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

2  
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12  
13  
14 **Abbreviated title:** Maternal obesity and programmed adiposity

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24 **Key words:** high-fat diet, visceral adiposity, gene expression, adipocyte size, Developmental  
25 Origin of Health and Disease, dimorphism.

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27

28 **Abstract**

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

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53 **Introduction**

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

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

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

80 Little is known about the programming mechanisms that may account for long-lasting  
81 perturbation of adipogenesis and WAT metabolism in offspring from obese dams. To unravel  
82 the underlying mechanisms, several animal models of maternal obesity have been developed  
83 using high-fat (HF) or cafeteria diet applied during the preconception, gestation and/or  
84 lactation periods in dams (Williams *et al.* 2014). These studies confirmed that maternal  
85 obesity has common long-term metabolic consequences sensitizing the offspring to metabolic  
86 syndrome features. In particular, maternal obesity at conception programs enhanced  
87 adipogenesis and lipogenesis from the fetal period to adulthood resulting in higher WAT mass  
88 and larger adipocytes (Muhlhausler & Smith 2009; Borengasser *et al.* 2013; Murabayashi *et*  
89 *al.* 2013). Overfeeding during lactation and/or postweaning periods leads to accelerated  
90 growth and consistently worsens adipogenesis and lipogenesis programming (Desai & Ross  
91 2011; Guberman *et al.* 2013; Masuyama & Hiramatsu 2014). Programmed upregulation of the  
92 key adipogenic factor PPAR $\gamma$  is one characteristic features of fat expansion in offspring of  
93 obese dams (Samuelsson *et al.* 2008; Muhlhausler & Smith 2009; Sen & Simmons 2010;  
94 Dahlhoff *et al.* 2014; Desai *et al.* 2015). Obesity-prone offspring rats from obese mothers also  
95 exhibited modified fatty acid composition within WAT (Benkalfat *et al.* 2011). However, in  
96 rodents, few studies have examined depot- and sex-specific consequences of maternal obesity  
97 in offspring's WAT and there is little agreement among them (Sun *et al.* 2012; Ornellas *et al.*  
98 2013; Dahlhoff *et al.* 2014; Masuyama & Hiramatsu 2014).

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

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

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128 **Materials and methods**

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130 *Animals*

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132 Four-week-old virgin female Wistar rats (1 month) were purchased from Charles River

133 Laboratories (L'Arbresle, France) and were housed in individual cages in a humidity-

134 controlled room with a 12:12-h light-dark cycle. Food and water were available *ad libitum*.

135 After two weeks of acclimatization on a control (C) diet (3.85 kcal/g with 10% of total

136 calories as fat consisting of soybean oil (5.6%) and lard (4.4%), 70% as carbohydrate and

137 20% as protein; D12450J, Research Diets, New Brunswick, NJ, USA), female rats were fed

138 either a HF diet (5.24 kcal/g with 60% of total calories as fat consisting of soybean oil (5.6%)

139 and lard (54.4%), 20% as carbohydrate and 20% as protein; D12492, Research Diets, New

140 Brunswick, NJ, USA) or a C diet for 16 weeks (n=12 per group). After 14 weeks of HF diet,

141 20-week-old female (5 months) rats were subjected to an oral glucose tolerance test (OGTT).

142 Plasma levels of insulin, leptin and corticosterone were also measured after 16h overnight

143 fasting in both groups. After mating with a male rat fed a C diet, 22-week-old pregnant

144 females were transferred into individual cages with free access to water and continued on

145 their respective diets (C or HF diet) throughout gestation and lactation. Maternal body weight

146 was measured weekly until delivery. At parturition, pups were weighed and sexed. Litter size

147 was adjusted to 8 pups per dam (four males and four females). During lactation, body weights

148 of dams and pups were assessed on postnatal days (PND) 1, 4, 7, 11, 14, 17 and 21. At

149 weaning (PND21), dams were sacrificed and glycemia as well as plasma levels of leptin

150 insulin and corticosterone were determined after 16h overnight fasting. To obviate any litter

151 effects, animals used for each experiment were randomly chosen in different litters and only a

152 limited number of animals (1 to 2 males and females) was used from each litter. After

153 weaning, male (M) and female (F) offspring from C or HF dams were housed individually

154 with free access to water and C diet, divided into four groups (CM, CF, HFM and HFF (n= 16



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

159

#### 160 ***Food intake and metabolic parameters***

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

168

#### 169 ***Oral glucose tolerance test***

170 For OGTT, rats were fasted overnight. Basal blood glucose level defined as T0 was  
171 determined using a glucometer (Glucotrend 2, Roche Diagnostics, France) before oral glucose  
172 administration (2g/kg of body weight). Tail vein blood glucose was then measured at 0, 30,  
173 60, 90 and 120 min after administration.

174

#### 175 ***Endocrine parameters***

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

180 levels were determined by a competitive enzyme immunoassay (Immunodiagnostic Systems  
181 Ltd, Boldon, U.K). Plasma insulin concentrations were measured by ELISA (DRG,  
182 International, Inc. USA). Plasma apelin content was determined by ELISA (Phoenix  
183 Pharmaceuticals). The assay sensitivity was 0.07 ng/mL (insulin), 0.04 ng/mL (leptin), 0.1  
184 ng/mL (adiponectin), 0.55 ng/mL (corticosterone) and the intra-and inter-assay coefficients of  
185 variation were 4% and 9.1% (insulin), 5.4% and 7.3% (leptin), 4.4% and 6.1% (adiponectin),  
186 4.9% and 7.8% (corticosterone), respectively. Assay kits were used to determine the contents  
187 of plasma triglycerides and total cholesterol (61238 Triglyceride Enzymatique PAP100,  
188 61218 Cholesterol Liquide, BioMérieux, France) as well as free cholesterol and free fatty acid  
189 (FFA) (references 279-47106 and 999-75406, Wako Chemicals, Neuss, Germany). Each  
190 sample was measured in duplicate.

191

### 192 ***Plasma and tissue collections***

193 36-week-old rats (9 months) were rapidly weighed and killed by decapitation between  
194 9 and 10 a.m after 16h overnight fasting. Trunk blood samples were collected into prechilled  
195 tubes containing EDTA (20  $\mu$ L of a 5% solution) and centrifuged at 4,000 g for 10 min at  
196 4°C. Plasma was stored at -20°C. Several tissues (brown adipose tissue, liver, heart, kidney,  
197 adrenal gland) as well as gonadal (GWAT) and perirenal (PWAT) fat pads were weighed,  
198 frozen in liquid nitrogen and stored at -80°C. For histology experiments, animals were fixed  
199 by intracardiac perfusion using buffered 4% paraformaldehyde solution.

200

### 201 ***Gene Expression Analysis***

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

205 manufacturer's recommendations. The yield of total RNA was quantified on a Multiskan Go  
206 Microplate Spectrophotometer (Thermo Scientific, Illkirch, France). The quality of total RNA  
207 was assessed by determining the 260/280 and the 260/230 absorbance ratio and by agarose gel  
208 electrophoresis. First-strand cDNAs were synthesized using ThermoScript RT Kit (Invitrogen,  
209 Life Technologies, France). Relative expression levels of RNA per sample were quantified by  
210 SYBR Green assay on a Roche Light Cycler 480 sequence detection assay (Roche  
211 Biochemicals, Meylan, France). Primers sequences are presented in Table 1. For each  
212 transcript, PCR was performed in duplicate with 10 µl final reaction volumes with 1 µl of  
213 cDNA, 8 µl of QuantiTect SYBR Green Master mix (Qiagen, Courtaboeuf, France) and 0.5 µl  
214 of each primer set (Table 1). PCR was conducted using the following cycle parameters : 10  
215 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  
216 pool of cDNA from control tissues was used as a standard for quantitative correction. All  
217 cDNA samples were applied in dilution of 1:10 to obtain results within the range of the  
218 standard. Analysis of transcript level was carried out using first the determination of the  
219 threshold cycle Ct for each reaction corrected by the efficiency. The level of gene expression  
220 was normalized to the reference gene transcript cyclophilin A RNA.

221

### 222 ***Morphometric analysis of adipose tissue***

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

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

233  
234 ***Statistical analysis***

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

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256 **Results**

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

259 HF-fed female rats gained more weight than C females (ANOVA  $P < 0.0001$ ) (Fig.1A).  
260 After 14 weeks of HF diet, females had about 2 fold higher plasma leptin concentration ( $4.3 \pm$   
261  $0.6$  ng/mL *versus*  $2.2 \pm 0.4$  ng/mL,  $P < 0.05$ ) compared with C females (Table 2). No  
262 difference was observed in basal glycemia, plasma insulin and corticosterone levels.  
263 However, HF-fed females showed a more pronounced increase in glucose levels during  
264 OGTT with a higher area under the curve (AUC) (Fig.1B), reflecting impaired glucose  
265 tolerance (ANOVA  $P < 0.0001$ ). HF-fed dams displayed a 20% increase in body weight at the  
266 end of the gestation (Fig.1A). At weaning, HF-fed dams exhibited about 3 fold higher plasma  
267 leptin ( $2.5 \pm 0.4$  ng/mL *versus*  $0.9 \pm 0.2$  ng/mL,  $P < 0.05$ ) and corticosterone levels ( $796.2 \pm$   
268  $187$  ng/mL *versus*  $278.1 \pm 86.8$  ng/mL,  $P < 0.05$ ) (Table 2) while showing a marked increase in  
269 all fat pads weights (data not shown). No variation was observed in glycemia or plasma  
270 insulin levels.

271

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

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

280

281

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

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

294

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

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

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

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

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

315

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

317 As shown in representative photographs (Fig.6A), PWAT of HF male offspring  
318 exhibited an increase in average adipocyte area (Fig.6B) and total cell number (Fig.6C)  
319 compared with C rats. Adipocytes measuring  $7500 \mu\text{m}^2$  or less represented 65% of all  
320 adipocytes in C male offspring whereas they represented only 40% of all adipocytes in HF  
321 male offspring. This indicates that maternal HF diet decreased the frequency of small-sized  
322 adipocytes (Fig.6D). In particular, the proportion of adipocytes measuring  $2500 \mu\text{m}^2$  or less  
323 displayed a marked 6-fold decrease in HF male offspring. In addition, these animals showed  
324 greater percentage of large sized adipocytes ( $7500\text{-}40000 \mu\text{m}^2$ ) (60% *versus* 35%) compared  
325 with C rats. Although no changes in average adipocyte area (Fig.7A, B) and total cell number  
326 (Fig.7C) were observed in GWAT of HF males, a marked 5-fold reduction in frequency of 0-  
327  $2500$  size adipocytes similarly occurred (Fig.7D). In contrast, both fat pads of HF female  
328 offspring (Fig.6 and 7) showed no major changes.

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

333 Maternal obesity led to pronounced changes in PWAT gene expression in HF adult  
334 male offspring. RT-qPCR data showed that leptin mRNA content was increased (+ 1.5 fold)  
335 in HF male rats compared to C animals (Fig.8A). This is in agreement with the increased  
336 serum leptin levels (Table 4). In PWAT, maternal obesity resulted in increased mRNA levels  
337 for genes promoting *de novo* lipogenesis such as fatty acid synthase (FAS, + 1.6 fold) and  
338 sterol regulatory element-binding protein-1 (SREBP1, + 1.7 fold) in HF male offspring. In  
339 addition, mRNA expression levels of genes involved in adipogenesis such as peroxisome  
340 proliferator-activated receptor gamma (PPAR $\gamma$ ) and 11 $\beta$ -hydroxysteroid dehydrogenase type  
341 1 (11 $\beta$ -HSD1) were decreased (- 1.3 and - 1.5 fold, respectively) in HF male offspring *versus*  
342 C rats (Fig.8A). By contrast, maternal obesity did not affect GWAT mRNA expression  
343 profiles to the same extent in HF male rats, except for a down-regulation of Ob-Rb (Fig.9A).  
344 Compared to HF males, less change in gene expression profile was observed in HF female  
345 adult offspring in both fat pads. HF female offspring still showed increased C/EBP $\alpha$   
346 mRNA expression levels (+ 2 fold) in PWAT (Fig.8B) and lower adiponectin (- 1.4 fold) and  
347 GR (- 1.4 fold) mRNA expression levels in GWAT (Fig.9B). This was consistent with  
348 differences in fat depot weights and plasma leptin concentrations observed in HF male *versus*  
349 female offspring (Tables 3 and 4).

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357 **Discussion**

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

372           In our model, HF adult offspring might have developed a modification of WAT's  
373 sensitivity to circulating hormones. Indeed, perirenal WAT depot exhibited changes in  
374 lipogenic and adipogenic pathways that may favor triglyceride storage in mature adipocytes.  
375 Consistent with increased fat deposition, HF adult male offspring displayed higher serum  
376 leptin concentration. Hyperleptinemia may be interpreted as a leptin-resistant state (Kirk *et al.*  
377 2009; Sun *et al.* 2012). First, leptin is known to activate adipogenesis by promoting  
378 preadipocyte differentiation (Bol *et al.* 2008; Guo *et al.* 2009). However, despite  
379 hyperleptinemia, the number of small adipocytes was markedly decreased in both depots of  
380 HF adult male offspring. In agreement with these findings, we observed that PPAR $\gamma$  gene  
381 expression was downregulated. Our findings disagree with other studies that reported an

382 upregulation of PPAR $\gamma$  contents in WAT along with enhanced adipogenesis in obese-prone  
383 offspring from malnourished dams (Samuelsson *et al.* 2008; Muhlhausler & Smith 2009; Sen  
384 & Simmons 2010; Desai *et al.* 2015). This discrepancy may be due to differences in the  
385 establishment of epigenetic marks during adipogenesis and/or hormonal environment, tissue  
386 sensitivity as well as inflammatory status in adipose tissue of adult offspring (Breton *et al.*,  
387 2013). The decrease in gene expression might be seen as an adaptive mechanism to limit fat  
388 accumulation (Lukaszewski *et al.* 2011). Indeed, an increase in the lipogenic capacity of  
389 adipose tissue is expected during the “dynamic phase of obesity”, when fat stores are rapidly  
390 expanding. However, during long lasting and stable obesity, the decreased expression of  
391 lipogenic genes may prevent a further development of fat mass (Ortega *et al.* 2010). Further  
392 experiments on the kinetic of fat deposition and the transcriptional profile of lipogenic genes  
393 during the development of WAT are needed to address this question. In agreement with this  
394 hypothesis, several studies described a relationship between obesity and lower expression  
395 and/or activity of PPAR $\gamma$  in visceral WAT. These modifications appear to be strongly  
396 associated with the pathogenesis of metabolic syndrome (Zhang *et al.* 1996; Fujiki *et al.*  
397 2009). Second, given the antilipogenic leptin action on mature adipocytes (Huan *et al.* 2003;  
398 Jiang *et al.* 2009), HF adult male offspring had a trend towards reduced leptin receptor  
399 contents and did not show any suppression of FAS gene expression, but instead a marked  
400 increased expression. In accordance with these findings, we reported that hyperleptinemic rat  
401 offspring from undernourished dams exhibited impaired leptin sensitivity with reduced  
402 pSTAT3 in WAT (Lukaszewski *et al.* 2011).

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

407 observed in WAT of HF offspring (Borengasser *et al.* 2013). HF adult male offspring also  
408 displayed hypercorticonemia. Several lines of evidence prompted us to explore whether  
409 sensitivity of WAT to glucocorticoid (GC) was modified in offspring. First, GC alone or in  
410 interaction with insulin regulates the differentiation of preadipocytes and lipogenic genes  
411 (Campbell *et al.* 2011). Second, a close link between chronic excess of plasma GC levels  
412 and/or increased GC sensitivity within WAT (i.e., modifications of GR, MR, 11 $\beta$ -  
413 hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) and 11 $\beta$ -hydroxysteroid dehydrogenase  
414 type 2 (11 $\beta$ -HSD2) contents) and fat expansion has been observed in offspring from  
415 malnourished dams (Gnanalingham *et al.* 2005; Lukaszewski *et al.* 2011; Guo *et al.* 2013).  
416 Third, increased expression of GR and 11 $\beta$ -HSD1 in visceral adipose tissue has been  
417 associated with the development of obesity in rats overfed during lactation (Boullu-Ciocca *et*  
418 *al.* 2008). We observed a depot-specific downregulation of 11 $\beta$ -HSD1 mRNA in PWAT in  
419 HF adult offspring as previously reported in 3-month-old offspring from obese mice  
420 (Samuelsson *et al.* 2008). We also showed that the ratio between 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2  
421 expression that controls local balance between active and inactive GC metabolites (Lee *et al.*  
422 2014) was decreased. As described in obesity-prone progeny from undernourished dams, it  
423 may diminish intratissular GC responsiveness and represent an adaptive mechanism to  
424 counteract excess fat storage (Lukaszewski *et al.* 2011). We cannot exclude that HF offspring  
425 may have decreased energy expenditure. Indeed, additional programming mechanisms such as  
426 elevated free fatty acid transport and/or lower lipolysis/ $\beta$ -oxydation activities within WAT  
427 might account for increased triglyceride storage (Dahlhoff *et al.* 2014).

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

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

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

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

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

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

482 It may cause epigenetic modifications that reprogram offspring's adipose tissue. Differences in  
483 fat cell embryonic origin, development, genetic and hormonal sensitivity may result in a depot-  
484 specific programming effects that may predispose offspring to higher adiposity (Öst &  
485 Pospisilik 2015). Indeed, maternal obesity in mice induces increased gene expression of Zfp423  
486 with lower promoter methylation levels in fetal offspring (Yang *et al.* 2013). Similarly,  
487 weanling rats from obese dams display increased Zfp423 and C/EBP $\beta$  mRNA expression levels  
488 with alterations in DNA methylation of CpG sites (Borengasser *et al.* 2013). Maternal HF diet  
489 during pregnancy also results in histone modifications within leptin and adiponectin promoter  
490 regions with gene expression modifications in mouse offspring (Masuyama & Hiramatsu  
491 2012).

492 Few studies have reported that maternal obesity programs metabolic alterations and  
493 adiposity differently in a sex-dependent manner in progeny (Sun *et al.* 2012; Ornellas *et al.*  
494 2013; Dahlhoff *et al.* 2014; Masuyama & Hiramatsu 2014). The basis of the sex-specific  
495 programming effects remain unclear but could reflect direct interactions between nutritional  
496 signals and sex hormones in tissues of the developing fetus (Aiken & Ozanne 2013). In  
497 human, numerous studies suggest that sex differences in fetal growth in response to adverse  
498 pregnancy conditions are likely to be mediated by sex-specific adaptation of the placenta  
499 (Clifton 2010). Similarly, sex-specific programming effects in rat offspring from obese dams  
500 might be due to sex-specific differences in placental response to maternal obesity (Reynolds  
501 *et al.* 2015). Epigenetic mechanisms may also contribute to placental programming in a  
502 dimorphic manner. Thus, the consumption of HF diet during pregnancy appears to differently  
503 affect placental methylation and placental gene expression patterns in male and female mice  
504 offspring (Gallou-Kabani *et al.*, 2010). Thus, sex-specific differences in term of epigenetic  
505 modulations may be associated with developmentally programmed phenotypes. It is possible  
506 that postnatal hormonal milieu, which is different between male and female offspring, modify

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

511

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744 **Figure legends**

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746 **Figure 1: Maternal phenotype.** Body weight of dams fed either a C or a HF diet during  
747 preconception, gestation and lactation (A). Concentration of blood glucose during OGTT after  
748 14 weeks of diet and area under the curve (B) (n= 12 per group). \* Effect of maternal HF diet  
749 vs. maternal C diet (\*\*,  $P<0.01$ ; \*\*\*,  $P<0.001$ ).

750

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

754

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

761

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

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767

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

772

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

779

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

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

792

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

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