

Prenatal stress increases the obesogenic effects of a high-fat-sucrose diet in adult rats in a sex-specific manner

(Running head: Prenatal stress and High Fat/Sugar diet)

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ABSTRACT

Stress during pregnancy can induce metabolic disorders in adult offspring. To analyze the possible differential response to a high-fat-sucrose (HFS) diet in offspring affected by prenatal

stress (PNS) or not, pregnant Wistar rats (n=11) were exposed to a chronic-mild-stress during the third week of gestation. This aimed to model a chronic depressive-like state that develops over time in response to stress, involving exposure of rats to a series of mild and unpredictable stressors. Control dams (n=11) remained undisturbed. Adult offspring were fed chow or HFS diet (20% protein, 35% carbohydrate, 45% fat) for 10 weeks. Changes in adiposity, biochemical profile and retroperitoneal adipose tissue gene expression by real-time polymerase chain reaction were analyzed. An interaction was observed between HFS and PNS concerning visceral adiposity, with higher fat mass in HFS-fed stressed rats, although this reached statistical significance only in females. HFS modified lipid profile and increased insulin resistance biomarkers, while PNS reduced insulin levels and the HOMA index. HFS diet intake increased gene (mRNA) expression of leptin and apelin and decreased cyclin-dependent kinase inhibitor 1A (Cdkn1a) and fatty-acid synthase (Fasn), whereas PNS increased fasn and stearyl-CoA desaturase1 (Scd1). An interaction between diet and PNS was observed for adiponutrin (Adpn) and peroxisome proliferator-activated receptor- γ coactivator1- β (Ppargc1a) gene expression: Adpn was increased by the PNS only in HFS-fed rats, whereas Ppargc1a was increased by PNS only in chow-fed rats. With these results it can be concluded that experience of maternal stress during intrauterine development can enhance predisposition to obesity induced by a HFS diet intake.

Key words: Adiponutrin, Adipose tissue, Diet-induced obesity, Early-life stress, Fecal corticosterone, Ingenuity.

INTRODUCTION

Obesity can be described as a clinical condition in which an excess of body fat is accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems (Haslam and James 2005). This chronic disease is one of the most serious public health burdens around the world (Marti, et al. 2008). The increasing prevalence of obesity is not only attributable to genetic factors (Moleres, et al. 2009) or to the high intake of foods rich in fat and sugars (Astrup, et al. 2008), but also to lifestyle and environmental factors such as adverse early life experiences including prenatal stress (PNS), which can program physiological systems and behavior in later life (Brunton and Russell 2010).

From human and animal studies, the “Barker Hypothesis” proposed that the intrauterine environment plays a significant role in the health of the offspring, such that exposure to limited resources in utero induces in the offspring maladaptive responses to the ample postnatal nutritional environment, contributing to development of obesity and diabetic features (Hales and Barker 1992). However, not only the nutritional status of the mother can affect her offspring, but also stress conditions arising from socioeconomic or psychosocial factors, which have been associated with the development of obesity-related conditions including excessive visceral fat deposition, insulin resistance, dyslipidemia, hypertension, and cardiovascular disease in humans (Rosmond and Bjorntorp 1999).

Indeed, PNS in rodents has been implicated in altered stress response (Brunton 2010), increased anxiety-like behavior (Miyagawa, et al. 2011) and cognitive impairment (Li, et al. 2008), but little is known about the consequences of PNS on energy homeostasis. Moreover, there are controversial results in experimental studies combining hypercaloric diets and stress, as it has been described that either a reduction (Paternain, et al. 2011; Tamashiro, et al. 2007) or an increase (Kuo, et al. 2007; Tamashiro, et al. 2009) in body weight may occur in response to stress, which may depend on the applied stress paradigms, but also on the species and strains, or the type and composition of the diet. Based on this information, we hypothesized that maternal stress during the prenatal stage could predispose offspring to obesity in adulthood. For this purpose, we have evaluated the peripheral effects of a high-fat-sucrose diet (HFS) intake in adulthood, after experiencing a chronic stressful situation during development in the uterus.

MATERIAL AND METHODS

Animals and experimental design

Timed-pregnant Wistar rats on gestation day 9 supplied by Harlan Iberica (Barcelona, Spain) were individually housed in a temperature (21 ± 1 °C) and humidity ($55 \pm 5\%$) controlled room on a 12-h light/dark cycle (lights on 08:00h-20:00h) with food and water freely available. Dams were weighed, and food intake was measured daily throughout gestation.

The day all the litters were born was designated postnatal day 0 (PND 0) and all pups were sexed by assessing ano-genital distance. In this study only litters that contained 8-10 pups were included. Specifically, after all rats delivered, 3 dams had to be excluded because they were not pregnant and 3 litters (2 in non-stressed group and 1 in stressed group) were eliminated for having less than 8 pups. Finally, 14 litters were used for the study, 7 in non-stressed group and 7 in stressed group. Rats were weaned on PND 21.

At 2 months old, offspring of both genders were randomized by weight and assigned into two dietary groups: standard chow diet (20% protein, 67% carbohydrate, 13% fat; 2014 Tekland Global 14% Protein Rodent Maintenance Diet, Harlan Iberica, Barcelona, Spain) (C group, n=8 and CE group, n=8) and HFS diet (20% protein, 35% carbohydrate, from which 17% was sucrose, 45% fat from Research Diets, Inc. (D12451)) (HFS, n=8 and HFSE group, n=8). Rats had ad libitum access to water and food during the dietary treatment (10 weeks) and body weight was recorded once a week.

In the 9th week of dietary treatment and after a fasting period of 12 h, rats were intraperitoneally injected with glucose at 1g/kg body weight (Lomba, et al. 2010a) in order to carry out an Intraperitoneal Glucose Tolerance Test (IPGTT). A drop of blood from a tail vein was collected by needle-prick at 0, 15, 30, 60, and 120 min after glucose administration in order to determine glucose concentrations with a Glucosemeter device (Roche Diagnostic, Mannheim, Germany).

After rats were killed by decapitation without anesthesia (end of week 10 of diet treatment), trunk blood was collected to obtain the serum for biochemical measurements; serum was separated after centrifugation of clotted blood and stored at -80°C. Liver, different adipose

depots such as subcutaneous (Sc), retroperitoneal (Rp), perigonadal (Periovaric (Peri) in female and Epididymal (Epi) in male) and mesenteric (Mes), gastrocnemius and extensor digitorum longus muscles and thymus were carefully dissected, weight and stored immediately at -80°C for further analysis. Visceral fat was calculated as the sum of perigonadal, retroperitoneal and mesenteric fat pads. All the procedures were performed according to national and institutional guidelines of the Animal Care and Use Committee at the University of Navarra.

Unpredictable Prenatal Stress Paradigm

Randomly selected pregnant female rats (n=10) were exposed to an unpredictable stress paradigm during the final eight days of gestation (days 14-21). Each stressor was applied during the light cycle unless noted otherwise. We selected a variable stress paradigm for our studies to prevent the rats from habituating to the stressor (Barnum, et al. 2007). Stress was restricted to the 3rd week of gestation because the neuronal circuits regulating the HPA axis and energy homeostasis, including the hypothalamus, are rapidly developing during this period (Weinstock 2001). The remaining control dams (n=10) were exposed to normal animal room husbandry practices in the animal facility. The stress paradigm consisted of 1h restraint in a cage with dimensions of 3.6x9x10cm; 10 minute swim stress in water at room temperature (22-24°C) in a vertical plexiglass cylinder (height: 45 cm, diameter: 19 cm) filled with 28–30 cm water; 12 h wet bedding; 6 h intermittent bell (10 db, 1 s/10 s); 18 h food deprivation during dark phase of light cycle; overnight illumination. A schedule for the application of the stressors is shown in Table 1. All stressed rats received exactly the same stressors.

Body composition analysis

On PND 28, at 2 months old (before starting the dietary treatment) and before the sacrificing day (after the 10 weeks of dietary treatment), whole body composition (fat and lean tissues) was determined using nuclear magnetic resonance technology with an Echo MRI Analyzer system (Echo Medical Systems, Houston, Texas, EEUU) (Nixon, et al. 2010).

Serum measurements

Circulating glucose was measured with a HK-CP kit (ABX diagnostic, Montpellier, France) using automatized PENTRA C200 equipment (HORIBA Medical, Montpellier, France). Serum leptin (Linco Research, St. Charles, MO, USA), insulin (Mercodia AB, Uppsala, Sweden), and monocyte chemoattractant protein-1 concentrations (MCP-1; an inflammatory marker (Melgarejo, et al. 2009)) (Invitrogen, Carlsbad, CA, USA) were determined by enzyme-linked-immunosorbent assay (ELISA) using an automatized TRITURUS equipment (Grifols International S.A., Barcelona, Spain). Homeostasis model assessment (HOMA) is an index that estimates insulin-resistance based on the relationship between the fasting plasma insulin concentration and the glucose concentration. It was calculated as fasting plasma glucose (mM) multiplied by fasting serum insulin (µU/mL) divided by 22.5, as described elsewhere (Paternain et al. 2011). Serum corticosterone concentration was determined using a commercially available enzyme immunoassay kit (Enzo Life Sciences, Farmingdale, NY). Inter- and intra-assay variability for each assay respectively, were as follows: leptin, 3.0–5.7% and 2.0–4.6%; insulin, 8.5–9.4% and 1.4–4.6%; and corticosterone, 7.8-13.1% and 6.6-8.0%. The sensitivities of these assays were 0.04 ng/mL for leptin, ≤0.15 µg/L for insulin and 26.99pg/ml for corticosterone.

Determination of fecal corticosterone

Twenty four hour fecal samples were collected in the 4th, 5th, 6th and 7th week of dietary treatment and stored at -20 °C until analyzed. Rats were moved to a clean cage for 24h, and then moved to another clean cage. The total 24 h fecal samples were powdered and corticosterone concentrations were determined in 0.2 g of dust-like fecal material, by ethanol extraction followed by a corticosterone enzyme immunoassay (EIA kit; Enzo Life Sciences, Farmingdale, NY). Inter- and intra-assay variability for this assay were 7.8-13.1% and 6.6-8.0% respectively, and it had a sensitivity of 26.99pg/ml. The results are reported as the total mass of corticoid content in the 24h fecal sample according to a previously published protocol (Paternain et al. 2011).

Real-Time Polymerase Chain Reaction (PCR)

Total RNA and DNA were isolated from retroperitoneal white adipose tissue (WAT) according to AllPrep® DNA/RNA manufacturer's instruction (Qiagen, Germantown, MD, USA). The cDNA was synthesized using RT² First Strand Kit (Qiagen, Germantown, MD, USA). Finally, quantitative real-time PCR of 48 genes recognized to be related to obesity and glucocorticoid metabolism (Table 5) was performed following manufacturer's recommendations using ABI PRISM 7900 HT Fast Real-Time PCR System. Obesity related genes were explored with RT² qPCR Primer Assay (Qiagen, Germantown, MD, USA), while glucocorticoid metabolism genes were analyzed with Taqman probes for rats (Applied Biosystems, Austin, TX, USA). The gene expression levels were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control. Fold change between the groups was calculated using the $2^{-\Delta\Delta Ct}$ method (Paternain et al. 2011). They were considered differentially expressed if the mRNA values showed fold-change of at least 1.5 and also satisfied $p < 0.05$.

Molecular network generation using Ingenuity pathways analysis

Networks were generated with all 48 analyzed genes and corresponding fold changes through Ingenuity pathways analysis (Ingenuity Systems, <http://www.ingenuity.com>, Redwood City, CA, USA), limiting the number of networks and eligible molecules per network to 25 and 35, respectively. Networks were algorithmically generated based on their connectivity and ranked by score (negative exponent of the right-tailed Fisher's exact test result). Molecules are represented as nodes, and the biological relationship between two nodes as an edge (line). Nodes are displayed using various shapes that represent the functional class of the gene product, whereas edges describe the nature of the relationship between the nodes, as defined in Ingenuity Systems.

Statistical analysis

All results are expressed as mean \pm standard error of the mean (SEM). Data were evaluated by two-way ANOVA (Diet and PNS), repeated-measures ANOVA (RM-Anova) or Student's t tests for independent samples as appropriate. Subsequent comparisons between groups were made with Bonferroni test procedures. A level of probability set at $p < 0.05$ was used as statistically significant. All analyses were performed using SPSS 15.0 packages for Windows (Chicago, IL, USA).

RESULTS

Dam and offspring measurements

There were no significant differences in maternal body weight between the groups before stress exposure (data not shown). However, the stress paradigm during the last week of gestation (gestation days 14–21) resulted in a lower maternal body weight (g) (mean \pm SEM, non-stress: 318.2 \pm 13.5 vs. stress: 284.3 \pm 2.9, Student's t-test: $p < 0.01$), although there were no marked differences in food intake (Kcal/day) (non-stress: 82.7 \pm 5.4 vs. stress: 75.1 \pm 1.8).

On the last day of the stress paradigm, dams' fecal corticosterone content (ng/24h) was lower in the stressed group than in the control group (non-stress: 2.0 \pm 0.2 vs. stress: 1.3 \pm 0.1, Student's t-test: $p < 0.01$).

Maternal stress significantly reduced offspring birth-weight and this effect lasted until the pups were 7 days old. Maternal stress also resulted in shorter body length, only in female offspring, and a lower body fat mass, only in male offspring (Supplementary Table 1).

Body measurements and food intake in adult offspring

The HFS diet treatment induced the expected obesity model in offspring of both sexes (Table 2), which was reflected in a higher final body weight and fat mass deposition. Interestingly, rats subjected to PNS and fed with HFS diet in adulthood (HFSE group) gained more body fat mass than HFS rats (Figure 1), although only in females did this reach statistical significance (Figure 1b) (Males: Main effect of the diet: $F_{3, 30} = 105.471$, $p < 0.001$, $n = 7-8$; Females: Main effect of the diet: $F_{3, 31} = 157.425$, $p < 0.001$; Main effect of the PNS: $F_{3, 31} = 4.901$, $p < 0.05$; Interaction (Diet \times PNS): $F_{3, 31} = 5.720$, $p < 0.05$, $n = 8$),

Regarding different WAT depots (Figure 2), there was an increase in all measured depots with HFS diet in both sexes (Males: Main effect of diet in Rp WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Epi WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Mes WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Visceral WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$) and Sc WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$); Females: Main effect of diet in Rp WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Peri WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Mes WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Visceral WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$) and Sc WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), but a sex-specific response induced by PNS was observed. Hence, there were no changes due to PNS in male rats (Figure 2a), while in females (Figure 2b) an interaction was observed between HFS diet and PNS in visceral WAT depots (Interaction (Diet \times PNS) in Rp WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$), Mes WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$) and Visceral WAT ($F_{3, 30} = 105.471$, $p < 0.001$, $n = 8$)) indicating that PNS induced more visceral adiposity in female rats.

Male and female rats that experienced PNS had a greater food intake than the control groups but interestingly, we only observed a significant effect of the HFS diet in male rats (Table 2). Moreover, both sexes fed the HFS diet had greater energy efficiency than rats on normal diet (Table 2).

Regarding other organ weights, the HFS diet intake induced a decrease of liver and total muscle weight (calculated as the sum of gastrocnemius and extensor digitorum longus weight),

but only in male rats (Main effect of diet (F3, 30= 40.910, $p<0.001$, $n=7-8$). There were no differences in thymus weight among the groups (Supplementary Figure 1)

Biochemical biomarkers in adult offspring

Biochemical measurements after 10 weeks of dietary treatment confirmed that the HFS diet intake induced changes leading to features commonly associated with obesity and metabolic syndrome, although these changes were more evident in female rats. These modifications were significant increases in serum leptin and glucose concentrations and a decrease in lipid profile markers (FFA in males; triglycerides, cholesterol, HDL, FFA in females; Table 3). Insulin resistance biomarkers (serum insulin concentration, HOMA) were also increased by HFS diet in males and females, but PNS decreased these biomarkers significantly, except in PNS males (Table 3).

At 9 weeks of dietary treatment, an IPGTT was performed (Figure 3). RM-ANOVA of these data demonstrated that blood glucose concentration during the IPGTT varied significantly with time (Main effect of time, $F_5= 40.990$, $p<0.05$). Moreover, a main effect of diet ($F_1, 28= 68.844$, $p<0.001$, $n=8$) and interaction (Diet \times PNS) ($F_1, 28= 11.059$, $p<0.01$, $n=8$) were observed. Further analysis indicated that after 12h of fasting rats fed HFS showed higher baseline glucose concentrations, and this effect was also observed at 15 min and 30 min. Regarding chow fed rats, the glucose clearance was faster in PNS rats. Overall, although the HFS diet intake induced a slower glucose clearance, PNS during the last week of gestation further slowed glucose clearance, indicating that PNS could worsen metabolic alterations in diet induced obesity.

The analysis of serum MCP-1 was assessed as an inflammatory marker, but no significant changes were observed (Table 3).

Corticosterone in adult offspring

The analysis of corticosterone or the mRNA expression levels of genes related with the glucocorticoids signaling were carried out only in female rats. The results showed that terminal serum corticosterone concentrations in females were not altered by HFS diet or by the stress paradigm (Figure 4a). Regarding the analysis of corticosterone content in feces over 24h, in weeks 4, 5, 6, and 7 of dietary treatment were also not affected by the treatment (Figure 4b).

Analysis of mRNA expression for different genes related to the metabolism or action of glucocorticoids in retroperitoneal WAT in female rats showed that 11 β -Hydroxysteroid dehydrogenase type 2 gene expression was significantly increased by HFS diet ($p<0.01$, Table 4); expression of 11 β -Hydroxysteroid dehydrogenase type 1, 11 β -Hydroxysteroid dehydrogenase type 2, Mineralocorticoid receptor and Glucocorticoid receptor (GR) mRNAs was not different among the groups (Table 4).

Obesity related genes in adult offspring

In this experimental model 9 out of 48 studied genes were differentially expressed retroperitoneal WAT as a result of HFS diet or PNS in female rats (Table 4). HFS induced an increase in mRNA expression for Apelin (Apln), and Leptin (Lep), and a decrease in Cyclin-

dependent kinase inhibitor 1A (Cdkn1a) and Fatty-acid synthase (Fasn). Furthermore, PNS increased mRNA expression for Fasn and Stearoyl-CoA desaturase1 (Scd1), while PNS decreased Interleukin-6 (Il6) mRNA expression levels. Statistically significant interactions between HFS diet intake and PNS were observed for Adpn and Peroxisome proliferator-activated receptor- γ coactivator1- α (Ppargc1a) mRNA expression. Regarding Adpn mRNA, an increase in PNS females was observed only in rats fed with HFS diet, and for Ppargc1a mRNA, PNS increased expression only in chow-fed females.

Ingenuity analysis of differentially expressed genes in WAT (Table 4) showed statistically implicated 3 main pathways related to the endocrine system, cell cycle and lipid metabolism in the effects of PNS and HFS diet. Supplementary Figure 2 shows the integrated network affected by HFS diet intake, and Supplementary Figure 3 shows the integrated network affected by PNS.

DISCUSSION

The environment during fetal development is important for the health of adult offspring, as exposure to nutritional shortages in pregnancy increases predisposition to development of obesity and diabetes mellitus type 2 in the offspring (Hales and Barker 1992). However, stressful situations also have been associated with the development of obesity-related alterations (Rosmond and Bjorntorp 1999). Moreover, it has been reported that Immune stress in late pregnant rats reduced gestational length and the number of viable pups born (Paris, et al. 2011). Thus, in the current study we examined the peripheral effects of a HFS diet intake on adult rats exposed to PNS.

It has been reported that there is a circadian variation of the corticosterone levels throughout rat pregnancy (Atkinson and Waddell 1995). In this study the stress exposure in late pregnancy reduced maternal weight during the period of the treatment and restricted early postnatal growth of the pups in line with previous findings (Franko, et al. 2010; Woods 2006) and this reduced maternal weight during the treatment could be due in part to the reduced weight of the fetuses and may also have reflected the differences in concentration of stress hormones (Franko et al. 2010). Moreover, lower fecal corticosterone levels in dams were observed in the last day of the stress paradigm in agreement with many other studies (Douglas, et al. 2003; Douglas, et al. 2005; Neumann, et al. 1998). These studies reported that in the rat corticosterone secretory responses in pregnancy is attenuated as a mechanism for minimizing exposure of the fetuses and neonate to glucocorticoids in the peripartum period and that the central mechanisms underlie this reduced response.

In relation to the effects in adult offspring, as expected, the intake of a hypercaloric diet induced changes in several obesity-related phenotypical variables (Lomba, et al. 2010b). In particular the weight of white fat pads was increased, leading to a higher final body weight. Regarding the biochemical measurements, HFS diet intake also induced alterations leading to common features associated with obesity and the metabolic syndrome, such as increased serum glucose, insulin and leptin concentrations, and increased HOMA, although the effects were more apparent in female rats. The decrease in lipid profile observed (decreased serum concentrations of triglycerides, HDL and FFA), in contrast with the increase detected in human beings, is commonly found in rat models of diet-induced obesity (Lomba et al. 2010b).

However, the PNS paradigm induced an increase in food intake and a higher fat mass gain only in HFS diet fed rats, indicating that PNS could worsen diet-induced obesity. PNS induced a decrease in insulin resistance biomarkers, which is in agreement with previous studies (Delaunay, et al. 1997; Lambillotte, et al. 1997; Solas, et al. 2010) that reported an inhibition of insulin secretion from pancreatic β -cells due to glucocorticoids. Although tendencies for these changes were observed in both sexes, only in female rats was statistical significance reached indicating that PNS can have sex-dependent effects in rats.

Neither ovariectomy nor orchietomy during adulthood change the weight gain or food intake response to restraint stress (Garcia-Caceres, et al. 2010), which suggests that post-pubertal gonadal steroids do not underlie the sex difference observed in this stress response. Indeed, some aspects of neuroendocrine stress axis function are responsive to post-pubertal sex steroid manipulations, while others are not (Patchev, et al. 1999). However, it is well known that sex steroids have organizational effects on the developing hypothalamus that result in structural changes that underlie sexually dimorphic endocrine responses including stress, growth and reproduction (Koehl, et al. 2009; McCarthy, et al. 2008). Thus, ovariectomized female rats gain significantly more weight than control females, and orchietomized males gain less weight than control males (Roepke 2009). Moreover, gender is one major variable that appears to confer differential vulnerability to stress. Darnaudery et al. (Darnaudery and Maccari 2008) and Simpson et al. (Simpson and Kelly 2012) commented that male and females differ in physiological and behavioral responses to stressors. Despite the knowledge that in humans, women are more susceptible than men to stress-related mental illnesses such as major depression (Lewinsohn, et al. 1998; Swaab and Hofman 1995; Weinstock 1999), many of the relevant studies in this field have been conducted in male rodents, and less information is available on the responses of females to stressors. Therefore, to investigate effects of PNS in diet-induced obesity, a deeper analysis was performed only in female rats.

PNS in rodents has also been implicated in altered stress responsivity of adult offspring (Brunton 2010), and to analyze the hypothalamo-pituitary-adrenal (HPA) axis response, serum and fecal corticosterone were measured. We did not observe any statistically significant changes in serum corticosterone concentration (after 10 weeks of diet treatment). However, the serum concentrations of corticosterone are of limited value because they were determined after behavioral manipulations. In this context, fecal corticosterone has advantages to study the changes in glucocorticoid release (Cavigelli, et al. 2005; Paternain et al. 2011). However, no changes were observed in corticosterone levels in feces over 24h in any of analyzed weeks (week 4, 5, 6, 7 of diet treatment). It had been reported that basal plasma corticosterone levels are elevated in prenatally stressed female rats (Ward, et al. 2000; Weinstock, et al. 1998). However, Kay et al. (Kay, et al. 1998) failed to report increased basal HPA activity following prenatal stress. These differences may result from differences between the prenatal stress paradigms used as well as variation in the time of day when blood samples were taken, as prenatal stress has been shown to induce a phase shift in the circadian corticosterone rhythm in adult offspring (Koehl, et al. 1997; Koehl, et al. 1999). Also, there were no changes in retroperitoneal WAT, after 10 weeks of diet treatment, in mRNA levels of different genes related to the metabolism of glucocorticoids, except that HFS diet intake increased 11 β -hsd2 mRNA expression levels. This enzyme is one of the enzymes modulating local glucocorticoid effects, regulating the access of 11 β -hydroxyglucocorticoids to its receptor

by converting corticosterone to the GR-inactive form dehydrocorticosterone (Kershaw, et al. 2005). This outcome is consistent with our previous study, which found enhanced 11 β -hsd2 gene expression in subcutaneous WAT in male Wistar rats with high-fat diet-induced obesity (Milagro, et al. 2007).

It is noteworthy that some of the results presented in this study can be affected by the sex hormone cycle of the females, which was not assessed in this study, as in many other studies on early life effects (Kalinichev, et al. 2002; Lehmann, et al. 1999; McIntosh, et al. 1999; Wigger and Neumann 1999). Estrogen may alter or interact with HPA axis regulation of corticosterone release and cognitive function, and it has been suggested that estrogen in females may protect against the effects of corticosterone (Luine 2002).

Regarding the obesity related genes, Ingenuity analysis revealed that the expression of genes involved in lipogenesis was affected by both dietary treatment and PNS. Thus, HFS diet intake increased *Lep* (Milagro, et al. 2009) and *Apln* (Fernandez-Galilea, et al. 2011) mRNA expression. However, *Cdkn1a* (Boque, et al. 2009) and *Fasn* (Lomba et al. 2010a) mRNA levels were downregulated by HFS diet. Given that the HFS diet intake increased the body WAT mass, it was unexpected to observe that the expression of these genes was downregulated in adipose tissue. However, these results can be explained, because at the time of measurement (week 10 of HFS diet) the rats could be at a late stage of obesity and the fat mass longer expanding (Diraison, et al. 2002). Nevertheless, PNS also increased lipogenic genes, such as *Fasn* (Drake, et al. 2010) and *Scd1* (Hu, et al. 2004), and decreased *Il6* mRNA levels (Ishii-Yonemoto, et al.). Interestingly, an interaction between the dietary treatment and PNS was observed in *Adpn* and *Ppargc1a* gene expression. *Adpn* is an adipose-specific transmembrane protein that is regulated by energy balance (Baulande, et al. 2001) and has been postulated to be the part of the adipose-specific energy homeostasis sensor (Johansson, et al. 2006). Thus, in an increase in *Adpn* gene expression with PNS was observed only when these rats were also fed with HFS diet. Moreover, *Ppargc1a* gene expression was also increased with PNS, but only when these rats were fed normal chow. Furthermore, Wyrwoll et al. (Wyrwoll, et al. 2008) described that female offspring of dexamethasone-treated mothers had increased skeletal muscle expression of *Ppargc1a*, which contributes to the insulin-sensitizing effects of *Pparg* activators as a transcriptional coactivator (Finck and Kelly 2006). Hence, the upregulation of this gene may be a homeostatic response to disturbances in insulin signaling, which is in agreement with the current work. Overall, these results reinforce the idea that PNS predisposes offspring to greater WAT gain in adulthood.

CONCLUSION

There is evidence that the conditions during the intrauterine period are critical for development of the offspring since disturbances in this stage can induce important biological changes that persist into adulthood (Hales and Barker 1992). In agreement, the general conclusion of the present work is that experience of stress during intrauterine development leads to adult rats having a higher predisposition to obesity induced by HFS diet intake, but this was found to be statistically significant only for females, indicating that PNS can have sex-dependent effects in rats. A deeper analysis performed in white adipose tissue from female rats indicated that altered *Fasn*, *Scd1*, *Adpn* and *Ppargc1a* gene expression regulation could be

implicated in a permanent effect of PNS in these rats, any epigenetic mechanisms involved remain to be explored.

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Declaration of interests

There are no conflicts of interest in this study to report.

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