Journal of Thermal Biology 53 (2015) 143–150

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

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

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ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form
1 October 2015
Accepted 2 October 2015
Available online 17 October 2015

Keywords:
Rats
Intrauterine programming
Maternal undernutrition
Tissue accretion

ABSTRACT

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

1. Introduction

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

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

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http://dx.doi.org/10.1016/j.jtherbio.2015.10.002
0306-4565/

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

2. Materials and methods

2.1. Maternal environment

All animal work was approved by the Iowa State University Animal Care and Use Committee. A total of 24, first parity timed pregnant OB Sprague–Dawley rats (189.0 ± 5.9 g BW) were obtained from Charles River Laboratories (Wilmington, MA) on d2 of gestation. Dams were fed a finely ground standard commercial chow diet (Harlan 2018; Harlan; Woodland, CA; 18% CP) and housed in individual cages (0.2 m × 0.4 m) in the Zumwalt Environmental Chambers at Iowa State University. Pregnant rats were exposed to thermoneutral (TN; constant 22.2 ± 0.1 °C; 34.6 ± 0.1% relative humidity (RH); $n=8$), HS (cyclical 30.0 daytime and 34.0 °C nighttime; 20.1 ± 0.1% RH; $n=8$), or pair-fed as a percent of BW to HS mothers in TN conditions (PFTN; constant 22.2 ± 0.1 °C; 34.6 ± 0.1% RH; $n=8$) to eliminate the confounding effects of dissimilar FI. Thermoneutral temperature was selected based on recommendations that rats be maintained at 20–24 °C (Hau and Shapiro, 2011), and pregnant dams were provided nesting material (Nestlets™ Nesting Material; Ancare; Radnor, PA) to reduce the potential for cold stress (Gaskill et al., 2013). Because rats are nocturnal species (Southern et al., 1946), peak HS was during the nighttime (dark from 0800–2000 h) when dams were active and baseline HS was during the daytime (light from 2000–0800 h) when dams were sleeping in order to match their circadian rhythm with the temperature cycles used in previous experiments with diurnal pregnant sows (Johnson et al., 2015b,c). Maternal environmental treatments began and ended on d3 and d18 of gestation, respectively, for TN and HS dams and on d4 and d19 of gestation, respectively, for PFTN mothers (Fig. 1), since by experimental design PFTN mothers were limit-fed to HS dams. To minimize the risk of post-parturition maternal cannibalism of offspring, and to ensure all progeny were exposed to similar lengths of *in utero* stress, all measurements ended and all dams were fed *ad libitum* and exposed to TN conditions (constant 22.2 ± 0.1 °C; 34.6 ± 0.1%RH) from d19 (TN, HS) or d20 (PFTN) of gestation until parturition.

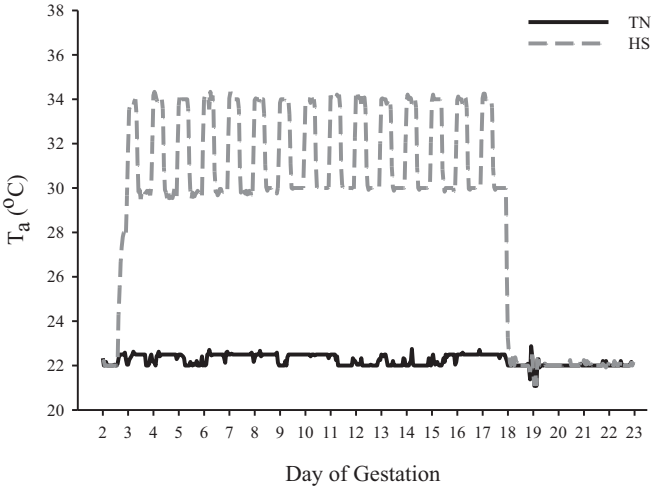

Fig. 1. Ambient temperature (T_a; °C) by day of gestation in pregnant Sprague–Dawley rats. Abbreviations are: maternal thermoneutral environment (TN; constant 22.2 ± 0.1 °C), and maternal heat stress environment (HS; cyclical 30.0 daytime to 34.0 °C nighttime).

Rectal temperatures (T_{re}) were obtained on all dams at 1600 h (peak heat) on alternate days of the *in utero* treatment period using a calibrated and lubricated digital thermometer (Safety 1st; Model #TH050) inserted approximately 1 cm into the rectum of restrained dams. Tail skin temperature (T_{skin}) was measured twice daily (0800, 1600 h) using a calibrated infrared thermometer (Model 42505, Extech Instruments, Waltham, MA). Body weight, FI, and water intake (WI) were determined daily (0800 h) for all dams. At parturition, pup number and average individual pup birth weight (total weight of litter/pup number) were recorded for each dam. To eliminate the confounding effects of dissimilar gestation length and litter size on offspring phenotypes, pups were selected from only 12 dams ($n=4$/treatment) with similar gestation lengths (22 d) and litter sizes (10.9 ± 0.9 pups) at parturition. All analyses of maternal data were performed using only the 12 selected dams.

2.2. Postnatal environment

The Iowa State University Animal Care and Use Committee approved all procedures involving rat offspring. Between parturition and weaning (d 23 postnatal life), all pups were exposed to TN conditions (20–24 °C) as recommended for neonatal rats (Hau and Schapiro, 2011). Female offspring were removed from the dams and euthanized on d4 of postnatal life to collect tissue for a separate experiment. The remaining male pups were balanced ($n=5$/dam) to prevent postnatal milk consumption differences and excess male offspring were euthanized. At weaning, two male offspring per pregnant dam were randomly selected from *in utero* TN (IUTN; $n=8$), *in utero* HS (IUHS; $n=8$), and *in utero* PFTN (IUPFTN; $n=8$) mothers and housed in individual cages (0.2 m × 0.4 m) in TN (21.8 ± 0.1 °C; 28.3 ± 0.2% RH) conditions in the Kildee Hall small animal holding rooms at Iowa State University. By experimental design, gestation length was similar (22 d) in all selected offspring to eliminate the confounding effects of dissimilar gestation length on the direct effects of *in utero* environment. Feed was provided *ad libitum* at weaning in jars as a finely ground standard commercial chow diet (Harlan 2018; Harlan; Woodland, CA; 18% CP). Body weight was recorded weekly and FI was measured every third day to determine average daily FI and BW gain. Average daily FI was similar ($p=0.16$; 4.2 g) between *in utero* treatments on the first 3 d (d 23–26 postnatal life) after weaning. An initial body scan was performed on anesthetized offspring (100 mg/mL ketamine and 10 mg/mL xylazine; administered at a dose of 0.2 mL/100 g BW IP) using a Hologic Discovery A dual-energy X-ray absorptiometer (DXA; Hologic, Inc.; Bedford, MA) in small animal mode with V8.26a:3 software. Offspring were scanned after an overnight fast on d26 (68.4 ± 0.7 g BW), d46 (190.5 ± 4.6 g BW), and d66 of postnatal life (302.9 ± 7.5 g BW) to determine percent fat, lean and bone mineral content (BMC) in individual animals. Based on tissue content, total tissue quantity and accretion rates were calculated as we have previously described (Johnson et al., 2015b,c) with the following formula: (Final content, g of tissue – initial content, g of tissue) / days between DXA scans.

2.3. Tissue collection

Immediately following the final DXA scan (d66 of postnatal life), offspring were euthanized by anesthesia injection (100 mg/mL ketamine and 10 mg/mL xylazine; administered at a