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# Depot- and sex-specific effects of maternal obesity in offspring's adipose tissue

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**Abbreviated title**: Maternal obesity and programmed adiposity

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## Abstract

According to the Developmental Origin of Health and Disease (DOHaD) concept, alterations of nutrient supply in the fetus or neonate result in long-term programming of individual body weight set-point. In particular, maternal obesity, excessive nutrition and accelerated growth in neonates have been shown to sensitize offspring to obesity. The white adipose tissue may represent a prime target of metabolic programming induced by maternal obesity. In order to unravel the underlying mechanisms, we have developed a rat model of maternal obesity using a high-fat (HF) diet (containing 60% lipids) before and during gestation and lactation. At birth, newborns from obese dams (called HF) were normotrophs. However, HF neonates exhibited a rapid weight gain during lactation, a key period of adipose tissue development in rodents. In males, increased body weight at weaning (+ 30%) persists until 3 months of age. Nine-month-old HF male offspring were normoglycemic but showed mild glucose intolerance, hyperinsulinemia and hypercorticosteronemia. Despite no difference in body weight and energy intake, HF adult male offspring were predisposed to fat accumulation showing increased visceral (gonadal and perirenal) depots weights and hyperleptinemia. However, only perirenal adipose tissue depot exhibited marked adipocyte hypertrophy and hyperplasia with elevated lipogenic (i.e., SREBP1, FAS, leptin) and diminished adipogenic (i.e., PPARγ, 11β-HSD1) mRNA levels. By contrast, very few metabolic variations were observed in HF female offspring. Thus, maternal obesity and accelerated growth during lactation program offspring for higher adiposity via transcriptional alterations of visceral adipose tissue in a depot- and sex-specific manner.

## Introduction

The rising prevalence of obesity in the world is considered a global epidemic (Popkin *et al.* 2012). Obesity is characterized by accumulation and functional alterations of white adipose tissue (WAT) predisposing the individuals to increased risk of metabolic pathologies (Sun *et al.* 2011). The expansion of WAT results from hyperplasia (increase in adipocyte number) and/or hypertrophy (increase in adipocyte size) along with modifications of tissue sensitivity to circulating hormones (Björntorp 1971). Obesity is the result of a complex interaction between genetic and environmental factors (Bouchard 2009). According to the Developmental Origin of Health and Disease (DOHaD) concept also called "developmental programming" or "conditioning" (Barker 2004; Hanson & Gluckman 2014), alterations of nutrient supply in the fetus or neonate result in long-term programming of individual body weight set-point. Epidemiological studies initially showed that maternal undernutrition leading to fetal growth restriction is associated with higher adiposity in adulthood (Ravelli *et al.* 1999). Clinical studies have also been shown that maternal obesity, excessive nutrition and accelerated growth in neonates sensitize offspring to obesity (Leddy *et al.* 2008).

Thus, WAT may represent a prime target of metabolic programming induced by maternal obesity. Perturbations to the perinatal nutrient supply may affect adipocyte development, leading to persistent alterations in their number and functional properties (Lukaszewski *et al.* 2013; Lecoutre & Breton 2014, 2015). Indeed, in fetuses and neonates, adipocyte stem cells are still plastic and potentially sensitive to maternal factors (Tang & Lane 2012). In humans, the number of adipocyte is set early in life and is a major determinant of fat mass in adulthood (Spalding *et al.* 2008). The timing of adipose tissue development, which differs between species, determines the window of vulnerability to potential adverse environment. In rodents, adipose tissue growth and adipogenesis mainly take place during the last week of gestation and accelerate throughout lactation whereas in larger mammals, these

processes occur before birth. However, there is now convincing evidence that adipogenesis occurs throughout the life time (Muhlhausler & Smith 2009).

Little is known about the programming mechanisms that may account for long-lasting perturbation of adipogenesis and WAT metabolism in offspring from obese dams. To unravel the underlying mechanisms, several animal models of maternal obesity have been developed using high-fat (HF) or cafeteria diet applied during the preconception, gestation and/or lactation periods in dams (Williams *et al.* 2014). These studies confirmed that maternal obesity has common long-term metabolic consequences sensitizing the offspring to metabolic syndrome features. In particular, maternal obesity at conception programs enhanced adipogenesis and lipogenesis from the fetal period to adulthood resulting in higher WAT mass and larger adipocytes (Muhlhausler & Smith 2009; Borengasser *et al.* 2013; Murabayashi *et al.* 2013). Overfeeding during lactation and/or postweaning periods leads to accelerated growth and consistently worsens adipogenesis and lipogenesis programming (Desai & Ross 2011; Guberman *et al.* 2013; Masuyama & Hiramatsu 2014). Programmed upregulation of the key adipogenic factor PPARγ is one characteristic features of fat expansion in offspring of obese dams (Samuelsson *et al.* 2008; Muhlhausler & Smith 2009; Sen & Simmons 2010; Dahlhoff *et al.* 2014; Desai *et al.* 2015). Obesity-prone offspring rats from obese mothers also exhibited modified fatty acid composition within WAT (Benkalfat *et al.* 2011). However, in rodents, few studies have examined depot- and sex-specific consequences of maternal obesity in offspring's WAT and there is little agreement among them (Sun *et al.* 2012; Ornellas *et al.* 2013; Dahlhoff *et al.* 2014; Masuyama & Hiramatsu 2014).

In the present study, we examined whether maternal obesity differently programs adipocyte number and morphology using a model of maternal obesity in rats fed a HF diet prior to and during gestation and lactation. We also profiled gene expression in two visceral fat depots (gonadal and perirenal WAT) in adult offspring of both sexes. Here, we demonstrate

that maternal obesity and accelerated growth during lactation program offspring for higher adiposity via transcriptional alterations of visceral adipose tissue in a depot- and sex-specific manner.

## Materials and methods

### *Animals*

Four-week-old virgin female Wistar rats (1 month) were purchased from Charles River Laboratories (L'Arbresle, France) and were housed in individual cages in a humidity-controlled room with a 12:12-h light-dark cycle. Food and water were available *ad libitum*. After two weeks of acclimatization on a control (C) diet (3.85 kcal/g with 10% of total calories as fat consisting of soybean oil (5.6%) and lard (4.4%), 70% as carbohydrate and 20% as protein; D12450J, Research Diets, New Brunswick, NJ, USA), female rats were fed either a HF diet (5.24 kcal/g with 60% of total calories as fat consisting of soybean oil (5.6%) and lard (54.4%), 20% as carbohydrate and 20% as protein; D12492, Research Diets, New Brunswick, NJ, USA) or a C diet for 16 weeks (n=12 per group). After 14 weeks of HF diet, 20-week-old female (5 months) rats were subjected to an oral glucose tolerance test (OGTT). Plasma levels of insulin, leptin and corticosterone were also measured after 16h overnight fasting in both groups. After mating with a male rat fed a C diet, 22-week-old pregnant females were transferred into individual cages with free access to water and continued on their respective diets (C or HF diet) throughout gestation and lactation. Maternal body weight was measured weekly until delivery. At parturition, pups were weighed and sexed. Litter size was adjusted to 8 pups per dam (four males and four females). During lactation, body weights of dams and pups were assessed on postnatal days (PND) 1, 4, 7, 11**,** 14, 17 and 21. At weaning (PND21), dams were sacrificed and glycemia as well as plasma levels of leptin insulin and corticosterone were determined after 16h overnight fasting. To obviate any litter effects, animals used for each experiment were randomly chosen in different litters and only a limited number of animals (1 to 2 males and females) was used from each litter. After weaning, male (M) and female (F) offspring from C or HF dams were housed individually with free access to water and C diet, divided into four groups (CM, CF, HFM and HFF (n= 16

per group) and weighed weekly until 9 months of age. Animal use authorization by the French Ministry of Agriculture (No. 04860) has been granted to our laboratory for experimentation with rats. Experiments were conducted in accordance with the principles of laboratory animal care (European Communities Council Directive of 1986, 86/609/EEC).

***Food intake and metabolic parameters***

Food consumption was recorded weekly from weaning to adulthood until sacrifice in the four groups. Food intake of rats was measured once a day at the beginning of the light phase (9 a.m.) by subtracting the uneaten food from the initial amount. Weight-related energy intake is defined as the energy content of the food ingested (Kcal) expressed relative to body weight (g). 24-week-old (6 months) offspring were placed in metabolic cage (Bioseb, Vitrolles, France). After an acclimatization period, food intake was recorded for each 24 hours period during one week to investigate light/dark phase food intake rhythm.

***Oral glucose tolerance test***

For OGTT, rats were fasted overnight. Basal blood glucose level defined as T0 was determined using a glucometer (Glucotrend 2, Roche Diagnostics, France) before oral glucose administration (2g/kg of body weight). Tail vein blood glucose was then measured at 0, 30, 60, 90 and 120 min after administration.

***Endocrine parameters***

Plasma hormone levels were evaluated in 30-week-old (7 months) and 36-week-old offspring (9 months) at sacrifice. Blood glucose was determined as described above. Plasma leptin and adiponectin concentrations were measured with murine ELISA kits (Diagnostic Systems Laboratories, Inc.USA ; AdipogenInc, Korea, respectively). Plasma corticosterone

levels were determined by a competitive enzyme immunoassay (Immunodiagnostic Systems Ltd, Boldon, U.K). Plasma insulin concentrations were measured by ELISA (DRG, International, Inc. USA). Plasma apelin content was determined by ELISA (Phoenix Pharmaceuticals). The assay sensitivity was 0.07 ng/mL (insulin), 0.04 ng/mL (leptin), 0.1 ng/mL (adiponectin), 0.55 ng/mL (corticosterone) and the intra-and inter-assay coefficients of variation were 4% and 9.1% (insulin), 5.4% and 7.3% (leptin), 4.4% and 6.1% (adiponectin), 4.9% and 7.8% (corticosterone), respectively. Assay kits were used to determine the contents of plasma triglycerides and total cholesterol (61238 Triglyceride Enzymatique PAP100, 61218 Cholesterol Liquide, BioMérieux, France) as well as free cholesterol and free fatty acid (FFA) (references 279-47106 and 999-75406, Wako Chemicals, Neuss, Germany). Each sample was measured in duplicate.

***Plasma and tissue collections***

36-week-old rats (9 months) were rapidly weighed and killed by decapitation between 9 and 10 a.m after 16h overnight fasting. Trunk blood samples were collected into prechilled tubes containing EDTA (20 μL of a 5% solution) and centrifuged at 4,000 g for 10 min at 4°C. Plasma was stored at -20°C. Several tissues (brown adipose tissue, liver, heart, kidney, adrenal gland) as well as gonadal (GWAT) and perirenal (PWAT) fat pads were weighed, frozen in liquid nitrogen and stored at -80°C. For histology experiments, animals were fixed by intracardiac perfusion using buffered 4% paraformaldehyde solution.

***Gene Expression Analysis***

GWAT and PWAT gene expression levels were determined in the four groups using RT-qPCR as previously validated (Lukaszewski *et al.* 2011). Briefly, total RNA was extracted and purified using RNeasy lipid tissue kit (Qiagen, Courtaboeuf, France) according to the

manufacturer's recommendations. The yield of total RNA was quantified on a Multiskan Go Microplate Spectrophotometer (Thermo Scientific, Illkirch, France). The quality of total RNA was assessed by determining the 260/280 and the 260/230 absorbance ratio and by agarose gel electrophoresis. First-strand cDNAs were synthesized using ThermoScript RT Kit (Invitrogen, Life Technologies, France). Relative expression levels of RNA per sample were quantified by SYBR Green assay on a Roche Light Cycler 480 sequence detection assay (Roche Biochemicals, Meylan, France). Primers sequences are presented in Table 1. For each transcript, PCR was performed in duplicate with 10 µl final reaction volumes with 1 µl of cDNA, 8 µl of QuantiTect SYBR Green Master mix (Qiagen, Courtaboeuf, France) and 0.5 µl of each primer set (Table 1). PCR was conducted using the following cycle parameters : 10 minutes at 95°C, and 40 three-steps cycles of 15 s at 95°C, 20 s at 60°C and 30 s at 72°C. A pool of cDNA from control tissues was used as a standard for quantitative correction. All cDNA samples were applied in dilution of 1:10 to obtain results within the range of the standard. Analysis of transcript level was carried out using first the determination of the threshold cycle Ct for each reaction corrected by the efficiency. The level of gene expression was normalized to the reference gene transcript cyclophilin A RNA.

***Morphometric analysis of adipose tissue***

Fat pad mass as well as cell-size distributions were measured. GWAT and PWAT from the four groups (n=6 per group) were post-fixed for 24 h in 4% paraformaldehyde in phosphate buffer saline and embedded in paraffin. Fixed tissues were then cut into serial 10 µm sections, mounted on gelatin-coated slides and stained with hematoxylin of Groat and phloxin (2%), according to standard laboratory protocols. Sections were examined using light microscopy (Leica DM IRE2) and photomicrographs were captured at x20 magnification. The surface of adipocytes was evaluated in ten randomly selected fields of vision for a total of at

least 250 adipocytes using Image J software (NIH, USA). Total cell number is a direct measure reflecting hyperplasia. The number of cells was estimated using the formula as previously described (Lemonnier 1972).

***Statistical analysis***

All data are expressed as means ± standard error of the mean (S.E.M.). Statistical analysis was carried out using GraphPad Prism5 (GraphPad, San Diego, CA, USA). A direct comparison between a pair of groups was made using an unpaired Student's t test or a two-way analysis of variance (ANOVA) for repeated measures followed by a Bonferroni post hoc test, where appropriate. P values $<0.05$ was considered statistically significant.

## Results

### *Effects of HF diet on maternal parameters*

HF-fed female rats gained more weight than C females (ANOVA $P<0.0001$) (Fig.1A). After 14 weeks of HF diet, females had about 2 fold higher plasma leptin concentration (4.3 ± 0.6 ng/mL *versus* 2.2 ± 0.4 ng/mL, $P<0.05$) compared with C females (Table 2). No difference was observed in basal glycemia, plasma insulin and corticosterone levels. However, HF-fed females showed a more pronounced increase in glucose levels during OGTT with a higher area under the curve (AUC) (Fig.1B), reflecting impaired glucose tolerance (ANOVA $P<0.0001$). HF-fed dams displayed a 20% increase in body weight at the end of the gestation (Fig.1A). At weaning, HF-fed dams exhibited about 3 fold higher plasma leptin (2.5 ± 0.4 ng/mL *versus* 0.9 ± 0.2 ng/mL, $P<0.05$) and corticosterone levels (796.2 ± 187 ng/mL *versus* 278.1 ± 86.8 ng/mL, $P<0.05$) (Table 2) while showing a marked increase in all fat pads weights (data not shown). No variation was observed in glycemia or plasma insulin levels.

### *Effects of maternal obesity on offspring growth during lactation*

Maternal obesity did not impact the litter size (C: 10 ± 2 pups *versus* HF: 9 ± 3 pups) or the birthweight of offspring (C: 6.2 ± 0,1 g *versus* HF: 6.3 ± 0,1 g). However, both sexes of HF neonates exhibited rapid weight gain during lactation (ANOVA $P<0.0001$) (Fig.2). Post hoc analysis revealed difference in body weight from PND10 in male offspring (Fig.2A) and from PND17 in female offspring (Fig.2B). At weaning, HF male offspring exhibited a 30% increase whereas HF female offspring only showed a 10% increase in body weight (Fig.2A, B).

***Effects of maternal obesity on offspring growth and energy intake from weaning to adulthood***

Weaned offspring were fed a C diet until 9 months of age. The body weight difference between C and HF male rats persisted until 12 weeks of age (Fig.3A). Among females, body weight equalized between C and HF rats as early as one week after weaning (Fig.3B). In both sexes, HF rats exhibited similar weight-normalized energy intake during adulthood suggesting they were not hyperphagic (Fig.3C, D). However, HF 4-week-old female offspring exhibited significantly increased weight-normalized energy intake as compared to C rats (Fig.3D). This transient post-weaning hyperphagia may reflect female-specific regulations of food intake from milk to solid diet. HF 6-month-old rats placed in metabolic cages exhibited a lower food intake during the dark phase and a higher food intake during the light phase resulting in modified light/dark-phase food intake rhythm (data not shown).

***Effects of maternal obesity on adult offspring metabolic parameters***

At 7 months of age, C and HF offspring had comparable fasting blood glucose concentrations. During OGTT, HF males displayed increased glucose levels at 30 min ($P$<0.01) (Fig.4A) with a trend towards higher AUC ($P$=0.09) (Fig.4B) reflecting mild glucose intolerance. No difference was observed in HF female rats (Fig.4C, D). In HF male rats, serum corticosterone concentration was about 2 fold higher than in C animals after fasting (188.7 ± 27.94 ng/mL *versus* 90.42 ± 14.18 ng/mL, $P$<0.01) and feeding (57.4 ± 9.4 ng/mL *versus* 23 ± 3.4 ng/mL $P$<0.05) conditions (Fig.5A). No difference was observed in HF female rats (Fig.5B).

Unlike females, HF 9-month-old male rats displayed increased PWAT (45.8 ± 2.4 mg/g BW *versus* 39.2 ± 2.6 mg/g BW, $P$<0.05) and GWAT weights, when normalized to body weight (35 ± 1.6 mg/g BW *versus* 30 ± 1.7 mg/g BW, P<0.05) compared with C rats (Table

3). These findings were consistent with an increase in plasma leptin levels (Table 4). HF females exhibited a decrease in interscapular brown fat pad weight, but this was not observed in HF males (1.54 ± 0.16 mg/g BW *versus* 1.97 ± 0.1 mg/g BW, $P<0.05$) (Table 3).

At 9 months of age, HF male rats had about 1.5 fold higher plasma insulin (43.63 ± 3.79 μu/mL *versus* 33.47 ± 3.23 μu/mL, $P<0.05$) and leptin concentrations (15.06 ± 1.25 ng/mL *versus* 10.15 ± 1.11 ng/mL, $P<0.05$) compared with C rats whereas no difference was observed in HF females (Table 4). The increased HOMA-IR index (+ 38.5 %, $P<0.05$) suggests that HF male rats had decreased insulin sensitivity compared to C rats.

***Effects of maternal obesity on adult offspring adipose tissue morphometric parameters***

As shown in representative photographs (Fig.6A), PWAT of HF male offspring exhibited an increase in average adipocyte area (Fig.6B) and total cell number (Fig.6C) compared with C rats. Adipocytes measuring 7500 $\mu m^2$ or less represented 65% of all adipocytes in C male offspring whereas they represented only 40% of all adipocytes in HF male offspring. This indicates that maternal HF diet decreased the frequency of small-sized adipocytes (Fig.6D). In particular, the proportion of adipocytes measuring 2500 $\mu m^2$ or less displayed a marked 6-fold decrease in HF male offspring. In addition, these animals showed greater percentage of large sized adipocytes (7500-40000 $\mu m^2$) (60% *versus* 35%) compared with C rats. Although no changes in average adipocyte area (Fig.7A, B) and total cell number (Fig.7C) were observed in GWAT of HF males, a marked 5-fold reduction in frequency of 0-2500 size adipocytes similarly occurred (Fig.7D). In contrast, both fat pads of HF female offspring (Fig.6 and 7) showed no major changes.

***Effects of maternal obesity on adult offspring adipose tissue gene expression profile***

Maternal obesity led to pronounced changes in PWAT gene expression in HF adult male offspring. RT-qPCR data showed that leptin mRNA content was increased (+ 1.5 fold) in HF male rats compared to C animals (Fig.8A). This is in agreement with the increased serum leptin levels (Table 4). In PWAT, maternal obesity resulted in increased mRNA levels for genes promoting *de novo* lipogenesis such as fatty acid synthase (FAS, + 1.6 fold) and sterol regulatory element-binding protein-1 (SREBP1, + 1.7 fold) in HF male offspring. In addition, mRNA expression levels of genes involved in adipogenesis such as peroxisome proliferator-activated receptor gamma (PPARγ) and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) were decreased (- 1.3 and - 1.5 fold, respectively) in HF male offspring *versus* C rats (Fig.8A). By contrast, maternal obesity did not affect GWAT mRNA expression profiles to the same extend in HF male rats, except for a down-regulation of Ob-Rb (Fig.9A). Compared to HF males, less change in gene expression profile was observed in HF female adult offspring in both fat pads. HF female offspring still showed increased C/EBPα mRNA expression levels (+ 2 fold) in PWAT (Fig.8B) and lower adiponectin (- 1.4 fold) and GR (- 1.4 fold) mRNA expression levels in GWAT (Fig.9B). This was consistent with differences in fat depot weights and plasma leptin concentrations observed in HF male *versus* female offspring (Tables 3 and 4).

## Discussion

The main finding of this study is that maternal obesity has long-lasting consequences on visceral WAT of adult rat offspring in a depot- and sex-specific manner. In particular, we showed that HF adult male offspring exhibit greater visceral fat pad weights with adipocyte hypertrophy and hyperplasia, despite no difference in body weight and energy intake. Our findings disagree with other studies (Kirk *et al.* 2009; Nivoit *et al.* 2009; Desai *et al.* 2014) reporting that maternal obesity prior and throughout pregnancy and lactation programs hyperphagia and marked increased body weight in adult rat offspring. The discrepancy between our results and those of others may reflect differences in fetal and/or postnatal programming. These differences may depend on the duration of maternal HF feeding, the dietary lipid content (percentage of lipids *versus* carbohydrates), the lipid composition (saturated *versus* insaturated) and the palatability (presence of sweetened condensed milk) of the diet and therefore the severity of maternal obesity (i.e., weight gain, hormonal status, inflammation grade, etc…). It may also depend on the difference of genetic background of rat strain used (Wistar *versus* Sprague Dawley) (Zambrano *et al.* 2010).

In our model, HF adult offspring might have developed a modification of WAT's sensitivity to circulating hormones. Indeed, perirenal WAT depot exhibited changes in lipogenic and adipogenic pathways that may favor triglyceride storage in mature adipocytes. Consistent with increased fat deposition, HF adult male offspring displayed higher serum leptin concentration. Hyperleptinemia may be interpreted as a leptin-resistant state (Kirk *et al.* 2009; Sun *et al.* 2012). First, leptin is known to activate adipogenesis by promoting preadipocyte differentiation (Bol *et al.* 2008; Guo *et al.* 2009). However, despite hyperleptinemia, the number of small adipocytes was markedly decreased in both depots of HF adult male offspring. In agreement with these findings, we observed that PPARγ gene expression was downregulated. Our findings disagree with other studies that reported an

upregulation of PPARγ contents in WAT along with enhanced adipogenesis in obese-prone offspring from malnourished dams (Samuelsson *et al.* 2008; Muhlhausler & Smith 2009; Sen & Simmons 2010; Desai *et al.* 2015). This discrepancy may be due to differences in the establishment of epigenetic marks during adipogenesis and/or hormonal environment, tissue sensitivity as well as inflammatory status in adipose tissue of adult offspring (Breton *et al.*, 2013). The decrease in gene expression might be seen as an adaptive mechanism to limit fat accumulation (Lukaszewski *et al.* 2011). Indeed, an increase in the lipogenic capacity of adipose tissue is expected during the "dynamic phase of obesity", when fat stores are rapidly expanding. However, during long lasting and stable obesity, the decreased expression of lipogenic genes may prevent a further development of fat mass (Ortega *et al.* 2010). Further experiments on the kinetic of fat deposition and the transcriptional profile of lipogenic genes during the development of WAT are needed to address this question. In agreement with this hypothesis, several studies described a relationship between obesity and lower expression and/or activity of PPARγ in visceral WAT. These modifications appear to be strongly associated with the pathogenesis of metabolic syndrome (Zhang *et al.* 1996; Fujiki *et al.* 2009). Second, given the antilipogenic leptin action on mature adipocytes (Huan *et al.* 2003; Jiang *et al.* 2009), HF adult male offspring had a trend towards reduced leptin receptor contents and did not show any suppression of FAS gene expression, but instead a marked increased expression. In accordance with these findings, we reported that hyperleptinemic rat offspring from undernourished dams exhibited impaired leptin sensitivity with reduced pSTAT3 in WAT (Lukaszewski *et al.* 2011).

Moreover, HF adult male offspring exhibited hyperinsulinemia with elevated SREBP1 and FAS mRNA levels in PWAT, two genes that are known to be upregulated by insulin levels. This suggests that adipose tissue remains sensitive to insulin. This is in agreement with a greater insulin-induced AKT phosphorylation and the up-regulation of lipogenic pathways

observed in WAT of HF offspring (Borengasser *et al.* 2013). HF adult male offspring also displayed hypercorticosteronemia. Several lines of evidence prompted us to explore whether sensitivity of WAT to glucocorticoid (GC) was modified in offspring. First, GC alone or in interaction with insulin regulates the differentiation of preadipocytes and lipogenic genes (Campbell *et al.* 2011). Second, a close link between chronic excess of plasma GC levels and/or increased GC sensitivity within WAT (i.e., modifications of GR, MR, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) contents) and fat expansion has been observed in offspring from malnourished dams (Gnanalingham *et al.* 2005; Lukaszewski *et al.* 2011; Guo *et al.* 2013). Third, increased expression of GR and 11β-HSD1 in visceral adipose tissue has been associated with the development of obesity in rats overfed during lactation (Boullu-Ciocca *et al.* 2008). We observed a depot-specific downregulation of 11β-HSD1 mRNA in PWAT in HF adult offspring as previously reported in 3-month-old offspring from obese mice (Samuelsson *et al.* 2008). We also showed that the ratio between 11β-HSD1 and 11β-HSD2 expression that controls local balance between active and inactive GC metabolites (Lee *et al.* 2014) was decreased. As described in obesity-prone progeny from undernourished dams, it may diminish intratissular GC responsiveness and represent an adaptive mechanism to counteract excess fat storage (Lukaszewski *et al.* 2011). We cannot exclude that HF offspring may have decreased energy expenditure. Indeed, additional programming mechanisms such as elevated free fatty acid transport and/or lower lipolysis/β-oxydation activities within WAT might account for increased triglyceride storage (Dahlhoff *et al.* 2014).

At birth, HF offspring had a normal birthweight and, then, exhibited a rapid weight gain during lactation, a key period of adipose tissue development. Adipocyte stem cells are also very sensitive to maternal factors during this developmental period (Tang & Lane 2012). Adipocyte number expansion that is set earlier in obese individual may be a major

determinant for increased fat mass in adulthood (Spalding *et al.* 2008). Obesity may arise from increased lipid storage in mature adipocytes during the perinatal period. In line with these findings, we showed that maternal obesity predisposes adult offspring to adiposity by increasing the number of adipocytes and the average fat cell volume. The accelerated postnatal growth in offspring is frequently associated with persisting adiposity throughout life. Several models have shed light on the importance of energy intake and milk composition during the lactation period for adipose tissue programming. Indeed, pups from mothers exposed to HF diet only during lactation (Sun *et al.* 2012; Desai *et al.* 2014; White & Tchoukalova 2014) and neonates reared in small litters, representing a model of postnatal overfeeding (Boullu-Ciocca *et al.* 2008), also displayed persistent hypertrophic adipocytes with enhanced adipogenic and lipogenic mRNA expression levels. However, maternal obesity prior to conception and gestation is also able to program similar outcomes during the embryonic period. Indeed, despite normal fetal weight, fetus from mice fed a HF diet prior and throughout pregnancy displayed larger adipocytes (Murabayashi *et al.* 2013; Umekawa *et al.* 2015) and increased mRNA expression levels of Zfp423, a key transcriptional factor initiating adipogenic commitment (Yang *et al.* 2013). Adult mouse offspring also exhibited increased mRNAs levels of several genes involved in *de novo* lipogenesis and lipid droplet size in visceral WAT (Dahlhoff *et al.* 2014). Similarly, obesity-prone rat offspring from obese dams induced by intragastric HF diet feeding displayed an increase in adipogenic and lipogenic pathways (Shankar *et al.* 2008).

Finally, we showed that maternal obesity sensitizes adult rat offspring to increased visceral adiposity in a depot- and sex-specific manner. Indeed, among GWAT and PWAT, only the latter shows marked programming features in HF male offspring. By contrast, very few variations were observed in WAT of HF female offspring. In line with these findings, studies have previously demonstrated the heterogeneity of the adipose lineage. All adipogenic

stem cells and adipocytes do not behave equally during adipogenesis. Indeed, each fat depot has an unique developmental gene expression signature (Yamamoto *et al.* 2010). Fat stem cells are influenced by the anatomic location of the depot and/or the hormonal microenvironment, as well as aging, gender, and metabolic health (Williams *et al.* 2014). Thus, intrinsic genetic depot-specific differences in adipose stem cells result in different adipogenic potential, gene expression profile, growth rate and biological properties (i.e., hormone sensitivity) between visceral and subcutaneous fat pads, but also between each specific visceral fat pad. The fact that different adipocyte precursors might determine the development and the function of specific fat pads led to the notion that each WAT depot could be considered a separate mini-organ (Berry *et al.* 2013).

Among programming mechanisms, inappropriate hormone levels during the perinatal period are a key factor leading to persistent deregulation of energy homeostasis in progeny. It may result in long-term fat expansion with permanent changes in plasma hormone levels in adult offspring (Breton 2013). Consistent with this notion, maternal obesity prolonged and amplified the plasma leptin surge in offspring in a sex-specific manner (Kirk *et al.* 2009; Masuyama & Hiramatsu 2014). Maternal HF diet during lactation was also associated with increased insulin and leptin levels in milk (Vogt *et al.* 2014). Leptin which displays differential morphogenesis effects on male and female adipocytes (Guo *et al.* 2009) might account for WAT's programming dimorphism. However, despite the marked lactation effect in HF male *versus* female neonates, gender specific-modifications of plasma hormone levels and/or adipose tissue hormonal sensitivity remain to be determined.

Maternal obesity may also affect epigenetic mechanisms during adipogenesis. These modifications might be persistent and have long-term effects on the expression of adipogenic and lipogenic genes. We hypothesize that maternal obesity affects offspring’s energy and hormonal status modifying activity of the enzymatic components of the epigenetic machinery.

It may cause epigenetic modifications that reprogram offspring's adipose tissue. Differences in fat cell embryonic origin, development, genetic and hormonal sensitivity may result in a depot-specific programming effects that may predispose offspring to higher adiposity (Öst & Pospisilik 2015). Indeed, maternal obesity in mice induces increased gene expression of Zfp423 with lower promoter methylation levels in fetal offspring (Yang *et al.* 2013). Similarly, weanling rats from obese dams display increased Zfp423 and C/EBPβ mRNA expression levels with alterations in DNA methylation of CpG sites (Borengasser *et al.* 2013). Maternal HF diet during pregnancy also results in histone modifications within leptin and adiponectin promoter regions with gene expression modifications in mouse offspring (Masuyama & Hiramatsu 2012).

Few studies have reported that maternal obesity programs metabolic alterations and adiposity differently in a sex-dependent manner in progeny (Sun *et al.* 2012; Ornellas *et al.* 2013; Dahlhoff *et al.* 2014; Masuyama & Hiramatsu 2014). The basis of the sex-specific programming effects remain unclear but could reflect direct interactions between nutritional signals and sex hormones in tissues of the developing fetus (Aiken & Ozanne 2013). In human, numerous studies suggest that sex differences in fetal growth in response to adverse pregnancy conditions are likely to be mediated by sex-specific adaptation of the placenta (Clifton 2010). Similarly, sex-specific programming effects in rat offspring from obese dams might be due to sex-specific differences in placental response to maternal obesity (Reynolds *et al.* 2015). Epigenetic mechanisms may also contribute to placental programming in a dimorphic manner. Thus, the consumption of HF diet during pregnancy appears to differently affect placental methylation and placental gene expression patterns in male and female mice offspring (Gallou-Kabani *et al*., 2010). Thus, sex-specific differences in term of epigenetic modulations may be associated with developmentally programmed phenotypes. It is possible that postnatal hormonal milieu, which is different between male and female offspring, modify

the programming of adipose tissue induced by maternal obesity. This may result in gender-specific outcomes in relation to different sex-steroids (Dunn *et al.* 2011). Thus, a better knowledge of the epigenome changes in response to maternal obesity may provide a promising way forward to reverse adverse programming of adiposity.

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## Figure legends

**Figure 1: Maternal phenotype.** Body weight of dams fed either a C or a HF diet during preconception, gestation and lactation (A). Concentration of blood glucose during OGTT after 14 weeks of diet and area under the curve (B) (n= 12 per group). * Effect of maternal HF diet *vs*. maternal C diet (**, $P<0.01$; ***, $P<0.001$).

**Figure 2: Rat offspring growth curves from birth to weaning.** Body weight of male (A) and female (B) from C or HF dams (n=16 per group). * Effect of maternal HF diet *vs.* maternal C diet (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

**Figure 3: Rat offspring growth curves and food intake from weaning to adulthood.** Body weights of male (A) and female (B) from C or HF dams. Weekly weight-related energy intake of male (C) and female (D) from C or HF dams (n=16 per group). Weight-related energy intake is defined as the energy content of the food ingested (Kcal) expressed relative to body weight (g). * Effect of maternal HF diet *vs.* maternal C diet (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

**Figure 4: Oral glucose tolerance test in 7-month-old adult offspring.** Time course of plasma glucose in male (A) and female (C) from C or HF dams (n=16 per group). The respective area under the curve (AUC) are shown (B, D). * Effect of maternal HF diet *vs.* maternal C diet (**, $P<0.01$).

**Figure 5: Plasma corticosterone concentrations in fasted and fed conditions in 7-month-old adult offspring.** Plasma levels in male (A) and female (B) from C or HF dams (n=16 per group). Effect of maternal HF diet *vs*. maternal C diet (***, $P<0.001$). # Effect of fasted condition *vs.* fed condition (#, $P<0.001$).

**Figure 6: Morphometric analysis of perirenal white adipose tissue in 9-month-old offspring.** Representative photomicrographs of paraffin-embedded sections (scale bars = 100 μm) (A), average area (B), total cell number (C) and percentage of adipocytes in a given size range (area in $\mu m^2$) (D) in male and female offspring from C or HF dams (n=6 per group).* Effect of maternal HF diet *vs.* maternal C diet (*, $P<0.05$; **, $P<0.01$). # Effect of male *vs*. female (#, $P<0.05$; ##, $P<0.01$).

**Figure 7: Morphometric analysis of gonadal white adipose tissue in 9-month-old offspring.** Representative photomicrographs of paraffin-embedded sections (scale bars = 100 μm) (A), average area (B), total cell number (C) and percentage of adipocytes in a given size range (area in $\mu m^2$) (D) in male and female offspring from C or HF dams (n=6 per group).* Effect of maternal HF diet *vs.* maternal C diet (*, $P<0.05$; **, $P<0.01$). # Effect of male *vs*. female (#, $P<0.05$; ##, $P<0.01$).

**Figure 8: mRNA expression levels of perirenal white adipose tissue in 9-month-old offspring.** Gene expression was measured in male (A) and female (B) by quantitative real-time PCR, corrected for the mean expression of reference gene (cyclophilin A) (n = 10 per group). Gene symbols are detailed in Table 1. * Effect of maternal HF diet *vs.* maternal C diet (*, $P<0.05$; **, $P<0.01$).

**Figure 9: mRNA expression levels of gonadal white adipose tissue in 9-month-old offspring.** Gene expression was measured in male (A) and female (B) by quantitative real-time PCR, corrected for the mean expression of reference gene (cyclophilin A) (n = 10 per group). Gene symbols are detailed Table 1. * Effect of maternal HF diet *vs.* maternal C diet (*, $P<0.05$).