribosome), and phosphorylate 4E-BP1, respectively. Activation of the S6 ribosomal protein allows mRNA to attach to the ribosome for translation, while 4E-BP1 phosphorylation unbinds eIF4E from the 5' capped mRNA's, allowing them to be transcribed (Anthony and McNurlan, 2013).

Transcription begins the process of protein synthesis in which an mRNA chain, is generated with one strand of the DNA double helix within the nucleus (Anthony and McNurlan, 2013). The DNA is “unzipped” by helicase leaving the single nucleotide chain open to be copied. RNA polymerase reads the DNA strand from the 3' to the 5' end while mRNA is transcribed from the 5' to 3' ends (after eIF4E is unbound). The actual synthesis of protein is known as translation, which occurs in the endoplasmic reticulum or cytosol on ribosomes. During this phase, mRNA is decoded to produce a specific polypeptide according to the genetic code. This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein. It occurs in four phases: activation, initiation, elongation, and termination. During activation, the correct amino acid is joined to the correct tRNA (transfer RNA). Initiation involves a small subunit of the ribosome (S6 ribosomal protein) binding to the 5' end of the mRNA. Elongation occurs when the next aminoacyl-tRNA in line binds to the ribosome along with GTP and an elongation factor. Termination occurs when a stop codon releases the polypeptide chain. Following translation, proteins fold into their secondary and tertiary structures and are released to perform their specific function.

Proteolysis is the breakdown of proteins into smaller polypeptides by the hydrolysis of the peptide bond. While some proteins (collagen and hemoglobin) are relatively resistant to degradation, those important in regulatory function or damaged are readily destroyed (Anthony and McNurlan, 2013). Amino acid degradation involves the removal of nitrogen from an amino acid, and the catabolism of the carbon skeleton (Anthony and McNurlan, 2013). As a result, the carbon skeleton provides energy either directly via oxidation or through the formation of glucose and fatty acids (Anthony and McNurlan, 2013). Additionally, amino acid carbon skeletons are recycled and used to form new amino acids. Protein can be targeted for degradation by either the ubiquitin-proteasome pathway or by lysosomal degradation (Anthony and McNurlan, 2013). The ubiquitin-proteasome pathway functions by binding ubiquitin to target abnormal or regulatory proteins for degradation. These “marked” proteins are then degraded by a large (26 subunit) multicatalytic protease complex known as a proteasome (Anthony and McNurlan, 2013). The ubiquitin-proteasome pathway is important in the accelerated protein degradation that accompanies many catabolic states. Lysosomal protein degradation functions mainly to degrade hormones (i.e. insulin) and eliminate their signal. In this process, vesicles are formed as portions of the cell membrane enclose a portion of extracellular matrix by endocytosis. These newly formed vesicles will then fuse with organelles (primarily lysosomes) within the cell and are enzymatically (cathepsins B, D, L, H) degraded (Anthony and McNurlan, 2013). Although very effective in eliminating membrane bound hormones, this process is less selective than the ubiquitin-proteasome pathway since entire portions of the cellular membrane are engulfed non-selectively (Anthony and McNurlan, 2013).

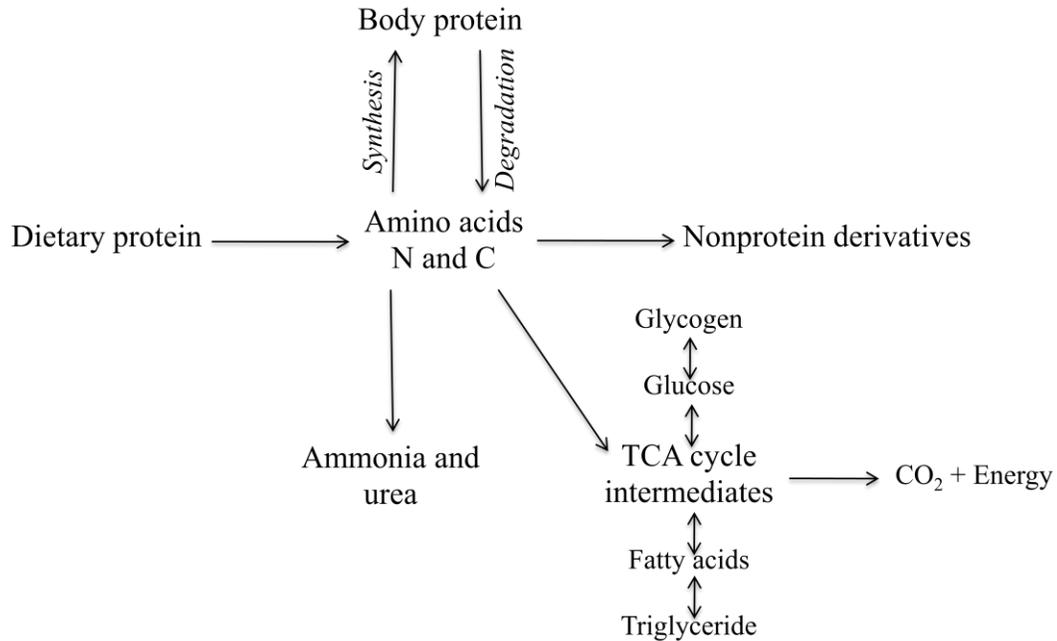


Figure 1.1: Protein turnover; Adapted from Anthony and McNurlan, 2013.

Heat Stress and Protein Metabolism

During periods of inadequate nutrient intake or disease, skeletal muscle amino acids are mobilized by protein catabolism to provide substrates to support energy metabolism (Rhoads et al., 2013). Similarly, HS can negatively affect post-absorptive protein metabolism as indicated by changes in the quantity of carcass lean tissue in growing livestock species (Collin et al., 2001; Brown-Brandl et al., 2004; Lu et al., 2007). This is because muscle protein synthesizing machinery and RNA/DNA synthetic capacity are reduced (Streffer, 1982), while skeletal muscle catabolism is increased (Bianca, 1965; Yunianto et al., 1997; Wheelock et al., 2010). Although it is known that protein breakdown is increased during HS, it is unclear if this is a result of enhanced rates of protein catabolism or a direct result of heat-induced muscle damage (Rhoads et al., 2013). It has been demonstrated that heat-stressed pigs (Pearce et al., 2013a), cows (Shwartz et al., 2009), and heifers (Ronchi et al., 1999) have increased plasma urea nitrogen concentrations compared to

thermal neutral controls (Rhoads et al., 2013). A more accurate indicator of muscle catabolism is circulating 3-methyl histidine or creatine, both of which are increased in heat-stressed poultry (Yunianto et al., 1997), rabbits (Marder et al., 1990), pigs (Pearce et al., 2013a), lactating cows (Schneider et al., 1988), and exercising men (Febbraio et al., 2001). In addition, evidence indicates that HS directly alters protein metabolism by decreasing milk protein synthesis in dairy cattle compared to their pair-fed counterparts (Rhoads et al., 2009). The reduction in protein synthesis and subsequent increase in catabolism is perplexing since HS causes an increase in plasma insulin, a promoter of protein synthesis (Baumgard and Rhoads, 2013). Table 1.6 demonstrates the impact of heat stress on biomarkers of proteolysis.

Table 1.6: The impact of heat stress on blood biomarkers of proteolysis.

Biomarker	Species	Response	Reference
Plasma urea nitrogen	Pigs	Increase	6
	Heifers	Increase	7
	Cows	Increase	4, 9, 10, 12
	Cows	Decrease	11
	Humans	Increase	3
	Rats	Increase	2
3-Methyl histidine	Poultry	Increase	13
	Rabbits	Increase	5
	Pigs	Increase	6
	Cows	Increase	4, 8
	Humans	Increase	1
Creatine	Poultry	Increase	13
	Rabbits	Increase	5
	Pigs	Increase	6
	Cows	Increase	8, 11
	Humans	Increase	3

1. Febbraio et al., 2001

2. Francesconi and Hubbard, 1986

3. Hart et al., 1980

4. Kamiya et al., 2006

5. Marder et al., 1990

6. Pearce et al., 2013a

7. Ronchi et al., 1999

8. Schneider et al., 1988

9. Settivari et al., 2007

10. Shwartz et al., 2009;

11. Srikandakumar and Johnson, 2004

12. Wheelock et al., 2010

13. Yunianto et al., 1997

Lipid

Lipids play a major role in the normal function of living organisms. They serve as an energy source, structural components of membranes, lubricants, and as signaling molecules (Sul, 2013). Although a variety of lipids are consumed in the diet, non-polar triacylglycerols (TAG) represent the largest portion and provide the essential fatty acids (Brenna and Sacks, 2013). All triacylglycerols contain fatty acids esterified to glycerol and are important sources of fatty acids for oxidation to supply energy, essential fatty acids, and the gluconeogenic substrate glycerol (Brenna and Sacks, 2013). In animals, lipids are stored in specialized cells called adipocytes and their fatty acid composition consists primarily of palmitate (16:0), stearate (18:0), and oleate (18:1 cis 9; Brenna and Sacks, 2013).

Digestion and Absorption

Following lipid ingestion, fats enter the stomach via the esophagus and form large lipid droplets. Lipid droplets enter the duodenum then form into micelles. The low pH triggers the release of secretin and cholecystokinin to stimulate the release of bicarbonate (increase pH), bile (detergent), and pancreatic lipases (Brannon et al., 2013). Bile acts on micelles to increase the surface area so that pancreatic lipases can effectively hydrolyze TAG to monoacylglycerols (MAG). Monoacylglycerols are incorporated into the mixed micelle (MAG, diacylglycerols, peptides, other dietary constituents), and travel to the brush border to enter the enterocyte via passive diffusion down a concentration gradient, or by carrier mediated diffusion through use of fatty acid transport protein 4, which guarantees absorption of fatty acids in times of reduced dietary intake (Brannon et al., 2013). Once in the enterocyte, fatty acids containing greater than 10 – 12 carbons (long chain fatty acids) are re-esterified into TAG via the monoacylglycerol pathway using MAG acyltransferase, while

those containing fewer than 10 – 12 carbons (short chain fatty acids) pass directly into portal circulation (Brannon et al., 2013). Re-esterified TAGs enter the endoplasmic reticulum (ER) and are collected as large fat particles. There, they receive a layer of apolipoproteins to stabilize them in an aqueous environment, forming a lipoprotein (Brannon et al., 2013). Lipoproteins are pinched off the ER and fuse with the Golgi apparatus where a carbohydrate is attached to the protein coat forming the chylomicron (CM), which is exocytosed into the lymphatic system (Brannon, et al., 2013). Once in the lymphatic vessels, CM are eventually released into the bloodstream (aortic arch) at a slow rate to prevent large changes in lipid concentration of peripheral blood (Brannon et al., 2013).

Synthesis and Oxidation

The synthesis of lipids, or lipogenesis, occurs by either dietary intake of fat or by *de novo* lipogenesis in the liver (i.e. humans) or adipose tissue (i.e. pigs, cattle; Figure 1.2). Lipogenesis occurs when an organism is in a “fed state” and blood glucose and insulin levels are elevated. During this process, CM and very low-density lipoproteins (VLDL) attach to lipoprotein lipase (LPL) on the surface of adipocytes, which acts as a dual receptor and enzyme to hydrolyze TAG in the CM and VLDL to three free fatty acids (FFA) and one glycerol (Sul, 2013). The three FFA enter the adipocyte while the glycerol travels to the liver to be metabolized. Concurrently, insulin is bound to its receptor resulting in dephosphorylation of hormone sensitive lipase (HSL), and simultaneously promoting uptake of glucose by the GLUT-4 transporter (Sul, 2013). Glucose is converted to glyceraldehyde-3-phosphate by glycolysis, and then combines with FFA to form new TAG in the adipocyte.

Lipolysis involves the hydrolysis of TAG into FFA (Berg et al., 2007; Sul, 2013). Hormones that initiate lipolysis are epinephrine, cortisol, norepinephrine, and glucagon, and

these act through G-protein coupled receptors (β -adrenergic receptors) on the surface of adipocytes. Lipolytic hormones are released during the “fasted state” when blood glucose and insulin levels are low. Once these hormones attach to their respective receptors, they cause activation of adenylate cyclase, which increases the production of cyclic AMP (cAMP) to phosphorylate protein kinase A (PKA; Berg et al., 2007; Sul, 2013). Protein kinase A phosphorylates HSL (activates) and Acetyl CoA carboxylase (ACC; deactivates). Hormone sensitive lipase hydrolyzes TAG into glycerol and FFA. Glycerol is transported to the liver and is converted to glycerol – 3 – phosphate by glycerol kinase, then dihydroxyacetonephosphate, then to glyceraldehyde-3-phosphate (Berg et al., 2007; Sul, 2013). Free fatty acids bind to serum albumin and are transported to tissues for conversion into Acetyl – CoA (2-carbons) by β -oxidation to enter the TCA cycle (Berg et al., 2007; Sul, 2013).

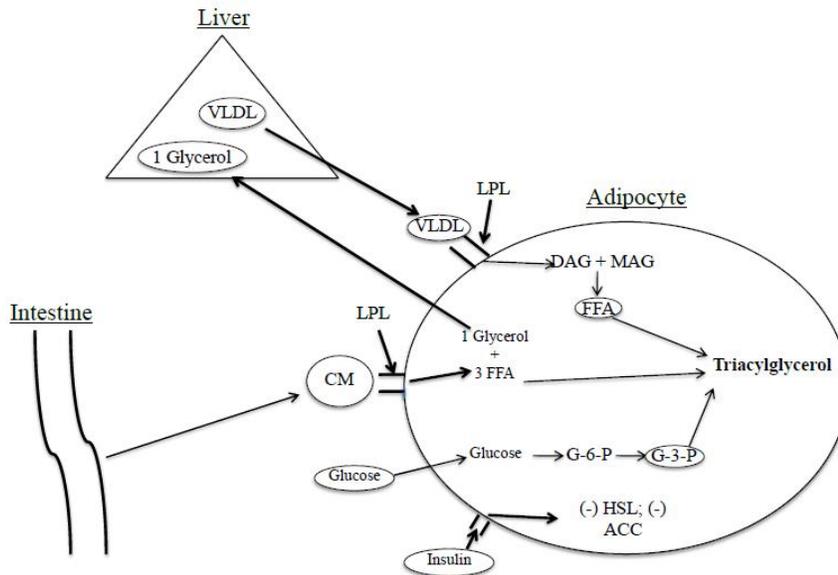


Figure 1.2: Lipogenesis; Adapted from Sul, 2013.

Heat Stress and Lipid Metabolism

Production and observational data indicates that HS impacts lipid metabolism differently than would be expected based on calculated whole body energy balance (Rhoads et al., 2013). Additionally, carcass data indicates that both chickens (Geraert et al., 1996) and pigs (Collin et al., 2001; Brown-Brandl et al., 2004) have increased lipid retention when reared in HS conditions and the effects in chickens are most pronounced in the abdominal fat depot (Yunianto et al., 1997). Interestingly, plasma non-esterified fatty acids (NEFA) concentrations are typically reduced in HS exposed sheep and cattle, despite marked reductions in feed intake (Sano et al., 1983; Ronchi et al., 1999; Shwartz et al., 2009) and especially when compared to pair-fed controls (Rhoads et al., 2009). Alterations in carcass lipid composition and serum metabolites agree with rodent results indicating that HS reduces *in vivo* lipolytic rates and *in vitro* lipolytic enzyme activity (Torlinska et al., 1987). Considering that HS causes a well-described increase in stress and catabolic hormones (i.e. epinephrine, cortisol, glucagon; Beede and Collier, 1986), it is surprising that lipolytic capacity is reduced (Baumgard and Rhoads, 2013).

Changes in lipid metabolism during HS may be a result of increased insulin concentration and/or insulin sensitivity, as it is a potent antilipolytic and lipogenic hormone (Vernon, 1992). In addition, reduced NEFA mobilization may allow for increased circulating insulin as excessive NEFAs cause pancreas β -cell apoptosis (Nelson et al., 2001). Previous studies have demonstrated enhanced insulin sensitivity in rodents (DeSouza and Meier, 1993), pigs (Hall et al., 1980), growing steers (O'Brien et al., 2010), and lactating cows (Wheelock et al., 2010). Moreover, HS increases adipose tissue lipoprotein lipase (Sanders et al., 2009), suggesting that adipose tissue of hyperthermic animals has an

increased capacity to uptake and store intestinal and hepatic derived FFA (Baumgard and Rhoads, 2013). This implies that heat-stressed animals have a limited ability to mobilize adipose tissue with a corresponding increased capacity for lipogenesis, resulting in greater adipose accretion. Table 1.7 shows the impact of heat stress on biomarkers of lipid metabolism.

Table 1.7: The impact of heat stress on biomarkers of lipid metabolism.

Parameter	Species	Response	Reference
<u>Blood</u>			
Cholesterol	Cattle	Decrease	8, 16
	Humans	No change	4
	Pigs	No change	3
Non-esterified fatty acids	Sheep	Decrease	15
	Cattle	Decrease	11, 12, 17, 19
	Rodents	Decrease	2, 6, 7
	Chickens	Decrease	1
	Pigs	Decrease	9
Triglycerides	Rodents	Decrease	5
	Sheep	Decrease	10
	Quail	Decrease	13
<u>Tissue</u>			
Lipoprotein lipase	Rodents	Increase	14
	Cattle	Increase	18

1. Bobek et al., 1997

2. Burger et al., 1972

3. Fletcher et al., 1988

4. Francesconi et al., 1976

5. Francesconi and Hubbard, 1986

6. Frankel et al., 1968

7. Frascella et al., 1977

8. Fuquay, 1981

9. Mitev et al., 2005

10. Nazifi et al., 2003

11. Rhoads et al., 2009

12. Ronchi et al., 1999

13. Sahin et al., 2002

14. Sanders et al., 2009

15. Sano et al., 1983

16. Shehab-El-Deen et al., 2010

17. Shwartz et al., 2009

18. Tao et al., 2013

19. Wheelock et al., 2010

Bioenergetics

Bioenergetics is the study of the flow and transformation of energy in and between living organisms, and between living organisms and their environment. There are four forms of energy in the living organism (chemical, mechanical, electrical, and thermal); however, only chemical energy can be used to create mechanical energy by living organisms. Per the first law of thermodynamics, energy cannot be created or destroyed but can only be converted or transformed from one form to another. In other words, all consumed energy must be partitioned towards metabolism and growth, or waste (i.e. urine, feces, and gas production; Oresanya et al., 2005; Patience, 2012; Figure 1.3). Thermal stress and muscle mass can influence how an animal partitions energy. When animals are cold-stressed, they generate more thermal energy due to enhanced shivering and non-shivering thermogenesis (Kleiber, 1961; Young, 1981), resulting in less net energy available for growth compared to TN conditions (Figure 1.3). Conversely, HS reduces net energy for growth by presumably increasing maintenance costs due to heat mitigation efforts (Kleiber, 1961; Fuquay, 1981), and reducing the consumption of gross energy (Baumgard and Rhoads, 2013; Figure 1.3). Further, lean tissue content can influence maintenance costs as muscle mass is expensive to maintain due to constant protein turnover.

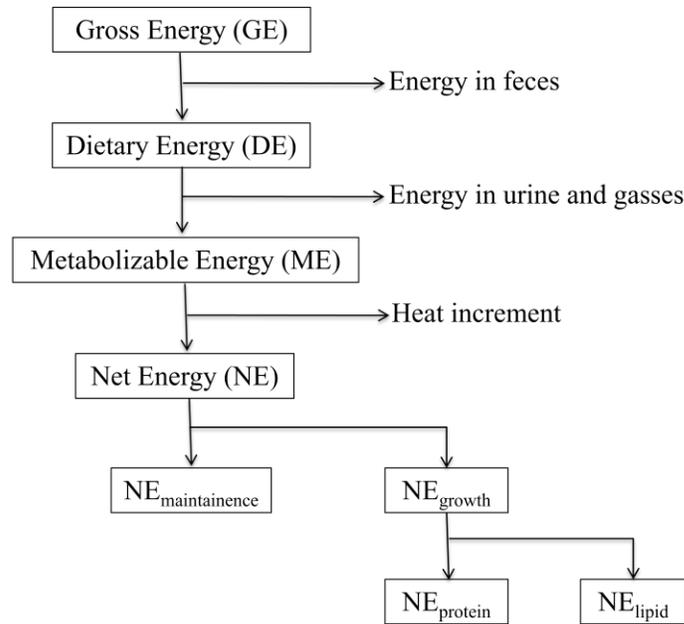


Figure 1.3: Schematic of energy utilization. Adapted from Oresanya et al., 2005 and Patience, 2012.

Porcine Tissue Accretion

In pigs, the rate of lean tissue growth increases after 25 kg of live weight, reaches a plateau (60 - 70 kg BW) then declines thereafter (Schinckel and Richert, 1996; Frank et al., 1998). High lean growth genotypes achieve their maximum lean growth potential later and maintain greater lean growth rates to heavier weights (Frank et al., 1998). A second concept in swine growth is that as feed intake increases, a linear response in lean growth and fat accretion rate occurs. Once pigs reach their maximal lean growth potential, a plateau in lean growth occurs and the rate of adipose deposition is increased (Schinckel and Richert, 1996). Due to the propensity for adipose accretion, the most efficient lean growth can be achieved when pigs consume 95-100% of the energy needed to reach their lean growth potential (Frank et al., 1998). As pigs grow, the slope for lean accretion becomes less steep so that at even 70-80% of normal feed intake maximum lean growth is achieved (Schinckel and

Richert, 1996). This suggests that as pigs reach heavier weights (68 – 110 kg), the slope of lean gain to energy intake decreases rapidly as the slope of adipose gain to energy intake increases (Schinckel and Richert, 1996).

Adipose tissue requires substantially more energy to deposit than lean, and the energy cost of protein gain is less (10.03 Mcal/kg lean) than that of adipose accretion (11.65 Mcal/kg adipose; Patience, 2012). Additionally, due to the water associated with lean tissue accretion, adipose requires three times more energy per kg than fat-free lean growth (Schinckel and Richert, 1996). As pigs age and become heavier, their lean growth potential is reduced while their feed intake increases above that needed for their maximum genetic potential, resulting in a linear increase in adipose accretion (Frank et al., 1998). Further, pigs with high lean growth potential reach their maximum lean growth at heavier weights, maximizing their ability to accrete skeletal muscle.

Prenatal Insults

Prenatal insults can have lasting effects on offspring growth (Tao et al., 2012), behavior (Shiota and Kayamura, 1989), metabolism (Chen et al., 2010) and can be teratogenic (Graham et al., 1998). Experimental models such as intrauterine growth retardation indicate that future growth parameters (Foxcroft et al., 2006; 2009), body composition (Pinney and Simmons, 2010), and post-absorptive metabolism can be markedly altered by gestational insults (Chen et al., 2010). Additionally, Hales and Barker (1992) first introduced the idea of a “thrifty phenotype” in response to fetal nutrient insufficiency, whereby fetal malnutrition causes maladaptive programming resulting in glucose-sparing mechanisms that persist in the offspring. *In utero* HS may also imprint future

thermotolerance to a heat load, and this has been demonstrated in unicellular organisms (Estruch, 2006), insects (Sorensen et al., 2001), and birds (Tzschentke, 2007; Piestun et al., 2008) in response to elevated temperatures. Further, although prenatal insults can result in negative postnatal phenotypes, they may pre-condition offspring to harsh environmental conditions and could be beneficial to future livestock production.

Epigenetics

Epigenetics is literally defined as “above the genome,” and is the study of DNA modifications capable of impacting gene expression, and subsequently the cellular phenotype that is not a result of differences in DNA base pair sequence (Reik, 2007). It occurs via DNA methylation or histone modifications during developmental progression and cellular differentiation towards specific cell lineages (Reik, 2007). Functionally, epigenetic modifications can improve the plasticity of the genome to improve responses to specific environmental conditions, and may provide postnatal protection against a negative environmental insult (Reik, 2007). Further, timing of the gestational insult relative to key developmental events may provide a greater impact on the future performance and epigenetic modifications in offspring (Reik, 2007).

DNA methylation is a stable, long-lasting DNA modification that can be inherited by offspring or acquired throughout an animal’s lifetime (Jones and Takai, 2001). Methylation occurs by the enzymatic transfer of a methyl group from the methyl donor S-adenosyl methionine, through the action of DNA methyltransferases (Jones and Takai, 2001). This often occurs by addition of a methyl group to the cytosine of CpG dinucleotide, the cytosine and guanine-rich regions located upstream of gene promoters (Jones and Takai, 2001). When these regions are hypermethylated, gene expression is typically suppressed through

recruitment of DNA binding proteins that interfere transcription factors (Jones and Takai, 2001). Additionally, methylation is maintained by DNA methyltransferases and is essential for copying existing methylation patterns during replication and development (Klose and Bird, 2006). Methylation may also occur in response to environmental stress and can potentiate a long-term or imprinted response to stress (Baumgard et al., 2012).

Histone modifications epigenetically modify phenotypes by altering histone interactions with DNA and control of histone structure (Turner, 2007); however, unlike DNA methylation, histone modifications can be reversible or static (Turner, 2007). Acetylation is catalyzed by histone acyltransferases and acts to weaken the histone-DNA bond and promote transcription by targeting lysine residues (Turner, 2000). Histone acetylation can be reversed by histone deacetylases to restore the interaction between DNA and histones causing repression of transcription (Turner, 2000). Furthermore, modification of histones can impact histone-DNA binding and further regulate histone modifications.

Maternal Heat Stress

Epigenetic programming and hormonal alterations in prenatally heat-stressed offspring resemble phenotypes of animals affected by intrauterine growth retardation (Baumgard et al., 2012). Phenotypic changes are likely mediated by altered metabolism and uterine blood flow. Negative effects of maternal HS range from developmental defects (Graham et al., 1998) to altered response to HS during future growth. In some non-agricultural species, exposure to prenatal HS causes a reduction in birth weight, average daily gain, and smaller brain weights that last into maturation (Jonson et al., 1976; Shiota and Kayamura, 1989). Although the specific cause of suboptimal performance in offspring

exposed to prenatal HS is currently unknown, it is likely that epigenetic regulation plays a key role in the imprinting of future phenotypes.

How prenatal HS exposure impacts postnatal performance is poorly understood, but is likely a result of epigenetic programming. Epigenetic programming is significantly influenced by differences in DNA methylation of CpG islands (Klose and Bird, 2006), that when negatively impacted during development can have lasting implications on gene expression (Bernal and Jirtle, 2010) and can have a potentially significant impact on lifetime production (Foxcroft et al., 2009). These epigenetic modifications in chromatin structure (which can last short periods or lifelong) influence the condensation of the DNA by reducing the recruitment and ability of DNA binding proteins, such as RNA polymerases, to interact with and transcribe genes from the genome. Intrauterine modifications via DNA methylation can also occur in temporal and tissue specific manners (Schneider et al., 2010), as numerous genes and quantitative trait loci associated with body composition, and growth and development are subject to epigenetic regulation in mammals (Thomsen et al., 2004).

In addition to intrauterine effects, HS during early postnatal development has been shown to cause histone modifications that extend into adulthood. This insult can imprint thermotolerance to HS later in life, and has been demonstrated to increase survivability to a future heat load in *Drosophila buzzatti* (Sorenson et al., 2001), or improves the future ability to remain euthermic during a thermal insult in birds (Tzschentke, 2007; Piestun et al., 2008). In chickens, HS at 3 days of age conferred protection against acute HS-related mortality during adulthood, presumably by increasing H3K9 acetylation and H3K9 demethylation (Yahav and McMurtry, 2001). This epigenetic protective effect has been characterized by analysis of histone modifications and resulting epigenetic memory (Kisliouk et al., 2010).

Histone modifications in response to chronic HS exposure have also been reported in rodents (Tetievsky and Horowitz, 2010); however, animals were exposed to HS only after birth. In this study, histone H3 phosphorylation and subsequent H4 acetylation occurred in the promoters of HSP-70 and HSP-90 protein, likely resulting in increased cryoprotection by heat shock proteins. The impact of *in utero* heat stress on postnatal phenotypes is demonstrated in Table 1.8.

Table 1.8: The impact of *in utero* heat stress on postnatal phenotypes.

Postnatal phenotype	Species	Response	Reference
Growth performance	Cow	Decrease	13
	Rodent	Decrease	11
Teratogenicity	Pig	Increase	2
	Guinea pig	Increase	1, 6
	Rodent	Increase	4, 7, 11
	Monkey	Increase	5
	Human	Increase	8, 9
Thermal tolerance	Yeast	Increase	3
	Insects	Increase	12
	Poultry	Increase	10, 15
	Rodents	Increase	14

1. Edwards, 1969	9. Miller et al., 1978
2. Edwards et al., 2003	10. Piestun et al., 2008
3. Estruch, 2006	11. Shiota and Kayamura, 1989
4. Germain et al., 1985	12. Sorenson et al., 2001
5. Hendrickx et al., 1979	13. Tao et al., 2012
6. Jonson, 1976	14. Tetievsky and Horowitz, 2010
7. Lary et al., 1983	15. Tzschentke, 2007
8. McDonald, 1961	

Maternal Nutrient Restriction

Maternal nutrient restriction negatively affects postnatal phenotypes, and was described in a cohort study of offspring born during the Dutch famine (Ravelli et al., 1976; Barker et al., 1993). In these studies, offspring whose mothers were nutrient restricted during

pregnancy were more likely to become obese and diabetic and have cardiovascular disease later in life (Ravelli et al., 1976, 1999; Roseboom et al., 2006), and these negative effects were most pronounced when nutrient restriction occurred in early compared to late gestation (Roseboom et al., 2006; Table 1.9). Nutrient restriction is also associated with intrauterine growth retardation (IUGR) and is evident by the reduced birth weight of offspring born of mothers who receive inadequate nutrition (Roseboom et al., 2006). Intrauterine growth retardation is caused by insufficient fetal nutrient and oxygen supply and placental development (Foxcroft et al., 2006). In this model of maternal nutrient restriction, epigenetic programming can markedly alter metabolism in the liver (MacLennan et al., 2004), and insulin secretion (Thompson et al., 2010). As consequence, IUGR and malnutrition prior to weaning causes impaired beta cell development in rodents (Garofano et al., 1998), growth potential and carcass quality in pigs (Foxcroft et al., 2006, 2009), and altered immune function (Collier et al., 2011). Furthermore, reduced feed intake may be partially responsible for the negative effects of prenatal HS on postnatal development, however this has yet to be substantiated.

Table 1.9: Phenotypic consequences of maternal nutrient restriction exposure during early, mid and late gestation¹.

Late gestation	Mid gestation	Early gestation
- Glucose intolerance	- Glucose intolerance	- Glucose intolerance
	- Microalbuminuria	- Atherogenic lipid profile
	- Obstructive airway disease	- Altered blood coagulation
		- Obesity
		- Stress sensitivity
		- Coronary heart disease
		- Breast cancer

¹Adapted from Roseboom et al., 2006.

Elevated Maternal Glucocorticoids

During postnatal development, exposure to HS and nutrient restriction causes a variety of endocrine disruptions. These include elevated insulin concentrations and sensitivity (Torlinska et al., 1987; O'Brien et al., 2010; Wheelock et al., 2010; Pearce et al., 2013a), reductions in thyroid hormone levels (Collier et al., 1982), and a marked increase in circulating cortisol, norepinephrine and epinephrine during acute HS exposure (Collier et al., 1982; Baumgard and Rhoads, 2013; Rhoads et al., 2013). As a result of HS or nutrient restriction-induced maternal glucocorticoid production, gestating fetuses are at risk for elevated exposure (Einarsson et al., 1996), resulting in detrimental effects (i.e. altered body composition, cardiovascular disease, altered HPA-axis) during postnatal life.

Glucocorticoids act through receptors expressed in most fetal tissues and have potent effects on development from early embryonic stages (Cole, 1996; Speirs et al., 2004), and in the placenta where they are thought to mediate metabolic and anti-inflammatory effects (Sun et al., 1997). Furthermore, their effects are transduced upon the genome during early developmental stages (Seckl, 2004), and may program the metabolic traits induced by maternal/fetal under nutrition and prenatal stress (Xita and Tsatsoulis, 2010).

Exposure to elevated glucocorticoids *in utero* can negatively affect future post-absorptive metabolism. Numerous studies have discovered a correlation between lower birth weight and the development of metabolic disorders in adult life including hypertension, insulin resistance, and type 2-diabetes (Barker et al., 1993; Lithell et al., 1996; Table 1.10). Interestingly, the low-birth weight syndrome associated with prenatal overexposure to glucocorticoids resembles the rare Cushing's syndrome of glucocorticoid excess where affected individuals suffer disproportionately from type 2 diabetes and insulin resistance,

dyslipidaemia and hypertension (Seckl, 2004). Additionally, insulin resistance has been linked to elevated lipid synthesis and increased gluconeogenesis leading to increased visceral adiposity (Dazert and Hall, 2011), and is likely due to reduced mTOR signaling, which reduces protein synthesis and partitions energy towards lipogenesis (Laplante and Sabatini, 2009). Furthermore, elevated glucocorticoids can cause greater expression of uncoupling proteins 2 and 3 mRNA, which are regulated by β 3-adrenergic agonists and leptin (Gong, et al., 1997).

In addition to low birth weight, insulin resistance, and altered mTOR signaling, overexposure to prenatal glucocorticoids can have negative effects on brain programming and is associated with reduced brain weight and delayed maturation of neurons (Huang et al., 1999; Huang et al., 2001). The negative effects of glucocorticoid exposure on the brain are particularly evident during early fetal development and are due to reduced 11 beta-hydroxysteroid dehydrogenase type 2 (Seckl, 2004). This enzyme is highly expressed in the placenta starting at mid-gestation and is responsible for converting active maternal glucocorticoids into inert 11-keto forms (White et al., 1997). The hypothalamic-pituitary-adrenal axis (HPA-axis) is particularly sensitive to glucocorticoids and their gestational programming actions (Welberg and Seckl, 2001). Prenatal glucocorticoid exposure permanently increases basal plasma corticosterone levels in adult rats (Levitt et al., 1996; Welberg et al., 2001), and this is primarily due to a reduction in the density of glucocorticoid and mineralocorticoid receptors in the hippocampus resulting in a reduction in negative feedback (Seckl, 2004). HPA-axis disruption has also been observed in response to prenatal environmental challenges such as maternal under nutrition, suggesting that altered HPA

programming could be a common outcome of a stressful prenatal environment (Seckl, 2004).

Table 1.10 demonstrates the impact of *in utero* glucocorticoids on postnatal phenotypes.

Table 1.10: The impact of *in utero* glucocorticoids on postnatal phenotypes.

Postnatal phenotype	Species	Response	Reference
HPA-axis dysfunction	Rodents	Increase	11, 17
	Humans	Increase	6
	Sheep	Increase	8
Obesity	Humans	Increase	1, 5, 13
Skeletal muscle mass	Rodents	Decrease	7
Metabolic dysfunction	Humans	Increase	1, 5, 13
	Rodents	Increase	12, 14, 15
	Sheep	Increase	18
Cardiovascular disease	Human	Increase	9
	Baboon	Increase	10
	Sheep	Increase	3, 4, 16
	Rodents	Increase	2

1. Barker et al., 1993	10. Koenen et al., 2002
2. Benediktsson et al., 1993	11. Levitt et al., 1996
3. Berry et al., 1997	12. Lindsay et al., 1996
4. Dodic et al., 2002	13. Lithell et al., 1996
5. Entringer et al., 2008	14. Nytranda et al., 1998
6. Entringer et al., 2009	15. Sugden et al., 2001
7. Gokulakrishnan et al., 2012	16. Tangalakis et al., 1992
8. Hawkins et al., 2000	17. Welberg et al., 2001
9. Kari et al., 1994	18. Whorwood et al., 2001

Summary

Stress is a key-limiting factor to efficient animal production, and it negatively impacts health and development during all lifecycle stages. In particular, environmentally induced hyperthermia undermines substantial advances made by the global animal agriculture industries and jeopardizes the efficient production of high quality animal protein.

Traditionally, HS research has focused on the postnatal production consequences (i.e. FI,

BW, ADG, milk production) of HS. Furthermore, recent reports indicate that altered post-absorptive metabolism may be partially responsible for HS-induced decline in production efficiency. In addition to the postnatal impact of stress, intrauterine stressors (i.e. maternal under nutrition, intrauterine growth retardation) can have a lasting impact on offspring performance and postnatal metabolism. While many studies of *in utero* HS have investigated its teratogenic impact, there is limited knowledge of its effects on postnatal metabolism and bioenergetics in mammalian species.

To better understand the consequences of *in utero* HS on production agriculture, a series of studies were performed to determine its impact on postnatal thermal tolerance and bioenergetics (Chapters II and III), and nutrient partitioning (Chapters IV, V and VI). Pregnant first parity pigs and rats were exposed to either TN or HS conditions for the entire gestation, the first half, or second half of gestation. To account for differences in maternal nutrient intake, we utilized an *ad libitum* thermal neutral control group and a pair-fed thermal neutral control group of rats. Progeny were evaluated for differences in growth performance, nutrient partitioning, thermoregulation, and post-absorptive metabolism.

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CHAPTER II: EFFECTS OF MAMMALIAN *IN UTERO* HEAT STRESS ON ADOLESCENT BODY TEMPERATURE

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Abstract

In-utero hyperthermia can cause a variety of developmental issues, but how it alters mammalian body temperature during adolescence is not well-understood. Study objectives were to determine the extent to which *in-utero* hyperthermia affects future phenotypic responses to a heat load. Pregnant first parity pigs were exposed to thermal neutral (TN; cyclical 15 to 22°C; n = 8) or heat stress (HS; cyclical 27 to 37°C; n = 7) conditions during the entire gestation. Of the resultant offspring, 12 males (n=6, gestational TN (GTN) conditions; n = 6, gestational HS (GHS) conditions) were housed in TN conditions (constant 22.7 ± 2.5°C), and 12 males (n = 6 GTN, n = 6 GHS) were maintained in HS conditions (constant 34.7 ± 2.3°C) for 15 d. Adolescent pigs in HS conditions had increased ($P < 0.01$) rectal temperature (ΔT_{re} ; 0.33°C) and respiration rate (ΔRR ; 44 bpm) compared to TN pigs, regardless of gestational treatment. Within the HS environment, no gestational difference in RR was detected; however, GHS pigs had increased (0.26°C; $P < 0.01$) T_{re} compared to GTN pigs. As T_{re} increased, GTN pigs had a more rapid increase in RR (12 bpm/1°C; $P < 0.01$) compared to the GHS pigs. Adolescent HS decreased nutrient intake (25%; $P < 0.01$), and

body weight gain (34%; $P = 0.04$), but neither variable was statistically influenced by gestational treatments. In summary, *in-utero* HS compromises the future thermoregulatory response to a thermal insult.

Key words: heat stress, mammals, and epigenetics

Introduction

Environmentally induced hyperthermia or heat stress (HS), results from the imbalance between thermal energy flowing into and out of an animal [1], and negatively impacts health and development in all mammalian species studied. Typical HS responses include slower growth rates, altered metabolism, mortality and altered body composition characterized primarily by increased adipose tissue and reduced skeletal muscle mass [2-4]. Moreover, swine genetic selection for lean tissue accretion rates further compromises HS tolerance as synthesizing and maintaining enhanced muscle mass generates increased metabolic heat [5, 6]. Consequently, HS is likely one of the primary factors limiting animal protein production and if the frequency of severe hot weather increases as predicted [7] food security will continue to be compromised during the warm summer months, especially in developing countries [4].

In-utero hyperthermia negatively impacts a variety of fetal development parameters and can be teratogenic [8]. Furthermore, maternal hyperthermia can have a lasting imprint on offspring growth, behavior [9], and metabolism [10]. Heat stress during embryonic development either increases survivability to a future heat load in *Drosophila buzzatti* [11] or improves the future ability to remain euthermic during a thermal insult in birds [12, 13]. Further, thermal conditioning immediately following birth can imprint long-term

thermotolerance in rodents [14]. In the aforementioned reports, thermotolerance is defined as the ability to maintain a lower or safe body temperature in response to a future heat load. However, the extent to which *in-utero* HS affects future body temperature indices in mammals lacks thorough exploration. Study objectives were to characterize HS-induced body temperature indices in adolescent pigs exposed to differing *in-utero* thermal environments. We hypothesized that mammalian gestational HS would imprint future thermotolerance to a heat load.

Materials and Methods

Gestational Environments

The University of Missouri – Columbia Animal Care and Use Committee approved all animal procedures. Fifteen first parity crossbred females (Large White x Landrace) were exposed to thermal neutral (TN; cyclical 15°C nighttime and 22°C daytime; 55% RH; n = 8), or HS (cyclical 27°C nighttime and 37°C daytime; 67.5% RH; n = 7) conditions in the Brody environmental chambers at the University of Missouri – Columbia as previously described by Lucy and colleagues [15]. The environmental treatments began six days after insemination and continued until birth (114 d gestation) and resulted in a sustained increase (0.30°C) in body temperature in pregnant first parity pigs [15]. After parturition, all piglets were exposed to the same environmental conditions (26 to 32°C) as recommended for neonatal pigs [16]. At weaning, offspring from both TN and HS exposed pregnant first parity pigs were transported in an environmentally controlled trailer to Iowa State University for postnatal experiments.

Adolescent Environments

Iowa State University Institutional Animal Care and Use Committee approved all animal procedures. Between weaning and 15 wks of age all animals were co-mingled and fed *ad libitum* in thermal neutral conditions [based upon body weight; 16]. At 15 wks of age, adolescent pigs from gestational TN (GTN; n = 12; 67.9 ± 5.3 kg BW), and gestational HS (GHS; n = 12; 64.9 ± 3.7 kg BW) exposed pregnant first parity pigs were housed in individual pens (0.61 x 2.43 m; no wallowing access) in one of two environmentally-controlled rooms (TN and HS). Castrated males were selected to reduce the body temperature variability associated with gonadal steroids. In each room, relative humidity (%RH) and ambient temperature (T_a) were recorded every 30 minutes using two mounted data loggers (EI – WIN – USB, Lascar Electronics Ltd.) for the length of the experiment. Data loggers were positioned in opposite ends of the rooms to confirm that temperature was uniform throughout each room.

Twelve adolescent males (n = 6 GTN, and n = 6 GHS) were housed in constant TN conditions, and 12 adolescent males (n = 6 GTN, and n = 6 GHS) were maintained in constant HS conditions for 15 d. The TN room was maintained at $22.7 \pm 2.5^\circ\text{C}$ and 71.1 ± 10.0 % RH, while the HS room was maintained at a constant $34.7 \pm 2.3^\circ\text{C}$ and 50.4 ± 9.7 % RH. All pigs were fed a standard commercial diet *ad libitum* consisting primarily of corn and soybean meal formulated to meet or exceed nutritional requirements [17]. Nutrient intake was determined on d 7 and 15, while BW was determined on d -1, 7, and 15. Body weights were used to calculate average daily gain (ADG). Unrestrained respiration rate (RR), skin temperature, and rectal temperature (T_{re}), were measured four times daily (0800, 1200, 1600, 2000 h) on all animals. Respiration rate (breaths per minute: BPM) was

determined by counting flank movement over a one-minute interval. Skin temperature (T_{skin}) was determined by calculating the mean temperature of four sites measured at the ear (T_{ear}), shoulder (T_{shoulder}), rump (T_{rump}) and tail (T_{tail}) using a calibrated infrared thermometer (Model 42505, Extech Instruments, Waltham, MA). Rectal temperature was determined with a calibrated and lubricated thermistor thermometer (Welch Allyn SureTemp[®]Plus, Skaneateles Falls, NY) inserted approximately 10 cm into the rectum of unrestrained pigs. Due to the constant heat load, and lack of statistical hourly differences the hourly body temperature indices (RR, T_{skin} , T_{re}) were condensed into daily averages. A thermal circulation index (TCI) was calculated using T_{re} , temperature at one of the skin sites or T_{skin} , and T_{a} , and was used as an indicator of blood and heat transfer to a particular area of the skin under steady state thermal conditions described for agricultural species [18]:

$$\text{TCI} = (T_{\text{skin}} - T_{\text{a}}) / (T_{\text{re}} - T_{\text{skin}}).$$

Statistics

All data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Statistical model components included gestational environment (GTN; GHS), adolescent environment (TN; HS), day (1 – 15), and all possible interactions. All interactions, regardless of significance level were included in the model and pregnant first parity pig was used as a random effect. Initial body weight was used as a covariate for analysis of final body weight and body weight gain. For repeated analyses, each pig's respective parameter was analyzed using repeated measures with an auto-regressive covariance structure with day as the repeated effect. Data are presented as least squares means and statistical significance was defined as $P \leq 0.05$, and a tendency was defined as $0.05 < P \leq 0.10$.

Linear ($y = mx + b$) and quadratic models were created using JMP (SAS Institute Inc., Cary, NC) to determine the relationship between RR (y – axis) and T_{re} (x – axis). Slope and correlation coefficient values from each model were similar, so the linear regression was used for analysis. Slope was obtained for individual animals using all collected data points and analyzed using the mixed procedure in SAS 9.3 (SAS Institute Inc., Cary, NC), with slope as the dependent variable, and gestational environment (GTN; GHS), adolescent environment (TN; HS) and the interaction of gestational environment and adolescent environment as fixed effects. Pregnant first parity pig was used as a random effect. All interactions, regardless of significance level were kept in the model. Data are presented as least squares means and statistical significance was defined as $P \leq 0.05$, and a tendency was defined as $0.05 < P \leq 0.10$.

Results

Thermal Indices

There were no gestational differences detected in overall RR ($P > 0.14$), but there was a large RR increase (~2 fold; $P < 0.01$) for adolescent pigs in HS conditions compared to TN controls (Table I; Figure 1A). Regardless of gestational treatment, adolescent pigs in HS conditions had increased T_{re} ($P < 0.01$; 0.33°C ; Table I) compared to TN-housed pigs (Figure 1B). An overall gestational by adolescent interaction ($P < 0.01$) indicated that GHS pigs had a larger increase in T_{re} during adolescent HS compared to GTN pigs (0.50 vs. 0.16°C ; Table I; Figure 1C). A gestational environment by adolescent environment by day interaction was not observed for T_{re} (Figure 1B).

No overall differences were detected at any specific skin temperature site or T_{skin} when comparing gestational treatments; however, all were increased for adolescent pigs housed in HS conditions compared to TN conditions by 17 to 19% (Table I; Figure 2). There were also no gestational by adolescent interactions detected for T_{ear} , T_{shoulder} , T_{rump} , T_{tail} or T_{skin} . Thermal circulation index was similar in adolescent pigs from differing environments when measured at the ear and tail. However, TCI was increased 50 to 52% at the rump and T_{skin} in heat-stressed pigs compared to pigs in TN conditions. Overall, pigs from GHS had increased TCI_{tail} ($P = 0.03$; 16%) compared to GTN pigs, but this was primarily due to the gestational by adolescent interaction ($P = 0.02$) indicating that the GHS pigs had a larger increase (150%) during HS compared to GTN pigs (Table I). Adolescent HS tended ($P < 0.06$) to increase $\text{TCI}_{\text{shoulder}}$ (32%; Table I). There was no gestational effect or gestational by adolescent environment interaction detected for $\text{TCI}_{\text{shoulder}}$, TCI_{rump} , TCI_{ear} , or TCI_{skin} .

Regression Analysis

With regards to the relationship between T_{re} and RR, GTN pigs had a steeper slope (12 bpm/1°C) compared to GHS pigs ($P < 0.01$; Figure 3). No gestational environment by adolescent environment interaction differences were detected for this relationship ($P = 0.15$).

Growth Parameters

Overall nutrient intake did not differ between gestational treatments (2.52 kg/d), but it was reduced by 25% ($P < 0.01$) in the adolescent HS conditions compared to the TN controls (Table II). Animal growth was not affected by gestational thermal treatments (0.70 kg/d), but decreased 34% ($P = 0.04$) in HS pigs compared to those in TN conditions (Table II). There were no gestational or adolescent treatment effects ($P > 0.16$) detected for feed efficiency (0.27 kg gain/kg feed; Table II).

Discussion

Although HS is a serious economic burden and negatively impacts human health and animal welfare, it is also a primary constraint to efficient livestock growth and development. While this is the case in most geographical areas of North America, it is particularly true in tropical and sub-tropical regions of the world where many developing countries are located [19, 20]. As a result, these regions experience extended periods of HS compared to temperate climates (i.e., pigs could be exposed to HS during both gestation and the growing phase), making HS not only an economic issue, but also a serious food security and humanitarian concern [19]. Despite the fact that HS constitutes a well-documented physiological insult with global implications, understanding the epigenetic impact of HS during gestation on future progeny is not well-defined in mammalian species.

A variety of prenatal insults can have permanent effects on postnatal phenotypes and has been evaluated with regard to metabolic dysfunction [10] and teratogenicity [8]. Experimental models like intrauterine growth retardation indicate that future growth parameters [21, 22], body composition [23], and post-absorptive metabolism can be markedly altered by *in-utero* insults [10]. Hales and Barker [24] first introduced the idea of a “thrifty phenotype,” whereby fetal malnutrition causes maladaptive programming, which results in glucose-sparing metabolism that persists in the offspring. Similar effects can be observed during thermal stress where exposure of unicellular organisms [25], insects [11] and birds [12, 13, 26, 27] to elevated temperatures in previous generations or *in-ovo* alters postnatal survivability and/or body temperature indices. This “thermal conditioning” has also been demonstrated in rodents exposed to HS conditions shortly after birth [14]. This is particularly true for imprinting thermotolerance; defined as the enhanced ability to maintain a

lower or safer body temperature in response to a future heat load. However, the degree to which *in-utero* HS affects thermotolerance in mammals during adolescence is not well-understood. Herein, we demonstrate that *in-utero* HS impairs the future HS response in a mammalian model, which was contrary to our hypothesis that thermotolerance would have been imprinted.

No overall gestational differences were detected in any body temperature variable during adolescent TN conditions. However, during adolescent HS, pigs derived from heat-stressed first parity pigs had an increase in a variety of body temperature indices, most notably, core temperature (T_{re}). That gestational differences were only detected in HS conditions is not surprising, as epigenetic alterations impacting body temperature likely need a thermal insult to be expressed. This sort of HS-induced phenotype is similar to what has been reported in poultry [13]. In stark contrast to the aforementioned literature that suggests thermal “imprinting” increases future temperature tolerance, gestationally heat-stressed pigs actually became more hyperthermic during a future HS compared to GTN pigs (Figure 1B). Reasons for the species difference is not clear but of obvious biological and practical interest. Further, given the physiological and phylogenetic similarities between humans and pigs, these data could predict the human response to *in utero* HS.

The gestation difference in T_{re} during adolescent HS averaged 0.26°C and suggests that pigs originating from chronically heat-stressed pregnant first parity pigs are less able to adapt to a future HS compared to pigs derived from TN dams (Figure 1B). The increase in T_{re} observed in GHS pigs during adolescent HS is corroborated by numerical, but not statistically significant differences in T_{skin} (Figure 2). The primary numerical gestational treatment differences in T_{skin} occurred between the 4th and 9th day of the experiment, which

temporally coincides with the largest difference in T_{re} (Figure 1B). As expected, constant adolescent HS markedly increased RR as pigs (in the absence of opportunity for behavioral wetting) utilize panting is their primary route of latent heat loss [3, 28]. Despite GHS pigs having increased T_{re} during HS conditions, RR was similar between gestational treatments within the adolescent HS group. Regardless of the lack of gestation RR differences, the overall relationship (specifically the slope relationship) between T_{re} and RR was steeper (33%) for GTN compared to GHS pigs. In other words, the RR response to increasing T_{re} occurred more rapidly in the GTN pigs than for GHS pigs (Figure 3). The overall blunted respiratory response in GHS animals may have compromised the effectiveness of heat dissipation and resulted in a higher T_{re} . This may also be due to epigenetic modification(s) that produce an increase in their body temperature “set-point” where the GHS pigs experience a rise in T_{re} without a subsequent increase in RR or other heat dissipating mechanisms to mitigate the effect. An alternative explanation may be that GHS animals are more tolerant to a higher body temperature and do not need to initiate respiratory cooling mechanisms as quickly as controls.

Consistent with previous reports [3, 5, 29, 30], adolescent HS in this study caused a reduction in a variety of growth variables compared to TN conditions. Specifically, HS reduced final BW (6%), nutrient intake (25%), and BW gain (34%); however, gestational treatment did not statistically impact these variables. Feed efficiency was not influenced by gestational conditions, and surprisingly not affected by adolescent HS. However, this measure is based upon gross body weight change and does not take into account body composition differences, which are important since HS reorganizes nutrient partitioning priorities and the hierarchy of tissue synthesis [4].

The T_{re} increase in the GHS pigs may imply that gestational hyperthermia results in increased susceptibility to future heat loads. Further, the relationship between T_{re} and RR (Figure 3) suggests that GHS were less effective at utilizing enhanced respiration as a means to dissipate excess heat. Consequently, based upon typical measures of thermoregulation, it could be argued that gestational HS compromises tolerance to a future heat load. Yet, despite the increased T_{re} in response to adolescent HS, pigs derived from *in-utero* HS had similar nutrient intake compared to GTN pigs, suggesting that GHS pigs may have tolerated higher body temperatures better than controls (potentially programmed prenatally). Although no nutrient intake differences were observed for GHS and GTN pigs, ADG was numerically but not statistically reduced ($P > 0.15$) in GHS compared to GTN pigs by 215 g/d, regardless of adolescent environment. This reduction combined with a possible increase in core temperature set-point has many bioenergetic implications, which if maintained throughout life could result in significant economic losses for the swine industry. If GHS animals are prenatally programmed to maintain an increased core temperature set-point during a future heat load, this would theoretically increase maintenance costs which would have implications to nutrient partitioning, feed efficiency, feed intake, and barn throughput.

Although it is tempting to speculate that GHS animals have an increase in maintenance costs due to a greater body temperature (at least during HS), we only measured growth variables to provide a context for the body temperature measurements. Accordingly, the growth data needs to be interpreted with care, as determining the effects of gestational HS on future production was not a primary objective and would certainly require more measurements and animals per treatments to make meaningful conclusions. Additionally, whether or not these temperature differences are maintained (or augmented) during a diurnal

heat pattern compared to a constant heat load would be of significant interest. Clearly this experiment needs to be repeated with more of an emphasis placed on continuous body temperature indices obtained in both TN and HS conditions. Regardless, the results suggest that the well-documented decrease in nutrient intake and increase in RR associated with increased core body temperature [2, 3, 5, 28, 29, 31] may be partially influenced by fetal programming. Furthermore, the phenotypic responses to HS are variable [31, 32], and it is tempting to hypothesize that a portion of this variation may be due to epigenetic imprinting [28].

Conclusion

Maternal hyperthermia can have lasting effects on developmental patterns. Further, it can alter future growth and behavior and we have now demonstrated that it alters the body temperature response to a heat load. Specifically, animals that experienced *in-utero* HS became more hyperthermic in response to a future heat load. This has obvious bioenergetic implications in both human health and animal agriculture. A prerequisite to developing HS mitigation strategies is a better understanding of how HS epigenetically alters physiological responses throughout all stages of the life cycle. Further, how this compromised thermoregulatory response during HS influences metabolic function, future development, and bioenergetics is unknown and will be the focus of upcoming investigations.

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Declaration of Interest

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Author Contributions

J.S. Johnson and L.H Baumgard were responsible for experimental design and manuscript preparation. R.L. Boddicker, M.V. Sanz Fernandez, J.W. Ross, and R.P. Rhoads provided assistance with statistical analysis and experimental design. M.C. Lucy and T.J. Safranski performed maternal phase experiments.

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Table I. Effects of gestational and postnatal thermal environments on body temperature indices in growing pigs.

Parameter	Environments				SD	<i>P</i>		
	GTN-TN ¹	GHS-TN ²	GTN-HS ³	GHS-HS ⁴		G ⁵	P ⁶	G x P
RR ⁷ (bpm)	52	45	93	90	23	0.14	<0.01	0.52
T _{re} ⁸ (°C)	39.35 ^a	39.27 ^a	39.51 ^b	39.77 ^c	0.31	0.15	<0.01	<0.01
T _{ear} (°C)	30.87	30.64	36.51	36.77	2.23	0.96	<0.01	0.39
T _{shoulder} (°C)	31.60	31.43	36.82	37.12	1.90	0.79	<0.01	0.26
T _{rump} (°C)	31.36	31.30	37.33	37.50	1.85	0.86	<0.01	0.61
T _{tail} (°C)	31.42	31.31	36.99	37.27	1.85	0.66	<0.01	0.30
T _{skin} ¹⁰ (°C)	31.32	31.17	36.91	37.19	1.75	0.78	<0.01	0.33
TCI _{ear} ¹¹	1.09	1.02	1.24	0.92	1.48	0.14	0.84	0.30
TCI _{shoulder}	1.21	1.16	1.71	1.42	3.07	0.29	0.06	0.32
TCI _{rump}	1.19	1.10	1.58	1.90	3.66	0.46	<0.01	0.11
TCI _{tail}	1.15 ^a	1.14 ^a	0.84 ^a	2.10 ^b	2.74	0.03	0.25	0.02
TCI _{skin}	1.12	1.07	1.51	1.78	2.07	0.38	<0.01	0.20

¹Gestational thermal neutral pigs in postnatal thermal neutral conditions²Gestational heat stress pigs in postnatal thermal neutral conditions³Gestational thermal neutral pigs in postnatal heat stress conditions⁴Gestational heat stress pigs in postnatal heat stress conditions⁵Gestational environment⁶Postnatal environment⁷Respiration rate⁸Temperature⁹Rectal¹⁰Average skin temperature¹¹Thermal circulation index^{a,b,c} *P* < 0.05

Table II. Effects of gestational and postnatal thermal environments on production parameters in growing pigs.

Parameter	Environments				SD	<i>P</i>		
	GTN-TN ¹	GHS-TN ²	GTN-HS ³	GHS-HS ⁴		G ⁵	P ⁶	G x P
Initial BW (kg)	68.5	66.3	67.3	63.5	4.6	0.17	0.31	0.68
Final BW (kg)	80.1	77.5	76.4	72.5	6.3	0.16	0.04	0.70
NI (kg/d) ⁸	2.87	2.89	2.24	2.07	0.56	0.71	<0.01	0.61
BW gain (kg/d) ⁷	0.92	0.75	0.68	0.42	0.29	0.16	0.04	0.70
Gain:Feed (kg/kg) ⁹	0.34	0.25	0.32	0.15	0.12	0.10	0.16	0.34

¹Gestational thermal neutral pigs in postnatal thermal neutral conditions

⁶Postnatal environment

²Gestational heat stress pigs in postnatal thermal neutral conditions

⁷Body weight gain

³Gestational thermal neutral pigs in postnatal heat stress conditions

⁸Nutrient intake

⁴Gestational heat stress pigs in postnatal heat stress conditions

⁹Feed efficiency

⁵Gestational environment

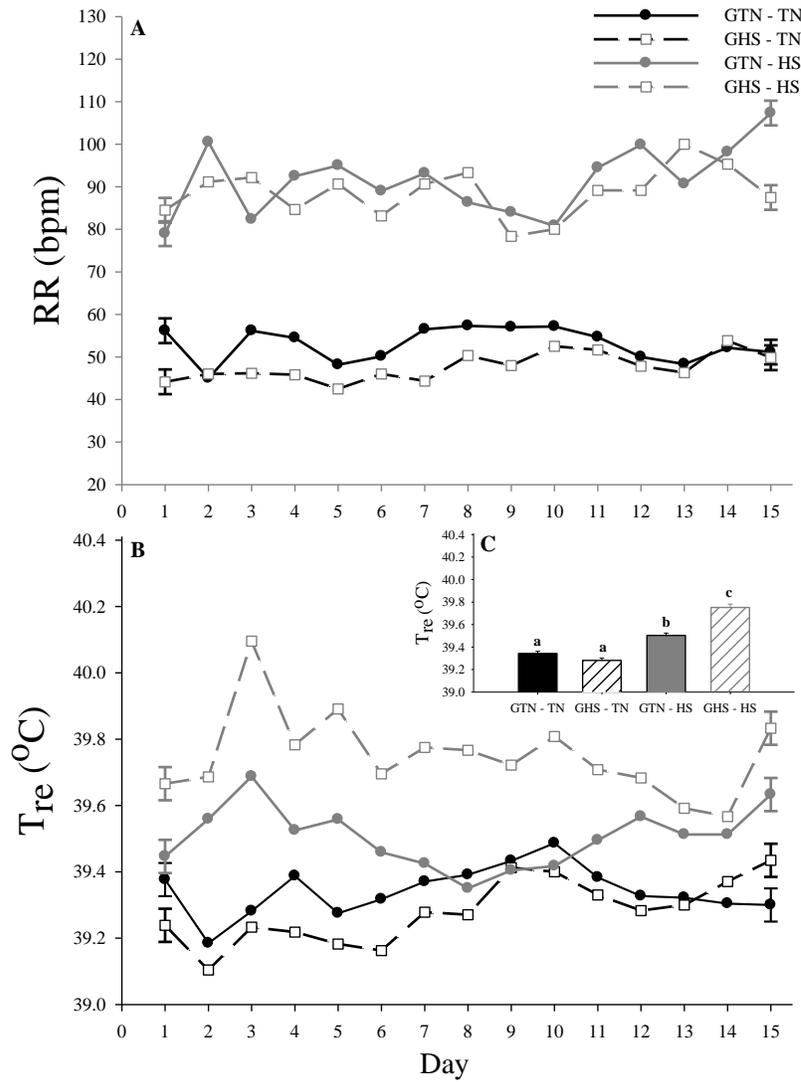


Figure 1: Effects of gestational and adolescent thermal environments on the temporal changes in (A) respiration rate (RR), (B) rectal temperature (T_{re}), and (C) T_{re} averaged by gestational and adolescent environment in growing pigs. Gestational heat stress (GHS), gestational thermal neutral (GTN), adolescent thermal neutral conditions (TN), adolescent heat stress conditions (HS). Error bars on d1 and d15 of the line indicate ± 1 SEM. Different letters (a,b,c) above vertical bars in 1C indicate significant differences ($P < 0.05$).

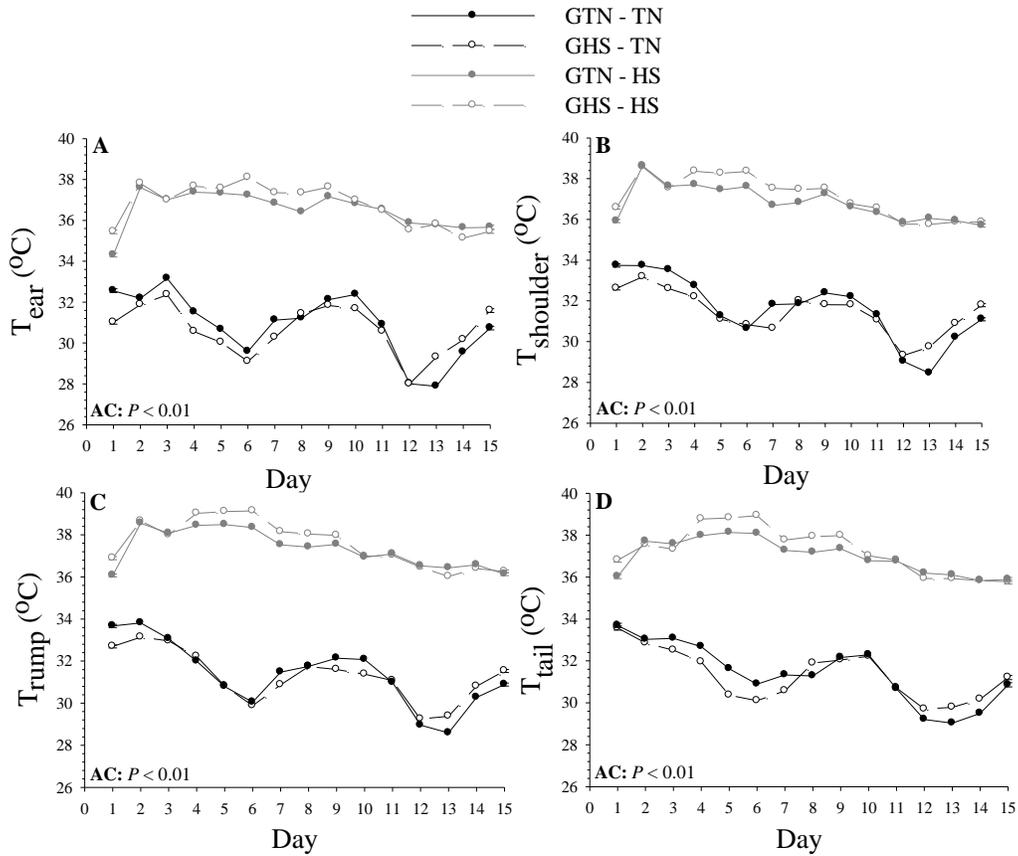


Figure 2: Effects of gestational and adolescent thermal environments on the temporal changes in (A) ear skin temperature (T_{ear}), (B) shoulder skin temperature (T_{shoulder}), (C) rump skin temperature (T_{rump}), and (D) tail skin temperature (T_{tail}) in growing pigs. Gestational heat stress (GHS), gestational thermal neutral (GTN), adolescent thermal neutral conditions (TN), adolescent heat stress conditions (HS), adolescent conditions (AC). Error bars on d1 and d15 of the line indicate ± 1 SEM. P -values in each figure represent T_{skin} differences comparing adolescent TN and HS exposed animals regardless of gestational treatment.

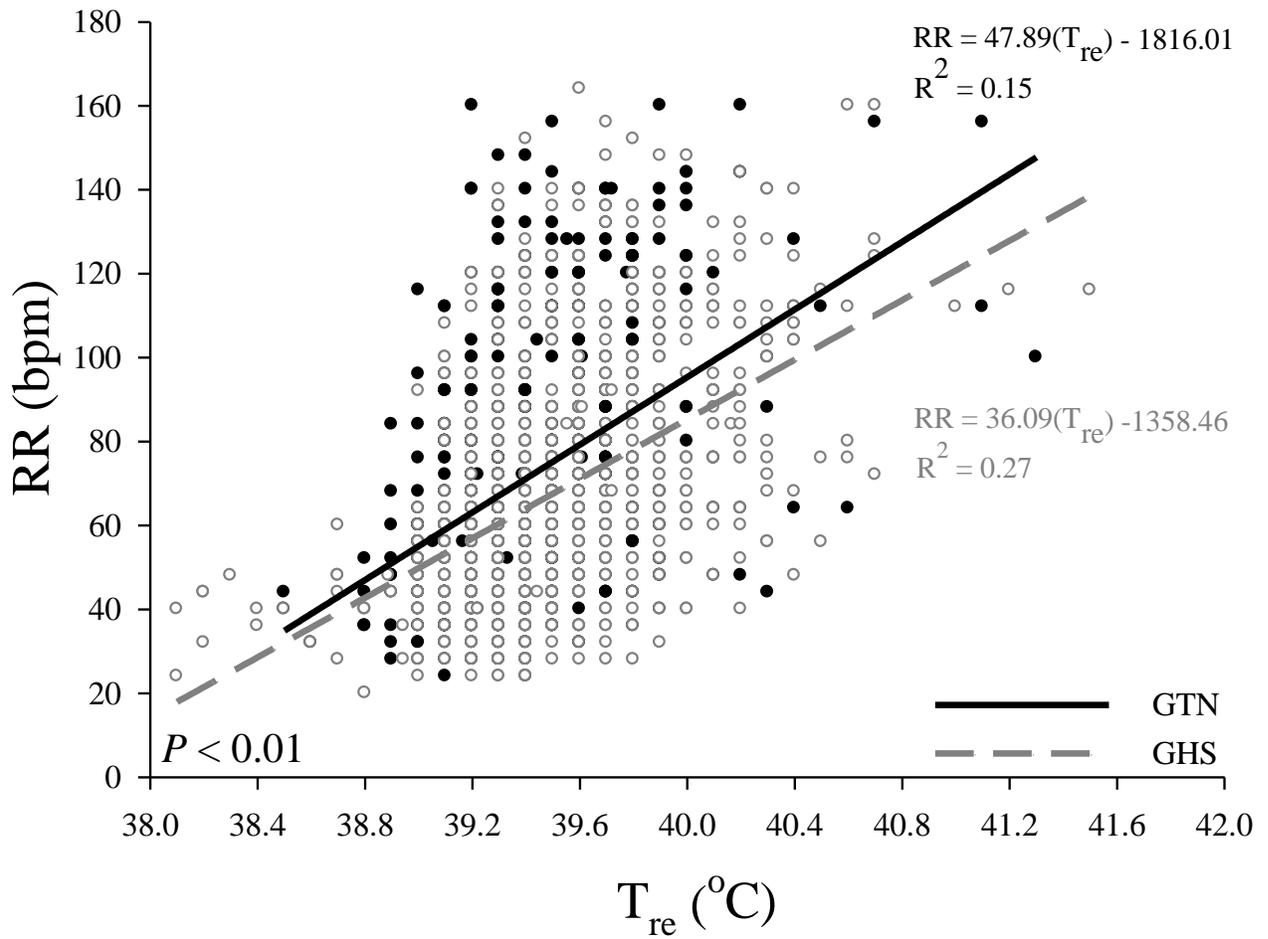


Figure 3: Linear regression ($y = mx + b$) of respiration rate (RR) as a function of rectal temperature (T_{re}) for gestational thermal neutral (GTN) and gestational heat stress (GHS) exposed pigs, regardless of adolescent environment. Coefficient of determination (R^2), and slope (m) is presented for each regression line.

CHAPTER III: *IN UTERO* HEAT STRESS INCREASES POSTNATAL CORE BODY TEMPERATURE IN PIGS

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Abstract

In utero heat stress (IUHS) negatively impacts postnatal development, but how it alters future body temperature parameters and energetic metabolism is not well-understood. Objectives were to characterize future temperature indices and bioenergetic markers in pigs originating from differing *in utero* thermal environments during both postnatal thermal neutral (TN) and cyclical heat stress (HS) exposure. First parity pregnant pigs (n = 13) were exposed to one of four ambient temperature (T_a) treatments [HS (cyclic 28 to 34°C) or TN (cyclic 18 to 22°C)], applied for the entire gestation (SHHS; TNTN), HS the first half (HSTN), or second half (TNHS) of gestation. Twenty-four offspring (30 ± 3 kg BW; n=6 SHHS, n=6 TNTN, n=6 HSTN, n=6 TNHS) were housed in TN ($21.7 \pm 0.7^\circ\text{C}$) conditions, and then exposed to two separate but identical HS periods (HS1 = 6 d; HS2 = 6 d; cycling 28 to 36°C). Core body temperature (T_{core}) was assessed every 15 minutes using surgically implanted temperature recorders. Regardless of *in utero* treatment, T_{core} increased during both HS periods ($p < 0.01$; 0.58°C). In TN, IUHS pigs had elevated ($p < 0.01$; 0.36°C) T_{core}

compared to TNTN controls. During HS1 and HS2, IUHS pigs had increased T_{core} ($p < 0.04$; 0.20 and 0.16°C, respectively) compared to TNTN controls. No *in utero* area under the T_{core} response curve differences were detected within either HS1 or HS2 ($p > 0.35$). Although unaffected by *in utero* environments, the ratio of total plasma thyroxine to triiodothyronine was reduced ($p < 0.01$) during HS1 and HS2 (39 and 29%, respectively) compared to TN. In summary, pigs originating from IUHS maintained an increased body temperature compared to TNTN controls regardless of external ambient temperature, and this thermal differential may have practical implications in animal agriculture.

Keywords: *in utero* heat stress, pigs, thermal imprinting, thermal regulation

Introduction

Although advances in technology (i.e. cooling systems and management practices) have partially ameliorated the negative effects of heat stress (HS), growth performance continues to be reduced while morbidity and mortality are increased in almost all agriculturally important species during the warm summer months (4, 9, 12, 21). Further, genetic selection for traditional production traits (i.e. enhanced lean tissue accretion) compromises HS-tolerance as synthesizing and maintaining muscle mass generates increased metabolic heat (8). Consequently, HS is one of the primary factors limiting profitable animal protein production and if the frequency of severe hot weather increases as predicted (29), the sustainability of some animal industries may be regionally threatened. Although primarily an economic concern in developed countries, HS and future climate change are food security and humanitarian issues in third-world nations (4).

While *in utero* HS (IUHS) negatively impacts fetal development and can be teratogenic (18), it either increases survivability to a future heat load in unicellular organisms (13) and *Drosophila buzzatti* (40), or improves the future ability to remain euthermic during a thermal insult in birds (35, 42). In contrast to the apparent improved thermoregulatory imprinting in the aforementioned reports, we recently reported that *in utero* HS actually increased future core body temperature (T_{core}) in pigs during exposure to a constant postnatal heat-load (21). Since this initial experiment utilized a constant HS challenge and T_{core} was only monitored four times daily, it is unknown whether this body temperature differential remains expressed during a diurnal HS-pattern or when T_{core} is monitored more frequently. Further, the mechanism(s) by which this temperature differential occurs are unknown and may be linked to an altered postnatal metabolite/endocrine profile. Therefore, study objectives were to fully characterize circadian body temperature indices and key bioenergetic metabolite/endocrine profiles in pigs exposed to differing *in utero* thermal environments during both postnatal TN and cyclical HS. Based on our previous research, we hypothesized that *in utero* HS would imprint an increase in the future T_{core} of mammalian offspring.

Materials and Methods

In utero environments

The University of Missouri Animal Care and Use Committee approved all animal procedures involving pregnant first parity pigs (protocol #6815). Thirteen pregnant first parity pigs (Large White x Landrace; GPK1 x GPK4; Choice Genetics USA; Des Moines, IA) were housed in the Brody Environmental Chambers at the University of Missouri and exposed to one of four ambient temperature (T_a) treatments. Thermal environments were

applied as either TN (cyclical 18°C nighttime and 22°C daytime; 62.4% RH), or HS (cyclical 28°C nighttime and 34°C daytime; 77.0% RH) conditions beginning six days after insemination (Duroc; Swine Genetics International; Cambridge, IA) and continued for most of gestation (108 d gestation; HSHS; TNTN), HS for the first half (54 d gestation; HSTN), or second half (54 d gestation; TNHS) of gestation. Although HS treatment increased ($p < 0.01$; 0.11°C) dam body temperature, maternal feed intake (FI) did not differ between *in utero* environments ($p > 0.42$; TNTN: 6.33 ± 0.19 kg/d, HSHS: 6.91 ± 0.11 kg/d, HSTN: 6.20 ± 0.19 kg/d, TNHS: 6.81 ± 0.09 kg/d) as all dams were limit-fed to prevent excessive maternal weight gain (standard industry practice; (7)). After parturition, all offspring were exposed to the same environmental conditions (26 to 32°C) as recommended for neonatal pigs (14). After weaning, offspring were transported in an aluminum livestock trailer with wood shavings (standard practice; 6 h) to Iowa State University.

Postnatal environments

Iowa State University Institutional Animal Care and Use Committee approved all animal procedures (protocol #7431-S). Between weaning and eight weeks of age all offspring were co-mingled and given water and feed *ad libitum* (based primarily on corn and soybean meal) in TN conditions (based upon body weight; (14)). At eight weeks of age, 24 male pigs from *in utero* TNTN ($n = 6$; 30.8 ± 1.0 kg BW), HSHS ($n = 6$; 27.9 ± 1.3 kg BW), HSTN ($n = 6$; 30.7 ± 0.7 kg BW), and TNHS ($n = 6$; 29.4 ± 1.4 kg BW) were housed in individual pens (0.76 x 1.82 m; no wallowing access) inside one of six (6.10 x 3.05 m) environmentally-controlled chambers (one pig/treatment/chamber) at the ISU Zumwalt Station Climate Change research facility. Castrated males were selected to reduce the body temperature variability associated with gonadal steroid production. In each chamber, relative

humidity (%RH) and ambient temperature (T_a) were recorded every five minutes using two mounted data loggers (El – WIN – USB; accuracy: $\pm 1.0^\circ\text{C}$; Lascar Electronics Ltd.; Erie, PA) for the duration of the experiment. Although T_a was controlled and evenly dispersed throughout each chamber, %RH was ungoverned.

Twenty-four males ($n = 6$ per treatment) were subjected to the following postnatal environmental conditions for 16 d. From d 1 – 2, all pigs were in TN conditions (constant $22.0 \pm 0.1^\circ\text{C}$; $22.5 \pm 4.3\%RH$; Fig. 1) to establish baseline body temperature measurements. All pigs were then exposed to cycling HS for six days (HS1; d 3 – 8; 28.0°C nighttime and 36.0°C daytime; $23.2 \pm 9.5\%RH$), followed by two days of TN (washout; d 9 – 10; $22.3 \pm 0.1^\circ\text{C}$; $38.0 \pm 3.7\%RH$), and then a second cycling HS period for six days (HS2; d 11 – 16; 28.0°C nighttime and 36.0°C daytime; $30.5 \pm 8.2\%RH$; Fig. 1). Data from the washout period were not included in the final analyses. All pigs were fed a standard commercial diet *ad libitum* consisting primarily of corn and soybean meal formulated to meet or exceed nutritional requirements (33). Feed intake was measured daily, and body weight (BW) was determined on d -1, 2, 9, and 16. Body weights were used to calculate average daily BW gain (ADG). Unrestrained respiration rate (RR) and skin temperatures were obtained four times daily (0800, 1300, 1600, and 2000 h). Respiration rate (breaths per minute: BPM) was determined by counting flank movement over one minute. Skin temperature (T_{skin}) was determined by calculating the mean temperature of four-shaved sites measured at the ear (T_{ear}), shoulder (T_{shoulder}), rump (T_{rump}) and tail (T_{tail}) using a calibrated infrared thermometer (Model 42505; accuracy: $\pm 5.0^\circ\text{C}$; Extech Instruments; Waltham, MA). Core temperature (T_{core}) was determined every 15 minutes using calibrated sterile thermochron temperature recorders (iButton; accuracy: $\pm 0.1^\circ\text{C}$; Dallas Semiconductor; Maxim[®]; Irving, TX) implanted

within the intraperitoneal cavity of individual pigs using sterile non-absorbable polyamide suture (Braunamid; B. Braun Medical Ltd., Sheffield, UK) seven days prior to HS treatment. For implantation, pigs were anesthetized by an intramuscular injection of a telazol (500 mg), ketamine (250 mg), and xylazine (250 mg) cocktail, administered at 1 mL per 23 kg BW. Following anesthesia, a six cm incision was made on the abdomen, five cm lateral to the linea alba, and sterile temperature recorders were sutured intraperitoneally to the abdominal muscle wall. Following surgery, pigs were administered an antibiotic (Excede[®], Zoetis, Florham Park, NJ) every three days per manufacturer's instructions. At the conclusion of the experiment, pigs were humanely euthanized by captive-bolt and thermochron temperature recorders were removed.

Blood sampling and analysis

Blood (10 mL) was obtained on all pigs via jugular venipuncture (BD[®] vacutainers; Franklin Lakes, NJ; K₃EDTA; lithium heparin) on d 2 of TN (1500 h), and at peak heat (1500 h) on d 2 and d 5 of both the HS1 and HS2 periods. Plasma was harvested by centrifugation at 4°C and 2500 x g, aliquoted and stored at -80°C. Glucose concentration was immediately determined from whole blood collected in lithium heparin tubes using a Vet Scan iStat[®] C68+ cartridge (Abaxis, Inc.; Union City, CA). An ELISA kit was used to determine plasma insulin (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden) and plasma heat shock protein 70 (HSP-70) concentrations (Porcine HSP 70 ELISA Kit; NEO Biolab; Cambridge, MA), following the manufacturer's instructions. Commercially available kits were used to determine plasma non-esterified fatty acid (NEFA; Autokit NEFA; Wako Chemicals USA, Richmond, VA) and plasma urea nitrogen (PUN; Urea Nitrogen Reagent; TECO Diagnostics, Anaheim, CA) concentrations. Total plasma

thyroxine (T₄) and triiodothyronine (T₃) concentrations were determined using a commercially available radioimmunoassay kit (ICN Biomedical Inc., Carson, CA). The intra-assay coefficients of variation were 4.7, 7.7, 5.5, 3.4, 4.1, and 3.7%, for insulin, NEFA, PUN, HSP-70, T₄, and T₃, respectively. The inter-assay coefficients of variation were and 5.2, 3.1, 9.6, and 7.2% for insulin, NEFA, PUN, and HSP-70, respectively. Systemic insulin sensitivity was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR; (glucose (mmol/L) * insulin (mg/L))/450; 19)

Statistics

The magnitude of the daily T_{core} increase was determined by calculating area under the T_{core} curve (AUC) for individual pigs during every day of HS1 and HS2 using average T_{core} of individual pigs during TN as a baseline. The AUC was calculated as a linear trapezoidal summation between successive T_{core} measurements and time coordinates after correcting for the mean baseline T_{core} during TN (calculations described by Baumgard et al., 2002 (3)). Rate of the daily T_{core} increase was determined using the slope of T_{core} as a function of hour (0800 – 1500 h) by linear regression ($y = mx + b$), and was calculated for individual pigs on every day of HS1 and HS2. Time to peak T_{core} was calculated from 0800 h to the time point when daily maximum T_{core} was the greatest, and was determined for individual pigs during every day of HS1 and HS2. Average T_{core} from 0800 – 2000 h and 2100 – 0700 h was calculated for individual pigs daily during HS1 and HS2. Hours that T_{core} of individual pigs remained above a threshold body temperature (e.g. 39.0, 39.5, 40.0, 40.5, and 41.0°C) were calculated during HS1 and HS2.

All data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). When analyzing all data within the TN period, only *in utero* environment

(TNTN; HSHS; HSTN; TNHS) was included as a statistical model component. The statistical model for the TN period was: $Y_{ij} = \mu + IU_i + e_{ij}$. Where, Y = parameter of interest, μ = mean, IU = *in utero* environment, and e = residual. Data analyses within the HS1 and HS2 periods were conducted with statistical model components including *in utero* environment (TNTN; HSHS; HSTN; TNHS), days within a HS period (d 1 – 6) and all possible interactions. The statistical model for HS1 and HS2 was: $Y_{ijk} = \mu + IU_i + D_j + IU*D_{ij} + e_{ijk}$. Where, Y = parameter of interest, μ = mean, IU = *in utero* environment, D = days within a HS period, and e = residual. For each of the TN, HS1 and HS2 period analyses, preplanned statistical comparisons were conducted for TNTN controls vs. pigs that received *in utero* HS during the first half of gestation (HSHS and HSTN pigs), and TNTN controls vs. all *in utero* HS (IUHS) treatments combined (HSHS, HSTN, and TNHS) using the CONTRAST statement of SAS. All interactions, regardless of significance level were included in the model, and dam was used as a random effect. For repeated analyses of HS1 and HS2 data, each pig's respective parameter was analyzed using repeated measures with an auto-regressive covariance structure with days within HS1 or HS2 as the repeated effect.

To compare data across periods, data within each period were averaged for individual pigs and statistical model components included *in utero* environment (TNTN; HSHS; HSTN; TNHS), period (TN; HS1; HS2), and all possible interactions. The statistical model for period analyses was: $Y_{ijk} = \mu + IU_i + P_j + IU*P_{ij} + e_{ijk}$. Where, Y = parameter of interest, μ = mean, IU = *in utero* environment, P = period, and e = residual. Preplanned statistical comparisons were conducted for TNTN controls vs. pigs that received *in utero* HS during the first half of gestation (HSHS and HSTN pigs), and TNTN controls vs. all IUHS treatments combined (HSHS, HSTN, and TNHS) using the CONTRAST statement of SAS. All

interactions, regardless of significance level were included in the model, and dam was used as a random effect.

Linear ($y = mx + b$) and quadratic ($y = mx + mx^2 + b$) models were created using JMP (SAS Institute Inc., Cary, NC) to determine the relationship between T_{core} (y - axis) and time (x - axis). Slope and correlation coefficient values from each model were similar, so the linear regression was used for analysis. Slope was obtained for individual animals using all collected data points and analyzed using PROC MIXED in SAS 9.3 (SAS Institute Inc., Cary, NC), with slope as the dependent variable, and *in utero* environment (TNTN; HSHS; HSTN; TNHS), period (TN; HS1; HS2) and the interaction of *in utero* environment and period as fixed effects. The statistical model for linear regression analyses was: $Y_{ijk} = \mu + IU_i + P_j + IU * P_{ij} + e_{ijk}$. Where, Y = parameter of interest, μ = mean, IU = *in utero* environment, P = period, and e = residual. Preplanned statistical comparisons were conducted for TNTN controls vs. pigs that received *in utero* HS during the first half of gestation (HSHS and HSTN pigs), and TNTN controls vs. all IUHS treatments combined (HSHS, HSTN, TNHS) using the CONTRAST statement of SAS. Dam was used as a random effect. All interactions, regardless of significance level were kept in the model. Data are presented as least squares means and statistical significance was defined as $p \leq 0.05$, and a tendency was defined as $0.05 < p \leq 0.10$.

Results

Thermal indices

Core temperature

During the TN period, average T_{core} did not differ amongst IUHS treatments, but HSHS, HSTN, and TNHS pigs had increased average T_{core} ($p < 0.01$; 0.34, 0.44, and 0.29°C, respectively) compared to TNTN controls (Table 1; Fig. 2). Average T_{core} increased ($p < 0.01$) in IUHS pigs, and pigs that received *in utero* HS during the first half of gestation (0.29 and 0.32°C, respectively) compared to TNTN controls within TN (Table 1). Minimum T_{core} was increased ($p < 0.01$) in HSHS, HSTN, and TNHS pigs (0.40, 0.52, and 0.36°C, respectively) versus TNTN controls during TN conditions (Table 1; Fig. 2). Maximum T_{core} tended to be increased ($p < 0.08$) in HSHS, HSTN, and TNHS pigs (0.27, 0.36, and 0.23°C, respectively) compared to TNTN controls during TN (Table 1).

In HS1, average T_{core} of IUHS pigs was increased ($p < 0.04$; 0.20°C, respectively) when compared to TNTN controls (Table 1; Fig. 2). Maximum T_{core} increased ($p < 0.04$; 0.42°C) in IUHS compared to TNTN controls in HS1 (Table 1). Between 0800 and 2000 h, average T_{core} tended to increase ($p < 0.10$; 0.17°C) in IUHS compared to TNTN pigs during HS1 (Table 1). From 2100 to 0700 h, average T_{core} tended to increase ($p < 0.08$; 0.27°C) in IUHS pigs during the first half of gestation compared to TNTN controls within HS1 (Table 1). Average T_{core} was numerically increased ($p > 0.14$) in HSHS, HSTN, and TNHS pigs (0.17, 0.25, and 0.17°C, respectively) compared to TNTN controls during HS1 (Table 1; Fig. 2).

During HS2, average T_{core} was increased ($p < 0.04$; 0.16°C) in IUHS compared to TNTN pigs (Table 1; Fig. 2). Maximum T_{core} tended to increase ($p < 0.08$; 0.19°C) in IUHS

compared to TNTN controls during HS2 (Table 1; Fig. 2). No differences in minimum T_{core} ($p > 0.95$; 39.64°C) were detected between *in utero* treatments during HS2 (Table 1).

Between 2100 and 0700 h, average T_{core} was increased ($p < 0.01$) in HSHS, HSTN, and TNHS pigs (0.15, 0.26 and 0.34°C , respectively) compared to TNTN controls in HS2 (Table 1). Average T_{core} was numerically increased ($p > 0.10$) in HSHS, HSTN, and TNHS pigs (0.13, 0.11, and 0.24°C , respectively) compared to TNTN controls during HS2 (Table 1; Fig. 2).

During HS1 and HS2, no *in utero* treatment differences ($p > 0.34$) were detected in average T_{core} AUC, average T_{core} slope (Fig. 3), or average T_{core} peak (Table 1). Regardless of *in utero* treatment, average T_{core} slope was reduced ($p < 0.01$; 20%) during HS2 compared to HS1 (Fig. 3). During HS1, average T_{core} was greater than 39.0 , 39.5 , 40.0 and 41.0°C for 10.4, 9.8, 6.5, and 2.7 more h ($p < 0.01$), respectively, compared to the HS2 period; however, no *in utero* or *in utero* by period differences were detected.

Respiration rate

During TN, HS1 and HS2, no *in utero* differences ($p > 0.07$) in RR were detected (Table 2). Regardless of *in utero* treatment, RR was increased ($p < 0.01$) during HS1 (117 ± 2 bpm) and HS2 (111 ± 6 bpm) compared to TN (54 ± 2 bpm), but no HS period differences were detected.

Skin temperature

No *in utero* treatment temperature differences ($p > 0.23$) were observed at any skin site during TN and HS1 (Table 2). During HS2, T_{shoulder} of HSTN pigs was reduced ($p < 0.03$; 0.43°C) compared to TNTN, HSHS, and TNHS pigs (Table 2). Regardless of *in utero* treatment, skin temperatures were increased at all measured sites ($p < 0.01$) during HS1 and

HS2 (5.41 and 4.63°C, respectively) compared to the TN period (Table 2). No *in utero* treatment by period interaction was detected at any measured skin site.

Growth parameters

Initial BW (23.1 kg), final BW (36.2 kg) and ADG (0.82 kg/d) were similar ($p > 0.44$) between all *in utero* treatments (Table 3). No *in utero* differences ($p > 0.18$) were detected for FI during the TN (1.59 kg/d), HS1 (1.37 kg/d) or HS2 (1.65 kg/d) periods (Table 3; Fig. 4). Regardless of *in utero* environment, an increase in FI ($p < 0.02$) was detected on d 4 compared to all other days in HS2 (Table 3; Fig. 4). Feed intake was reduced during HS1 ($p < 0.01$) compared to TN and HS2 (13.8 and 17.0%, respectively); however, no FI differences ($p > 0.05$; 1.62 kg/d) were detected comparing TN and HS2 (Table 3; Fig. 4).

Blood parameters

Plasma insulin concentrations in HSTN pigs ($0.10 \pm <0.01$ ng/mL) tended to be increased ($p < 0.09$) compared to TNTN ($0.07 \pm <0.01$ ng/mL), HSHS ($0.06 \pm <0.01$ ng/mL), and TNHS ($0.08 \pm <0.01$ ng/mL) pigs. No *in utero* treatment differences ($p > 0.16$) were detected for glucose (119.7 ± 4.1 mg/dL). No *in utero* treatment differences were observed for HOMA-IR (1.33 ± 0.21 AU), NEFA (59.5 ± 5.5 mEq/L), PUN (6.8 ± 0.5 mg/dL), HSP-70 (3.76 ± 0.49 ng/mL), T_4 (31.07 ± 3.44 ng/mL), T_3 (0.65 ± 0.05 ng/mL), or the ratio of T_4 to T_3 (2.51 ± 0.02).

Regardless of *in utero* environment, insulin was reduced ($p < 0.01$) on HS1-D2, HS1-D5, HS2-D2, and HS2-D5 (66.7, 60.0, 46.7, and 60.0% respectively) compared to TN, and was increased on HS2-D2 (30%) compared to HS1-D2, HS1-D5, and HS2-D5 (Table 4). HOMA-IR was reduced ($p < 0.01$) on HS1-D2, HS1-D5, HS2-D2, and HS2-D5 (64.5, 63.3, 46.5, and 63.3% respectively) compared to TN, and was increased on HS2-D2 (47.8%)

compared to all other HS periods (Table 4). Plasma urea nitrogen was increased ($p < 0.04$) during HS2-D5 (12%) compared to all other periods (Table 4). Serum HSP-70 was reduced ($p < 0.01$) on HS1-D2, HS1-D5, HS2-D2, and HS2-D5 (18.7, 19.2, 19.2, and 24.7%, respectively) compared to TN (Table 4). Circulating T_4 was reduced ($p < 0.01$) on HS1-D2, HS1-D5, HS2-D2, and HS2-D5 (35.0, 12.8, 12.3, and 9.6%, respectively) compared to TN, and was reduced on HS1-D2 (24.4%) compared to all other HS periods (Table 4). Plasma T_3 was reduced ($p < 0.01$) on HS1-D2, HS1-D5, HS2-D2, and HS2-D5 (54.7, 53.8, 41.5, and 42.4%, respectively) compared to TN, and was reduced (21.0%) during HS1-D2 and HS1-D5 compared to HS2-D2 and HS2-D5 (Table 4). The ratio of T_3 to T_4 was reduced ($p < 0.01$) on HS1-D2, HS1-D5, HS2-D2, and HS2-D5 (31.7, 46.6, 33.1, and 25.2% respectively) compared to TN, and reduced on HS1-D5 (24.0%) compared to all other HS periods (Table 4). No *in utero* environment by period interactions were detected.

Discussion

While animal welfare and productivity are negatively affected by HS in most geographical areas of North America, HS is of particular concern in tropical and sub-tropical regions of the world where many developing countries are located (29, 32). As a consequence, these regions can experience extended periods of extreme ambient temperatures compared to temperate climates (i.e., animals could be exposed to HS during both gestation and postnatal life), especially if the frequency of severe hot weather events increase as predicted (29). Although hyperthermia causes well-documented physiological insults with global implications, the impact of *in utero* HS on the phenotypes of future progeny is not well-understood in mammalian species.

Prenatal HS increases survivability to a future heat load in unicellular organisms (13) and *Drosophila buzzatti* (40), or improves the future ability to remain euthermic during a thermal insult in birds (35, 42). In addition, “thermal conditioning” has been demonstrated in rodents exposed to HS shortly after birth (41). In contrast to the aforementioned reports, we recently reported that exposure to *in utero* HS increased future T_{core} in pigs exposed to constant postnatal HS (21). Whether this T_{core} differential remains during exposure to a diurnal HS pattern or when T_{core} is monitored more frequently is unknown. In the present study, an average T_{core} increase of 0.24°C was detected in IUHS pigs compared to TNTN controls that was sustained during the TN (0.36°C), HS1 (0.20°C), and HS2 (0.16°C) periods. This altered phenotype is similar to what we have reported in pigs (21); however, the present experiment demonstrates that T_{core} differences exist regardless of external ambient temperature. Reasons for the reduced T_{core} differential between IUHS and TNTN pigs during HS1 and HS2 compared to TN are unclear, but may be due to increased efforts by all treatments to reduce their body temperature (i.e., RR increased in all treatments during HS1 and HS2 compared to TN; data in text in Results section) in response to thermal stress. In addition, the average T_{core} increase (compared to TNTN controls) was more pronounced (35% increase) during the TN period in pigs exposed to HS during the first half of gestation (HSHS and HSTN) compared to those exposed to HS during the last half of gestation (TNHS; Table 1; Fig. 2). That *in utero* differences were most evident in HSTN and HSHS pigs (i.e., early gestation HS) is not surprising, as most prenatal imprinting likely occurs in early pregnancy when rapid embryonic cellular differentiation occurs (1, 36). While mechanism(s) responsible for the increased T_{core} in IUHS pigs are not apparent, it may be due to increased heat production resulting from an increase in mitochondrial uncoupling protein

activity (37) and futile cycling (28), or increased whole-body adipose tissue observed in IUHS pigs (22) that may insulate against efficient heat dissipation. Further, since reduced head and brain size are associated with *in utero* HS (18), and has been observed in IUHS pigs by our lab (22, 23), augmented hypothalamic function may be an alternative explanation as it is thought to tightly control body temperature in mammalian species (6, 10).

Despite the increased average T_{core} in IUHS pigs, RR and skin temperatures were similar amongst all *in utero* treatments. Although it is counterintuitive that pigs with increased T_{core} would have similar RR and skin temperature as those with a reduced T_{core} in HS conditions, these results agree with our previous observations (21), and could be indicative of similar hyperthermic response as both the magnitude and rate of the daily T_{core} increase was similar in all *in utero* treatments during HS1 and HS2 (Table 1). While no statistically significant increase was observed, the increase in T_{core} in IUHS pigs was corroborated by a numerical increase in T_{skin} during TN (Table 2). Since the numerical T_{skin} increase (0.36°C) was the same as the average T_{core} differential (0.36°C) in IUHS pigs compared to TNTN controls during TN, it could indicate that body heat produced by IUHS pigs was sufficiently dissipated through the skin surface since increased skin temperature is associated with enhanced body heat dissipation (5). However, because no significant differences were observed, it cannot be said with certainty that heat dissipation remained uncompromised in IUHS pigs.

No metabolite/endocrine profile differences were detected when comparing *in utero* treatments. Serum HSP-70 and total plasma thyroid hormone concentrations were similar in IUHS pigs and TNTN controls, which is slightly surprising since elevated HSP-70 gene expression is often associated with increased T_{core} (16, 30, 31, 34), and thyroid hormones are

key regulators of energy metabolism (2, 24) and thermogenesis (39). Although these observations may imply that IUHS and TNTN pigs share a similar stress response (as indicated by serum HSP-70 levels), a lack of thyroid hormone differences may suggest that basal metabolic rate and heat production are similar amongst *in utero* treatments. However, body heat production is not solely dependent on thyroid hormone secretion (25), and there are a variety of other mechanisms (e.g., protein turnover, futile cycling, uncoupling protein activation, subcutaneous adipose tissue, etc.) that may be responsible for increased T_{core} .

While no *in utero* differences were detected, total circulating T_4 , T_3 and the ratio of active T_3 to inactive T_4 were reduced during all HS periods compared to TN (Table 4). The reduction in total circulating thyroid hormones may indicate a decrease in metabolic rate (2, 24) and thermogenesis (39), and could signify HS-induced reductions in maintenance costs as suggested in a previous study by our lab (22, 23). We previously determined that HSP-70 gene expression in muscle tissue is increased in heat-stressed pigs (34), and others have described increased HSP-70 gene expression in epithelial cells (27) and liver tissue (38) in response to HS. In addition, although the function of circulating HSP-70 is not well-understood, a recent study by Gaughan and colleagues (16) determined that circulating HSP-70 concentrations are increased in response to chronically elevated T_{core} in cattle, and HSP-70 is released into extracellular fluid by cultured rat embryo cells in response to thermal stress (20). However, in the present study, serum HSP-70 concentrations were reduced in both postnatal HS periods compared to TN conditions, regardless of *in utero* treatment. Reasons for this reduction are unclear, but may result from increased intracellular use of heat shock proteins to perform their cytoprotective function during periods of acute (e.g., current study) versus chronic (16) thermal stress (as reviewed by Kregel (26)). An alternative

explanation is that a blood collection-induced stress response may have artificially elevated circulating HSP-70 concentrations during TN (first blood collection), since HSP-70 levels are increased in response to stressful stimuli (e.g., hypoxia, energy depletion; as reviewed by Kregel (26)), and act as “danger signals” to initiate stress response programs in surrounding cells (17).

Heat stress causes a well-documented decrease in FI and growth parameters of agriculturally important livestock species (15). In the present study, FI was reduced (14%) during HS1 compared to TN in all treatments; however, in HS2, FI was similar to TN levels. The lack of a FI reduction during HS2 is likely an indication of HS acclimation as indicated by a reduced T_{core} response (i.e., decreased slope of the line) to increasing ambient temperature in all *in utero* treatments during HS2 compared to HS1 (Fig. 3). Although no *in utero* treatment differences in feed efficiency (FE) were detected in the present study (Table 3), we recently reported a reduction in FE in IUHS pigs compared to IUTN controls (22). Reasons for this discrepancy are unclear, but it is possible that FE differences may have been detected if the current experiment had more animals per treatment.

In accordance with our previous report (21), chronically elevated T_{core} was detected in IUHS pigs compared to TNTN controls (Table 1; Fig. 2). In contrast to the aforementioned report (21), increased T_{core} in the current study was observed regardless of postnatal environment, and while implementing a diurnal pattern of HS. The T_{core} increase, regardless of external ambient temperature could verify that *in utero* HS imprints a permanent elevation in postnatal body temperature, and if this differential were maintained throughout the pig’s life, the consequences are presumably to increase basal metabolic rate (11) and potentially lifetime maintenance costs as previously suggested (21). However, the specific

mechanism(s) of the T_{core} increase are currently unknown and any implications towards greater fasting heat production and increased maintenance costs would need to be confirmed by indirect calorimetry.

Perspectives and Significance

In utero hyperthermia can permanently impact postnatal phenotypes and alter future growth and behavior. We have now demonstrated that it can impact future mammalian body temperature, regardless of postnatal environment. Specifically, animals experiencing *in utero* HS maintained a greater T_{core} during the entire experiment, and this was especially true for pigs heat-stressed in the first half of gestation. Reasons for the time-point difference are unclear but may be due to genomic imprinting that likely occurs in early pregnancy during the period of rapid cellular differentiation. Chronically increased T_{core} has obvious bioenergetic implications in animal agriculture, and adds to our knowledge of how HS alters physiological responses throughout all stages of the lifecycle. Further, how this augmented body temperature influences metabolism and future development is unknown and will be the focus of future investigations.

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Declaration of Interest

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Author Contributions

J.S. Johnson and L.H Baumgard were responsible for experimental design and manuscript preparation. M.V. Sanz Fernandez, J.T. Seibert, J.W. Ross, and R.P. Rhoads provided assistance with statistical analysis, lab work, and experimental design. M.C. Lucy and T.J. Safranski performed maternal phase experiments. T.H. Elsasser and S. Kahl analyzed thyroid hormone concentration.

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Table 1: Effects of *in utero* heat stress on core body temperature in growing pigs.

Parameter	Environment				SEM	<i>p</i>				
	TNTN ¹	HS ²	HSTN ³	TNHS ⁴		IU ⁵	D ⁶	IU x D	Contrast 1 ⁷	Contrast 2 ⁸
TN period										
T _{core} ⁹ (°C)	39.59 ^a	39.93 ^b	40.03 ^b	39.88 ^b	0.08	0.01	-	-	0.01	0.01
T _{core} MAX ¹⁰ (°C)	39.92	40.19	40.28	40.15	0.09	0.07	-	-	0.01	0.01
T _{core} MIN ¹¹ (°C)	39.25 ^a	39.65 ^b	39.77 ^b	39.61 ^b	0.09	0.01	-	-	0.01	0.01
RR ⁹ (bpm)	51	56	51	58	2	0.08	-	-	0.23	0.07
HS1 period										
T _{core} (°C)	40.32	40.49	40.57	40.49	0.09	0.15	0.01	0.84	0.03	0.02
T _{core} MAX (°C)	41.00	41.17	41.28	41.28	0.09	0.16	0.01	0.15	0.07	0.03
T _{core} MIN (°C)	39.59	39.74	39.76	39.67	0.10	0.67	0.01	0.71	0.23	0.29
T _{core} (0800 – 2000h) ¹²	40.36	40.52	40.53	40.54	0.08	0.40	0.01	0.47	0.12	0.09
T _{core} (2100 – 0700h) ¹³	40.28	40.45	40.64	40.42	0.12	0.21	0.01	0.96	0.07	0.10
T _{core} AUC ¹⁴ (°C x h)	8.63	8.33	8.20	7.41	0.53	0.62	0.01	0.99	0.67	0.41
T _{core} Slope ¹⁵ (°C/h)	0.19	0.19	0.18	0.22	0.02	0.81	0.01	0.08	0.90	0.85
T _{core} Peak ¹⁶ (h)	7.7	7.5	7.6	7.7	0.1	0.63	0.03	0.13	0.47	0.68
HS2 period										
T _{core} (°C)	40.28	40.41	40.39	40.52	0.08	0.11	0.01	0.22	0.12	0.03
T _{core} MAX (°C)	40.81	40.96	40.96	41.07	0.09	0.25	0.01	0.21	0.17	0.07
T _{core} MIN (°C)	39.63	39.65	39.68	39.59	0.12	0.96	0.01	0.67	0.84	0.97
T _{core} (0800 - 2000h)	40.26	40.35	40.25	40.42	0.09	0.56	0.01	0.54	0.75	0.49
T _{core} (2100 - 0700h)	40.30 ^a	40.45 ^b	40.56 ^b	40.64 ^b	0.07	0.01	0.01	0.24	0.02	0.01
T _{core} AUC (°C x h)	8.29	8.76	6.90	7.79	0.39	0.35	0.01	0.36	0.81	0.79
T _{core} Slope (°C/h)	0.14	0.16	0.14	0.18	0.03	0.67	0.29	0.85	0.78	0.54
T _{core} Peak (h)	7.3	7.3	7.0	7.4	0.2	0.67	0.54	0.24	0.56	0.70

¹Thermal neutral conditions during entire gestation²Heat stress conditions during entire gestation³Heat stress conditions during first half of gestation⁴Heat stress conditions during second half of gestation⁵*In utero* environment⁶Day within a period⁷HS² and HSTN vs TNTN⁸Heat stress conditions during any part of gestation vs TNTN⁹Temperature¹⁰Maximum daily temperature¹¹Minimum daily temperature¹²Core body temperature from 0800 to 2000 h¹³Core body temperature from 2100 to 0700 h¹⁴Area under the daily core temperature curve¹⁵Slope of the core temperature increase (0800 – 1500h)¹⁶Hours to peak daily core temperature^{a,b,c} $p < 0.05$

Table 2: Effects of *in utero* heat stress on thermoregulation parameters in growing pigs.

Parameter	Environment				SEM	<i>p</i>				
	TNTN ¹	HSHS ²	HSTN ³	TNHS ⁴		IU ⁵	D ⁶	IU x D	Contrast 1 ⁷	Contrast 2 ⁸
TN period										
RR ⁹ (bpm)	51	56	51	58	2	0.08	-	-	0.23	0.07
T ¹⁰ _{ear}	31.32	31.70	32.02	31.81	0.25	0.31	-	-	0.11	0.10
T _{shoulder}	32.38	32.73	32.46	32.96	0.21	0.27	-	-	0.44	0.21
T _{rump}	32.20	32.38	32.28	32.68	0.32	0.72	-	-	0.74	0.52
T _{tail}	32.09	32.26	32.33	32.63	0.31	0.67	-	-	0.60	0.40
T _{skin} ¹¹	31.99	32.27	32.27	32.52	0.23	0.51	-	-	0.37	0.22
HS1 period										
RR (bpm)	121	122	111	115	6	0.59	0.01	0.18	0.68	0.58
T _{ear}	36.57	36.94	36.99	37.14	0.21	0.28	0.01	0.49	0.13	0.06
T _{shoulder}	37.22	37.38	37.33	37.54	0.16	0.57	0.18	0.56	0.52	0.31
T _{rump}	37.18	37.18	37.28	37.37	0.14	0.71	0.01	0.86	0.78	0.56
T _{tail}	36.74	36.79	36.73	37.03	0.12	0.27	0.01	0.32	0.86	0.42
T _{skin}	36.94	37.07	37.09	37.27	0.12	0.29	0.01	0.54	0.36	0.15
HS2 period										
RR (bpm)	114	116	105	107	6	0.59	0.01	0.58	0.81	0.65
T _{ear}	36.72	36.84	36.73	36.80	0.16	0.94	0.01	0.72	0.74	0.70
T _{shoulder}	37.05 ^a	37.14 ^a	36.70 ^b	37.20 ^a	0.13	0.03	0.01	0.58	0.42	0.81
T _{rump}	37.02	37.11	36.89	37.18	0.15	0.51	0.01	0.31	0.91	0.81
T _{tail}	36.67	36.77	36.45	36.88	0.14	0.19	0.01	0.67	0.72	0.88
T _{skin}	36.85	36.96	36.70	37.01	0.13	0.38	0.01	0.81	0.91	0.79

¹Thermal neutral conditions during entire gestation²Heat stress conditions during entire gestation³Heat stress conditions during first half of gestation⁴Heat stress conditions during second half of gestation⁵*In utero* environment⁶Day within a period⁷HSHS and HSTN vs TNTN⁸Heat stress conditions during any part of gestation vs TNTN⁹Respiration rate¹⁰Temperature¹¹Average skin temperature^{a,b,c} $p < 0.05$

Table 3: Effects of *in utero* heat stress on growth parameters in growing pigs.

Parameter	Environment				SEM	<i>p</i>				
	TNTN ¹	HS ²	HSTN ³	TNHS ⁴		IU ⁵	D ⁶	IU x D	Contrast 1 ⁷	Contrast 2 ⁸
Initial BW ⁹ (kg)	24.2	21.8	23.7	22.7	1.2	0.52	-	-	0.38	0.34
Final BW (kg)	35.9	35.8	37.0	36.2	0.6	0.45	-	-	0.47	0.42
ADG ¹⁰ (kg/d)	0.80	0.79	0.87	0.82	0.04	0.45	-	-	0.98	0.96
TN period										
Feed intake (kg)	1.43	1.49	1.72	1.70	0.20	0.62	-	-	0.57	0.44
Feed intake % BW ¹¹	6.32	6.66	7.25	7.41	0.49	0.34	-	-	0.29	0.16
Gain : Feed ¹² (kg/kg)	0.66	0.51	0.49	0.50	0.11	0.57	-	-	0.23	0.20
HS1 period										
Feed intake (kg)	1.39	1.29	1.43	1.36	0.11	0.79	0.26	0.99	0.77	0.69
Feed intake % BW	5.04	5.12	5.21	5.05	0.33	0.98	0.01	0.99	0.77	0.83
Gain : Feed (kg/kg)	0.60	0.61	0.64	0.66	0.03	0.55	-	-	0.19	0.29
HS2 period										
Feed intake (kg)	1.60	1.52	1.81	1.66	0.10	0.19	0.02	0.92	0.61	0.59
Feed intake % BW	4.91	5.14	5.41	5.37	0.39	0.80	0.06	0.95	0.47	0.40
Gain : Feed (kg/kg)	0.51	0.53	0.54	0.48	0.03	0.75	-	-	0.61	0.85

¹Thermal neutral conditions during entire gestation²Heat stress conditions during entire gestation³Heat stress conditions during first half of gestation⁴Heat stress conditions during second half of gestation⁵*In utero* environment⁶Day within a period⁷HS² and HSTN vs TNTN⁸Heat stress conditions during any part of gestation vs TNTN⁹Body weight¹⁰Average daily gain¹¹Feed intake percentage of body weight¹²Feed efficiency

Table 4: Effects of postnatal heat stress on bioenergetic parameters in growing pigs.

Parameter	Period					SEM	<i>p</i>
	TN ¹	HS1-D2 ²	HS1-D5 ³	HS2-D2 ⁴	HS2-D5 ⁵		P ⁶
Glucose (mg/dL)	122.8	120.3	114.6	119.1	121.8	3.2	0.26
Insulin (ng/mL)	0.15 ^a	0.05 ^b	0.06 ^b	0.08 ^c	0.06 ^b	<0.01	0.01
HOMA-IR ⁷ (AU)	2.54 ^a	0.90 ^b	0.93 ^b	1.36 ^c	0.93 ^b	0.17	0.01
Insulin : Glucose	0.12 ^a	0.04 ^b	0.05 ^b	0.07 ^c	0.05 ^b	<0.01	0.01
NEFA ⁸ (mEq/L)	61.4	61.6	67.2	51.3	56.0	5.4	0.27
PUN ⁹ (mg/dL)	6.5 ^a	7.0 ^{ab}	6.2 ^a	6.9 ^{ab}	7.4 ^b	0.4	0.04
HSP-70 ¹⁰ (ng/mL)	4.49 ^a	3.65 ^b	3.63 ^b	3.63 ^b	3.38 ^b	0.28	0.01
T ₄ ¹¹ (ng/mL)	36.12 ^a	23.36 ^b	31.50 ^c	31.68 ^c	32.67 ^c	1.98	0.01
T ₃ ¹² (ng/mL)	1.06 ^a	0.48 ^b	0.49 ^b	0.62 ^c	0.61 ^c	0.04	0.01
T ₃ : T ₄	3.57 ^a	2.44 ^b	1.90 ^c	2.39 ^b	2.67 ^b	0.13	0.01

¹Thermal neutral period²Day two of the first heat stress period³Day five of the first heat stress period⁴Day two of the second heat stress period⁵Day five of the second heat stress period⁶Period⁷Homeostatic model assessment of insulin resistance⁸Non-esterified fatty acid⁹Plasma urea nitrogen¹⁰Heat shock protein 70¹¹Thyroxine¹²Triiodothyronine^{a,b,c} *p* < 0.05

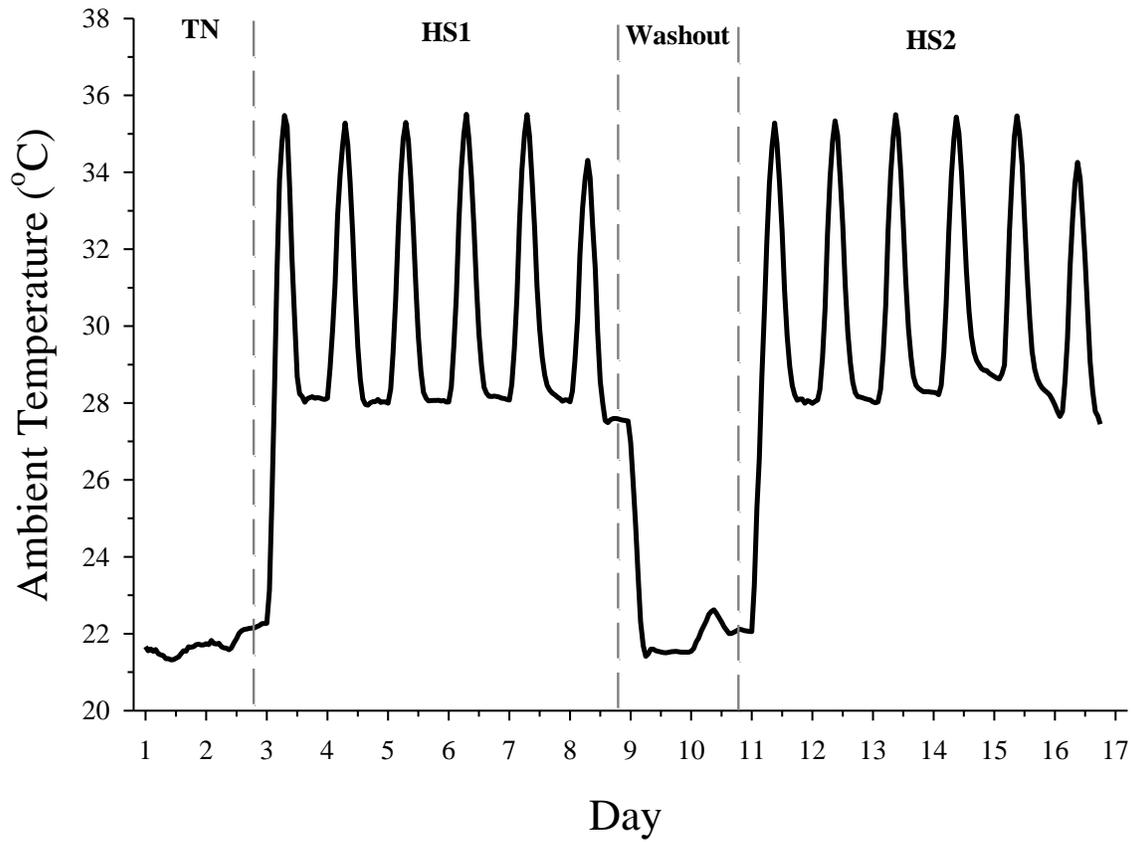


Figure 1: Ambient temperature (°C) by day of study. Abbreviations are: thermal neutral period (TN; constant $21.7 \pm 0.7^\circ\text{C}$), first heat stress period (HS1; 28.0 to 36.0°C), washout (constant $22.3 \pm 0.1^\circ\text{C}$), second heat stress period (HS2; 28.0 to 36.0°C).

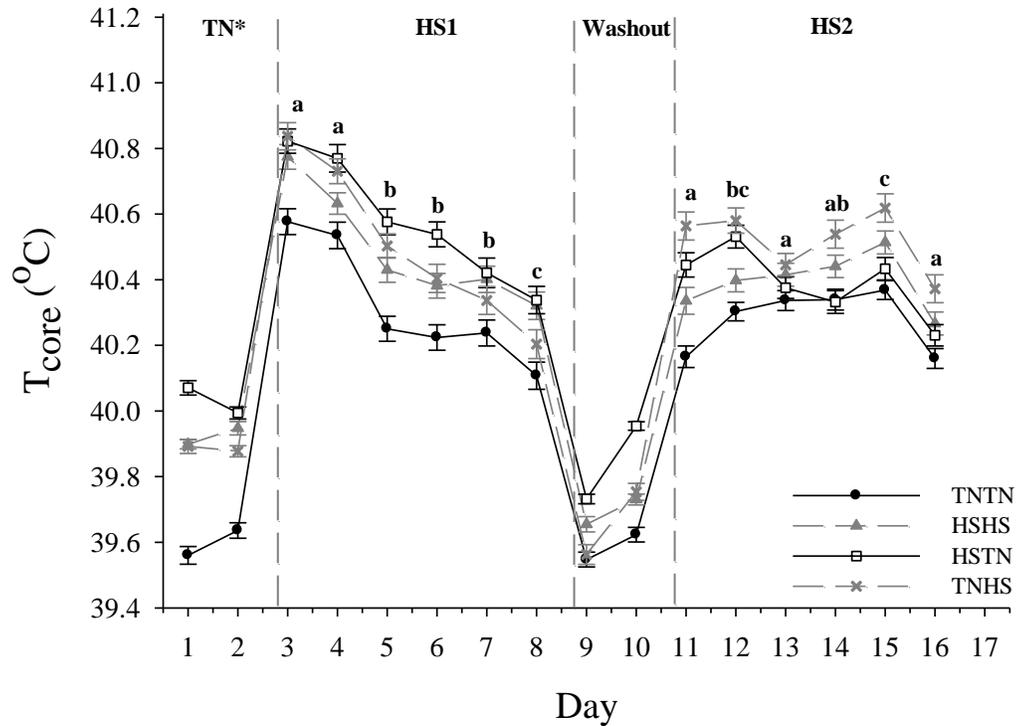


Figure 2: Effects of *in utero* environment on the temporal changes in core body temperature (T_{core}) averaged by *in utero* treatment and day of study in growing pigs. Abbreviations are: *in utero* thermal neutral conditions for entire gestation (TNTN), *in utero* heat stress conditions for entire gestation (HSHS), *in utero* heat stress conditions for first half of gestation (HSTN), *in utero* heat stress conditions for second half of gestation (TNHS), thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}\text{C}$), first heat stress period (HS1; cycling 28.0 to 36.0°C), washout (constant $22.3 \pm 0.1^{\circ}\text{C}$), second heat stress period (HS2; cycling 28.0 to 36.0°C). Error bars on d 1 and d 16 of treatment indicate standard error of the mean in each *in utero* environment. Letters (a,b,c) above points indicate significance ($p < 0.01$) comparing days within period. An asterisk (*) indicates significance ($p < 0.01$) across periods.

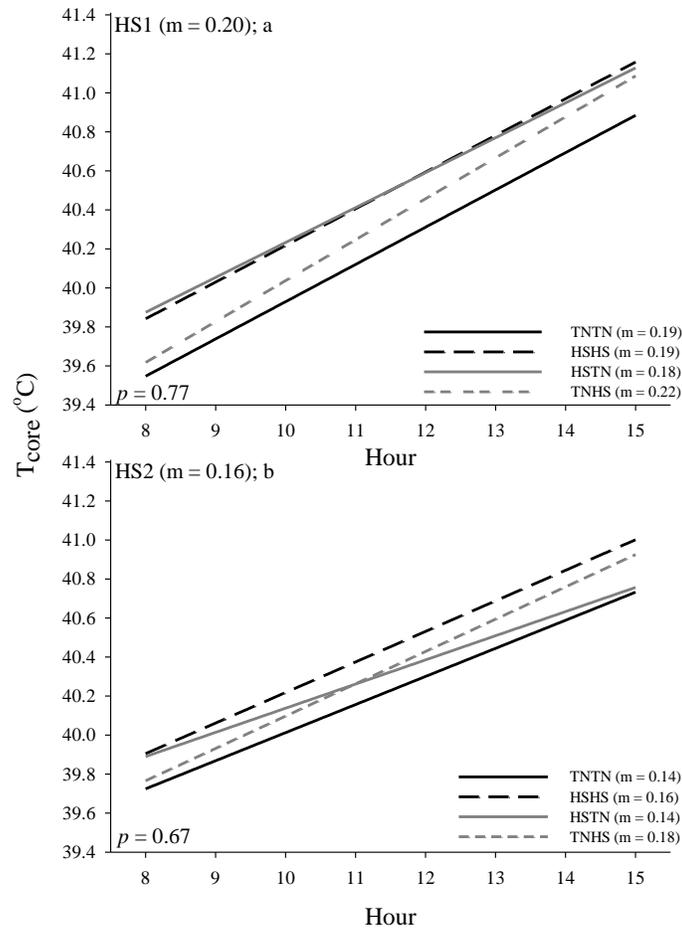


Figure 3: Linear regression ($y = mx + b$) of core temperature (T_{core}) as a function of hour (0800-1500h) for pigs exposed to *in utero* thermal neutral conditions for entire gestation (TNTN), *in utero* heat stress conditions for entire gestation (HSHS), *in utero* heat stress conditions for first half of gestation (HSTN), and *in utero* heat stress conditions for second half of gestation (TNHS). Abbreviations are: first heat stress period (HS1; cycling 28.0 to 36.0°C), second heat stress period (HS2; cycling 28.0 to 36.0°C). Letters (a,b) indicate differences between periods ($p < 0.05$). Slope (m) is presented for each period and *in utero* treatment.

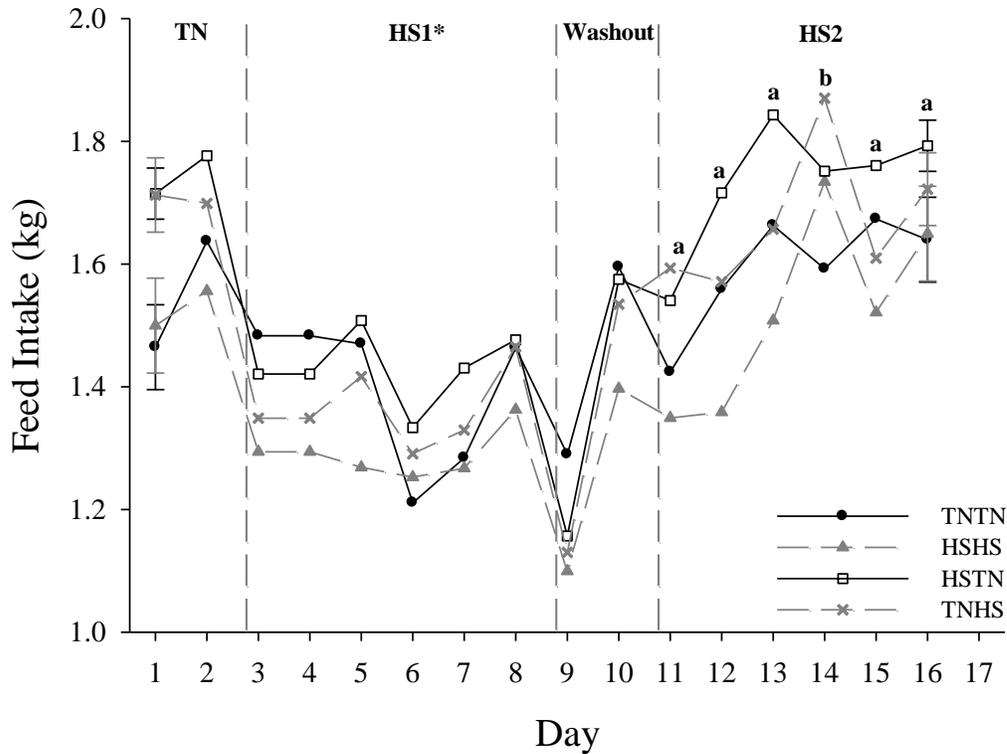


Figure 4: Effects of *in utero* environment on the temporal changes in feed intake (kg) averaged by *in utero* treatment and day of study in growing pigs. Abbreviations are: *in-utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat-stressed conditions for entire gestation (HSHS), *in-utero* heat-stressed conditions for first half of gestation (HSTN), *in-utero* heat-stressed conditions for second half of gestation (TNHS), thermal neutral period (TN; constant $21.7 \pm 0.7^\circ\text{C}$), first heat stress period (HS1; 28 to 36°C), washout (constant $22.3 \pm 0.1^\circ\text{C}$), second heat stress period (HS2; 28 to 36°C). Error bars on d 1 and d 16 of treatment indicate standard error of the mean in each *in utero* environment. Letters (a,b) above points indicate significance ($p < 0.05$) comparing days within period. An asterisk (*) indicates significance ($p < 0.05$) across periods.

**CHAPTER IV: EFFECTS OF *IN UTERO* HEAT STRESS AND CORE BODY
TEMPERATURE ON TISSUE ACCRETION DURING THE
GROWING PHASE (30 TO 60 KG) IN PIGS**

A paper submitted to *The Journal of Animal Science*

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Abstract

Environmentally induced heat stress (**HS**) negatively influences production variables in agriculturally important species. However, the extent to which HS experienced *in utero* affects nutrient partitioning during the rapid lean tissue accretion phase of postnatal growth is unknown. Study objectives were to compare future whole-body tissue accretion rates in pigs exposed to differing *in utero* and postnatal thermal environments when lean tissue deposition is likely maximized. Pregnant sows were exposed to thermal neutral (**TN**; cyclical 15°C nighttime and 22°C daytime; n = 9) or HS (cyclical 27°C nighttime and 37°C daytime; n = 12) conditions during their entire gestation. Twenty-four offspring from *in utero* TN (**IUTN**; n = 6 gilts, 6 barrows; 30.8 ± 0.2 kg BW) and *in utero* HS (**IUHS**; n = 6 gilts, 6 barrows; 30.3 ± 0.2 kg BW) were euthanized as an initial slaughter group (**ISG**). Following the ISG, 48 pigs from IUTN (n = 12 gilts, 12 barrows; 34.1 ± 0.5 kg BW) and IUHS (n = 12 gilts, 12 barrows; 33.3 ± 0.3 kg BW) were exposed to constant HS (34.1 ± 2.4°C) or TN (21.5 ±

2.0°C) conditions until they reached 61.5 ± 0.8 kg BW, at which point they were sacrificed and their body composition was determined. Homogenized carcasses were analyzed for nitrogen, crude fat, ash, water, and gross energy content. Data were analyzed using PROC MIXED in SAS 9.3. Rectal temperature and respiration rate increased ($P < 0.01$) during postnatal HS compared to TN (39.4 vs. 39.0°C and 94 vs. 49 bpm, respectively). Regardless of *in utero* environment, postnatal HS reduced ($P < 0.01$) feed intake (2.06 vs. 2.37 kg/d) and ADG (0.86 vs. 0.98 kg/d) compared to TN conditions. Postnatal HS did not alter water, protein, and ash accretion rates, but reduced adipose tissue accretion rates (198 vs. 232 g/d; $P < 0.04$) compared to TN-reared pigs. *In utero* environment had no effect on tissue deposition rates; however, IUHS pigs from the ISG had reduced liver weight ($P < 0.04$; 17.9%) compared to IUTN controls. In summary, postnatal HS reduced lipid accretion rates, but *in utero* HS did not appear to impact either lean or adipose tissue accretion during this specific growth phase.

Keywords: pigs, *in utero* heat stress, imprinting, and tissue accretion

Introduction

Heat stress (HS) reduces growth, alters carcass quality, and compromises efficiency, thus diminishing efforts by animal agriculture to produce high quality protein for human consumption (Baumgard et al., 2012). The negative effects of HS will likely become more pronounced as climate models predict an increase in extreme summer temperatures for most agricultural areas (Luber and McGeekin, 2008). Since basal heat production has markedly increased with genetic selection for enhanced lean tissue accretion (Brown–Brandl et al., 2004), some suggest faster growing animals are more sensitive to HS (Nienaber and Hahn,

2007). As a consequence, there is an urgent requirement to understand the mechanisms by which HS reduces animal performance.

While the detrimental effects of postnatal HS on tissue accretion have been well documented (Kouba et al., 2001; Collin et al., 2001a; Kerr et al., 2003), the extent to which *in utero* hyperthermia affects future animal performance is relatively unknown. If climate change lengthens summer duration and increases the frequency/intensity of heat waves, the number of animals gestating during the stressful warm times of the year will increase. Previous studies indicate prenatal stressors (non-thermal) can permanently alter growth (Foxcroft et al., 2006, 2009), post-absorptive metabolism (Chen et al., 2010; Pinney and Simmons, 2010), and body composition (Ravelli et al., 1976; Barker et al., 1993; Roseboom et al., 2006); however, the effects of maternal HS on offspring postnatal growth variables are unknown. Therefore, our study objectives were to determine the future rate and quantity of whole-body tissue accretion in pigs exposed to differing *in utero* and postnatal thermal environments. We hypothesized that *in utero* HS exposure would reduce future skeletal muscle accretion during the growing phase (30 to 60 kg BW), the period when lean tissue deposition dominates growth.

Materials and Methods

In utero environments

The University of Missouri Animal Care and Use Committee approved all procedures involving pregnant sows. Twenty-one first parity-crossbred sows (Large White x Landrace; GPK1 x GPK4; Choice Genetics USA; Des Moines, IA) were exposed to thermal neutral (TN; cyclical 15°C nighttime and 22°C daytime; 55% RH; n = 9), or HS (cyclical 27°C

nighttime and 37°C daytime; 67.5% RH; n = 12) conditions in the Brody Environmental Chambers at the University of Missouri (Lucy et al., 2012). Thermal treatments began six days after insemination (Duroc; Swine Genetics International; Cambridge, IA) and continued until farrowing (116.6 ± 0.5 d gestation; Lucy et al., 2012). Heat stress caused a sustained increase in rectal temperature (T_{re} ; $P < 0.05$; 0.3°C) in pregnant sows compared to TN controls (Lucy et al., 2012). Feed intake (**FI**) did not differ between treatments ($P > 0.15$; TN: 5.24 ± 0.07 kg/d and HS: 5.08 ± 0.08 kg/d) as both the HS and TN control pregnant sows were limit-fed to prevent excessive maternal weight gain (standard industry practice; Brendemuhl and Myer, 2009). Between parturition and weaning, all piglets were exposed to the same environmental conditions ($26 - 32^{\circ}\text{C}$) as recommended by FASS (2010). After weaning, all offspring (n = 253) were transported in an aluminum livestock trailer with wood shavings (standard practice; 6 h) to Iowa State University.

Postnatal environments

The Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals (protocol #7169-S). Between weaning and approximately 30 kg BW, all pigs were housed in TN conditions as recommended by FASS (2010), and allowed to consume water and feed (based primarily on corn and soybean meal) *ad libitum*. From the 253 pigs transported to Iowa State University, 24 pigs with no previous postnatal HS exposure from *in utero* thermal neutral (**IUTN**; n = 6 gilts, 6 barrows; 30.8 ± 0.2 kg BW), and *in utero* HS (**IUHS**; n = 6 gilts, 6 barrows; 30.3 ± 0.2 kg BW) were randomly selected and euthanized as part of an initial slaughter group (**ISG**). Following the ISG, 48 pigs from IUTN (n = 12 gilts, 12 barrows; 34.1 ± 0.5 kg initial BW) and IUHS (n = 12 gilts, 12 barrows; 33.3 ± 0.3 kg initial BW) were housed in individual pens (0.61 x 2.44 m) in one of

two environmentally controlled rooms. Pigs were evenly distributed within rooms based upon treatment. Within each room, ambient temperature (T_a) and relative humidity (%RH) were continuously recorded by two mounted data loggers (El – WIN – USB; accuracy: $\pm 1.0^\circ\text{C}$; Lascar Electronics Ltd.; Wiltshire, UK) every 30 min. Fans were utilized to prevent uneven temperature dispersion, and data loggers were positioned at opposite ends of the rooms to confirm uniform environmental conditions. Although T_a was controlled, %RH was ungoverned throughout the experiment.

Twelve barrows ($n = 6$ IUTN, 6 IUHS) and 12 gilts ($n = 6$ IUTN, 6 IUHS) were housed in constant TN conditions ($21.5 \pm 2.0^\circ\text{C}$; 68.1 ± 8.3 %RH), and 12 barrows ($n = 6$ IUTN, 6 IUHS) and 12 gilts ($n = 6$ IUTN, 6 IUHS) were maintained in constant HS conditions ($34.1 \pm 2.4^\circ\text{C}$; 48.9 ± 6.7 %RH) until they reached approximately 60 kg. All pigs were fed *ad libitum*, a standard commercial diet formulated to meet or exceed nutritional requirements (Table 1; NRC, 2012). Feed intake was determined weekly and BW was determined bi-weekly. Respiration rate (**RR**) and T_{re} were obtained twice daily (0800, 1600 h). Respiration rate (breaths per minute: **BPM**) was determined by counting flank movement over a one-minute interval. Rectal temperature was determined with a calibrated and lubricated thermistor thermometer (Welch Allyn SureTemp[®]Plus; accuracy: $\pm 0.1^\circ\text{C}$; Skaneateles Falls, NY) inserted approximately 10 cm into the rectum of unrestrained pigs.

Blood sampling and analysis

Blood (10 mL) was obtained from all pigs via jugular venipuncture (BD[®] vacutainers; Franklin Lakes, NJ; K₃EDTA; lithium heparin; serum) at 1500 h one day prior to sacrifice (in a fed state while still experiencing their respective environmental treatment) and stored on ice until processing; plasma and serum were then harvested by centrifugation at 2500 x g,

aliquoted and stored at -80°C. Glucose concentration was immediately determined from whole blood collected in lithium heparin tubes using a Vet Scan iStat® C68+ cartridge (Abaxis, Inc.; Union City, CA). Plasma insulin concentration was measured using an ELISA kit (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden), following the manufacturer's instructions. Commercially available kits were used to determine plasma non-esterified fatty acid (**NEFA**; Autokit NEFA; Wako Chemicals USA, Richmond, VA), plasma urea nitrogen (**PUN**; Urea Nitrogen Reagent; TECO Diagnostics, Anaheim, CA), and serum creatine kinase (**CK**) concentrations (Creatine Kinase-SL Assay; SEKISUI Diagnostics, Charlottetown, PE Canada). The intra- and inter-assay coefficients of variation were 3.8, 7.2, 6.4, 1.9%, and 8.0, 4.7, 8.3, 4.5% for insulin, NEFA, CK, and PUN, respectively. Quantification of insulin resistance was determined by the homeostatic model assessment of insulin resistance (**HOMA-IR**; (glucose (mmol/L) * insulin (mg/L))/450; Haffner et al., 1996), and the insulin to FI ratio ([insulin]/average daily feed intake throughout the entire experiment) was calculated for individual pigs using average daily FI over the entire experiment.

Serial slaughter and sub-sample analysis

All pigs utilized in the postnatal experiment were harvested after an overnight fast in TN conditions at 30.6 ± 0.2 kg BW (ISG), and 61.5 ± 0.8 kg BW (final slaughter group; **FSG**). Initial and final BW were selected in an attempt to capture the period of rapid lean accretion in commercially relevant growing pigs (Wagner et al., 1999; Van Milgen and Noblet, 2003). The ISG was euthanized with an intravenous barbiturate overdose (100 mg/kg BW; Nembutol®; Ovation Pharmaceuticals Inc.; Deerfield, IL). Since a similar BW at sacrifice was an objective, FSG pigs were harvested at different times (32.5 ± 4.5 d on trial).

At sacrifice, FSG pigs were electrically stunned and exsanguinated and blood was collected. For initial and final slaughter groups, stomach, intestinal, gallbladder and bladder contents were removed, and liver, spleen and total viscera weight (minus contents) was recorded. Whole carcass, head, viscera (minus contents of stomach, intestine, gallbladder, and bladder), and blood were weighed to determine empty body weight (**EBW**). Carcass, head, viscera, and blood of individual pigs were frozen, sectioned, passed twice through a mechanical grinder (Buffalo no. 66BX Enterprise; St. Louis, MO) and then passed four times through a Hobart 52 grinder with a 5-mm die (model #4046; Troy, OH) for homogenization. Ground pigs were sub-sampled, and sub-samples were immediately dried in a convection oven (101°C; 24 h) to a constant weight for determination of dry matter (**DM**) according to method 950.46 (AOAC Int., 2002). Dried sub-samples were ground through a 1-mm screen (Retsch ZM 100; Glen Mills Inc.; Clifton, NJ), and analyzed for ash, crude fat, nitrogen (**N**), and gross energy (**GE**). Ash was determined by drying at 600°C (24 h) to a constant weight according to method 923.03 (AOAC Int., 2002). Crude fat was determined by Soxhlet extraction according to method 991.36 (AOAC Int., 2002) using n-hexane as the solvent (Fischer Scientific; Fair Lawn, NJ). Nitrogen content was determined by combustion using a LECO TruMac N Nitrogen Determinator (Leco Corporation; St. Joseph, MI; model #630-300-300) according to method 992.15 (AOAC Int., 2002). Calibration of the LECO was conducted with an EDTA standard (known N content $9.56 \pm 0.03\%$; N content determined to be $9.58 \pm 0.06\%$), and crude protein was expressed as nitrogen * 6.25. Gross energy was determined using isoperibol bomb calorimetry (Parr 620 calorimeter; Parr Instrument Company; Moline, IL; model #6200), and benzoic acid was used as the calibration standard (known GE $6,318 \pm 18$ kcal/kg; GE determined to be $6,319 \pm 8$ kcal/kg). All chemical

analyses were carried out in duplicate, and repeated when the intra-duplicate coefficient of variation exceeded 3%.

Based on the sub-sample chemical content, total body composition was determined for water, ash, adipose, and protein using the EBW (Noblet et al., 1987). The average chemical composition of IUTN and IUHS pigs in the ISG was used to estimate the initial body composition of IUTN and IUHS pigs in the FSG, respectively. Within each individual pig, the accretion of water, adipose, protein, and ash were estimated by: (final content, g of tissue – estimated initial content, g of tissue) / days between harvest dates.

To determine carcass gain efficiency (**CE**), the average carcass weight of IUTN and IUHS pigs in the ISG was used to estimate the initial carcass weight of IUTN and IUHS pigs in the FSG, respectively. Within each individual pig, the accretion of carcass tissue was estimated by: (final carcass weight – estimated initial carcass weight)/days between harvest dates. Carcass gain efficiency was calculated by dividing carcass gain by average daily FI in individual pigs (carcass gain : FI).

Statistical analysis

All data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Statistical model components included *in utero* environment (IUTN; IUHS), postnatal environment (TN; HS), gender (M; F), and all interactions. Since no significant gender differences were detected, it was removed from the final analysis. All interactions, regardless of significance level were included in the model and dam was used as a random effect for all analyses. Average daily gain (**ADG**) of individual pigs calculated two weeks prior to the start of the postnatal treatment was used as a covariate for analysis of all body composition and growth variables. For repeated analysis of T_{re} and RR, each pig's

respective parameter was analyzed using repeated measures with an auto-regressive covariance structure with day as the repeated effect. Statistical significance was defined as $P \leq 0.05$, and a tendency was defined as $0.05 < P \leq 0.10$.

Results

Thermal indices

An overall increase ($P < 0.01$) in RR and T_{re} was detected in postnatal HS pigs compared to TN controls (94 ± 2 vs. 49 ± 2 bpm, Fig. 1A; 39.4 ± 0.1 vs $39.0 \pm 0.1^\circ\text{C}$, Fig. 1B, respectively). Neither *in utero* nor *in utero* by postnatal treatment differences were detected for RR or T_{re} .

Growth performance

Although not affected by *in utero* environments ($P > 0.50$; 2.29 kg/d), postnatal HS reduced ($P < 0.01$) FI by 13% compared to TN conditions (Table 2). Average daily gain was reduced ($P < 0.01$; 0.12 kg/d) in pigs in postnatal HS compared to TN conditions (Table 2). No postnatal feed efficiency (**FE**) differences were detected (0.42 kg gain/kg feed; Table 2), and neither ADG nor FE were influenced by *in utero* thermal treatment (Table 2). Regardless of *in utero* treatment, CE increased ($P < 0.01$; 12.5%) in postnatal HS compared to TN-exposed pigs; however, no *in utero* or *in utero* by postnatal treatment differences in CE ($P > 0.15$) were observed (Table 2). No *in utero*, postnatal, or *in utero* by postnatal treatment differences ($P > 0.10$) were detected in BW gain per Mcal of metabolizable energy (**ME**) consumed (0.12 kg/Mcal; Table 2).

Organ and carcass weights

In the ISG, absolute liver weight and liver weight as a percent of EBW were reduced ($P < 0.04$; 17.9 and 13.6%, respectively), and total viscera weight tended ($P < 0.10$) to be reduced (6.5%) in IUHS compared to IUTN pigs (Table 3). In the FSG, head weight was reduced ($P \leq 0.05$; 4.9%) in IUHS compared to IUTN pigs (Table 3). A decrease ($P < 0.01$) in total viscera (450 g), liver (80 g), spleen (20 g), and blood (190 g) weight was observed in pigs in postnatal HS compared to TN environments (Table 3). Total viscera, liver, and spleen weight as a percent of EBW were decreased ($P < 0.02$) 5.9, 7.6, and 15.8 %, respectively, in postnatal HS compared to TN control pigs (Table 3). No other *in utero* or postnatal organ and carcass weight differences were detected (Table 3).

Tissue composition and accretion

In utero HS pigs in postnatal HS conditions had reduced ($P < 0.03$; 56 g/d) water accretion compared to IUTN-HS pigs (Table 4). Regardless of *in utero* environment, postnatal HS pigs had reduced adipose tissue accretion ($P < 0.04$; 14.7%) compared to TN controls (Table 4; Fig. 2). No other *in utero* or postnatal tissue composition or accretion differences were observed (Table 4).

Blood analyses

In utero HS pigs in postnatal HS conditions tended ($P < 0.10$) to have reduced glucose concentrations (7.25 mg/dL) compared to IUTN-HS pigs (Table 5). Regardless of *in utero* treatment, PUN was reduced ($P < 0.04$; 17%) and [insulin]/FI was increased ($P \leq 0.05$; 30%) for pigs in postnatal HS compared to TN conditions (Table 5). No other *in utero* or postnatal treatment differences in blood variables were detected (Table 5).

Discussion

Despite cooling system advances and improved management practices, HS continues to compromise efficient high quality animal protein production. Although HS is primarily an animal welfare and economic issue in developed countries, it is a food security and humanitarian concern in regions that lack the resources to afford heat abatement technology (Battisti and Naylor, 2009). While the negative effects of HS on postnatal performance have been well documented (Renaudeau 2008, 2012; Baumgard and Rhoads, 2013), the impact of *in utero* HS on future progeny performance is ill defined. The uncertainty of environmental influence on future productivity will become more important if climate change intensifies the severity and frequency of heat waves and extends summer length.

Non-thermal prenatal stressors can permanently reduce growth (Foxcroft et al., 2006, 2009), alter post-absorptive metabolism (Pinney and Simmons, 2010; Chen et al., 2010), and influence body composition (Ravelli et al., 1976; Barker et al., 1993; Roseboom et al., 2006). The offspring's altered body composition and metabolism is generally characterized by increased adiposity and a metabolite/endocrine profile that resembles Type II diabetes. However, in the present study, *in utero* hyperthermia had no measureable impact on key body composition variables during the growing phase of postnatal growth, when protein accretion is ostensibly maximized (Wagner et al., 1999). Reasons for the lack of *in utero* treatment effects on postnatal lean and adipose tissue accretion are not clear, but may be the result of similar maternal FI (i.e. differences in dam nutrient intake may partly explain the aforementioned non-thermal effects), insufficient maternal hyperthermia (i.e. the heat load applied to the pregnant dams was inadequate to alter fetal programming), or the impact of *in utero* HS may

not be expressed until later in life when the rate of skeletal muscle accretion has slowed and adipose accretion is increased.

As reviewed by Graham and colleagues (1998), *in utero* hyperthermia caused microcephaly (reduced cranial size) in the present study, independent of postnatal treatment (Table 3). Since head size is likely associated with brain size, this observation could be indicative of micrencephaly (smaller brain size), which can negatively impact the central nervous system and future skeletal muscle development in non-porcine models (as reviewed by Graham et al., 1998). Additionally, liver weight and liver weight as a percent of EBW were reduced in IUHS pigs compared to IUTN pigs in the ISG, which may impact maintenance costs since reduced organ size (particularly liver and intestinal tissue) is correlated with decreased fasting heat production (Koong et al., 1982). Regardless of *in utero* environment, and similar to reports by others (Lefaucheur et al., 1989; Rinaldo and Le Dividich, 1991), pigs maintained in postnatal HS conditions had reduced total viscera mass and liver weight compared to those in postnatal TN conditions. Since the viscera (particularly the liver) has a high metabolic activity (Burrin et al., 1988; Van Milgen and Noblet, 2003), decreased splanchnic bed mass likely represents a strategy to minimize basal heat production in response to hyperthermia, possibly due to reduced visceral blood flow as described by others (Lambert et al., 2002; Leon and Helwig, 2010).

Although adipose tissue accretion was reduced (15%), protein deposition was similar (173 g/d) in postnatal HS pigs compared to TN controls (Fig. 2). Reduced adiposity corroborates results observed by Le Bellego and colleagues (2002), where both carcass fat content and back fat thickness were decreased in HS pigs compared to *ad libitum* fed TN controls. The observed reduction in adipose content was not unexpected since postnatal HS in

the present study negatively impacted FI and ADG (Table 2) as previously described in heat-stressed livestock species (Renaudeau et al., 2008; Baumgard et al., 2012; Pearce et al., 2013; Johnson et al., 2013a). Reduced FI limits energy available for growth, thus decreasing net energy available for adipose deposition, but it is unclear why protein accretion was not similarly affected. A likely explanation is that although FI was reduced, it was still adequate to allow for maximum lean tissue deposition during this growth phase (Van Milgen and Noblet, 2003).

No *in utero* thermal treatment differences were detected in circulating glucose, insulin, NEFA, PUN, and CK (Table 5). The lack of differences in glucose and NEFA agrees with our previous *in utero* HS reports (Boddicker et al., 2014), and were not unexpected, as we did not detect gross differences in body composition. However, we previously reported that *in utero* HS increases future circulating insulin concentration, regardless of postnatal thermal environments (Boddicker et al., 2014). Reasons why the current experiment did not corroborate our previous results are not clear, but may be due to infrequent blood sampling in the current study.

Circulating insulin, glucose, NEFA, and CK concentrations were similar in pigs in both postnatal environments. These data are somewhat surprising as insulin is frequently reported to increase while glucose and NEFA concentrations are decreased during HS, especially compared to pair-fed TN controls (Baumgard and Rhoads, 2013; Pearce et al., 2013). Additionally, HS pigs consumed 13% less feed compared to TN controls, and decreased FI normally reduces glucose and insulin levels, and increases circulating NEFA concentrations, since insulin secretion (a potent lipogenic and antilipolytic signal) is sensitive to changes in nutrient intake (Vernon, 1992). To better understand how HS influences the

insulin to FI relationship, we calculated the insulin to FI ratio and determined that HS-exposed pigs had increased circulating insulin per unit of FI by 30% compared to TN controls. These data agree with reports demonstrating that HS increases insulin secretion compared to TN environments (Baumgard and Rhoads, 2013; Pearce et al., 2013). Contrary to previous reports indicating that acute HS increases PUN in various species (as reviewed by Baumgard and Rhoads, 2013), chronic postnatal HS in the present study reduced PUN by 17% (Table 5). Discrepancies between data sets may be due to the length and severity of HS, since chronic HS reduces protein turnover and PUN relative to control treatments (Temim et al., 2000), and blood samples in the present study were obtained approximately five weeks after HS exposure began.

In general, early reports (Kleiber, 1961; NRC, 1981; Curtis, 1983) indicate that an animal's maintenance costs increase when ambient temperature exceeds the upper critical temperature. Increased HS-induced maintenance costs has specifically been reported in multiple species including cattle (McDowell et al., 1969; Beede and Collier, 1986), lambs (Ames and Brink, 1971), rodents (Collins et al., 1980), and pigs (Campos et al., 2014), and this increase is primarily attributed to greater energy costs associated with employing heat mitigating processes (i.e., panting, sweating) and enhanced chemical reaction rates as predicted by the Van't Hoff Arrhenius equation (Kleiber, 1961). However, some reports in heat-stressed pigs (Collin et al., 2001b; Renaudeau et al., 2013) describe reduced total heat and fasting heat production, and this is likely due to reductions in visceral mass caused by both the hyperthermia (Rinaldo and Le Dividich, 1991), and reduced FI (Koong et al., 1982). To gain a better appreciation for how HS alters bioenergetics, ME for maintenance ($ME_{\text{maintenance}}$) was estimated with the following equation (Patience, 2012): $ME_{\text{maintenance}} =$

$ME_{\text{intake}} - (ME_{\text{protein}} + ME_{\text{adipose}})$. Where, ME_{intake} = metabolizable energy in diet (Mcal/kg) * feed intake (kg/d), ME_{protein} = g protein gain/d * 10.03 kcal/g of protein gain, and ME_{adipose} = g adipose gain/d * 11.65 kcal/g of adipose gain (as reviewed by Patience, 2012). Assuming that the efficiency of protein and lipid gain and the efficiency of dietary energy use remained unaltered during HS, it appears that pigs exposed to postnatal HS (regardless of *in utero* environment) required approximately 588 kcal ME/d less energy for $ME_{\text{maintenance}}$.

The presumed increase in maintenance costs is the principal reason why HS likely decreases FE as is frequently reported in pig research articles (Kerr et al., 2003; Renaudeau et al., 2008), and reviews (NRC, 1981; Renaudeau et al., 2012). However, the effects of HS on FE are inconsistent as some studies report either no FE differences (Collin et al., 2001a; Johnson et al., 2013a), or actually improved FE (Lefaucheur et al., 1989) in HS compared to TN-exposed pigs. In agreement with the aforementioned reports, gross FE was similar amongst treatments (0.42 kg gain/ kg feed; Table 2). While reasons for this are unclear, it is possible that the reduced maintenance costs observed in the current study increased energy efficiency in HS-exposed pigs. Other indirect evidence also suggests that energy efficiency is enhanced during HS. First, the efficiency of converting dietary energy into body mass was increased during HS (i.e. the relationship between FI and BW gain is steeper in HS compared to TN controls; Fig. 3). Second, despite reduced FI, HS pigs retained 4.0% more ME_{intake} for growth ($ME_{\text{protein}} + ME_{\text{adipose}}$) compared to TN controls. These data suggest that although HS pigs consume less feed, a greater percentage of energy is partitioned toward growth, possibly due to reduced maintenance costs and overall heat production compared to TN controls. Although some inconsistencies exist within the literature regarding the effect of environmental HS on FE (possibly due to experimental differences), one explanation may be

differences between gross measurements of FE (BW gain : feed intake) and the efficiency of carcass tissue accretion (carcass gain : FI). For example, in the current experiment, although carcass and head weights were similar, total viscera weight was reduced (7.7%) in HS-exposed pigs compared to TN controls (Table 3), and this confirms reports by others (Lefaucheur et al., 1989; Rinaldo and Le Dividich, 1991). Therefore, although gross FE was similar, CE was actually increased ($P < 0.01$; 12.5%) in postnatal HS compared to TN-exposed pigs (Table 2). These data suggest that reductions in gross FE due to HS (NRC, 1981; Kerr et al., 2003; Renaudeau et al., 2008, 2012) may be a result of decreased visceral mass, and not due to a reduced rate of converting dietary nutrients into skeletal muscle and adipose tissue.

We have previously demonstrated increased postnatal core body temperature in pigs exposed to prenatal HS (Johnson et al., 2013a,b); though, no rectal temperature differences were detected between *in utero* treatments in the present study (Fig. 1B). These results are not surprising as identifying body temperature differences was not a primary objective, and detecting small changes in body temperature (a cyclical parameter) requires multiple measurements per day as demonstrated by our previous studies (Johnson et al., 2013a,b). However, it is noteworthy that both the head and the liver weighed less in the IUHS pigs and because these are both relatively large heat-producing systems it suggests our previous body temperature results (Johnson et al., 2013a,b) are due to other heat generating organs/tissues (assuming total heat production is related to mass).

Conclusion

While *in utero* programming can permanently modify future offspring development, the present study indicates that *in utero* HS had little effect on growth during the period of life primarily characterized by rapid lean tissue accretion. In contrast to *in utero* effects, postnatal HS reduced adipose accretion but had no effect on protein accretion rates. When considering that postnatal HS reduced visceral weight, HS pigs had increased efficiency of converting dietary nutrients into carcass tissue, and this has obvious energetic implications for production agriculture. Although it is possible that *in utero* HS may alter future nutrient partitioning later in life (i.e. when skeletal muscle deposition plateaus and adipose accretion is increased), it does not appear to influence tissue accretion during this particular growth phase.

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Author Contributions

J.S. Johnson and L.H Baumgard were responsible for experimental design and manuscript preparation. M.V. Sanz Fernandez, J.F. Patience, N.K. Gabler, J.W. Ross, and R.P. Rhoads provided assistance with statistical analysis, lab work, and experimental design. M.C. Lucy and T.J. Safranski performed maternal phase experiments. N.A. Gutierrez Cespedes assisted with maintenance costs calculations.

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Table 1. Ingredients and chemical composition of diet for growing pigs (as-fed basis).

Ingredients	---- % ----
Corn	60.6
Dehulled soybean meal	17.4
Dried distillers grains	18.1
45-30 vitamin and mineral premix ¹	2
Lysine	1.15
Threonine	0.114
Methionine	0.058
Tryptophan	0.024
Salt	0.65
Calculated chemical composition %	
DM	87
Crude protein	18.92
Crude fat	3.82
Crude fiber	3.70
SID Lysine	0.97
Calcium	0.60
Phosphorus	0.45
ME ² , Mcal/kg	3.31
NE ³ , Mcal/kg	2.45

¹Supplied per kilogram of diet: vitamin A, 8804 IU; vitamin D3, 1675 IU; vitamin E, 48 IU; vitamin K, 2.4 IU; choline, 5.5 mg; riboflavin, 4.6 mg; niacin, 23 mg; pantothenic acid, 18.2 mg; vitamin B₁₂, 30 µg; biotin, 1.6 µg; folic acid, 0.0005 mg; Zn, 158 ppm; Mn, 61 ppm; Fe, 177 ppm; Cu, 21 ppm; Se, 0.25 ppm. ^{2,3}Estimated using the NRC 2012 individual dietary ingredients.

Table 2: Effect of *in utero* and postnatal environment on growth parameters in growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
<u>Initial slaughter group (30 kg)</u>								
Final live BW ⁸ (kg)	30.8	30.3	-	-	0.2	0.15	-	-
EBW ⁹ (kg)	26.2	26.2	-	-	0.8	0.98	-	-
<u>Final slaughter group (60 kg)</u>								
Final live BW (kg)	62.1	61.9	62.3	59.5	0.8	0.06	0.23	0.07
EBW (kg)	58.8	58.1	58.9	55.8	0.9	0.05	0.32	0.17
Average daily gain (kg)	0.99	0.97	0.88	0.84	0.03	0.34	0.01	0.52
Feed intake (kg)	2.42	2.31	2.06	2.06	0.08	0.51	0.01	0.47
Gain : Feed ¹⁰ (kg/kg)	0.41	0.42	0.43	0.41	0.01	0.51	0.84	0.12
Carcass gain : Feed ¹¹ (kg/kg)	0.31	0.33	0.37	0.35	0.02	0.83	0.01	0.16
Gain : ME intake ¹² (kg/Mcal)	0.12	0.12	0.12	0.12	<0.01	0.49	0.75	0.11

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions

²*In utero* heat-stressed pigs in postnatal thermal neutral conditions

³*In utero* thermal neutral pigs in postnatal heat stress conditions

⁴*In utero* heat-stressed pigs in postnatal heat stress conditions

⁵Standard error of the mean

⁶*In utero* environment

⁷Postnatal environment

⁸Body weight

⁹Final live BW minus gastrointestinal contents

¹⁰Feed efficiency

¹¹Carcass weight gain per kilogram of feed intake

¹²Body weight gain per Mcal of ME intake

Table 3: Effect of *in utero* and postnatal environment on carcass and organ weights of growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
<u>Initial slaughter group (30 kg)</u>								
Weight								
Carcass (kg)	22.3	21.8	-	-	0.7	0.61	-	-
Total Viscera (kg)	4.15	3.88	-	-	0.11	0.09	-	-
Liver (kg)	0.95	0.78	-	-	< 0.01	0.03	-	-
Spleen (kg)	0.07	0.08	-	-	< 0.01	0.28	-	-
Percent of EBW ⁸								
Carcass % of EBW	84.3	84.8	-	-	0.4	0.35	-	-
Total Viscera % of EBW	15.7	15.2	-	-	0.4	0.34	-	-
Liver % of EBW	3.59	3.10	-	-	0.15	0.03	-	-
Spleen % of EBW	0.30	0.29	-	-	0.02	0.57	-	-
<u>Final slaughter group (60 kg)</u>								
Weight								
Carcass (kg)	45.9	45.3	46.4	43.8	0.9	0.07	0.61	0.21
Head (kg)	4.07	3.94	4.09	3.92	0.07	0.05	0.89	0.95
Total Viscera (kg)	5.85	5.90	5.53	5.33	0.11	0.48	0.01	0.22
Liver (kg)	1.00	0.99	0.94	0.89	0.02	0.14	0.01	0.17
Spleen (kg)	0.11	0.11	0.09	0.09	0.01	0.62	0.01	0.62
Blood (kg)	2.97	2.93	2.85	2.67	0.06	0.14	0.01	0.20
Percent of EBW								
Carcass % of EBW	78.0	78.0	78.8	78.6	0.3	0.72	0.06	0.78
Head % of EBW	7.07	6.75	6.96	6.93	0.01	0.29	0.74	0.14
Total Viscera % of EBW	10.0	10.2	9.4	9.6	0.2	0.29	0.01	0.92
Liver % of EBW	1.71	1.73	1.60	1.59	0.01	0.96	0.02	0.74
Spleen % of EBW	0.18	0.19	0.16	0.16	0.01	0.78	0.01	0.94
Blood % of EBW	5.1	5.1	4.8	4.8	0.1	0.93	0.07	0.91

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions²*In utero* heat-stressed pigs in postnatal thermal neutral conditions³*In utero* thermal neutral pigs in postnatal heat stress conditions⁴*In utero* heat-stressed pigs in postnatal heat stress conditions⁵Standard error of the mean⁶*In utero* environment⁷Postnatal environment⁸Final live body weight minus gastrointestinal contents

Table 4: Effect of *in utero* and postnatal environment on tissue accretion in growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
Initial slaughter group (30 kg)								
Percent of EBW ⁸								
Water % of EBW	71.2	70.9	-	-	3.6	0.53	-	-
Protein % of EBW	16.7	16.8	-	-	< 0.1	0.77	-	-
Adipose % of EBW	9.6	10.0	-	-	0.4	0.52	-	-
Ash % of EBW	2.5	2.4	-	-	< 0.1	0.79	-	-
Gross Energy (kcal/g)	6230	6221	-	-	36	0.86	-	-
Final slaughter group (60 kg)								
Percent of EBW								
Water % of EBW	63.1	63.7	65.2	63.8	0.6	0.56	0.13	0.10
Protein % of EBW	16.9	17.2	17.2	17.3	< 0.1	0.36	0.37	0.62
Adipose % of EBW	17.3	16.8	15.2	16.4	< 0.1	0.67	0.14	0.20
Ash % of EBW	2.5	2.6	2.4	2.7	0.2	0.44	0.98	0.61
Tissue accretion								
Water ⁹ (g/d)	568 ^a	592 ^a	594 ^a	538 ^b	18	0.39	0.52	0.03
Protein ¹⁰ (g/d)	171	177	175	168	7	0.97	0.74	0.23
Adipose ¹¹ (g/d)	240	224	192	204	14	0.88	0.04	0.28
Ash ¹² (g/d)	25	26	23	26	3	0.44	0.77	0.79
Adipose : Protein	1.41	1.29	1.13	1.24	0.09	0.99	0.12	0.18
Gross Energy (kcal/g)	6796	6782	6740	6720	76	0.85	0.44	0.96

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions²*In utero* heat-stressed pigs in postnatal thermal neutral conditions³*In utero* thermal neutral pigs in postnatal heat stress conditions⁴*In utero* heat-stressed pigs in postnatal heat stress conditions⁵Standard error of the mean⁶*In utero* environment⁷Postnatal environment⁸Final live body weight minus gastrointestinal contents⁹Water accretion per day¹⁰Protein accretion per day¹¹Adipose accretion per day¹²Ash accretion per day^{a,b,c} $P < 0.05$

Table 5: Effect of *in utero* and postnatal environment on blood parameters in growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
Glucose (mg/dL)	100.7	104.3	101.6	94.3	3.1	0.16	0.57	0.09
Insulin (ng/mL)	0.11	0.14	0.16	0.14	0.02	0.56	0.22	0.13
Insulin : Glucose	0.10	0.15	0.15	0.15	0.01	0.28	0.21	0.23
[Insulin] : Feed intake (kg) ⁸	4	6	7	6	1	0.56	0.05	0.12
HOMA-IR ⁹ (AU)	1.91	1.95	1.90	1.87	0.32	0.99	0.86	0.90
NEFA ¹⁰ (mEq/L)	80.7	82.0	81.2	86.8	11.4	0.77	0.81	0.85
Creatine Kinase (U/L)	198	194	196	225	24	0.61	0.54	0.48
PUN ¹¹ (mg/dL)	13.9	14.9	11.2	12.7	1.1	0.30	0.03	0.83

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions

²*In utero* heat-stressed pigs in postnatal thermal neutral conditions

³*In utero* thermal neutral pigs in postnatal heat stress conditions

⁴*In utero* heat-stressed pigs in postnatal heat stress conditions

⁵Standard error of the mean

⁶*In utero* environment

⁷Postnatal environment

⁸Insulin concentration per kilogram of feed intake

⁹Quantification of insulin resistance

¹⁰Non-esterified fatty acid

¹¹Plasma urea nitrogen

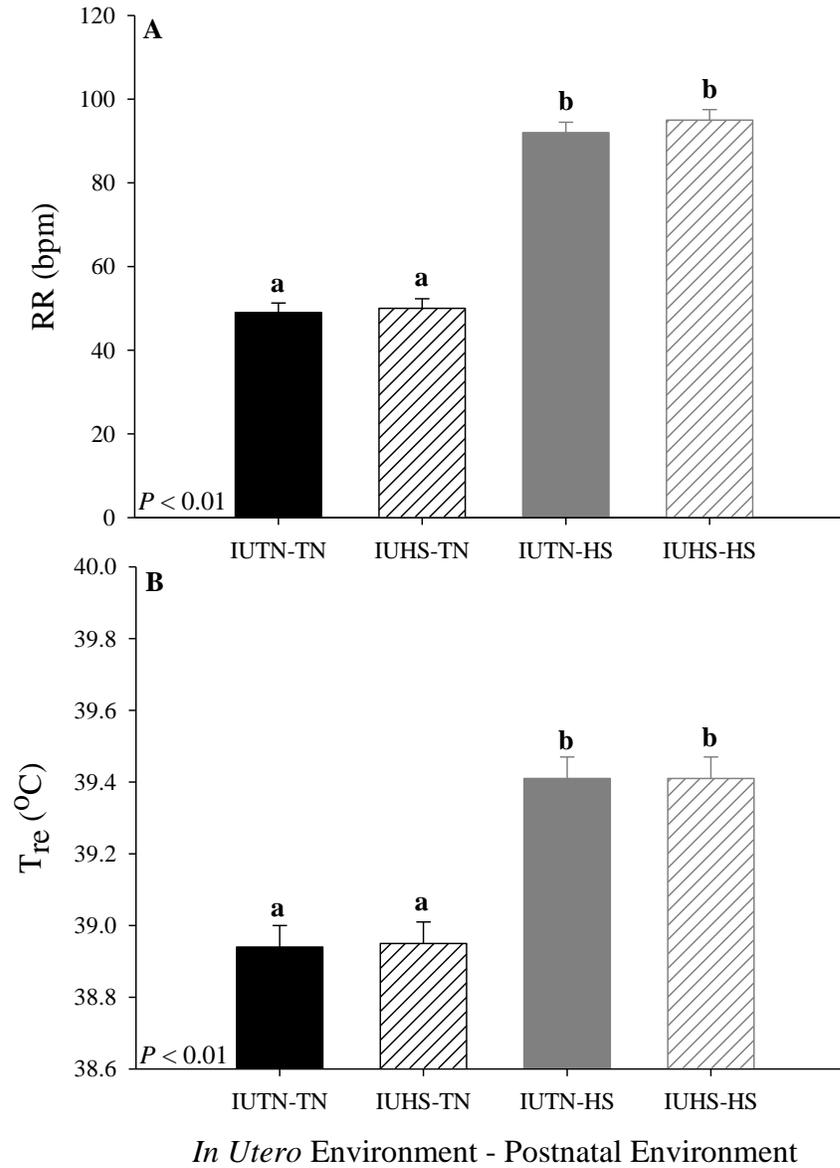


Fig. 1: Effects of *in utero* and postnatal thermal environments on (A) respiration rate (RR), and (B) rectal temperature (T_{re}) averaged by *in utero* and postnatal environment in growing pigs. Abbreviations are: *in utero* thermal neutral (IUTN), *in utero* heat-stressed (IUHS), postnatal thermal neutral conditions (TN), postnatal heat stress conditions (HS). Letters above bars (a,b) indicate significance ($P < 0.01$).

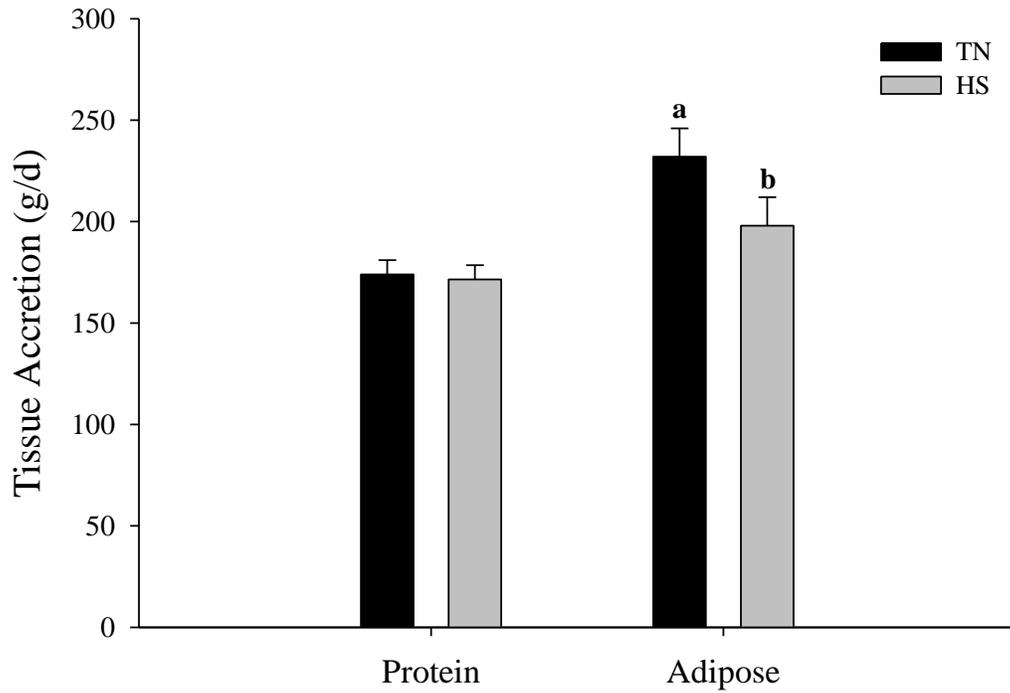


Fig. 2: Effect of postnatal thermal environments on protein accretion (g/d) and adipose accretion (g/d) in growing pigs. Abbreviations are: postnatal thermal neutral conditions (TN), postnatal heat stress conditions (HS). Letters above bars (a,b) indicate significance ($P < 0.05$).

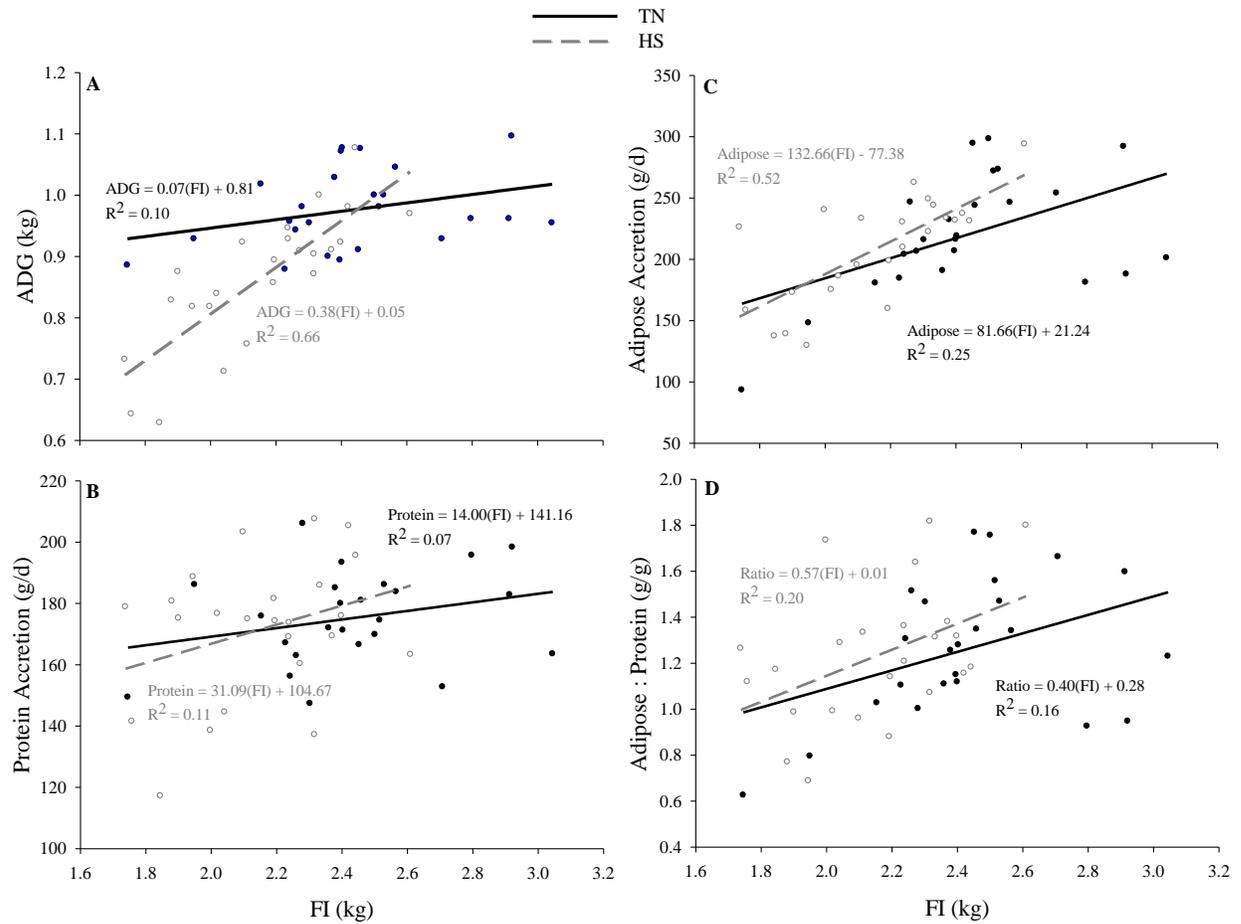


Fig. 3: Linear regression ($y = mx + b$) of (A) average daily gain (ADG) as a function of feed intake (FI), (B) protein accretion (g/d) as a function of FI, (C) adipose accretion (g/d) as a function of FI, and (D) the ratio of adipose to protein accretion/d as a function of FI. Abbreviations are: postnatal thermal neutral pigs (TN) and postnatal heat stress pigs (HS). Coefficient of determination (R^2), and slope (m) is presented for each regression line.

**CHAPTER V: *IN UTERO* HEAT STRESS ALTERS BODY COMPOSITION
DURING THE EARLY FINISHING PHASE (60 TO 80 KG) IN PIGS**

A paper submitted to *The Journal of Animal Science*

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Abstract

The detrimental effects of heat stress (**HS**) on animal productivity have been well-documented. However, whether *in utero* HS interacts with a future thermal insult to alter tissue deposition during the early finishing phase of pig growth is unknown. Study objectives were to compare the subsequent rate and quantity of whole-body tissue accretion in pigs exposed to differing *in utero* and postnatal thermal environments. Pregnant sows were exposed to thermal neutral (**TN**; cyclical 15°C nighttime and 22°C daytime; n = 9) or HS (cyclical 27°C nighttime and 37°C daytime; n = 11) conditions during gestation. Twenty-four offspring from *in utero* TN (**IUTN**; n = 6 gilts, 6 barrows; 62.4 ± 0.7 kg BW), and *in utero* HS (**IUHS**; n = 6 gilts, 6 barrows; 61.9 ± 0.8 kg BW) were euthanized as part of an initial slaughter group (**ISG**). After the ISG, 48 pigs from IUTN (n = 12 gilts, 12 barrows; 66.1 ± 1.0 kg BW) and IUHS (n = 12 gilts, 12 barrows; 63.4 ± 0.7 kg BW) were exposed to constant HS (34.4 ± 1.8°C) or TN (22.7 ± 2.5°C) conditions until they reached 80.5 ± 1.5 kg BW, at which point they were sacrificed and their body composition was determined. Homogenized carcasses were analyzed for nitrogen, crude fat, ash, water and gross energy

content. Data were analyzed using PROC MIXED in SAS 9.3. Rectal temperature and respiration rate were increased during postnatal HS compared to TN (39.6 vs. 39.3°C and 92 vs. 58 bpm, respectively; $P < 0.01$). Postnatal HS decreased ($P < 0.01$) feed intake (2.13 vs. 2.65 kg/d) and ADG (0.70 vs. 0.94 kg/d) compared to TN conditions, but neither variable was influenced by *in utero* environment. Whole-body protein and adipose tissue accretion rates were reduced in HS pigs compared to TN controls (126 vs. 164 g/d and 218 vs. 294 g/d, respectively; $P < 0.04$). Independent of postnatal environments, *in utero* HS reduced future protein accretion rates (16%; $P < 0.01$), and tended to increase adipose accretion rates (292 vs. 220 g/d; $P < 0.07$) compared to IUTN controls. The ratio of adipose to protein accretion rates increased (95%; $P < 0.01$) in IUHS pigs compared to IUTN controls. In summary, the future hierarchy of tissue accretion is altered by *in utero* HS, and this modified nutrient partitioning favors adipose tissue deposition at the expense of skeletal muscle during this specific phase of growth.

Keywords: pigs, *in utero* heat stress, adipose accretion, body composition

Introduction

Although advances in nutrition and production management (i.e. cooling systems and barn design) have partially ameliorated the negative effects of heat stress (**HS**) on animal agriculture, production losses continue during the warm summer months (St-Pierre et al., 2003; Baumgard et al., 2012). Sources of reduced revenue in the swine industry include: reduced growth, poor sow performance, increased morbidity and mortality, inconsistent market weights, and altered carcass composition (Brown-Brandl et al., 2004; Baumgard et al., 2012; Baumgard and Rhoads, 2013). Heat stress-related suboptimal production will

likely worsen as climate models predict an increase in extreme summer conditions for most U.S. pig-producing areas (Luber and McGeehin, 2008). Further, continued genetic selection for rapid lean growth increases basal heat production (Brown –Brandl et al., 2004), and this may compromise HS-tolerance (Nienaber and Hahn, 2007). Consequently, there is an urgent need to better understand the mechanisms by which HS hinders efficient pork production.

Normally, during insufficient nutrient intake, metabolic adaptations favor muscle growth at the expense of adipose tissue accretion (Le Dividich et al., 1980; Oresanya et al., 2008). However, when pigs and other animals are reared in HS conditions (a condition of voluntarily reduced feed intake) carcasses typically accrete more adipose tissue than energetically expected (Katsumata et al., 1990; Gerart et al., 1996; Kouba et al., 2001; Collin et al., 2001a). This postnatal HS-induced altered nutrient partitioning is just beginning to be characterized (as reviewed by Baumgard and Rhoads, 2013); however, the extent to which *in utero* hyperthermia affects future bioenergetic metabolism and tissue growth patterns remains largely unknown.

Previous studies evaluating non-thermal prenatal stressors reported permanent effects on growth (Foxcroft et al., 2006, 2009), post-absorptive metabolism (Chen et al., 2010; Pinney and Simmons, 2010), and nutrient partitioning (Ravelli et al., 1976; Barker et al., 1993), changes that can be broadly characterized by insulin resistance. Consequently, current study objectives were to compare the future rate and quantity of whole-body tissue accretion in pigs exposed to differing *in utero* and postnatal thermal environments. We hypothesized that *in utero* HS exposure would increase postnatal adipose tissue accretion rates at the expense of protein gain.

Materials and Methods

In utero environments

The University of Missouri Animal Care and Use Committee approved all animal procedures involving pregnant sows. Twenty, first parity-crossbred sows (Large White x Landrace; GPK1 x GPK4; Choice Genetics USA; Des Moines, IA) were exposed to thermal neutral (TN; cyclical 15°C nighttime and 22°C daytime; 55% RH; n = 9), or HS (cyclical 27°C nighttime and 37°C daytime; 67.5% RH; n = 11) conditions in the Brody Environmental Chambers at the University of Missouri (Lucy et al., 2012). Thermal treatments began six days after insemination (Duroc; Swine Genetics International; Cambridge, IA) and continued until farrowing (116.6 ± 0.5 d gestation; Lucy et al., 2012). Although heat-stressed pregnant sows had increased rectal temperature ($P < 0.05$; 0.3°C; Lucy et al., 2012) compared to TN controls, feed intake (FI) did not differ between thermal environments ($P > 0.15$; TN: 5.24 ± 0.07 kg/d and HS: 5.08 ± 0.08 kg/d) as all sows were limit-fed to prevent excessive maternal weight gain (standard industry practice; Brendemuhl and Myer, 2009). Between parturition and weaning, all piglets were exposed to the same environmental conditions (26 - 32°C) as recommended by FASS (2010). After weaning, all offspring (n = 253) were transported in an aluminum livestock trailer with wood shavings (standard practice; 6 h) to Iowa State University.

Postnatal environments

The Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals (protocol #7169-S). Between weaning and approximately 60 kg BW, all pigs were housed in TN conditions as recommended by FASS (2010), and allowed to consume water and feed (based primarily on corn and soybean meal) *ad libitum*.

From the 253 pigs transported to Iowa State University, 24 growing pigs with no previous postnatal HS exposure from *in utero* TN (**IUTN**; n = 6 gilts, 6 barrows; 62.4 ± 0.7 kg BW), or *in utero* HS (**IUHS**; n = 6 gilts, 6 barrows; 61.9 ± 0.8 kg BW) conditions were randomly selected and euthanized as part of an initial slaughter group (**ISG**). Following the ISG, 48 pigs from IUTN (n = 12 gilts, 12 barrows; 66.1 ± 1 kg initial BW) and IUHS (n = 12 gilts, 12 barrows; 63.4 ± 0.7 kg initial BW) were housed in individual pens (0.61 x 2.44 m) in one of two environmentally controlled rooms. Pigs were evenly distributed within rooms based upon treatment. Within each room, ambient temperature (T_a) and relative humidity (**%RH**) were continuously recorded by two data loggers (EI – WIN – USB; accuracy: $\pm 1.0^\circ\text{C}$; Lascar Electronics Ltd.; Wiltshire, UK) every 30 min. Circulating fans were utilized to prevent uneven temperature dispersion. Relative humidity within the rooms was ungoverned.

Twelve barrows (n = 6 IUTN, 6 IUHS) and 12 gilts (n = 6 IUTN, 6 IUHS) were housed in constant TN conditions ($22.7 \pm 2.5^\circ\text{C}$; 72.5 ± 10.9 %RH), and 12 barrows (n = 6 IUTN, 6 IUHS) and 12 gilts (n = 6 IUTN, 6 IUHS) were maintained in constant HS conditions ($34.4 \pm 1.8^\circ\text{C}$; 54.4 ± 9.2 %RH) until they reached approximately 80 kg. All pigs were given *ad libitum* access to a standard commercial diet formulated to meet or exceed nutritional requirements (Table 1; NRC, 2012). Feed intake was determined weekly and BW was determined bi-weekly. Respiration rate (**RR**) and rectal temperature (T_{re}) were obtained twice daily (0800, 1600 h). Respiration rate (breaths per minute: **BPM**) was determined by counting flank movement for one minute. Rectal temperature was determined with a calibrated and lubricated thermistor thermometer (Welch Allyn SureTemp[®] Plus; accuracy: \pm

0.1 °C; Skaneateles Falls, NY) inserted approximately 10 cm into the rectum of unrestrained pigs.

Blood sampling and analysis

Blood (10 mL) was obtained on all pigs via jugular venipuncture (BD[®] vacutainers; Franklin Lakes, NJ; K₃EDTA; lithium heparin; serum) at 1500 h one day prior to sacrifice (in a fed state and while still experiencing their respective postnatal environmental treatment) and stored on ice until processing; plasma and serum were then harvested by centrifugation at 2500 x g, aliquoted and stored at -80°C. Glucose concentration was immediately determined from whole blood collected in lithium heparin tubes using a Vet Scan iStat[®] C68+ cartridge (Abaxis, Inc.; Union City, CA). Plasma insulin concentration was measured using an ELISA kit (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden), following the manufacturer's instructions. Commercially available kits were used to determine plasma non-esterified fatty acid (**NEFA**; Autokit NEFA; Wako Chemicals USA, Richmond, VA), plasma urea nitrogen (**PUN**; Urea Nitrogen Reagent; TECO Diagnostics, Anaheim, CA), and serum creatine kinase (**CK**) concentrations (Creatine Kinase-SL Assay; SEKISUI Diagnostics, Charlottetown, PE Canada). The intra- and inter-assay coefficients of variation were 3.8, 7.2, 6.4, 1.9%, and 8.0, 4.7, 8.3, 4.5% for insulin, NEFA, CK, and PUN, respectively. Systemic insulin sensitivity was estimated using the homeostatic model assessment of insulin resistance (**HOMA-IR**; (glucose (mmol/L) * insulin (mg/L))/450; Haffner et al., 1996), and the insulin to FI ratio ([insulin]/average daily feed intake throughout the entire experiment) was calculated for individual pigs.

Serial slaughter and sub-sample analysis

All pigs utilized in the postnatal experiment were harvested after an overnight fast in TN conditions at 62.2 ± 0.7 kg BW (ISG), and 80.5 ± 1.5 kg BW (final slaughter group; **FSG**). Initial and final BW were selected to represent the early finishing phase in modern pigs. Since a similar BW at sacrifice was an objective, FSG pigs were harvested at different times (23.5 ± 3.5 d on trial).

At sacrifice, pigs were electrically stunned, exsanguinated, and blood was collected. Stomach, intestinal, gallbladder and bladder contents were removed, and liver, spleen and total viscera weight was recorded. Whole carcass, including head, viscera (minus contents of stomach, intestine, gallbladder, and bladder) and blood were weighed to determine empty body weight (**EBW**). Carcass, head, viscera and blood of individual pigs were frozen, sectioned, passed twice through a mechanical grinder (Buffalo no. 66BX Enterprise; St. Louis, MO), and then passed four times through a Hobart 52 grinder with a 5-mm die (model #4046; Troy, OH) for homogenization. Ground carcasses were sub-sampled, and sub-samples were immediately dried in a convection oven (101°C ; 24 h) to a constant weight for determination of dry matter (**DM**) according to method 950.46 (AOAC Int., 2002). Dried samples were ground through a 1-mm screen (Retsch ZM 100; Glen Mills Inc.; Clifton, NJ), and analyzed for ash, crude fat, nitrogen (**N**), and gross energy (**GE**). Ash was determined by drying at 600°C (24 h) to a constant weight according to method 923.03 (AOAC Int., 2002). Crude fat was determined by Soxhlet extraction according to method 991.36 (AOAC Int., 2002) using n-hexane as the solvent (Fischer Scientific; Fair Lawn, NJ). Nitrogen content was determined by combustion using a LECO TruMac N Nitrogen Determinator (Leco Corporation; St. Joseph, MI; model #630-300-300) according to method 992.15

(AOAC Int., 2002). Calibration of LECO was conducted with an EDTA standard (known N content $9.56 \pm 0.03\%$; N content determined to be $9.58 \pm 0.06\%$), and crude protein was expressed as nitrogen * 6.25. Gross energy was determined using isoperibol bomb calorimetry (Parr 620 calorimeter; Parr Instrument Company; Moline, IL; model #6200), and benzoic acid was used as the calibration standard (known GE $6,318 \pm 18$ kcal/kg; GE determined to be $6,319 \pm 8$ kcal/kg). All chemical analyses were carried out in duplicate, and repeated when the intra-duplicate coefficient of variation exceeded 3%.

Based on the sub-sample chemical content, total body composition was determined for water, ash, adipose, and protein using the EBW as described (Noblet et al., 1987). The average chemical composition of IUTN and IUHS pigs in the ISG was used to estimate the initial body compositions of IUTN and IUHS pigs in the FSG, respectively. Within each individual pig, the accretion of water, adipose, protein, and ash were estimated by: (final content, g of tissue – estimated initial content, g of tissue) / days between harvest dates.

To determine carcass gain efficiency (CE), the average carcass weight of IUTN and IUHS pigs in the ISG was used to estimate the initial carcass weight of IUTN and IUHS pigs in the FSG, respectively. Within each individual pig, the accretion of carcass tissue was estimated by: (final carcass weight – estimated initial carcass weight)/days between harvest dates. Carcass gain efficiency was calculated by dividing carcass gain by average daily FI for individual pigs (carcass gain : FI).

Statistical analysis

All data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Statistical model components included *in utero* environment (IUTN; IUHS), postnatal environment (TN; HS), gender (M; F), and all interactions. Gender was

removed from the final model because it was insignificant on all measured parameters. All interactions, regardless of significance level were included in the model and dam was used as a random effect for all analyses. Average daily gain (**ADG**) of individual pigs calculated two weeks prior to the start of treatment was used as a covariate for analysis of all body composition and growth performance measurements. For repeated analysis of T_{re} and RR, each pig's respective parameter was analyzed using repeated measures with an autoregressive covariance structure with day as the repeated effect. Data are presented as LSmeans and statistical significance was defined as $P \leq 0.05$, and a tendency was defined as $0.05 < P \leq 0.10$.

Results

Thermal indices

Regardless of postnatal treatment, RR tended ($P < 0.06$) to be reduced (5 bpm) in IUHS compared to IUTN pigs (Fig. 1A). Overall, a considerable increase ($P < 0.01$) in postnatal RR was observed in HS-exposed (92 ± 2 bpm) compared to TN (58 ± 2 bpm; Fig. 1A) pigs. No *in utero* by postnatal environment interaction in RR was detected ($P > 0.59$). While no overall *in utero* differences were detected, T_{re} increased ($P < 0.01$) in postnatal HS pigs ($39.6 \pm 0.1^\circ\text{C}$) compared to TN controls ($39.3 \pm 0.1^\circ\text{C}$; Fig. 1B).

Growth performance

Overall, FI did not differ between *in utero* treatment groups (2.39 kg/d), but postnatal HS reduced ($P < 0.01$) FI by 19.4% compared to TN controls (Table 2). No FI differences ($P > 0.33$) were observed for the *in utero* by postnatal environment interaction (Table 2). Overall, ADG was not affected by *in utero* treatment (0.81 kg/d); however, it was reduced (P

< 0.01; 0.23 kg/d) in postnatal HS compared to TN control pigs (Table 2). Pigs from *in utero* HS had overall reduced overall feed efficiency (**FE**; $P < 0.01$; 11.1%) compared to IUTN controls (Table 2). Although no *in utero* by postnatal interaction was detected, postnatal HS exposure decreased FE ($P < 0.02$; 9%) compared to postnatal TN conditions (Table 2). Regardless of postnatal treatment, CE was reduced ($P < 0.03$; 14.3%) in IUHS compared to IUTN pigs; however, no postnatal or *in utero* by postnatal treatment differences in CE ($P > 0.30$) were observed (Table 2). Body weight gain per Mcal of metabolizable energy (**ME**) consumed was reduced ($P < 0.03$; 9.5%) in IUHS compared to IUTN pigs, regardless of postnatal environment (Table 2). Overall, BW gain per Mcal of ME consumed was reduced ($P < 0.05$; 9.5%) in postnatal HS compared to TN-exposed pigs (Table 2). No *in utero* by postnatal treatment interaction was observed for BW gain per Mcal of ME consumed (Table 2).

Organ and carcass weights

No treatment differences ($P > 0.28$) were detected for FSG carcass weight (63.7 kg), spleen weight (0.13 kg), blood volume (3.36 kg), spleen weight as percent of EBW (0.16%), and blood volume as percent of EBW (4.28%; Table 3). *In utero* HS reduced ($P < 0.04$; 5.5%) head weight compared to control pigs, regardless of postnatal environment (Table 3). Total viscera and liver weights were reduced ($P \leq 0.05$) in postnatal HS pigs by 340 and 80 g, respectively, compared to TN controls (Table 3). As a percent of EBW; head, total viscera, and liver weights were decreased ($P < 0.01$; 3.9, 5.6, and 7.3%, respectively; Table 3) in postnatal HS pigs compared to TN controls. Overall, carcass weight as a percent of EBW was not influenced by *in utero* environment, but was increased ($P < 0.01$; 0.9%) in HS pigs compared to TN controls (Table 3).

Tissue composition and accretion

No *in utero* or postnatal environment differences ($P > 0.12$; Table 4) were detected in carcass percent water (60.5%), adipose (19.7%) or ash (2.6%). Carcass protein content decreased ($P < 0.01$; 3.8%) in IUHS compared to IUTN pigs, and increased ($P < 0.04$; 2.1%) in postnatal HS pigs compared to TN controls (Table 4). Water accretion was reduced ($P < 0.01$; 23%) in postnatal HS pigs compared to postnatal TN controls (Table 4). Accretion rates of protein, adipose, and ash from postnatal HS pigs were reduced ($P < 0.04$) by 39, 78, and 10 g/d, respectively, compared to pigs in postnatal TN conditions (Table 4). Overall, protein accretion rate was reduced ($P < 0.01$; 16%; Fig. 2A) in IUHS pigs compared to IUTN pigs, regardless of postnatal environment (Table 4). Adipose accretion rate tended ($P < 0.07$; Fig. 2B) to be increased in IUHS (292 g/d) pigs compared to IUTN (220 g/d) controls. Regardless of postnatal environment, the ratio of adipose to protein accretion was increased ($P < 0.01$; 95%) for IUHS compared to IUTN pigs (Fig. 2C; Table 4). While no *in utero* differences in whole-body GE content were detected, GE decreased ($P < 0.04$; -95 kcal/g) in postnatal HS pigs compared to TN controls (Table 4).

Blood analyses

Although not altered by *in utero* environments, blood glucose and plasma insulin were reduced ($P < 0.03$) in postnatal HS pigs compared to TN controls (7.5 mg/dL and 0.06 ng/mL, respectively; Table 5). There tended ($P < 0.09$) to be an *in utero* by postnatal interaction in the insulin to glucose ratio as it decreased (47%) in the IUTN-HS pigs but was maintained in the IUHS-HS pigs (Table 5). Regardless of *in utero* environment, PUN ($P < 0.03$; 13%) and HOMA-IR ($P < 0.01$; 40%) were reduced in postnatal HS pigs compared to TN controls (Table 5). The insulin to FI ratio was increased ($P \leq 0.05$; 20%) in IUHS-HS

compared to IUHS-TN pigs, and reduced (42.9%) for IUTN-HS compared to IUTN-TN controls (Table 5). No *in utero* or postnatal differences in NEFA (106.9 mEq/L), or CK concentrations (173.6 U/L) were detected (Table 5).

Discussion

Although the negative impacts of HS have been partially ameliorated by advances in cooling technology and improved management strategies (St-Pierre et al., 2003), it continues to constrain efficient livestock production (Baumgard and Rhoads, 2013). While the effects of HS are primarily evident during the summer months in temperate regions, tropical and sub-tropical regions can experience prolonged periods of HS, making it not only an economic issue, but also a food security and humanitarian concern (Battisti and Naylor, 2009; Muller et al., 2010). Despite the fact that HS causes well-documented postnatal physiological insults with global implications, the impact of HS during *in utero* development on future progeny performance is ill-defined.

Normally, during periods of insufficient FI, metabolic adaptations prioritize skeletal muscle growth at the expense of adipose tissue accretion (Le Dividich et al., 1980; Van Milgen and Noblet, 2003; Oresanya et al., 2008). However, during postnatal HS when FI is voluntarily restricted and insufficient to maintain optimal production, there is an increase in lipid retention in pigs (Kouba et al., 2001), poultry (Geraert et al., 1996) and rodents (Katsumata et al., 1990). This altered metabolic prioritization of tissue development during HS may in part be due to an increased capacity to retain and decreased ability to mobilize fat as demonstrated in a variety of species (as reviewed by Baumgard and Rhoads, 2013).

Prenatal stressors (i.e., maternal malnutrition, intrauterine growth retardation) can negatively affect future growth (Foxcroft et al., 2006, 2009), post-absorptive metabolism (Pinney and Simmons, 2010; Chen et al., 2010) and body composition (Ravelli et al., 1976; Barker et al., 1993), and these aforementioned phenotypes resemble HS-induced changes in postnatal productivity and metabolism (as reviewed by Baumgard and Rhoads, 2013). Consequently, the effects and mechanism(s) of non-thermal prenatal stressors may provide a physiological link between the pre- and postnatal effects of HS on nutrient partitioning. In the present study, *in utero* HS caused a 95% increase in adipose to protein deposition rates (Fig. 2C), and tended to increase adipose deposition (72 g/d; Fig. 2B). Increased carcass fat in IUHS pigs coincided with a 16% reduction in protein accretion (Fig. 2A). The overall increase in adiposity and reduction in protein accretion in IUHS pigs could be the result of a general *in utero* stress response that constrains lean tissue synthesis later in life as has been demonstrated in prenatally stressed rodents (Gokulakrishnan et al., 2012). Since postnatal FI did not differ between *in utero* thermal treatments, nutrient intake in excess of that needed to support lean tissue synthesis was likely partitioned into adipose tissue to a larger extent in IUHS pigs compared to IUTN controls. While this postnatal phenotype differs from what we have previously reported in younger pigs (30 to 60 kg; Johnson et al., 2014), different growth phases (specifically the predisposition to accrete adipose and lean tissue) may differentially respond to previous *in utero* thermal insults. Identifying which stages of future growth are most susceptible to *in utero* HS and discovering the mechanism(s) that mediate this response is of obvious interest.

The increase in adiposity and reduction in protein deposition in IUHS pigs indicates that *in utero* HS modifies future nutrient metabolism and the hierarchy of tissue accretion,

independent of subsequent environmental exposure. This prenatally induced phenotype could be detrimental to efficient animal protein synthesis, especially in pigs gestated during months and in areas that experience prolonged periods of extreme environmental conditions. Although the mechanism of action is currently unknown, this body composition differential is similar to what has been reported in response to maternal nutrient deprivation (Ravelli et al., 1976), intrauterine growth retardation (Desai et al., 2005), and increased maternal dietary fat intake (Tamashiro et al., 2009), where offspring had increased rates of obesity and reduced protein accretion during postnatal development. Although it is important to note that in the current experiment nutrient intake was similar amongst dams. Consequently, considering the similar phenotypic offspring responses in the aforementioned reports and that of *in utero* hyperthermia, it is tempting to speculate that any *in utero* stress, independent of origin/cause, may prenatally program offspring through common mechanisms.

Postnatal HS reduced the accretion rates of almost all tissues compared to postnatal TN-exposed pigs (Table 4). However, this likely mirrors the consequence of reduced FI (16%) and the resulting decrease in ADG (26%) in postnatal HS pigs compared to TN controls (Table 2). Presumably the reduced FI limited the net energy available for tissue synthesis, and as a consequence negatively impacted growth rates, resulting in reduced tissue accretion as has been previously described in heat-stressed pigs (Le Bellego et al., 2002; Kerr et al., 2003; Johnson et al., 2014).

As expected, postnatal HS in the present study reduced FI, ADG and FE compared to *ad libitum* TN conditions (Table 2). Although no *in utero* treatment differences were observed in ADG and FI, IUHS pigs had reduced FE (11%), CE (14%), and reduced BW gain per Mcal of ME consumption (10%) compared to IUTN controls. The reduced growth

efficiency parameters, despite similar ADG and FI, may be due to either increased maintenance costs or the reorganization of nutrient partitioning priorities. To estimate maintenance requirements in the present study, ME for maintenance ($ME_{\text{maintenance}}$) was calculated using the following equation (Patience, 2012): $ME_{\text{maintenance}} = ME_{\text{intake}} - (ME_{\text{protein}} + ME_{\text{adipose}})$. Where, $ME_{\text{intake}} = \text{metabolizable energy of diet (Mcal/kg)} * \text{feed intake (kg/d)}$, $ME_{\text{protein}} = \text{g protein gain/d} * 10.03 \text{ kcal/g of protein gain}$, and $ME_{\text{adipose}} = \text{g adipose gain/d} * 11.65 \text{ kcal/g of adipose gain}$ (as reviewed by Patience, 2012).

Utilizing these variables and assuming that the efficiency of protein and lipid gain and the efficiency of dietary energy use remained unaltered during HS, it appears that IUHS pigs required approximately 212 kcal ME/d less energy for $ME_{\text{maintenance}}$ compared to IUTN pigs, and this was not due to reductions in visceral mass (Table 3). Instead, it is possible that reduced $ME_{\text{maintenance}}$ in IUHS pigs resulted from decreased muscle mass (as indicated by reduced carcass protein content and deposition rate) and presumably reductions in protein turnover rates (Van Milgen and Noblet, 2003). Reduced FE in IUHS pigs is likely reflected by increased adipose and reduced lean accretion rates compared to IUTN controls (Fig. 2C), since depositing adipose is less efficient than lean tissue (as reviewed by Patience, 2012).

Independent of *in utero* environment, pigs exposed to postnatal HS conditions required about 433 kcal/d less energy for $ME_{\text{maintenance}}$ compared to TN controls, and this confirms our previous report (Johnson et al., 2014). The estimated decrease in maintenance requirements in our experiment agrees with other reports indicating heat-stressed animals typically have reduced circulating thyroid hormone concentrations (Sano et al., 1983; Prunier et al, 1997; Sanz Fernandez et al., 2014a), oxygen consumption (Hales, 1973) and heat production (Collin et al., 2001b; Kerr et al., 2003; Brown-Brandl et al., 2004; Renaudeau et

al., 2013), variables all associated with decreased maintenance costs. Although maintenance costs appear to be decreased, postnatal gross FE was also reduced by 9% in HS compared to TN controls. However, this reduction may be explained by differences between gross FE measurements (BW gain : FI) and the efficiency of carcass tissue gain (carcass gain : FI). Despite reduced gross FE, postnatal HS-exposed pigs had a numerical increase in CE (3.3%), and a 4.3% reduction in visceral mass compared to TN controls (Table 3). These data suggest that reductions in gross FE due to HS (NRC, 1981; Renaudeau et al., 2012) are a result of decreased visceral mass, and not due to a reduced rate of converting dietary nutrients into skeletal muscle and adipose tissue, further corroborating a recent report from our lab (Johnson et al., 2014). Further, biological systems may also be altered during HS (e.g. nutrient absorption, endocrine status), potentially compromising efficient growth independently of increased maintenance costs (Baumgard and Rhoads, 2013).

Despite the fact that marked phenotypic changes in postnatal body composition were detected in response to *in utero* HS, no significant bioenergetic differences (i.e. glucose, insulin, NEFA) were detected between IUHS and IUTN pigs (Table 5). Reasons for this are not clear but likely include the fact that only one blood sample per animal was obtained and body composition changes are a continuous accumulation over time. Blood samples are simply a snapshot in time and these bioenergetic parameters are influenced by numerous environmental factors including time relative to feeding. Postnatal HS reduced circulating glucose and insulin concentrations and plasma insulin concentration per kg FI, similar to reports by Rinaldo and Le Dividich (1991), and increased insulin sensitivity as denoted by a reduced HOMA-IR (Haffner et al., 1996; Table 5). The apparent HS-induced increase in insulin sensitivity corresponds with previous reports in rodents (Kokura et al., 2007), pigs

(Sanz Fernandez et al., 2014b), lactating cows (Wheelock et al., 2010) and humans (McCarty et al., 2009). The hyperthermia-induced increased insulin sensitivity might be one mechanism for the increased adiposity in HS-exposed pigs (Kouba et al., 2001; Collin et al., 2001a), as insulin is a potent lipogenic and antilipolytic hormone (Vernon, 1992).

We have previously demonstrated increased postnatal core body temperature in pigs exposed to prenatal HS (Johnson et al., 2013a,b). However, in the present study, no postnatal rectal temperature differences were detected between *in utero* treatments (Fig. 1B). These results are not surprising as identifying body temperature differences was not a primary objective, and detecting small differences in body temperature (a cyclical parameter) requires multiple measurements per day. Regardless, the increased body temperature in pigs gestated during *in utero* HS (Johnson et al., 2013 a,b) is presumably not the result of enhanced skeletal muscle mass (a relatively large contributor to total heat production; Van Milgen and Noblet, 2003) as our current data indicates reduced protein accretion rates in IUHS pigs. A more likely explanation for the elevated body temperature in IUHS pigs could be increased whole-body adipose tissue that may insulate against heat dissipation. Further, determining the mechanism(s) by which pigs gestated during *in utero* HS maintain a higher body temperature during postnatal life is of both bioenergetic and practical interest.

Similar to previous reports (Graham et al., 1998; Johnson et al., 2014), *in utero* HS caused microcephaly (reduced head size), and may be indicative of micrencephaly (reduced brain size), which negatively affects the central nervous system and is associated with reduced muscle tone as reviewed by Graham and colleagues (1998). Pigs housed in the postnatal HS environment also had reduced liver weight, liver weight as a percent of EBW, and total viscera weight as a percent of EBW, regardless of *in utero* environment, and this

confirms previous reports by others (Rinaldo and Le Dividich, 1991; Table 3). As the viscera (particularly the liver) has high metabolic activity (Burrin et al., 1988; Van Milgen and Noblet, 2003), it is not surprising that size of the splanchnic bed, and likely heat production would be decreased in response to HS, possibly due to reduced visceral blood flow as described by others (Lambert et al., 2002; Leon and Helwig, 2010). Regardless of why, the decrease in visceral mass agrees with our idea (Baumgard and Rhoads, 2013; Johnson et al., 2014), and others (Collin et al., 2001b; Renaudeau et al., 2013) that HS actually reduces maintenance costs and heat production in the growing pig.

In utero HS caused a multitude of postnatal phenotypic alterations, namely, an increase in adiposity with a concomitant decrease in protein accretion during the early finishing phase of pig growth. This shift in nutrient partitioning may have negative implications with regards to the amount of lean tissue ultimately synthesized by pigs gestated during the summer months. The negative consequences of *in utero* HS on postnatal body composition would not be apparent until the animals reached market weight during mid- to late spring. As a consequence, the impact of HS on the global pork industry may be much more extensive than originally thought. Future research attempting to identify the stage of gestation that is most susceptible to environmental hyperthermia may provide the foundation for developing mitigation strategies that minimize the negative consequences of summer HS.

Conclusion

In utero programming can permanently modify offspring metabolism and development, and we have now demonstrated that maternal hyperthermia alters the future hierarchy of tissue synthesis. Specifically, pigs experiencing *in utero* HS deposited less

skeletal muscle and more adipose tissue during the postnatal development stage characterized by rapid adipose accretion. These results have implications to the future performance and carcass quality of animals gestated in HS conditions and could compromise the efficiency of lean tissue production, especially in areas that experience prolonged periods of extremely high ambient temperatures. Consequently, the negative consequences of *in utero* HS on future livestock productivity needs to be considered when attempting to quantify the impact of climate change on animal agriculture.

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Author Contributions

J.S. Johnson and L.H Baumgard were responsible for experimental design and manuscript preparation. M.V. Sanz Fernandez, J.F. Patience, N.K. Gabler, J.W. Ross, and R.P. Rhoads provided assistance with statistical analysis, lab work, and experimental design. M.C. Lucy and T.J. Safranski performed maternal phase experiments.

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Table 1. Ingredients and chemical composition of diet for growing pigs (as-fed basis).

Ingredients	---- % ----
Corn	60.6
Dehulled soybean meal	17.4
Dried distillers grains	18.2
45-30 Vitamin and Mineral Premix ¹	2
Lysine	1.05
Threonine	0.114
Methionine	0.058
Tryptophan	0.024
Salt	0.65
Calculated chemical composition, %	
DM	89
Crude protein	18.39
Crude fat	4.21
Crude fiber	4.01
SID Lysine	0.84
Calcium	0.54
Phosphorus	0.44
ME ² , Mcal/kg	3.31
NE ³ , Mcal/kg	2.46

¹Supplied per kilogram of diet: vitamin A, 8804 IU; vitamin D3, 1675 IU; vitamin E, 48 IU; vitamin K, 2.4 IU; choline, 5.5 mg; riboflavin, 4.6 mg; niacin, 23 mg; pantothenic acid, 18.2 mg; vitamin B₁₂, 30 µg; biotin, 1.6 µg; folic acid, 0.0005 mg; Zn, 158 ppm; Mn, 61 ppm; Fe, 177 ppm; Cu, 21 ppm; Se, 0.25 ppm. ^{2,3}Estimated using the NRC 2012 individual dietary ingredients.

Table 2: Effect of *in utero* and postnatal environment on growth parameters in growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
<u>Initial slaughter group (60 kg)</u>								
Final live BW ⁸ (kg)	62.4	61.9	-	-	0.7	0.99	-	-
EBW ⁹ (kg)	58.4	58.6	-	-	0.9	0.92	-	-
<u>Final slaughter group (80 kg)</u>								
Final live BW (kg)	80.7	80.1	82.2	79.0	1.5	0.25	0.88	0.38
EBW (kg)	78.9	78.2	80.8	77.7	1.5	0.25	0.61	0.39
Daily BW gain (kg)	0.96	0.91	0.72	0.67	0.05	0.32	0.01	0.92
Feed intake (kg)	2.53	2.76	2.13	2.13	0.12	0.34	0.01	0.33
Gain:Feed ¹⁰ (kg/kg)	0.38	0.33	0.34	0.31	0.02	0.01	0.03	0.39
Carcass gain : Feed ¹¹ (kg/kg)	0.34	0.30	0.36	0.30	0.01	0.02	0.88	0.31
Gain : ME intake ¹² (kg/Mcal)	0.11	0.10	0.10	0.09	<0.01	0.02	0.04	0.29

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions

²*In utero* heat-stressed pigs in postnatal thermal neutral conditions

³*In utero* thermal neutral pigs in postnatal heat stress conditions

⁴*In utero* heat-stressed pigs in postnatal heat stress conditions

⁵Standard error of the mean

⁶*In utero* environment

⁷Postnatal environment

⁸Body weight

⁹Final live BW minus gastrointestinal contents

¹⁰Feed efficiency

¹¹Carcass weight gain per kilogram of feed intake

¹²Body weight gain per Mcal of ME intake

Table 3: Effect of *in utero* and postnatal environment on carcass and organ weights of growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
<u>Initial slaughter group (60 kg)</u>								
Weight								
Carcass (kg)	45.9	45.5	-	-	0.8	0.68	-	-
Head (kg)	4.10	3.90	-	-	0.08	0.21	-	-
Total Viscera (kg)	5.8	5.9	-	-	0.1	0.97	-	-
Liver (kg)	1.00	0.99	-	-	0.02	0.69	-	-
Spleen (kg)	0.11	0.11	-	-	0.01	0.80	-	-
Blood (kg)	2.97	2.93	-	-	0.07	0.78	-	-
Percent of EBW ⁸								
Carcass % of EBW	78.0	78.0	-	-	0.3	0.92	-	-
Head % of EBW	7.1	6.8	-	-	< 0.1	0.29	-	-
Total Viscera % of EBW	10.0	10.2	-	-	0.2	0.62	-	-
Liver % of EBW	1.71	1.73	-	-	0.04	0.99	-	-
Spleen % of EBW	0.18	0.19	-	-	0.01	0.72	-	-
Blood % of EBW	5.1	5.1	-	-	< 0.1	0.93	-	-
<u>Final slaughter group (80 kg)</u>								
Weight								
Carcass (kg)	63.1	63.1	65.6	63.0	1.3	0.35	0.29	0.28
Head (kg)	5.2	4.9	5.1	4.8	< 0.1	0.04	0.07	0.95
Total Viscera (kg)	7.2	6.9	6.9	6.6	0.2	0.18	0.05	0.99
Liver (kg)	1.15	1.11	1.08	1.03	0.03	0.21	0.03	0.93
Spleen (kg)	0.12	0.13	0.14	0.11	0.02	0.71	0.79	0.35
Blood (kg)	3.42	3.35	3.34	3.34	0.10	0.75	0.69	0.74
Percent of EBW								
Carcass % of EBW	80.0	80.6	81.1	81.0	0.3	0.51	0.01	0.19
Head % of EBW	6.5	6.3	6.2	6.1	< 0.1	0.16	0.01	0.29
Total Viscera % of EBW	9.1	8.9	8.5	8.5	0.2	0.61	0.01	0.39
Liver % of EBW	1.45	1.41	1.33	1.32	0.01	0.51	0.01	0.63
Spleen % of EBW	0.15	0.16	0.17	0.15	0.01	0.79	0.85	0.38
Blood % of EBW	4.3	4.3	4.2	4.3	0.1	0.85	0.57	0.34

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions²*In utero* heat-stressed pigs in postnatal thermal neutral conditions³*In utero* thermal neutral pigs in postnatal heat stress conditions⁴*In utero* heat-stressed pigs in postnatal heat stress conditions⁵Standard error of the mean⁶*In utero* environment⁷Postnatal environment⁸Final live body weight minus gastrointestinal contents

Table 4: Effect of *in utero* and postnatal environment on carcass composition and tissue accretion in growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
<u>Initial slaughter group (60 kg)</u>								
Percent of EBW ⁸			-	-			-	-
Water % of EBW	63.1	63.7	-	-	0.6	0.20	-	-
Protein % of EBW	16.9	17.2	-	-	< 0.1	0.13	-	-
Adipose % of EBW	17.3	16.8	-	-	0.7	0.28	-	-
Ash % of EBW	2.5	2.6	-	-	0.1	0.82	-	-
Gross Energy (kcal/g)	6796	6782	-	-	77	0.71	-	-
<u>Final slaughter group (80 kg)</u>								
Percent of EBW								
Water % of EBW	60.9	60.2	60.8	60.1	0.7	0.39	0.81	0.99
Protein % of EBW	17.1	16.6	17.6	16.8	< 0.1	0.01	0.04	0.46
Adipose % of EBW	19.0	20.7	18.9	20.3	< 0.1	0.82	0.13	0.83
Ash % of EBW	2.7	2.6	2.7	2.4	< 0.1	0.33	0.37	0.23
Tissue accretion								
Water ⁹ (g/d)	525	473	425	344	38	0.12	0.01	0.68
Protein ¹⁰ (g/d)	173	155	141	110	12	0.01	0.03	0.57
Adipose ¹¹ (g/d)	248	340	192	244	35	0.07	0.03	0.54
Ash ¹² (g/d)	36	29	28	18	6	0.27	0.04	0.73
Adipose : Protein	1.32	2.57	1.26	2.47	0.28	0.01	0.73	0.93
Gross Energy (kcal/g)	6861	6900	6757	6813	49	0.41	0.04	0.85

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions²*In utero* heat-stressed pigs in postnatal thermal neutral conditions³*In utero* thermal neutral pigs in postnatal heat stress conditions⁴*In utero* heat-stressed pigs in postnatal heat stress conditions⁵Standard error of the mean⁶*In utero* environment⁷Postnatal environment⁸Final live body weight minus gastrointestinal contents⁹Water accretion per day¹⁰Protein accretion per day¹¹Adipose accretion per day¹²Ash accretion per day

Table 5: Effect of *in utero* and postnatal environment on blood parameters in growing pigs.

Parameter	Environment				SEM ⁵	P		
	IUTN-TN ¹	IUHS-TN ²	IUTN-HS ³	IUHS-HS ⁴		IU ⁶	P ⁷	IU x P
Glucose (mg/dL)	95.6	92.4	88.3	84.8	2.1	0.12	0.01	0.95
Insulin (ng/mL)	0.17	0.15	0.08	0.13	0.03	0.72	0.03	0.15
Insulin : Glucose	0.17	0.16	0.10	0.15	0.03	0.62	0.09	0.09
[Insulin] : Feed intake (kg) ⁸	7 ^a	5 ^b	4 ^b	6 ^a	1	0.38	0.74	0.05
HOMA-IR ⁹ (AU)	2.17	1.89	0.99	1.45	0.35	0.83	0.01	0.23
NEFA ¹⁰ (mEq/L)	99.5	96.5	110.8	120.8	20.8	0.89	0.26	0.68
Creatine Kinase (U/L)	170	138	203	183	29	0.40	0.15	0.85
PUN ¹¹ (mg/dL)	14.5	14.2	12.2	12.7	1.0	0.96	0.02	0.66

¹*In utero* thermal neutral pigs in postnatal thermal neutral conditions

²*In utero* heat-stressed pigs in postnatal thermal neutral conditions

³*In utero* thermal neutral pigs in postnatal heat stress conditions

⁴*In utero* heat-stressed pigs in postnatal heat stress conditions

⁵Standard error of the mean

⁶*In utero* environment

⁷Postnatal environment

⁸Insulin concentration per kilogram of feed intake

⁹Quantification of insulin resistance

¹⁰Non-esterified fatty acid

¹¹Plasma urea nitrogen

^{a,b} $P < 0.05$

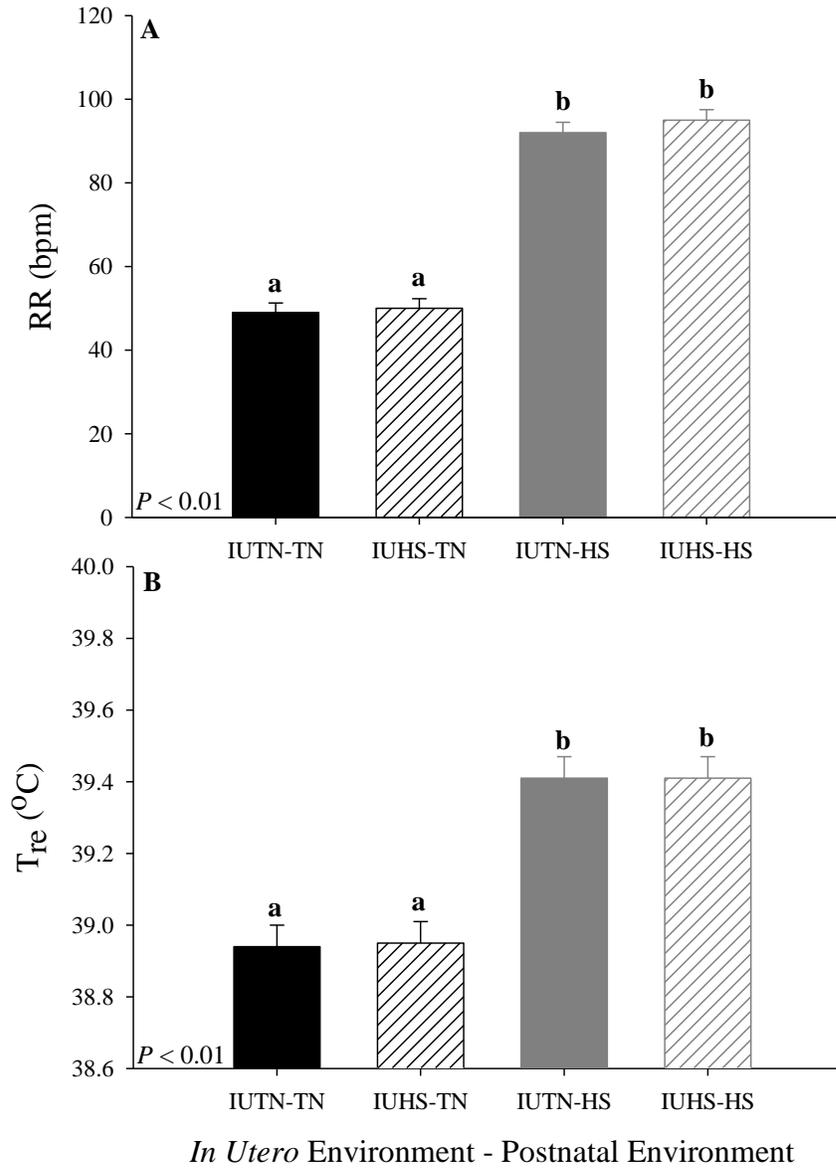


Fig. 1: Effects of *in utero* and postnatal thermal environments on (A) respiration rate (RR), and (B) rectal temperature (T_{re}), averaged by *in utero* and postnatal environment in growing pigs. Abbreviations are: *in utero* thermal neutral (IUTN), *in utero* heat-stressed (IUHS), postnatal thermal neutral conditions (TN), postnatal heat stress conditions (HS). Error bars indicated ± 1 SEM. Letters above bars (a, b) indicate significance ($P < 0.05$).

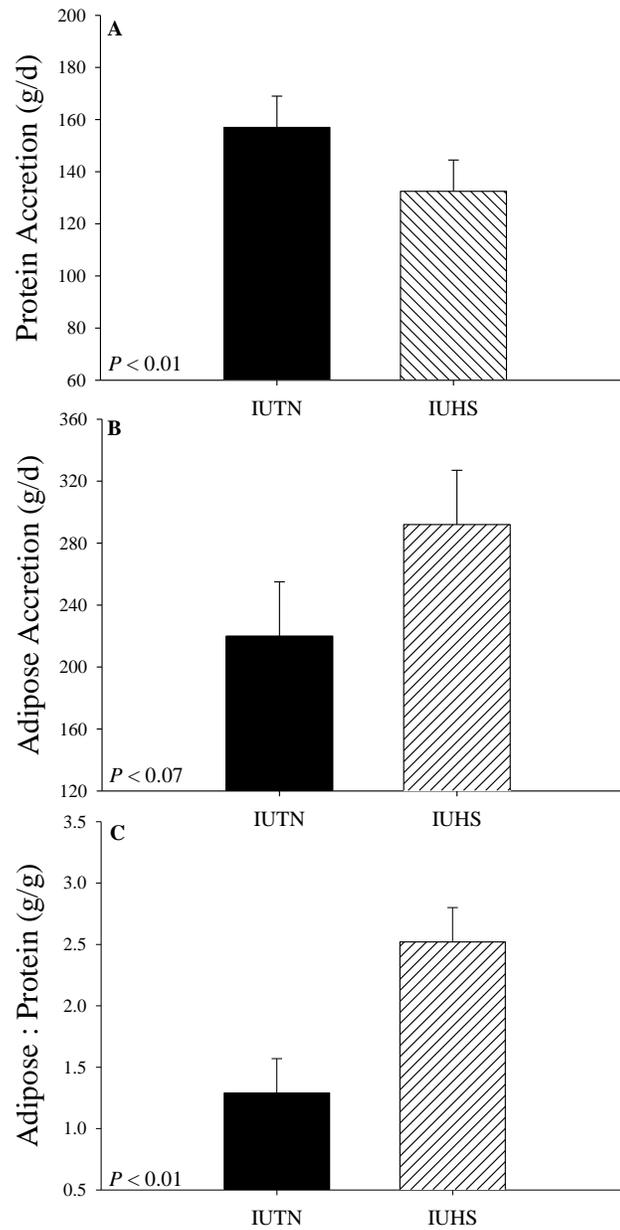


Fig. 2: Effect of *in utero* environments on (A) protein deposition (g/d), (B) adipose deposition (g/d), and (C) the ratio of adipose to protein deposition (g/g) averaged by *in utero* environment in growing pigs. Abbreviations are: *in utero* thermal neutral (IUTN), *in utero* heat-stressed (IUHS). Error bars indicate ± 1 SEM.

CHAPTER VI: THE IMPACT OF *IN UTERO* HEAT STRESS AND NUTRIENT RESTRICTION ON PROGENY BODY COMPOSITION

A paper to be published in *The Journal of Applied Physiology*

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Abstract

We have recently demonstrated that *in utero* heat stress (IUHS) alters future tissue accretion in pig progeny, but whether this transcends species, is indirectly due to reduced maternal feed intake (FI), or is due to the direct effect of heat stress (HS) is not clear. Study objectives were to compare the rate and quantity of tissue accretion in rats exposed to differing *in utero* thermal environments while eliminating the confounding effect of dissimilar maternal FI. On gestation d3, pregnant Sprague Dawley rats were exposed to either thermal neutral (TN; constant 22°C; n = 4), or HS conditions (cyclical 34°C nighttime and 30°C daytime; n = 4) until d18 of gestation. A third group was pair-fed to HS-counterparts in TN conditions (PFTN; constant 22°C; n = 4) from d4 until d19 of gestation. Overall, HS increased dam rectal temperature ($p < 0.01$; 1.3°C) versus TN and PFTN mothers, and reduced FI ($p < 0.01$; 33%) compared to TN controls. By experimental design, the magnitude of FI reduction was similar between HS and PFTN dams. Litter size was similar ($p > 0.96$; 10.9 pups/litter) between environmental treatments, but pup birth weight was reduced ($p < 0.04$; 15.4%) in HS compared to PFTN and TN dams. At d26 of life, two

male pups per dam [n = 8 *in utero* TN (IUTN); n = 8 IUHS; n = 8 *in utero* PFTN (IUPFTN)] were selected, and body composition was determined using dual-energy x-ray absorptiometry (DXA). Following the initial scan, all offspring were individually housed in TN conditions ($21.8 \pm 0.1^\circ\text{C}$) and DXA analyses were repeated on d46 and d66 of life. *In utero* treatment did not alter ($p > 0.81$) future offspring FI (18.6 g/d) or BW gain (5.8 g/d) from d26 to d66 of life. Body fat content and total adipose tissue were increased ($p < 0.01$; 11.2 and 13.1%, respectively) in IUPFTN compared to IUTN and IUHS offspring. *In utero* PFTN offspring had reduced body lean tissue ($p < 0.01$; 2.6%) compared to IUTN and IUHS counterparts. Body composition parameters did not differ between IUHS and IUTN offspring. *In utero* environmental treatments did not alter body bone mineral content. From d26 to d66 of life, the adipose to lean tissue accretion ratio was increased ($p < 0.01$; 19.2%) in IUPFTN compared to IUHS offspring, but was similar to IUTN progeny. Epididymal fat pad weight was increased ($p < 0.04$; 21.6%) in IUPFTN versus IUHS offspring, but no differences were observed when comparing IUPFTN and IUHS progeny to IUTN controls. In summary and in contrast to pigs, IUHS did not appear to impact future body composition; however, *in utero* feed restriction altered the future hierarchy of tissue accretion.

Keywords: rat, *in utero* heat stress, nutrient restriction, tissue accretion

Introduction

Sub-optimal productivity due to heat stress (HS) jeopardizes efficient animal production and threatens global food security (2). This is primarily explained by: decreased and inconsistent growth, poor reproductive performance, altered carcass composition, and increased morbidity and mortality (3, 5). The negative effects of HS will likely become more

evident as climate models predict an increase in extreme summer conditions for most agricultural areas (21). 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. Defining 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 intrauterine insults can have lasting effects on offspring performance (7, 19, 30). One extensively researched experimental model is maternal under-nutrition that can increase offspring lipid accretion and reduce lean tissue (1, 7, 28, 30). In addition, we recently reported (15) that *in utero* HS increases future adipose deposition at the expense of skeletal muscle mass in pigs. Similarities between the *in utero* under-nutrition and HS models, coupled with the fact that HS causes a well-documented reduction in feed intake in almost all species (3, 10, 35), lends itself to the hypothesis that the two *in utero* insults may be mechanistically linked. Study objectives were to investigate the direct and indirect effects 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 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* thermal neutral controls.

Materials and Methods

Maternal environment

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving pregnant rats (IACUC #7634-R). Twelve, first parity timed pregnant OB Sprague Dawley rats (201.5 ± 3.1 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 thermal neutral [TN; constant $22.2 \pm <0.1^\circ\text{C}$; $34.6 \pm 0.1\%$ relative humidity (%RH); n = 4], HS (cyclical 34.0°C nighttime and 30.0°C daytime; $20.1 \pm 0.1\%$ RH; n = 4), 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 = 4) to eliminate the confounding effects of dissimilar feed intake (FI). Within each room, ambient temperature (T_a) and %RH were continuously recorded by one mounted data logger (El – WIN – USB, Accuracy: $\pm 1.0^\circ\text{C}$; Lascar Electronics Ltd.; Wiltshire, UK) every five min. Although T_a was controlled, %RH was ungoverned throughout the experiment.

Maternal environmental treatments began and ended on d3 and d18 of gestation, respectively, for TN and HS mothers, and on d4 and d19 of gestation, respectively, for PFTN mothers (Fig. 1). 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 dams were fed *ad libitum* and exposed to TN conditions (constant $22.2 \pm <0.0^\circ\text{C}$; $34.6 \pm 0.1\%$ RH) from d19 (TN, HS) or d20 (PFTN) of gestation until parturition (d22). Rectal temperatures (T_{re}) were obtained on all dams at 1600 h every other day of the *in utero* treatment period using a

calibrated and lubricated digital thermometer (Safety 1st; Accuracy: $\pm 0.1^\circ\text{C}$; Model #TH050) inserted approximately 1 cm into the rectum. Tail skin temperature (T_{skin}) was measured twice daily (0800, 1600 h) using a calibrated infrared thermometer (Model 42505, Accuracy: $\pm 5.0^\circ\text{C}$; Extech Instruments, Waltham, MA). Body weight, FI, and water intake (WI) were determined daily (0800 h) on all dams. At parturition, individual pup birth weight and pup number was recorded for each dam. Between parturition and weaning, all pups were exposed to TN environmental conditions (20 - 24°C) as recommended for neonatal rats (12).

Postnatal environment

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving rat offspring (IACUC #7634-R). Two male offspring per pregnant dam were selected from *in utero* TN (IUTN; n = 8), *in utero* HS (IUHS; n = 8), and *in utero* PFTN (IUPFTN; n = 8) mothers at weaning (d23 of life) and housed in individual cages (0.2 m x 0.4 m) in TN ($21.8 \pm 0.1^\circ\text{C}$; $28.3 \pm 0.2\%$ RH) conditions. By experimental design, gestation length (22 d) was the same for all selected offspring. Body weight was determined weekly and FI measured every third day to determine average daily FI and BW gain. An initial body scan was performed on anesthetized offspring (100 mg/mL ketamine and 10 mg/mL xylazine; 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 at d26 (68.4 ± 0.7 g), d46 (190.5 ± 4.6 g BW), and d66 of life (302.9 ± 7.5 g BW) to determine percent fat, lean and bone mineral content of individual animals. Based on tissue content, total tissue quantity and accretion rates were calculated as we have previously described (15, 16):

$$(\text{Final content, g of tissue} - \text{initial content, g of tissue}) / \text{days between DXA scans}$$

Tissue collection

Following the final DXA scan (d66), offspring were humanely euthanized by anesthesia injection (100 mg/mL ketamine and 10 mg/mL xylazine; 0.2 mL/100 g BW IP) and 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.

Histology

Fixed samples of subcutaneous flank fat and visceral fat were sent to the Iowa State University Veterinary Diagnostic Laboratory for sectioning and hematoxylin and eosin staining. Adipocytes from each sample were imaged using Q-capture Pro 6.0 software (Qimaging[®], Surrey, BC, Canada), and adipocyte size was determined using ImageJ 1.47v software (National Institutes of Health; Bethesda, MD, USA).

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, and their interactions. Statistical model components for postnatal environment data included *in utero* environment (IUTN; IUHS; IUPFTN), DXA scan day (26; 46; 66), and their interactions. Dam was used as a random effect for all analyses of *in utero* environment variables. For repeated analyses, each rat's respective parameter was analyzed using repeated measures with an autoregressive covariance structure with 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 $0.05 < p \leq 0.10$.

Results

Maternal environment

Thermal indices

Over the entire maternal treatment period, T_{re} 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).

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). Over the entire maternal treatment period, maternal BW was similar ($p > 0.66$; Table 1) between treatments, but a maternal environment by day interaction was detected ($p < 0.01$; Fig. 3) as HS dams had reduced ($p < 0.01$; 22.6 g) BW from d10 to d19 of gestation compared to TN controls (Fig. 3), and PFTN dams had decreased BW ($p < 0.01$) compared to TN (41.4 g) and HS-exposed dams (18.8 g; Fig. 3). Overall, BW gain per gram of FI was increased ($p \leq 0.05$; 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 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 ($p >$

0.96; 10.9 pups/litter), individual pup birth weight was reduced ($p \leq 0.05$; 15.4%) in HS-exposed dams compared to PFTN and TN controls (Table 1).

Offspring variables

Growth

In utero PFTN offspring had increased BW ($p < 0.05$; 6.7%) compared to IUTN rats on d26 of life; however, no *in utero* treatment BW differences ($p > 0.84$) were detected on d46 (189.7 g) or d66 of life (302.9 g; Table 2). Overall, daily BW gain (5.8 g/d), FI (18.6 g/d), and feed efficiency (0.32 g gain per g feed intake) were similar ($p > 0.81$) in offspring from all *in utero* treatments (Table 2).

Tissue composition and accretion

Overall, IUFTN offspring had reduced percent lean ($p < 0.01$) compared to IUTN and IUHS progeny by 2.4 and 2.7%, respectively (Table 3; Fig. 4A). When considering all DXA scan days, percent adipose tissue was increased ($p < 0.01$) in IUPFTN compared to IUTN and IUHS offspring (10.6 and 12.5%, respectively; Table 3; Fig. 4B). Overall, IUPFTN offspring had increased ($p < 0.01$) grams of adipose tissue compared to IUTN and IUHS rats by 10.3 and 16.1%, respectively (Table 3). A DXA scan day effect was observed, regardless of *in utero* treatment, where percent lean decreased ($p < 0.01$; Fig. 4A) and both percent adipose tissue (Fig. 4B) and grams of adipose tissue increased ($p < 0.01$) as progeny matured (Table 3). No other body composition differences were detected comparing *in utero* environment, DXA scan day, or their interaction.

No *in utero* tissue accretion differences amongst treatments were detected from d26 to d46 of life ($p > 0.16$; Table 4), or d46 to d66 of life ($p > 0.54$; Table 4). From d26 to d66 of life, grams of lean tissue gain per gram of BW gain were reduced ($p < 0.05$; 3.8%) in

IUPFTN compared to IUHS rats; however, no differences were detected when compared to IUTN controls (Table 4). Adipose tissue gain per gram of BW gain from d26 to d66 of life increased ($p < 0.04$; 15.0%) in IUPFTN compared to IUHS offspring, but no differences were observed when compared to IUTN controls (Table 4). From d26 to d66 of life, the ratio of adipose to lean accretion increased ($p < 0.04$; 19.2%) in IUPFTN compared to IUHS offspring; however, no differences were detected comparing IUHS and IUPFTN offspring to IUTN controls (Table 4). No other *in utero* treatment differences in tissue accretion were observed (Table 4).

Organ and tissue weights

Epididymal fat pad weight was increased ($p \leq 0.05$; 0.77 g) in IUPFTN compared to IUHS rats (Table 5), but no differences were detected when comparing IUHS and IUPFTN offspring to IUTN controls (Table 5). *In utero* PFTN rats had increased ($p < 0.04$) epididymal fat pad weight as a percent of BW compared to IUTN and IUHS rats (17.1 and 21.8%, respectively; Table 5). Testis weight and testis weight as a percent of BW were increased ($p < 0.05$) in IUPFTN compared to IUTN and IUHS rats by 10.6 and 13.4%, and 7.4 and 11.5%, respectively (Table 5). Gastrocnemius muscle weight and gastrocnemius muscle weight as a percent of BW were similar ($p > 0.72$; 1.77 g and 0.58%, respectively) amongst *in utero* treatments (Table 5). Soleus muscle weight and soleus muscle weight as a percent of BW were similar ($p > 0.91$; 0.13 g and 0.04%, respectively) between *in utero* treatments (Table 5). No other organ and tissue weight differences were detected between *in utero* treatments (Table 5).

Histology

Adipocyte size was increased ($p < 0.01$) in subcutaneous adipose tissue in IUPFTN compared to IUTN and IUHS offspring by 83 and 92%, respectively (Table 5; Fig. 5). No adipocyte size differences in visceral adipose tissue ($p > 0.63$; 3895 ± 505.6 microns²; Table 5) were detected amongst *in utero* treatments.

Discussion

Heat stress jeopardizes efforts by animal agriculture industries to efficiently produce animal products for human consumption (3). 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 (21). Although the effects of HS on animal production during postnatal life are well-documented (10, 35), 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 (9, 15, 19). Specifically, prenatal under-nutrition, results in reduced lean content and greater adiposity in offspring (1, 28, 30). Likewise, *in utero* HS increases postnatal adipose accretion at the expense of skeletal muscle mass in pigs (15). Considering the well-documented energy intake reduction of most species in response to HS (10, 35), the negative intrauterine programming effects of prenatal HS may be an indirect result of maternal feed restriction. In the current study, despite similar maternal feed intake, IUPFTN offspring had increased adipose tissue mass compared to IUHS and IUTN progeny (Table 3). Increased adiposity of IUPFTN compared to IUHS offspring was accompanied by a slight reduction in total lean tissue content (Table 3), likely due to

modified postnatal nutrient partitioning resulting from either reduced protein synthesizing capacity (15, 36), or decreased glucose uptake by skeletal muscle as described in intrauterine growth restricted rodents by Ozanne and colleagues (25).

The stark differences in postnatal phenotypes described above may shed light on the mechanism by which HS affects bioenergetics. A previous study by our lab (26) reported enhanced 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 (19 g), 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). While specific mechanisms are unknown, increased BW gain and efficiency of converting dietary nutrients into tissue may be the result of HS-induced reductions in maintenance costs (15, 16) and heat production (17), thus sparing energy for additional growth. This could imply that although HS and PFTN mothers consumed similar amounts of gross energy, nutrient availability may have been greater in HS dams.

Subcutaneous adipocyte hypertrophy is closely linked with obesity (24, 32) and is a postnatal phenotypic consequence of *in utero* under-nutrition (11, 23). In the present study, subcutaneous adipocyte size was increased (87%) in IUPFTN compared to IUHS and IUTN progeny (Table 5; Fig. 5). A report by O'Connell and colleagues (24) indicates that the degree to which adipocytes can expand to accommodate excess lipid may be determined by intrauterine influences (i.e. maternal under-nutrition, *in utero* stress). As a result, hypertrophic adipocytes may indirectly influence metabolism by increasing incidence of

hyperinsulinemia because larger adipocytes are less sensitive to insulin action (13, 31), and may be an independent predictor of type II diabetes (20, 39).

Increased epididymal fat pad weight is frequently reported in obese rodent models (18), and may be a postnatal consequence of *in utero* under-nutrition (8). In accordance with the aforementioned reports, epididymal fat pad weight and epididymal fat pad weight as a percent of BW was increased (25 and 22%, respectively) in IUPFTN compared to IUHS offspring (Table 4). The increased epididymal fat pad weight corroborates the DXA data and because offspring FI and BW gain were similar, it is likely a consequence of preprogrammed nutrient partitioning that favors adipose deposition in IUPFTN progeny. In addition, testis weight and testis weight as a percent of BW was increased (12 and 9%, respectively) in IUPFTN versus IUTN and IUHS progeny (Table 4). These results agree with those by Rae and colleagues (27) where testis weight of sheep fetuses exposed to prenatal malnutrition was numerically increased. Causes for how or why prenatal nutrient restriction increases testis size is unknown, but may have practical implications to reproductive success in agriculturally important animals. Although total 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 likely explanation is that the decrease in lean content of IUPFTN offspring was not large enough to be detected by gross measurements of individual muscles mass in a small sample size.

Reduced birth weight is a well-documented effect of *in utero* under-nutrition (1, 28, 30, 34) and hyperthermia (29, 33, 37), 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 for IUHS rats, litter size and gestation length (by experimental design) were similar for all *in utero* treatments. Additionally, since no pup birth weight differences were observed for IUPFTN offspring, this indicates that reduced birth weight in IUHS rats was not a function of *in utero* restricted feed intake 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 (22, 29). Since fetal development was retarded despite the overall apparent increase in available nutrients in heat-stressed dams (as indicated by enhanced dam growth), it may imply that the previously reported (15) negative postnatal consequences of *in utero* HS in pigs are mediated by intrauterine growth retardation as opposed to maternal under-nutrition. Further, as low birth weight is a significant risk factor for the development of obesity in later life (34), IUHS offspring may have accumulated more adipose tissue if the experiment had lasted longer into life.

Although IUHS treatments reduced offspring birth weight, pup BW at weaning was similar to IUTN controls. This indicates that lactogenic capacity in heat-stressed mothers was likely not compromised and was copious enough to allow for “catch-up” growth in IUHS offspring. While IUPFTN conditions altered offspring body composition compared to IUHS treatment, no BW or FI differences were detected during postnatal growth (Table 3). 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 in IUPFTN offspring was surprising, since hyperphagia is a commonly reported postnatal consequence of prenatal under-nutrition (4, 38), possibly

mediated by altered expression of the appetite controlling peptides pro-opiomelanocortin and neuropeptide Y (6, 14). 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 (4, 38), 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 lab (15) indicates 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 30 to 34°C; pig = cycling 27 to 37°C), timing of the gestational insult (rat = 73% of gestation; pig = 95% of gestation), or because FI reductions were not expressed in heat-stressed compared to TN sows due to the standard swine industry practice of limit-feeding during gestation (15). Additionally, there may be species differences in how maternal HS affects fetal and postnatal development.

Perspectives and Significance

Previous reports by our lab indicate that *in utero* HS alters postnatal nutrient partitioning in pigs, and we hypothesized that this prenatal programming may be mediated by maternal feed restriction. However, our hypothesis was challenged by the current rodent model of *in utero* HS. 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 and heat

production (as indicated by enhanced dam growth). Although an apparent increase in available nutrients occurred in heat-stressed dams, IUHS birth weight was reduced, which may be indicative of intrauterine growth retardation and could predict 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. Furthermore, the mechanisms by which *in utero* HS alters postnatal nutrient partitioning are still unknown, and this experiment likely needs to be repeated in pigs.

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Declaration of Interest

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Author Contributions

J.S. Johnson and L.H. Baumgard were responsible for experimental design and manuscript preparation. R.P. Rhoads, J.W. Ross, J.T Selsby and A.F. Keating provided assistance with statistical analysis and experimental design. M. Abuajamieh assisted with data collection.

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Table 1: Effect of environmental treatment on maternal variables.

Parameter	Environment			SEM ⁴	<i>P</i>		
	TN ¹	HS ²	PFTN ³		M ⁵	D ⁶	M x D
T _{re} (°C) ⁷	37.2 ^a	38.5 ^b	37.2 ^a	0.1	0.01	0.01	0.01
T _{skin} (°C) ⁸	27.0 ^a	36.8 ^b	27.2 ^a	0.2	0.01	0.01	0.01
Body weight (g) ⁹	225.5	221.1	218.1	5.5	0.65	0.01	0.01
Body weight gain (g/d)	5.96 ^a	4.39 ^b	3.70 ^c	0.18	0.01	0.01	0.01
Feed intake (g/d)	21.0 ^a	14.1 ^b	13.7 ^b	0.6	0.01	0.01	0.15
FI as % of BW (g/g) ¹⁰	9.3 ^a	6.3 ^b	6.3 ^b	0.1	0.01	0.01	0.66
BW gain (g) : FI (g) ¹¹	0.28 ^{ab}	0.30 ^b	0.26 ^a	<0.01	0.05	0.01	0.01
Water intake (mL/d)	41.7 ^a	52.1 ^b	44.8 ^a	2.2	0.02	0.01	0.01
Birth weight ¹² (g)	7.5 ^a	6.3 ^b	7.4 ^a	0.3	0.05	-	-
Pup number	11.0	10.8	10.8	0.9	0.97	-	-

¹Thermal neutral²Heat-stressed³Pair-fed in thermal neutral conditions⁴Standard error of the mean⁵Maternal environment⁶Day of gestation⁷Rectal temperature⁸Tail skin temperature⁹Average body weight during gestation¹⁰Feed intake % of body weight¹¹Feed efficiency¹²Mean birth weight of individual pups^{a,b}*p* < 0.05

Table 2: Effect of *in utero* environment on rat offspring growth.

Parameter	Environment			SEM ⁴	<i>P</i> IU ⁵
	IUTN ¹	IUHS ²	IUPFTN ³		
Body weight					
Day 26 (g)	67.4 ^a	68.9 ^{ab}	71.9 ^b	0.9	0.04
Day 46 (g)	189.7	187.8	191.7	4.9	0.85
Day 66 (g)	302.8	300.4	305.6	7.5	0.88
Body weight gain (g/d)	5.9	5.7	5.8	0.2	0.82
Feed intake (g/d)	18.7	18.4	18.8	0.7	0.93
BW gain (g) : FI (g) ⁶	0.32	0.32	0.31	0.01	0.91

¹*In utero* thermal neutral²*In utero* heat-stressed³*In utero* pair-fed thermal neutral⁴Standard error of the mean⁵*In utero* environment⁶Feed efficiency^{a,b} $p < 0.05$

Table 3: Effect of *in utero* environment on postnatal body composition in rat offspring.

Parameter	Environment			SEM ⁴	P		
	IUTN ¹	IUHS ²	IUPFTN ³		IU ⁵	D ⁶	IU x D
Adipose %	17.9 ^a	17.6 ^a	19.8 ^b	0.4	0.01	0.01	0.25
Lean %	79.8 ^a	80.1 ^a	77.9 ^b	0.4	0.01	0.01	0.23
BMC ⁷ %	2.22	2.29	2.25	0.03	0.38	0.01	0.22
Adipose (g)	35.9 ^a	34.1 ^a	39.6 ^b	1.1	0.01	0.01	0.15
Lean (g)	147.0	148.1	147.6	3.5	0.97	0.01	0.92
BMC (g)	4.23	4.34	4.35	0.08	0.54	0.01	0.52

¹*In utero* thermal neutral²*In utero* heat-stressed³*In utero* pair-fed thermal neutral⁴Standard error of the mean⁵*In utero* environment⁶Day of DXA body scan⁷Bone mineral content^{a,b} $p < 0.05$

Table 4: Effect of *in utero* environment on postnatal tissue accretion in rat offspring.

Parameter	Environment			SEM ⁴	<i>P</i> IU ⁵
	IUTN ¹	IUHS ²	IUPFTN ³		
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 ^b	0.31 ^a	< 0.01	0.03
Adipose (g/g BW gain)	0.22 ^{ab}	0.20 ^b	0.23 ^a	< 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* thermal neutral²*In utero* heat-stressed³*In utero* pair-fed thermal neutral⁴Standard error of the mean⁵*In utero* environment⁶Day of life⁷Bone mineral content⁸Grams of tissue gain per gram of body weight gain^{a,b}*p* < 0.05

Table 5: Effect of *in utero* environment on postnatal carcass components in rat offspring.

Parameter	Environment			SEM ⁴	<i>P</i> IU ⁵
	IUTN ¹	IUHS ²	IUPFTN ³		
Weight					
Epididymal fat pad (g)	3.23 ^{ab}	3.05 ^b	3.82 ^a	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 (microns ²)	1803 ^a	1719 ^a	3293 ^b	370	0.01
Visceral adipocyte size (microns ²)	4158	4013	3513	506	0.64

¹*In utero* thermal neutral²*In utero* heat-stressed³*In utero* pair-fed thermal neutral⁴Standard error of the mean⁵*In utero* environment⁶Body weight^{a,b}*p* < 0.05

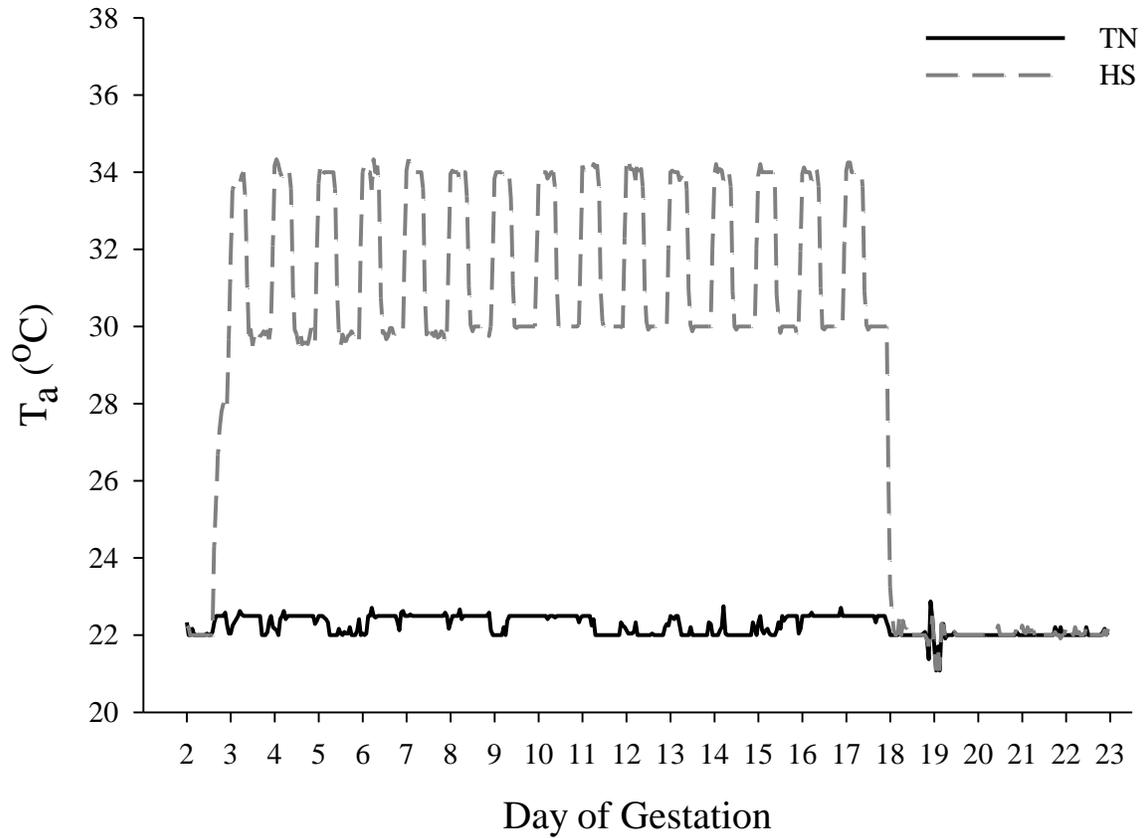


Figure 1: Ambient temperature ($^{\circ}\text{C}$) by day of gestation in pregnant Sprague Dawley rats. Abbreviations are: maternal thermal neutral environment (TN; constant $22.2 \pm <0.1^{\circ}\text{C}$), and maternal heat stress environment (HS; cyclical 30.0 to 34.0°C).

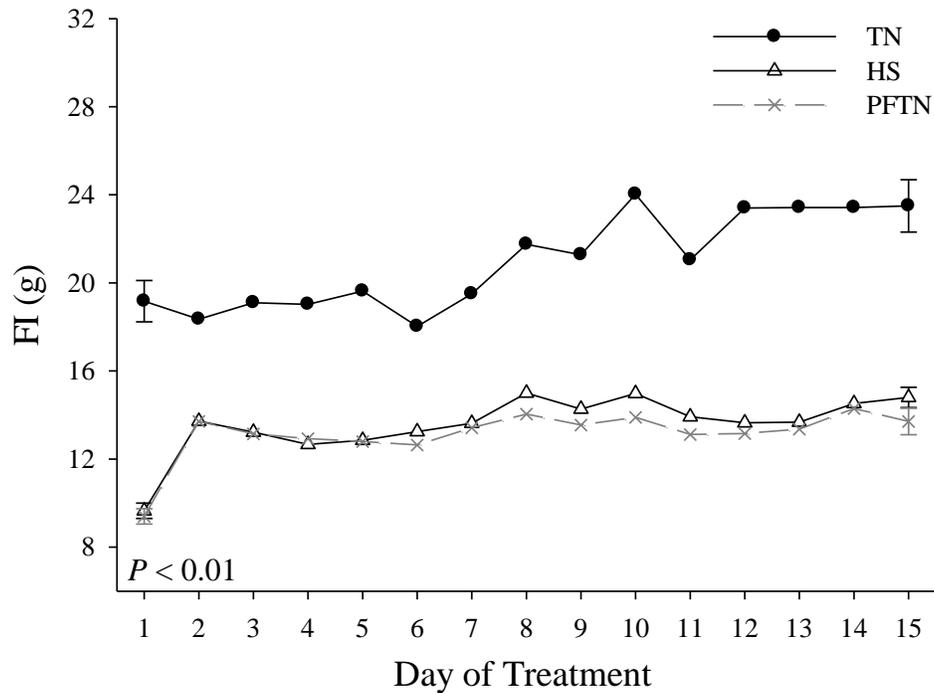


Figure 2: Effects of maternal environment on the temporal changes in feed intake (FI) in pregnant Sprague Dawley rats averaged by day of treatment. Abbreviations are: maternal thermal neutral environment (TN; constant $22.2 \pm <0.1^\circ\text{C}$), maternal heat stress environment (HS; cycling 30.0 to 34.0°C), 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.

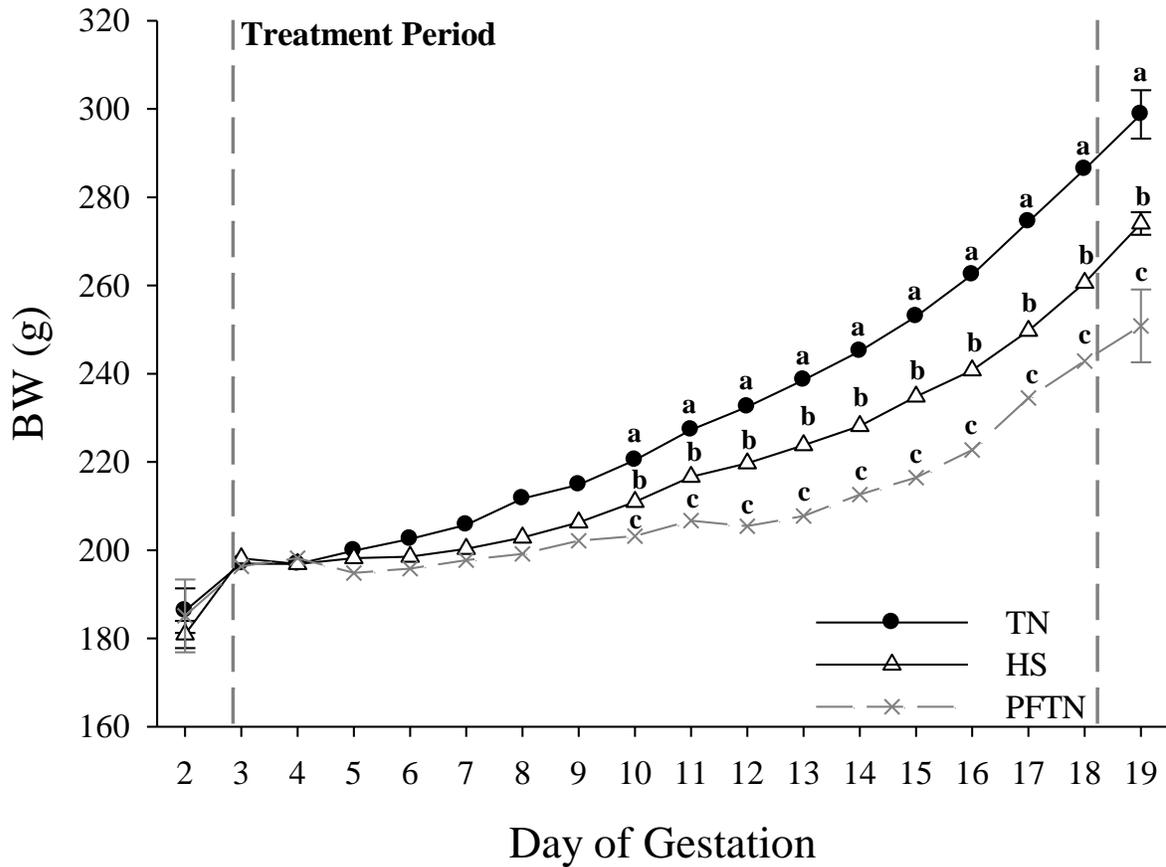


Figure 3: Effects of maternal environment on the temporal changes in body weight by day of gestation in pregnant Sprague Dawley rats. Abbreviations are: maternal thermal neutral environment (TN; constant $22.2 \pm <0.1^\circ\text{C}$), maternal heat stress environment (HS; 30.0 to 34.0°C), mothers pair-fed 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 ($p < 0.01$).

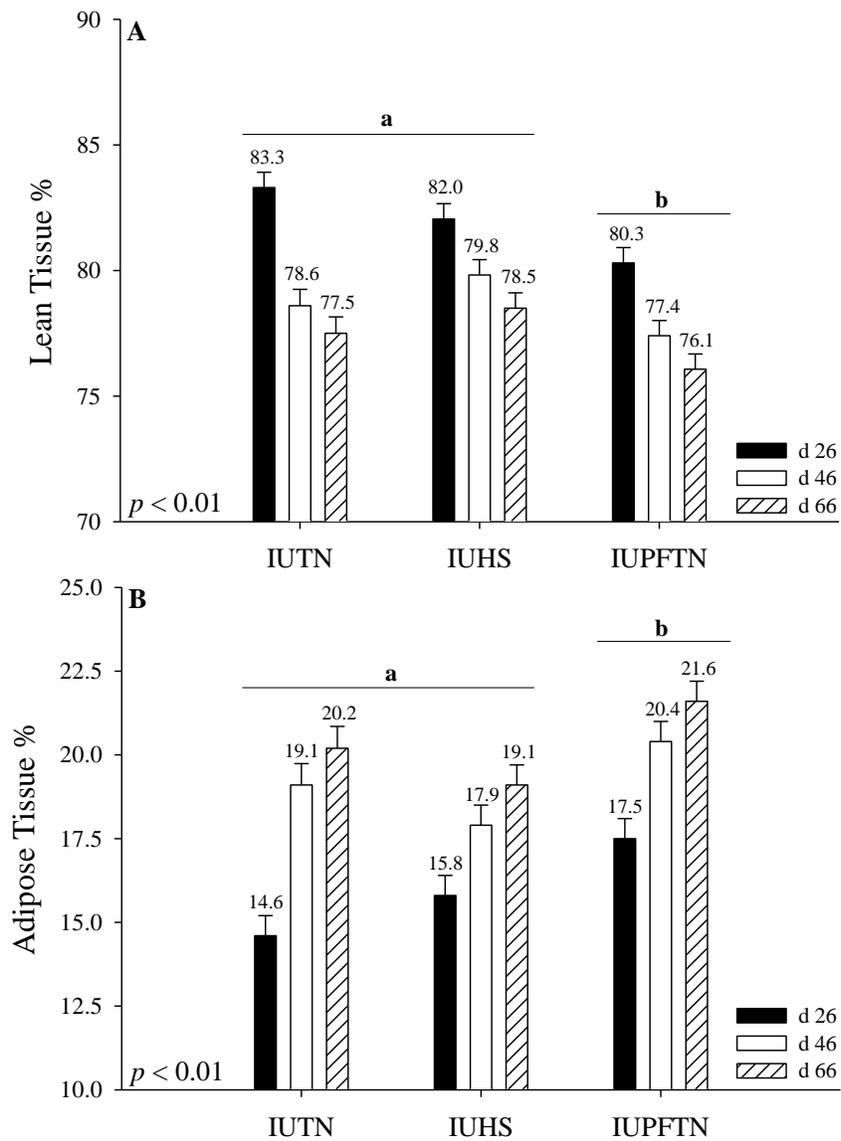


Figure 4: Effect of *in utero* environment on (A) percent lean tissue, and (B) percent adipose tissue as determined by DXA scans on d26, d46, and d66 of postnatal life. Abbreviations are: *in utero* thermal neutral environment (IUTN), *in utero* heat-stressed environment (IUHS), and *in utero* pair-fed to HS-exposed rats in TN conditions (IUPFTN). Numbers above error bars indicate tissue percent at each DXA scan day. Letters (a,b) indicate differences between *in utero* treatments.

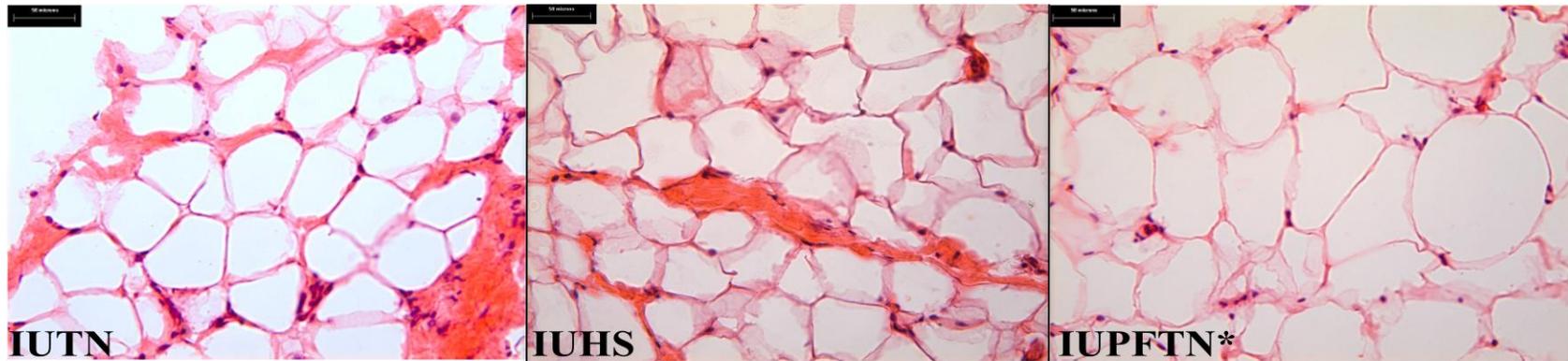


Figure 5: Estimation of adipocyte size in the subcutaneous adipose tissue of rat offspring. Abbreviations are: *in utero* thermal neutral environment (IUTN), *in utero* heat-stressed environment (IUHS), and *in utero* pair-fed to HS-exposed rats in TN conditions (IUPFTN). An asterisk (*) indicates significance ($p < 0.01$) comparing *in utero* treatments.

CHAPTER VII: SUMMARY

Environmentally induced hyperthermia results from an imbalance between thermal energy flowing into and out of an animal, and negatively impacts health and development in agriculturally important species. The economic impact of HS is substantial and will likely increase as climate models predict more extreme summer conditions for most animal-producing areas. Despite advances in heat abatement technology, HS continues to limit animal production and is an economic problem in developed countries and a food security and humanitarian concern in underdeveloped countries located in tropical and sub-tropical regions. Consequently, there is an urgent need to understand mechanisms by which HS compromises the efficient production of high quality animal protein during both pre- and postnatal development.

Although HS can negatively impact animals during postnatal life, exposure to HS during fetal development may have lifelong consequences that undermine considerable advances made by animal agriculture industries. Intrauterine stressors (i.e. maternal under nutrition, intrauterine growth retardation, heat stress) can have lasting effects on offspring performance, postnatal metabolism, growth performance, and can be teratogenic. While many studies of *in utero* HS have investigated its teratogenic impact, there is limited knowledge of its effects on postnatal metabolism and bioenergetics in mammalian species.

In the present studies, *in utero* HS caused multiple postnatal phenotypic changes that are detrimental to animal agriculture. Prenatal HS permanently increased core body temperature, and altered postnatal nutrient partitioning by prioritizing the deposition of adipose tissue over skeletal muscle during the lipid deposition phase of life. Both altered

phenotypes are likely to compromise efficient production as they come with considerable bioenergetic cost.

Previous reports indicate that prenatal exposure to HS in unicellular organisms, insects, and poultry species can infer increased postnatal thermal tolerance. However, we demonstrated in three studies that mammalian species (pigs) have a different postnatal thermoregulatory response to *in utero* HS. Specifically, pigs that experience *in utero* HS maintain a greater core body temperature, regardless of the postnatal ambient temperature, and this increase is likely not influenced by a reduced ability to dissipate body heat (i.e. reduced skin temperature and respiration rate). Chronically increased core body temperature has obvious bioenergetic implications in both human health and animal agriculture, as maintaining this core body temperature differential requires a substantial amount of energy. Further, these studies have added to our knowledge of how HS alters physiological responses throughout all stages of the lifecycle that is likely due to epigenetic imprinting.

A permanent increase in future core temperature caused by *in utero* HS may theoretically result from increased basal heat production, which implies increased metabolic rate and greater maintenance costs. Although it is difficult to determine the exact amount and source of the thermal energy being produced, calculations derived from Bruce and Clark (1979) may help predict the bioenergetic implications. The calculation is described as: Watts (Joules/sec) = $(\Delta T_{\text{core}} * SA) / (R_a + R_t)$. Where; ΔT_{core} is the difference in T_{core} comparing IUHS and TNTN pigs in TN conditions, SA is the total surface area of the pig (m^2), R_a is the external thermal resistance at skin exposed to air ($^{\circ}\text{Cm}^2/\text{W}$), and R_t is the tissue thermal resistance ($^{\circ}\text{Cm}^2/\text{W}$). Using this equation, and assuming that the average T_{core} differential is maintained throughout the pig's entire life, we can determine the additional energy in

Mcal/day that IUHS pigs have to utilize to maintain their core temperature differential. For our particular experiments, we determined that over the average lifespan of a market pig (approximately 175 days), an average of 7 Mcal of extra thermal energy would be produced. Although seemingly inconsequential, this elevated energy output may translate to an increase in maintenance costs and a possible reduction in feed efficiency, which could ultimately increase costs for producers and is detrimental to global food production. For example, assuming that 1 kg of feed contained 3.5 Mcal of metabolizable energy, an IUHS pig would have to consume 2 additional kg of feed to make up for extra thermal energy production. If feed costs are \$300 per 909 kg, this is an additional \$0.66/pig, and when considering a barn of 10,000 head, this could translate into thousands of lost dollars due to increased feed costs and extra days on feed.

Altered body composition is a well-documented consequence of intrauterine stress (i.e. under nutrition, intrauterine growth retardation), and can negatively impact lean content of carcasses. In the current studies, we demonstrated that *in utero* HS alters porcine body composition during the early finishing phase by reducing protein and increasing adipose tissue deposition. While the specific mechanisms for this altered phenotype are unknown, it is likely a result of a reduced capacity to synthesize protein that diverts excess consumed energy toward adipose tissue. In addition to *in utero* effects, and in contrast to previous literature, postnatal HS likely reduced the energy required for maintenance compared to thermal neutral controls. These findings could have implications toward the mechanisms by which HS reduces feed efficiency in livestock species and may indicate reduced visceral mass rather than reduced lean tissue accretion.

Since HS causes a well-documented reduction in feed intake of animals, a link may exist between the negative intrauterine programming effects of *in utero* feed restriction and HS. However, despite similar gross energy consumption of pair-fed and heat-stressed pregnant rats, only IUPFTN offspring appeared to display the negative phenotype generally associated with *in utero* under-nutrition. These results were unexpected and may provide greater insight into the bioenergetics of HS. Heat stress treatment increased maternal feed efficiency and BW gain compared to PFTN dams, likely due to reduced maintenance costs and heat production. Therefore, enhanced nutrient availability may have mitigated the negative intrauterine programming impact of maternal nutrient restriction. However, because birth weight of IUHS offspring was reduced compared to IUTN and IUPFTN progeny, it is likely that fetal development was still compromised, possibly as a result of reduced placental blood flow and intrauterine growth retardation. Further, while no postnatal alterations in body composition were observed for IUHS offspring, their reduced birth weight (and apparent intrauterine growth restriction) may predict increased rates of obesity in later stages of life.

Mechanisms influencing *in utero* HS-induced phenotypic alterations require further investigation. These include: determining the source of increased heat production and verification heat production is increased (using indirect calorimetry); establish protein synthesizing capacity of IUHS pigs; perform metabolic challenges in prenatally heat-stressed animals; determine the specific programming mechanism by which *in utero* HS alters postnatal body composition; determine the lifetime productivity of animals gestated during summer compared to winter months; and repeat the rodent experiment utilizing a greater *in utero* ambient temperature in the HS group. Data obtained in the current studies provides

original information about how HS affects mammalian species during all stages of the lifecycle. Further, these studies have provided invaluable information to producers that will influence future animal production and are the first of their kind to characterize the postnatal metabolic consequences of prenatal hyperthermia in mammals.

Table 7.1: The biological consequences of heat stress

Postnatal phenotype	Prenatal	Postnatal
Insulin	No change	Increase
Feed Efficiency	Decrease	Both
Core temperature	Increase	Increase
Protein accretion	Decrease	Decrease
Lipid accretion	Increase	Increase
Heat production	Increase	Decrease
Liver weight	Decrease	Decrease
Total viscera weight	No change	Decrease
Head size	Decrease	No change

APPENDIX

Table A-3.1. Ingredients and chemical composition of diet for growing pigs (as-fed basis).

Ingredients	---- % ----
Corn	60.6
Dehulled soybean meal	17.4
Dried distillers grains	18.1
45-30 vitamin and mineral premix ¹	2
Lysine	1.15
Threonine	0.114
Methionine	0.058
Tryptophan	0.024
Salt	0.65
Calculated chemical composition %	
DM	88.3
Crude protein	18.2
Crude fat	3.82
Crude fiber	3.68
Calcium	0.60
Phosphorus	0.45
ME ² , Mcal/kg	3.31
NE ³ , Mcal/kg	2.45

¹Supplied per kilogram of diet: vitamin A, 8804 IU; vitamin D3, 1675 IU; vitamin E, 48 IU; vitamin K, 2.4 IU; choline, 5.5 mg; riboflavin, 4.6 mg; niacin, 23 mg; pantothenic acid, 18.2 mg; vitamin B₁₂, 30 µg; biotin, 1.6 µg; folic acid, 0.0005 mg; Zn, 158 ppm; Mn, 61 ppm; Fe, 177 ppm; Cu, 21 ppm; Se, 0.25 ppm. ^{2,3}Estimated using the NRC 2012 individual dietary ingredients.

Table A-3.2: Effects of *in utero* heat stress on bioenergetic parameters in pigs.

Parameter	Environment				SEM	<i>p</i>		
	TNTN ¹	HS ²	HSTN ³	TNHS ⁴		IU ⁵	Contrast 1 ⁶	Contrast 2 ⁷
Glucose (mg/dL)	124.5	118.8	117.1	118.6	4.1	0.61	0.20	0.19
Insulin (ng/mL)	0.07	0.06	0.10	0.08	>0.01	0.08	0.65	0.57
HOMA-IR ⁸ (AU)	1.33	0.99	1.60	1.38	0.21	0.23	0.89	0.97
Insulin : Glucose	0.06 ^a	0.05 ^a	0.08 ^b	0.07 ^b	>0.01	0.04	0.39	0.35
NEFA ⁹ (mEq/L)	57.5	57.6	66.1	56.8	5.5	0.58	0.83	0.72
PUN ¹⁰ (mg/dL)	7.2	6.5	7.4	6.2	0.5	0.28	0.63	0.35
HSP-70 ¹¹ (ng/mL)	3.76	4.10	4.13	3.03	0.49	0.33	0.39	0.68
T ₄ ¹² (ng/mL)	32.6	32.08	32.04	27.54	3.44	0.68	0.90	0.61
T ₃ ¹³ (ng/mL)	0.71	0.71	0.63	0.56	0.05	0.17	0.58	0.25
T ₃ : T ₄	2.58	2.76	2.36	2.34	0.2	0.42	0.95	0.71

¹Thermal neutral conditions during entire gestation²Heat stress conditions during entire gestation³Heat stress conditions during first half of gestation⁴Heat stress conditions during second half of gestation⁵*In utero* environment⁶HS² and HSTN vs TNTN⁷Heat stress conditions during any part of gestation vs TNTN⁸Homeostatic model assessment of insulin resistance⁹Non-esterified fatty acid¹⁰Plasma urea nitrogen¹¹Heat shock protein 70¹²Thyroxine¹³Triiodothyronine

Table A-6.1. Nutrient content of Harlan 2018 rodent diet (as-fed basis).

Nutrients	---- % ----
Starch	41.2
Sucrose	4.9
Fructose	Trace
Crude Protein	18.6
Crude Fat	6.2
Vitamins ¹	1.0
Minerals ²	4.8
Energy (ME)	3.1

¹Supplied per gram of diet: vitamin A, 15.0 IU; vitamin D3, 1.5 IU; vitamin E, 110 IU; vitamin K, 50 µg; choline, 17 µg; riboflavin, 15 µg; niacin, 70 µg; pantothenic acid, 33 µg; vitamin B₁₂, 0.0008 µg; biotin, 0.004 µg; folic acid, 0.04 µg. ²Supplied per gram of diet: Zn, 70 ppm; Mn, 100 ppm; Fe, 200 ppm; Cu, 15 ppm; Se, 0.23 ppm.

Table A- 6.2: Effect of *in utero* environment on bioenergetic variables of fasted rats.

Parameter	Environment			SEM ⁴	<i>P</i>
	IUTN ¹	IUHS ²	IUPFTN ³		IU ⁵
Glucose (mg/dL)	438.5 ^{ab}	373.6 ^b	492.7 ^a	26.6	0.03
Insulin (ng/mL)	0.29 ^{ab}	0.28 ^b	0.32 ^a	0.01	0.03
Insulin : Glucose	0.06	0.08	0.07	0.01	0.15
NEFA ⁶ (mEq/L)	615.6	508.2	539.9	75.4	0.61
C-peptide (pmol/L)	389.7	298.7	495.8	60.1	0.11
Irisin (ug/mL)	1.69	1.63	1.93	0.14	0.32

¹*In utero* thermal neutral²*In utero* heat-stressed³*In utero* pair-fed thermal neutral⁴Standard error of the mean⁵*In utero* environment⁶Non-esterified fatty acids^{a,b}*p* < 0.05

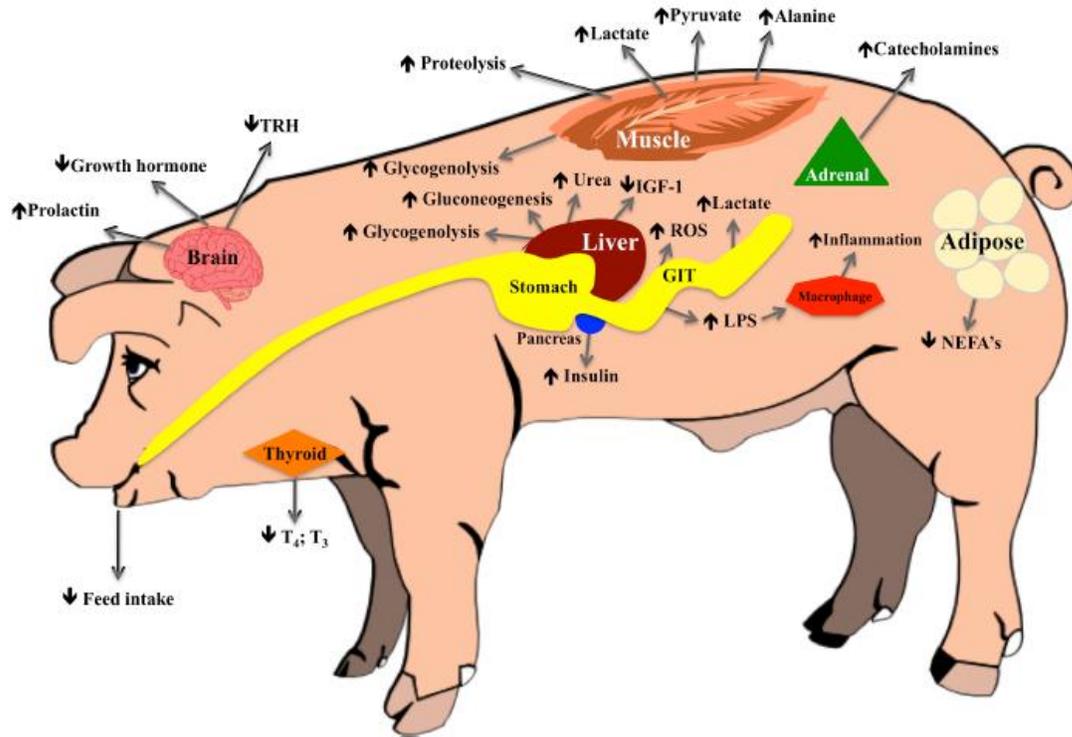


Figure A-1.1: Effects of heat stress on the metabolite/endocrine profile of growing pigs.

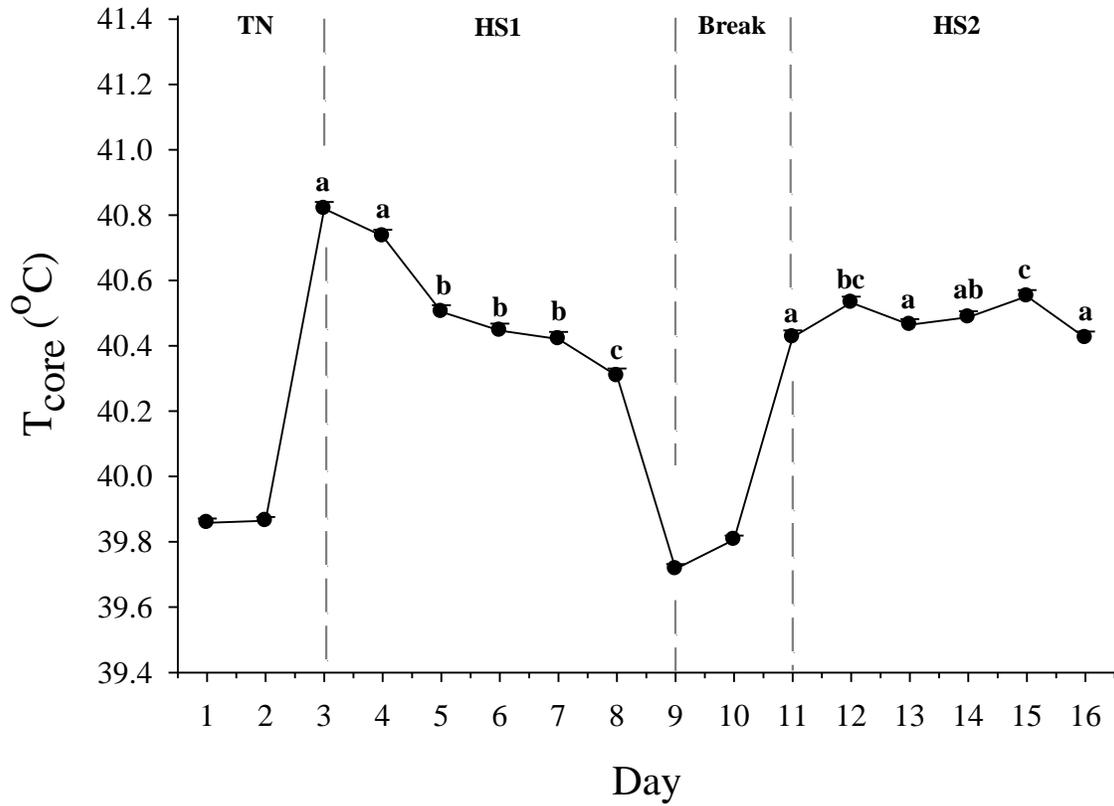


Fig. A-3.1: Temporal pattern of core body temperature (T_{core}) averaged by day of study in growing pigs. Thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}\text{C}$), first heat stress period (HS1; 28 to 36°C), second heat stress period (HS2; 28 to 36°C). Error bars on each point indicate ± 1 SEM. Letters above bars (a,b,c) indicate significance ($P < 0.05$) within a period (TN, HS1, HS2) only.

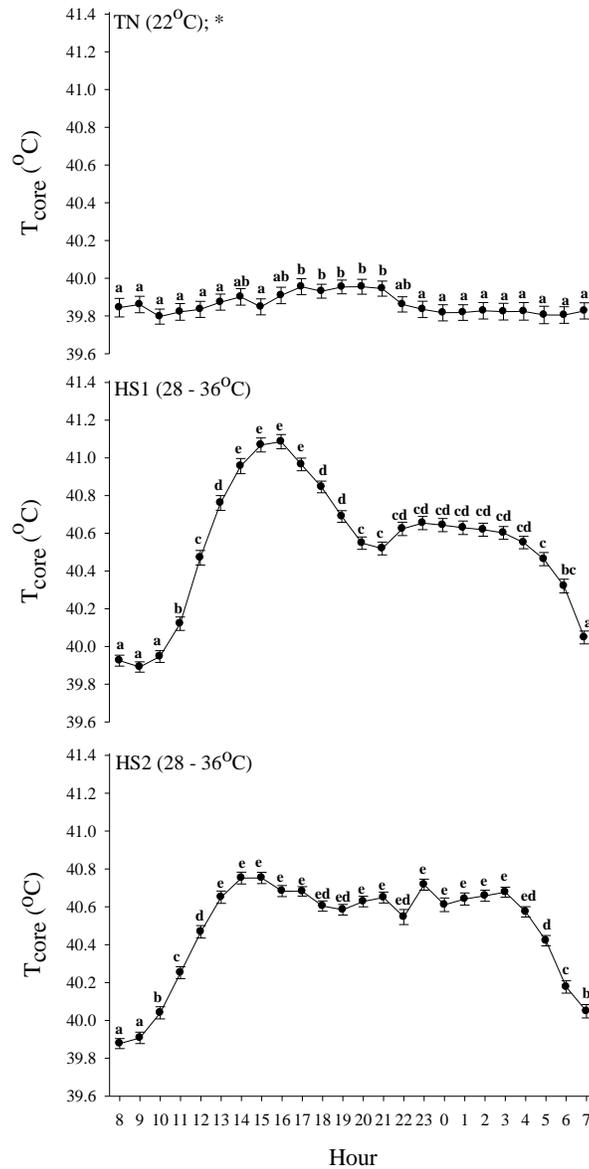


Figure A-3.2: Effects of gestational environment on the temporal changes in core body temperature (T_{core}) averaged by hour by period in growing pigs. **(A)** Thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}$ C), **(B)** first heat stress period (HS1; 28 to 36° C), **(C)** second heat stress period (HS2; 28 to 36° C). Error bars indicate ± 1 SEM. Letters (a,b,c,d,e) indicate hourly differences within a period ($p < 0.05$). An asterisk (*) indicates differences ($p < 0.05$) across periods.

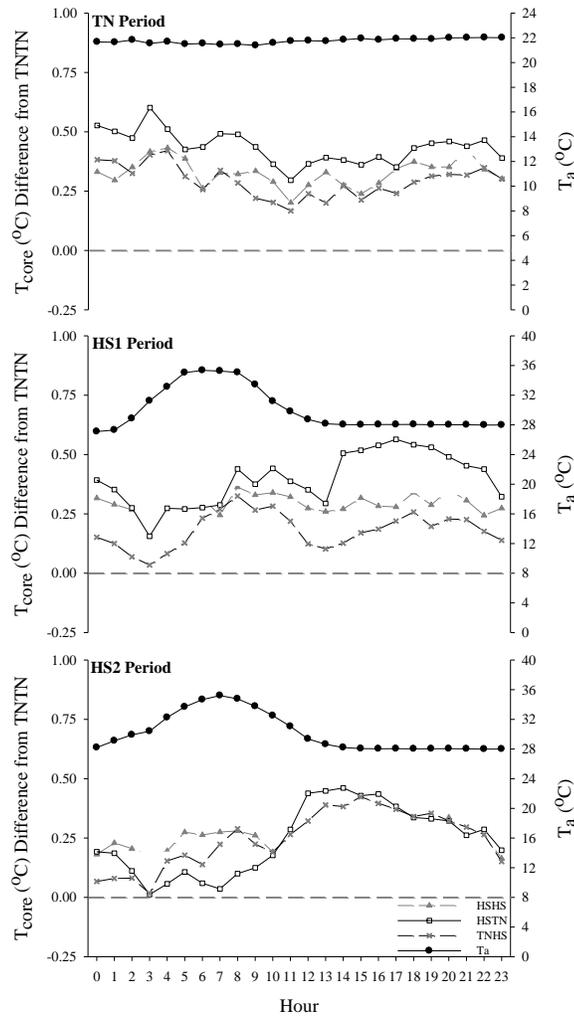


Fig. A-3.3: Difference in core body temperature (T_{core}) comparing pigs exposed to *in utero* heat stress versus thermal neutral controls averaged by hour in growing pigs. Thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}\text{C}$), first heat stress period (HS1; 28 to 36°C), second heat stress period (HS2; 28 to 36°C). *In-utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat stress conditions for entire gestation (HSHS), *in-utero* heat stress conditions for first half of gestation (HSTN), *in-utero* heat stress conditions for second half of gestation (TNHS), ambient temperature (T_a).

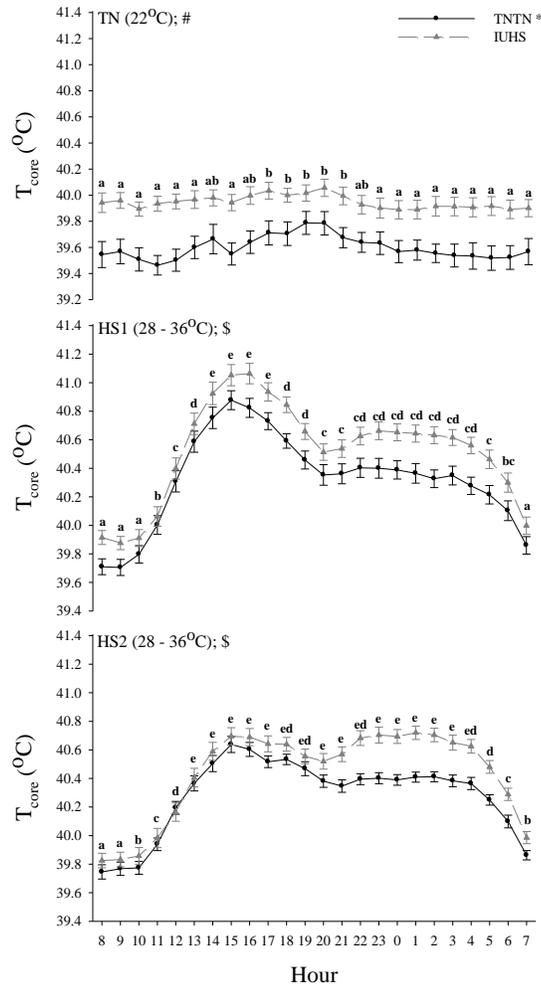


Figure A-3.4: Effects of *in utero* environment on the temporal changes in core body temperature (T_{core}) averaged by hour by period in growing pigs. Abbreviations are: thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}$ C), first heat stress period (HS1; 28 to 36° C), second heat stress period (HS2; 28 to 36° C), *in-utero* thermal neutral conditions for entire gestation (TNTN), *in utero* HS during any part of gestation (IUHS). Letters (a,b,c,d,e) indicate hourly differences within a period ($p < 0.01$). An asterisk (*) indicates differences ($p < 0.01$) between *in utero* treatments. Symbols (#, \$) indicate differences between periods ($p < 0.01$).

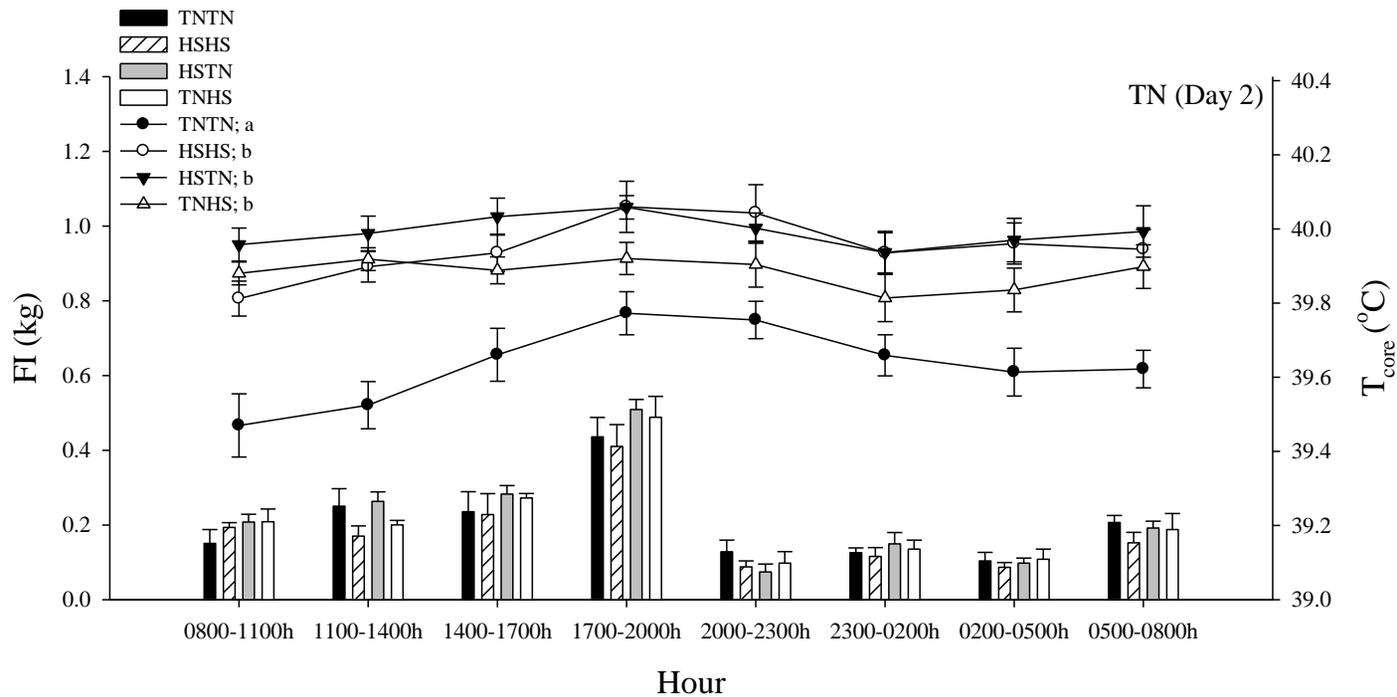


Fig. A-3.5: Effects of *in utero* environment on the temporal changes in feed intake (FI), and core body temperature (T_{core}) during the second day of the thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}\text{C}$) averaged by *in utero* treatment and hour in growing pigs. *In-utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat stress conditions for entire gestation (HSHS), *in-utero* heat stress conditions for first half of gestation (HSTN), *in-utero* heat stress conditions for second half of gestation (TNHS). Letters (a,b) next to TNTN, HSHS, HSTN and TNHS indicate significance ($P < 0.05$) for T_{core} across *in utero* treatment groups.

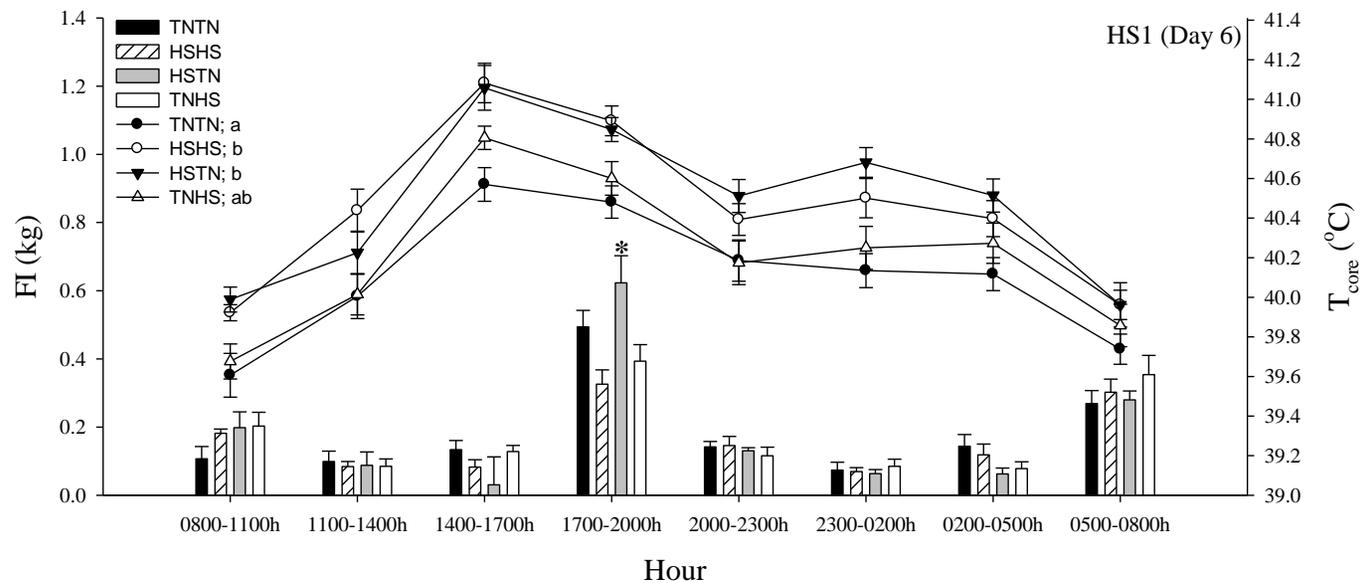


Fig. A-3.6: Effects of *in utero* environment on the temporal changes in feed intake (FI), and core body temperature (T_{core}) during the sixth day of the first heat stress period (HS1; 28 to 36°C) averaged by *in utero* treatment and hour in growing pigs. *In-utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat stress conditions for entire gestation (HSHS), *in-utero* heat stress conditions for first half of gestation (HSTN), *in-utero* heat stress conditions for second half of gestation (TNHS). Letters (a,b) next to TNTN, HSHS, HSTN and TNHS indicate significance ($P < 0.05$) comparing T_{core} across *in utero* treatment groups. Asterisk (*) above bars indicate significance ($P < 0.05$) comparing FI across *in utero* treatment groups.

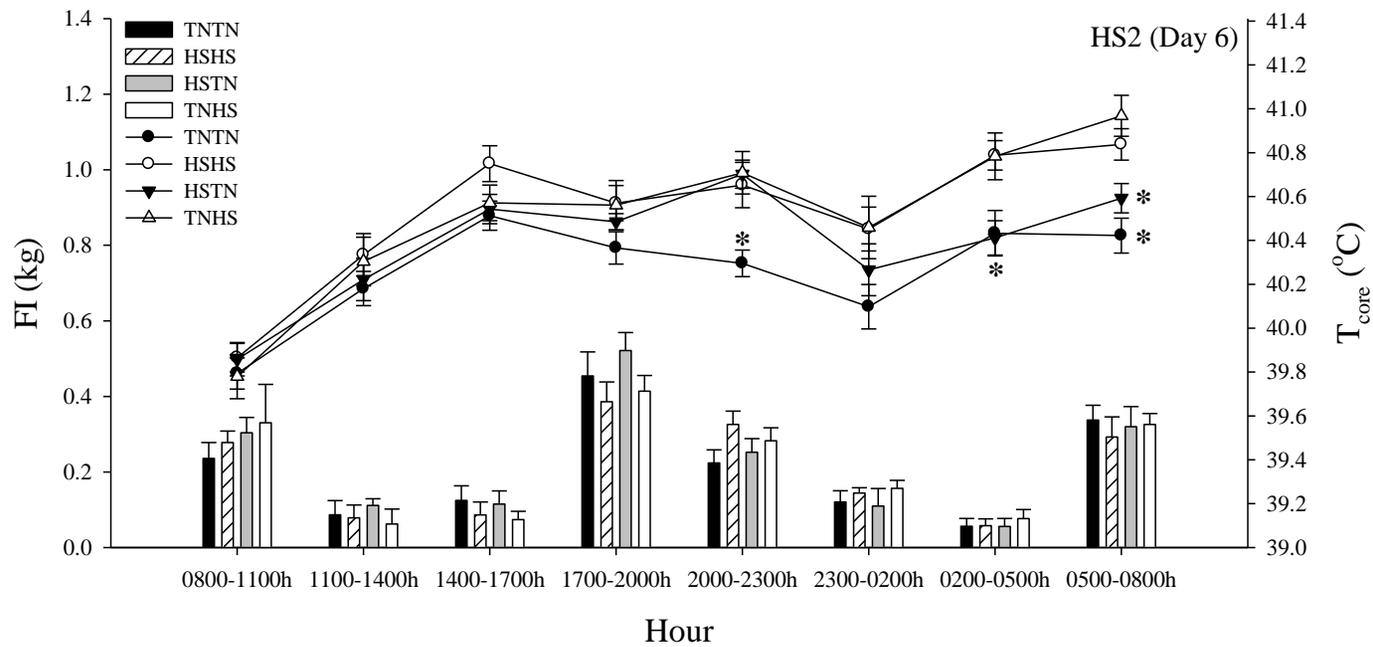


Fig. A-3.7: Effects of *in utero* environment on the temporal changes in feed intake (FI), and core body temperature (T_{core}) during the sixth day of the second heat stress period (HS2; 28 to 36°C) averaged by gestational treatment and hour in growing pigs. *In-utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat stress conditions for entire gestation (HSHS), *in-utero* heat stress conditions for first half of gestation (HSTN), *in-utero* heat stress conditions for second half of gestation (TNHS). Asterisk (*) indicate significance ($P < 0.05$) comparing T_{core} across *in utero* treatment groups by hour.

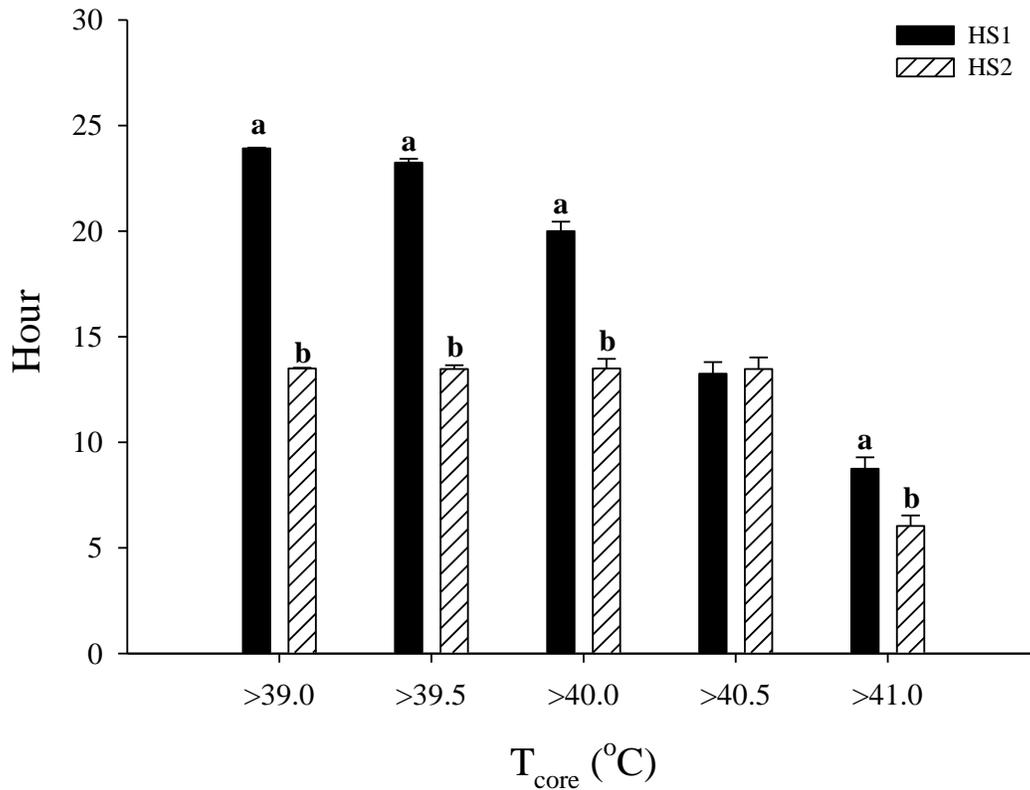


Figure A-3.8: Hours above a threshold core temperature in growing pigs comparing differences by period. Hours are calculated out of a total of 24. Abbreviations are: first heat stress period (HS1; 28 to 36°C), second heat stress period (HS2; 28 to 36°C). Error bars on each point indicate ± 1 SEM. Letters (a,b) above HS1 and HS2 bars indicate significance ($p < 0.05$) across periods.

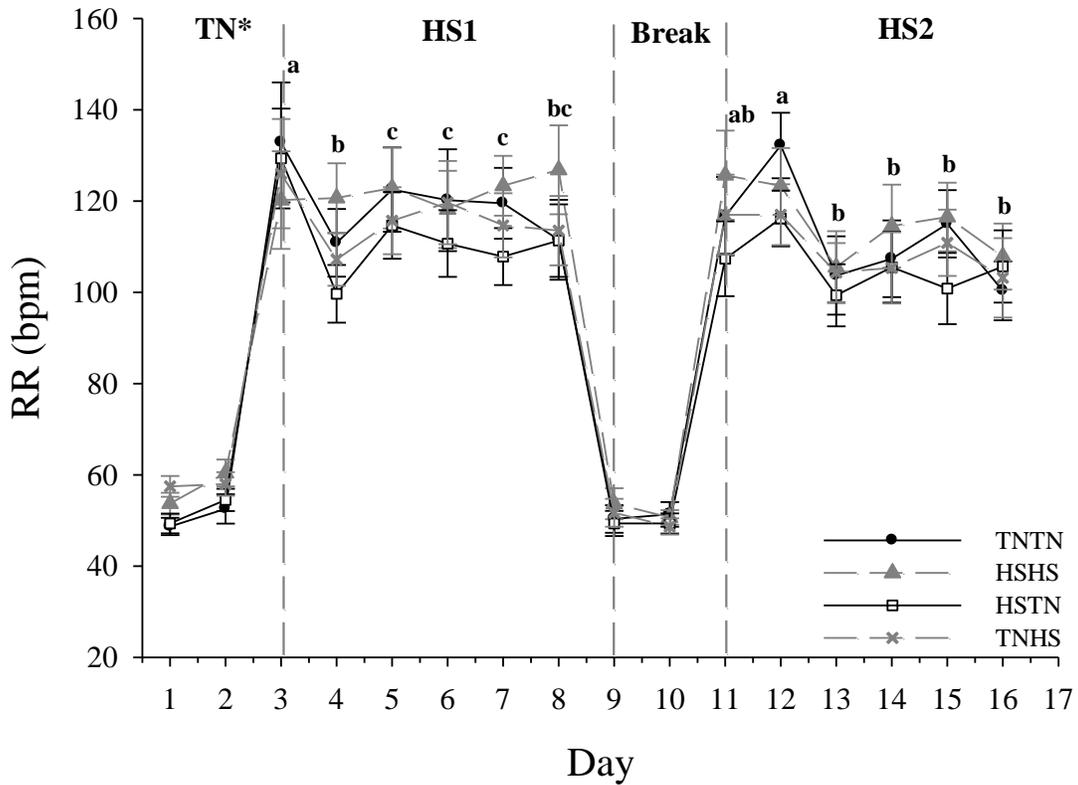


Figure A-3.9: Effects of *in utero* environment on the temporal changes in respiration rate (RR) averaged by *in utero* treatment and day of study in growing pigs. Abbreviations are: *in utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat stress conditions for entire gestation (HSHS), *in-utero* heat stress conditions for first half of gestation (HSTN), *in-utero* heat stress conditions for second half of gestation (TNHS), thermal neutral period (TN; constant $21.7 \pm 0.7^\circ\text{C}$), first heat stress period (HS1; 28 to 36°C), break (constant $22.3 \pm 0.1^\circ\text{C}$), second heat stress period (HS2; 28 to 36°C). Letters (a,b) above points indicate significance ($p < 0.05$) comparing days within period. An asterisk (*) indicates significance ($p < 0.05$) across periods.

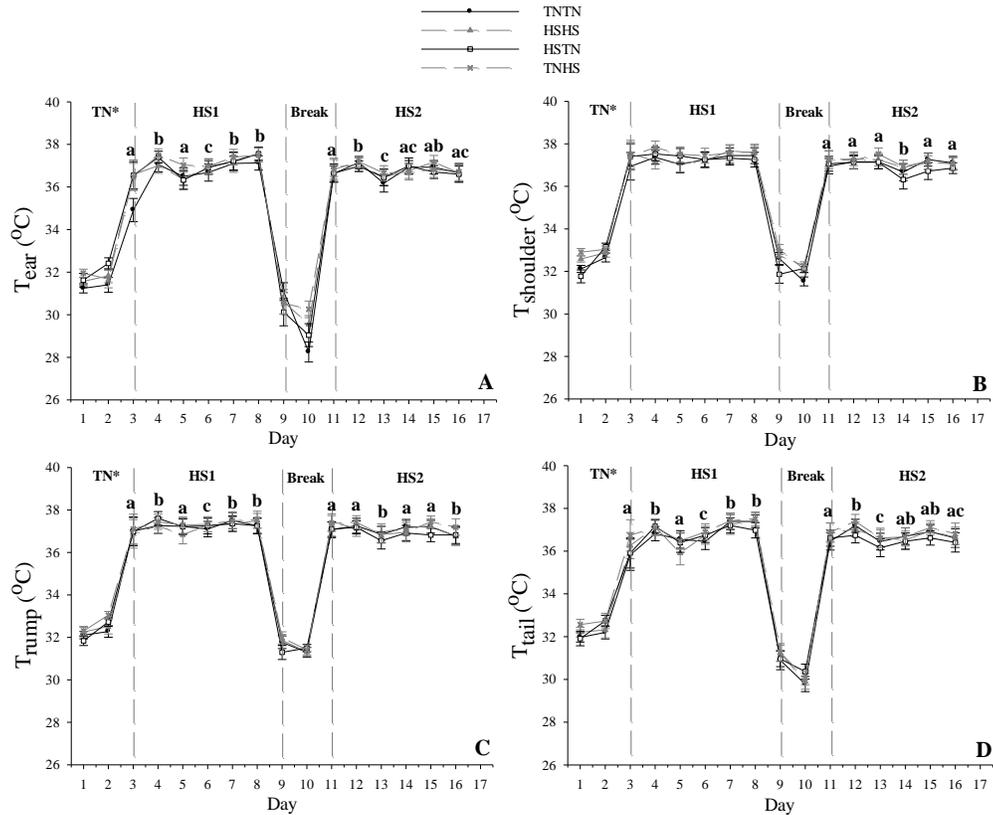


Figure A-3.10: Effects of *in utero* environment on the temporal changes in (A) ear skin temperature (T_{ear}), (B) shoulder skin temperature (T_{shoulder}), (C) rump skin temperature (T_{rump}), and (D) tail skin temperature (T_{tail}) averaged by *in utero* treatment and day of study in growing pigs. Abbreviations are: *in utero* thermal neutral conditions for entire gestation (TNTN), *in utero* heat stress conditions for entire gestation (HSHS), *in utero* heat stress conditions for first half of gestation (HSTN), *in utero* heat stress conditions for second half of gestation (TNHS), thermal neutral period (TN; constant $21.7 \pm 0.7^{\circ}\text{C}$), first heat stress period (HS1; 28 to 36°C), break (constant $22.3 \pm 0.1^{\circ}\text{C}$), second heat stress period (HS2; 28 to 36°C). Letters (a,b) above points indicate significance ($p < 0.05$) comparing days within period. An asterisk (*) indicates significance ($p < 0.05$) across periods.

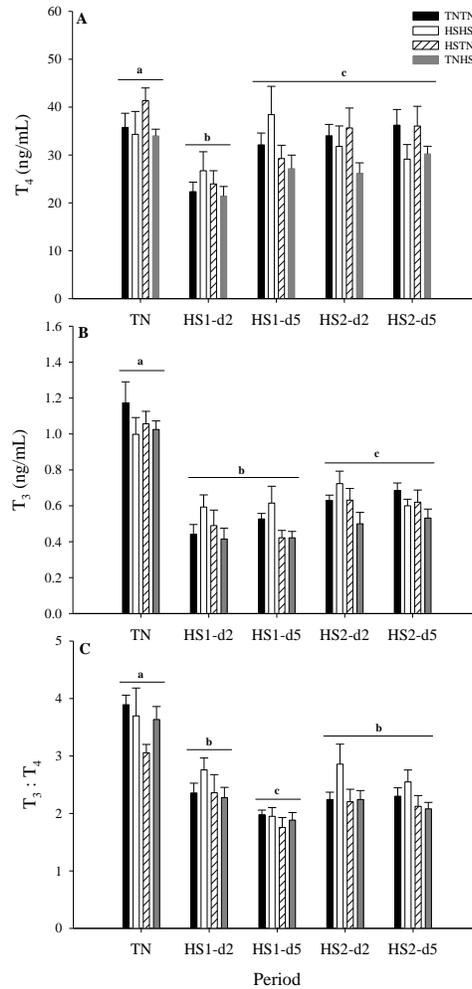


Figure A-3.11: Circulating levels of (A) thyroxine (T_4), (B) triiodothyronine (T_3), and (C) the ratio of T_3 to T_4 from serum obtained during the thermal neutral period (TN), day two of the first heat stress period (HS1-D2), day five of the first heat stress period (HS1-D5), day two of the second heat stress period (HS2-D2), and day five of the second heat stress period (HS2-D5). Abbreviations are: *in-utero* thermal neutral conditions for entire gestation (TNTN), *in-utero* heat stress conditions for entire gestation (HSHS), *in-utero* heat stress conditions for first half of gestation (HSTN), *in-utero* heat stress conditions for second half of gestation (TNHS). Error bars above each bar indicate ± 1 SEM. Letters (a,b) indicate significance ($p < 0.05$) across periods.

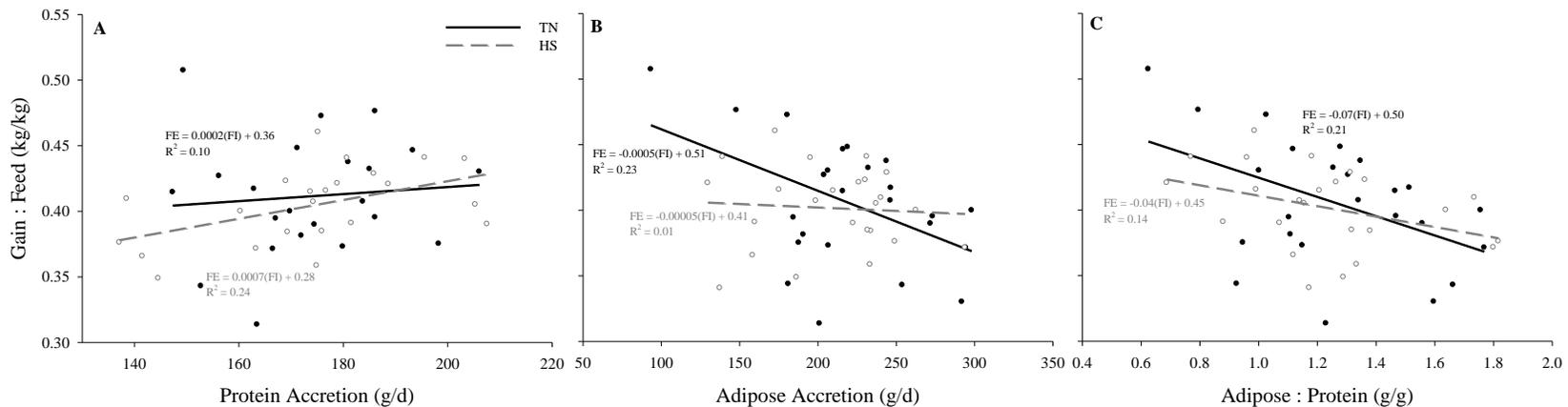


Fig. A-4.1: Linear regression ($y = mx + b$) of (A) feed efficiency (kg gain/kg feed) as a function of protein accretion (g/d), (B) feed efficiency (kg gain/kg feed) as a function of adipose accretion (g/d), and (C) feed efficiency (kg gain/kg feed) as a function of the ratio of adipose to protein accretion/d. Postnatal thermal neutral pigs (TN) and postnatal heat stress pigs (HS). Coefficient of determination (R^2), and slope (m) is presented for each regression line.

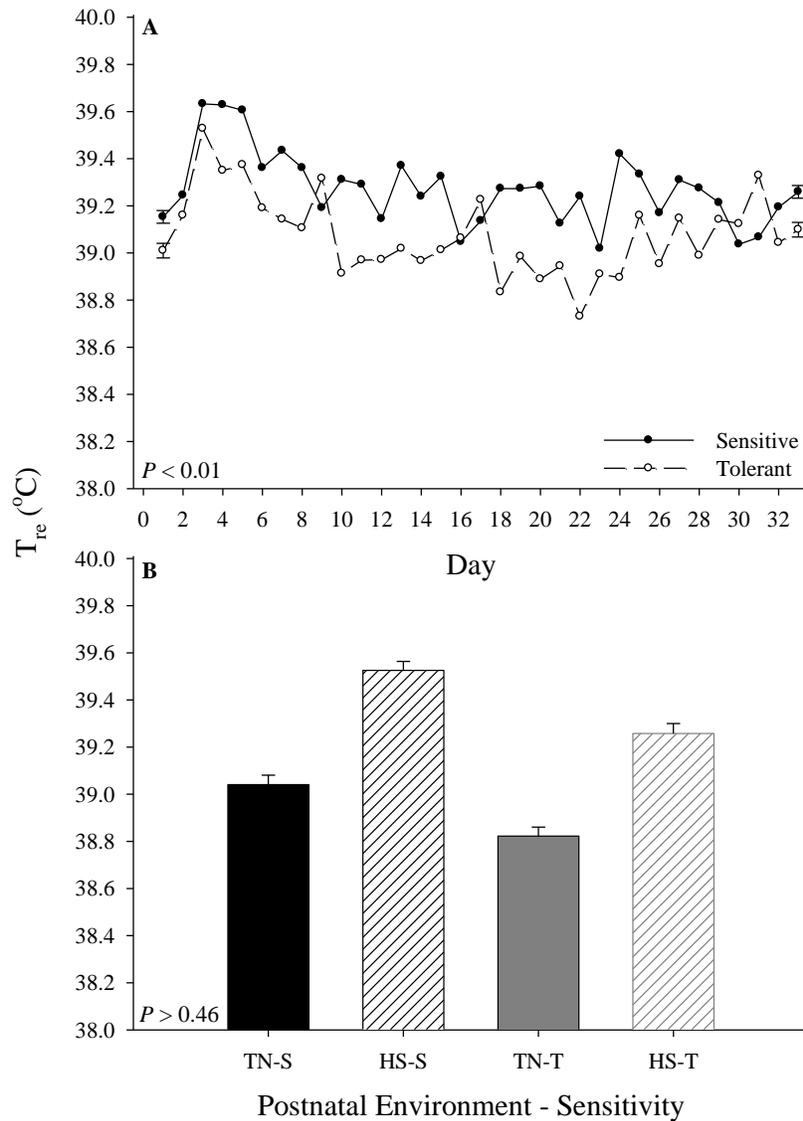


Figure A-4.2: Rectal temperature (T_{re}) response comparing (A) sensitivity index and (B) postnatal environment and sensitivity index interactions in growing pigs. Postnatal thermal neutral conditions (TN), postnatal heat stress conditions (HS), sensitive pigs with chronically elevated T_{re} (S; $39.3 \pm 0.1^\circ\text{C}$), and tolerant pigs with sustained lower T_{re} (T; $39.0 \pm 0.1^\circ\text{C}$). Error bars indicate ± 1 SEM.

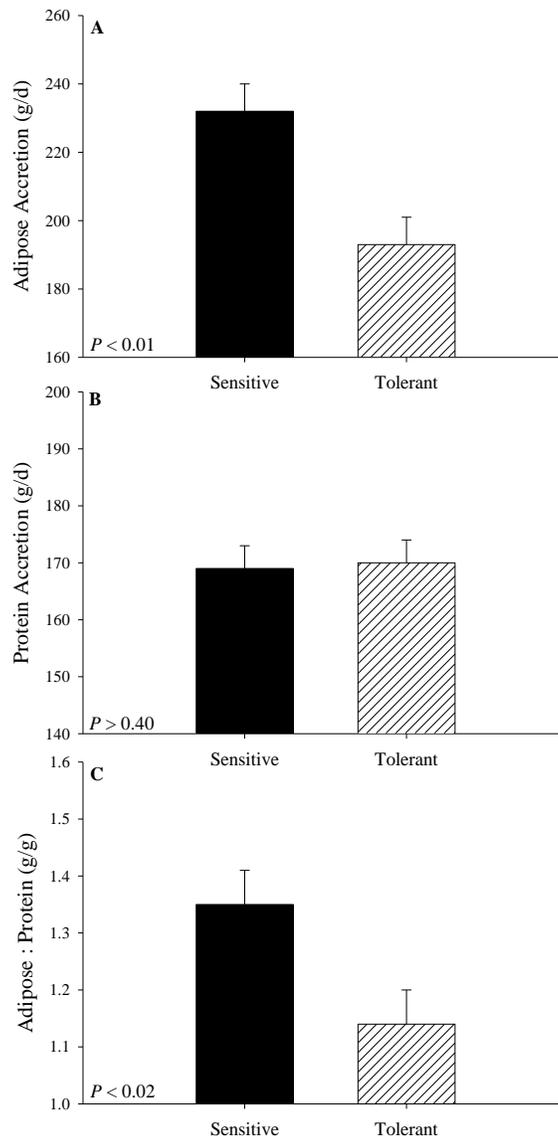


Figure A-4.3: Effect of chronically elevated rectal temperature (T_{re}) on (A) adipose accretion (g/d), (B) protein accretion (g/d), and (C) the ratio of adipose to protein accretion (g/g), averaged by sensitivity in growing pigs. Sensitive pigs with chronically elevated T_{re} ($39.3 \pm 0.1^\circ\text{C}$), and tolerant pigs with sustained lower T_{re} ($39.0 \pm 0.1^\circ\text{C}$; $P < 0.01$). Error bars indicate ± 1 SEM.

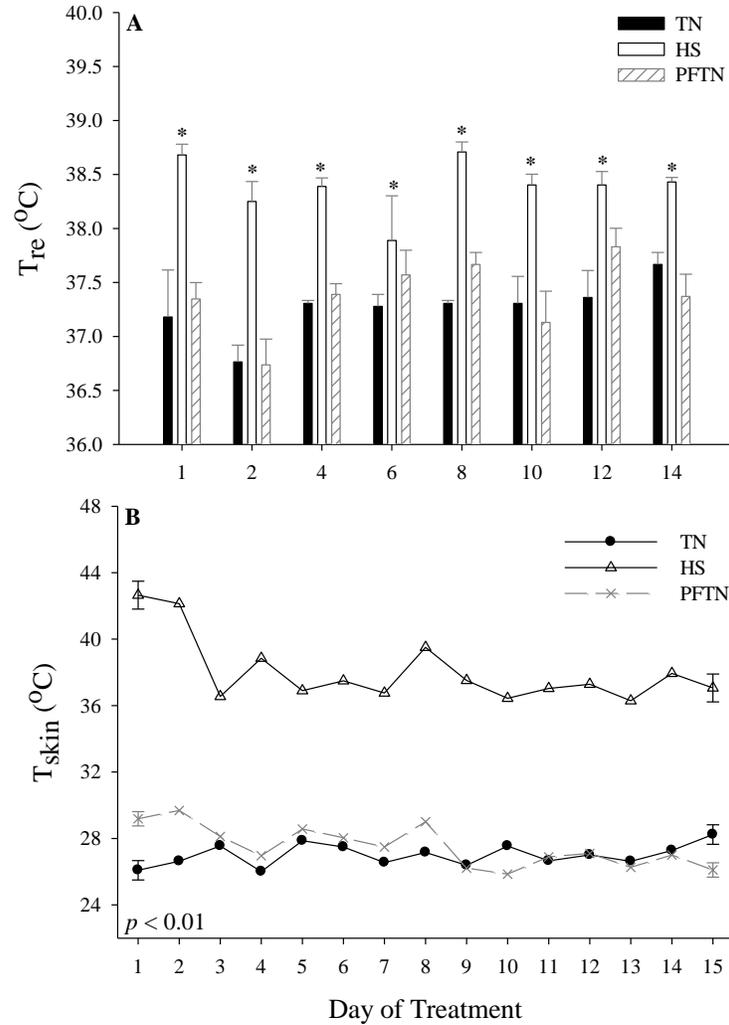


Figure A-6.1: Effects of maternal environment on the temporal changes in (A) core body temperature (T_{core}) averaged by day of treatment, and (B) tail skin temperature (T_{skin}) averaged by day of treatment in Sprague Dawley rats. Abbreviations are: thermal neutral treatment (TN; constant $22.2 \pm 0.0^\circ\text{C}$), heat stress treatment (HS; 30 to 34°C), pair-fed to HS-exposed rats in TN conditions (PFTN; constant $22.2 \pm 0.0^\circ\text{C}$). Error bars indicate ± 1 SEM. Asterisk (*) indicates differences ($p < 0.05$) between maternal treatments.

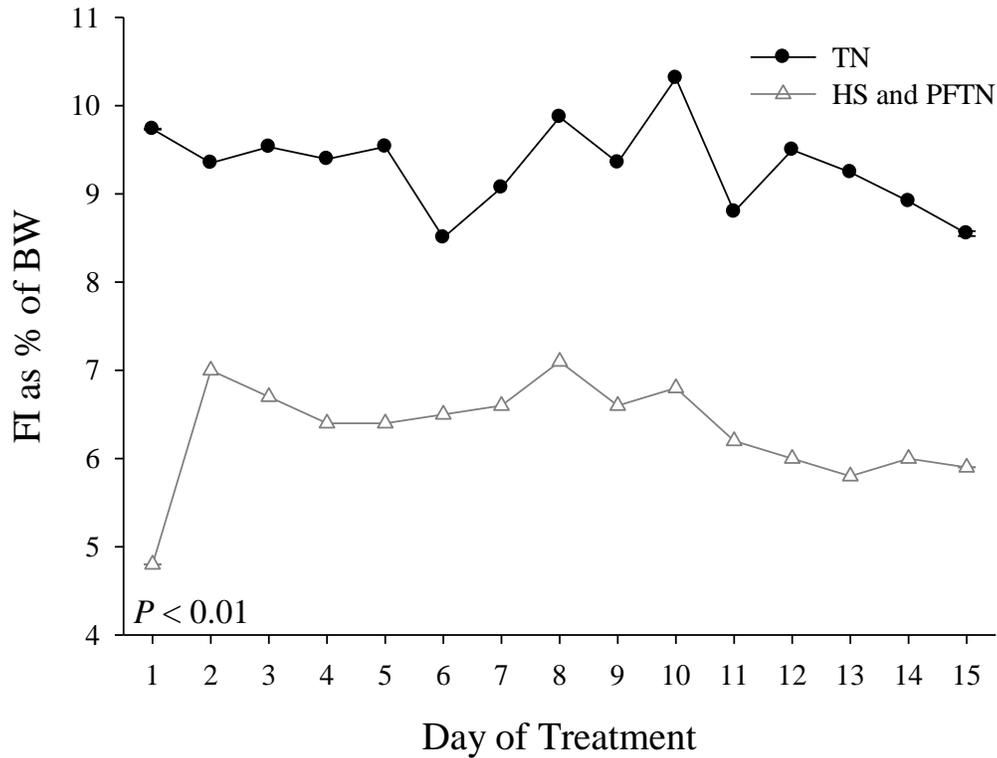


Figure A-6.2: Effects of maternal environment on the temporal changes in feed intake as a percent of BW in pregnant Sprague Dawley rats averaged by day of treatment.

Abbreviations are: thermal neutral environment (TN; constant $22.2 \pm <0.1^\circ\text{C}$), heat stress environment (HS; 30 to 34°C), pair-fed to HS-exposed rats in TN conditions (PFTN; constant $22.2 \pm <0.1^\circ\text{C}$). Standard error of the mean (0.1) is indicated by bars on d 1 and d 15 of treatment.

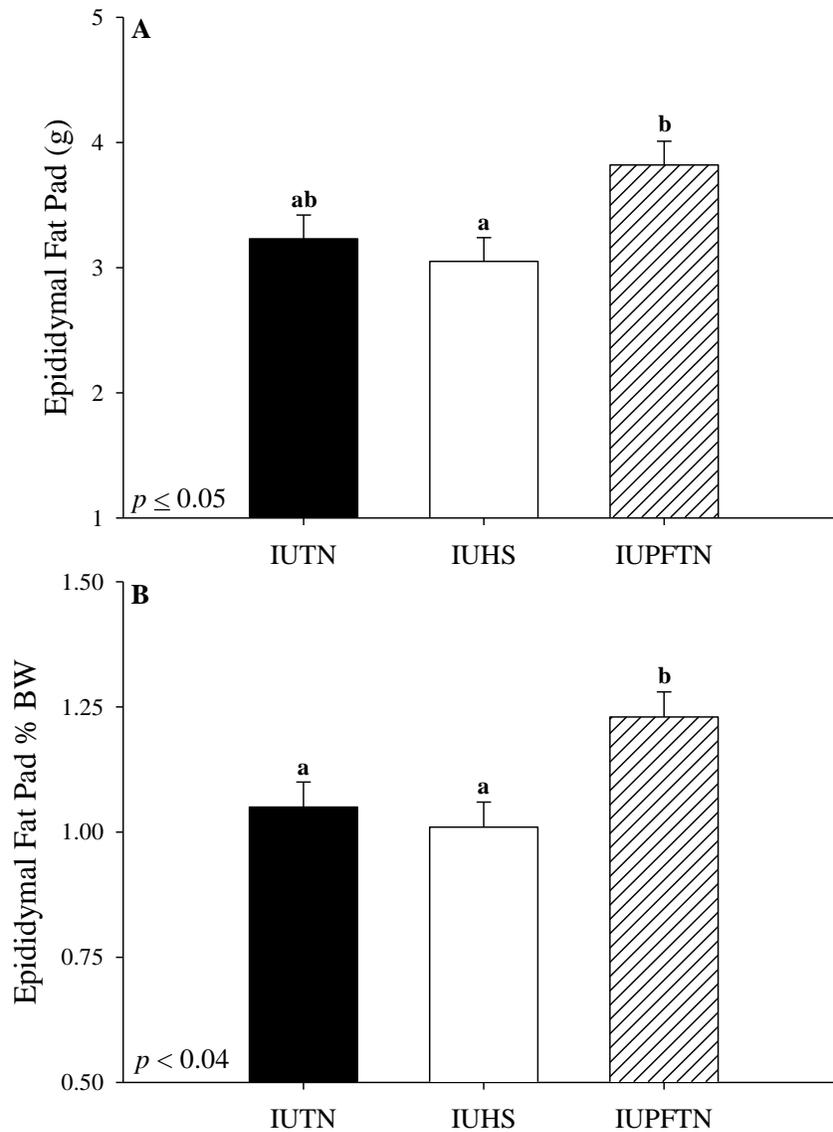


Figure A-6.3: Effect of *in utero* environment on (A) epididymal fat pad weight (g), and (B) epididymal fat pad weight as a percent of body weight. Abbreviations are: *in utero* thermal neutral treatment (IUTN), *in utero* heat-stressed treatment (IUHS), *in utero* pair-fed treatment (IUPFTN). Letters (a,b) indicates differences between *in utero* treatments

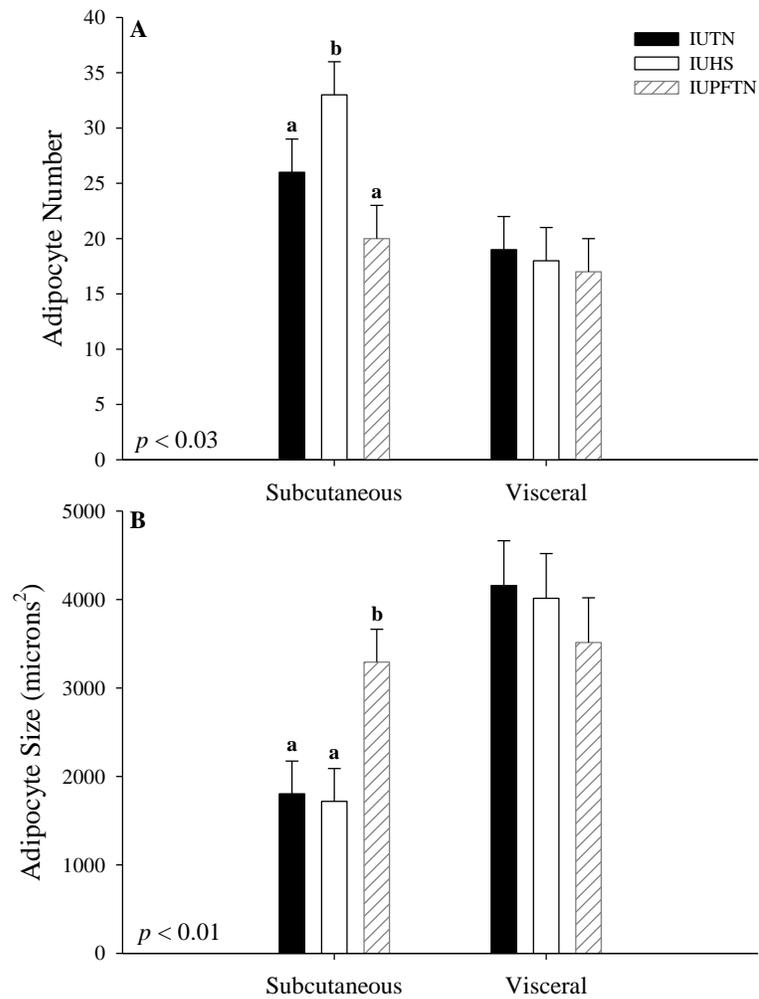


Figure A-6.4: Effect of *in utero* environment on (A) adipocyte number within the field of vision in subcutaneous and visceral adipose samples, and (B) adipocyte size (microns²) in subcutaneous and visceral adipose samples. Abbreviations are: *in utero* thermal neutral treatment (IUTN), *in utero* heat stress treatment (IUHS), *in utero* pair-fed treatment (IUPFTN). Error bars indicate ± 1 SEM. Letters (a,b) indicate differences between *in utero* treatments.

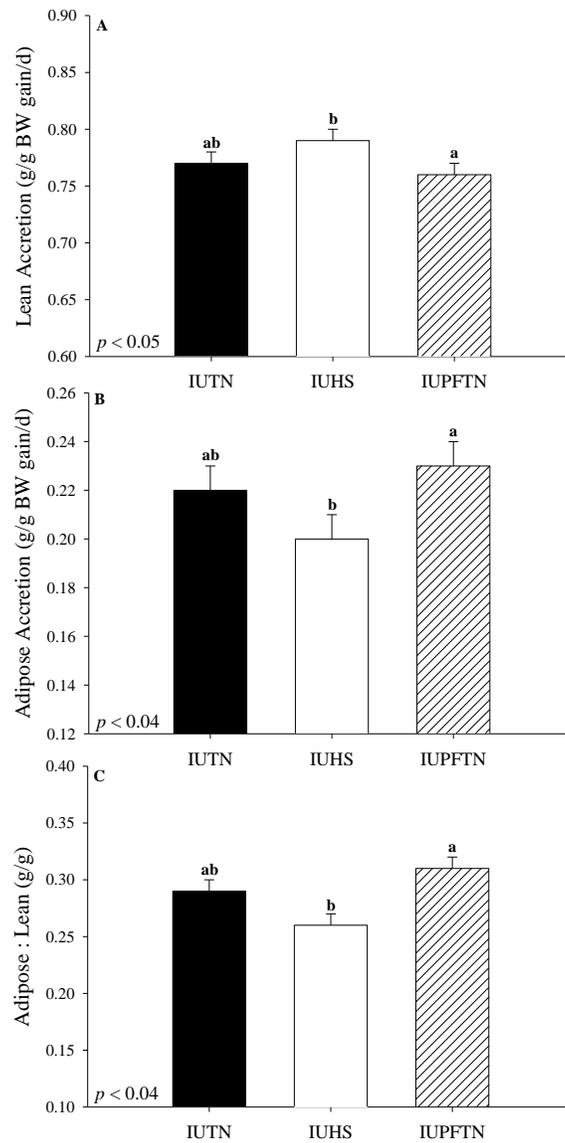


Figure A-6.5: Effect of *in utero* environment on postnatal (A) lean tissue accretion (g/g BW gain/d), (B) adipose tissue accretion (g/g BW gain/d), and (C) the ratio of adipose to lean tissue accretion (g/g) as determined by DXA scans on d 26 to 66 of postnatal life.

Abbreviations are: *in utero* thermal neutral environment (IUTN), *in utero* heat-stressed environment (IUHS), *in utero* pair-fed to HS-exposed rats in TN conditions (IUPFTN).

Letters (a,b) indicate differences between *in utero* treatments.