Altered adipose tissue metabolism in offspring of dietary obese rat dams

Nassira Batoul BENKALFAT*, Hafida MERZOUK*, Samira BOUANANE*, Sid-Ahmed MERZOUK†, Jérôme BELLINGER‡, Joseph GRESTI‡, Christian TESSIER‡ and Michel NARCE‡

*Laboratory of Physiology and Biochemistry of Nutrition, Department of Biology, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria, †Department of Technical Sciences, Faculty of Engineering, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria, and ‡INSERM UMR 866, ‘Lipids Nutrition Cancer’, University of Burgundy, Faculty of Life, Earth and Environment Sciences, 6 Bd Gabriel, Dijon 21000, France

ABSTRACT

To investigate further the mechanisms of developmental programming, we analysed the effects of maternal overnutrition and of postnatal high-fat feeding on adipose tissue metabolism in the offspring. Postnatal changes in serum adiponectin, leptin and TAG [triacylglycerol (triglyceride)] levels, adipose tissue TAGs, fatty acids and enzyme activities were determined in offspring of cafeteria-diet-fed dams during gestation and lactation, weaned on to standard chow or on to cafeteria diet. Obese rats showed higher adiposity (+35 % to 85 %) as well as a significant increase in serum glucose, insulin, leptin, adiponectin and TAG levels (P < 0.01) and adipose tissue LPL (lipoprotein lipase) and GPDH (glycerol-3-phosphate dehydrogenase) activities (P < 0.01), compared with control pups at weaning (day 21) and at adulthood (day 90). Adipose HSL (hormone-sensitive lipase) activity was increased only at day 90 (P < 0.05), and FAS (fatty acid synthase) activity remained unchanged. The proportions of SFAs (saturated fatty acids) and MUFAs (mono-unsaturated fatty acids) and the Δ⁹-desaturation index were significantly increased (P < 0.05), whereas PUFAs (polyunsaturated fatty acids) were decreased (P < 0.01) in serum and adipose TAGs of obese pups compared with controls. The cafeteria diet at weaning induced more severe abnormalities in obese rats. In conclusion, maternal overnutrition induced permanent changes in adipose tissue metabolism of the offspring. These pre-existing alterations in offspring were worsened under a high-fat diet from weaning to adulthood. Consequently, adipose adipokines and enzymes could provide a potential therapeutic target, and new investigations in this field could constitute strategies to improve the impact of early-life overnutrition.

INTRODUCTION

The developmental origins hypothesis proposes that the pathogenesis of many diet-related chronic diseases begins not in adulthood, but during early development. Several recent reports, including some from our laboratory, suggest that both maternal obesity and nutritional state in the early postnatal phase are important in promoting obesity in offspring [1–3]. Obesity is characterized by insulin resistance, alterations in carbohydrate and fat metabolism and by increased adipose tissue mass as well as increased TAG [triacylglycerol (triglyceride)] storage.
During the last several years, the role of the adipose tissue in contributing to obesity-associated cardiovascular and metabolic risk has gained much attention. Obesity may arise from increased size of individual adipose cells due to lipid accumulation or increased number of adipocytes arising from differentiation of adipose precursor cells to mature adipocytes [5]. In addition to serving as an energy store, adipocytes also secrete hormones or adipokines (e.g. leptin and adiponectin) that regulate energy balance, metabolism and the neuroendocrine response to altered nutrition [6]. To maintain lipid homeostasis, adipocytes carry out two reciprocal biochemical processes: lipogenesis and lipolysis. Thus body fat content is controlled, at least partially, by metabolism of adipose tissue itself [5,6].

It is well known that insulin plays a major role in modulation of key genes in lipid metabolism and TAG storage, including FAS (fatty acid synthase), LPL (lipoprotein lipase), HSL (hormone-sensitive lipase), adiponectin and leptin. FAS catalyses the synthesis of long-chain fatty acids, palmitate from acetyl-CoA and malonyl-CoA in the presence of NADPH [7]. Hyperinsulinaemic animals are characterized by enhanced adipose tissue FAS activity contributing to fat accretion in an obesity state [8]. Similarly, insulin increases FAS activity and gene transcription in human adipocytes [9]. Another key enzyme involved in TAG accumulation is LPL, which hydrolyses circulating TAG-rich lipoproteins such as VLDLs (very-low-density lipoproteins) and chylomicrons and provides substrates for fatty acid uptake into adipose tissue. LPL is increased in the adipose tissue of obese humans, as well as in different models of rodent obesity, and this increased adipose LPL has been implicated in the pathophysiology of obesity [10]. HSL is the principal regulator of NEFA (non-esterified ‘free’) fatty acid release from adipose tissue. HSL protein and mRNA expression, as well as maximum lipolytic capacity of adipocytes, has been reported to be reduced in obese men and women [11]. A positive relationship between fat cell size and HSL expression was seen with high-fat feeding in rats, where fat feeding was associated with an increase in adipocyte cell size and an increase in both basal and stimulated HSL activity [12]. The cytosolic enzyme GPDH (glycerol-3-phosphate dehydrogenase) catalyses the conversion of dihydroxyacetone phosphate derived from glucose into glycerol 3-phosphate, which is then acylated to form TAGs. Previous data indicated that elevated GPDH activity might contribute to the increase of triacylglycerol synthesis in adipose tissue of obese subjects, humans and rats [13,14].

Leptin, the product of the ob gene primarily expressed in adipose tissue, is an adipocyte hormone that is secreted in response to food intake and acts centrally as a satiety factor [15]. The amount of circulating leptin is highly correlated with adiposity [16]. Adiponectin may be the most relevant and promising adipokine with respect to a better understanding of the link between obesity and CHD (coronary heart disease) [17]. Adiponectin is exclusively produced by adipocytes, and its expression is inversely related to adipose tissue mass, particularly visceral adiposity. Contrary to other adipose-derived hormones, adiponectin circulates at relatively high concentrations in the bloodstream, accounting for 0.05% of total serum proteins, and is inversely associated with obesity, insulin resistance and cardiovascular disease [18].

There is considerable interest in the metabolism of adipose tissue in investigating some of the events leading to obesity. Thus, increasing evidence suggests that adipose tissue, especially visceral fat tissue, participates directly in the pathophysiology of obesity-related cardiovascular diseases [4].

Exposure to maternal obesity may be associated with ‘programmed’ alterations in the expression of genes, which are important in adipocyte differentiation and function and which may increase the risk of obesity and insulin resistance in animal models [1,3,19]. Maternal obesity is associated with increased adiposity and altered expression of genes in the adipose tissue of the fetus, including increased expression of LPL, adiponectin, leptin and the adipogenic factor PPARγ (peroxisome proliferator-activated receptor γ) [19]. These changes may be persistent, since rodent studies suggest that maternal obesity is associated with changes in gene expression in adipose tissue in adulthood [1,3,20]. Although the detrimental consequences of maternal obesity/overnutrition have been extensively studied, little attention has been directed towards offspring adipocyte metabolism throughout adulthood. In the present study, we explored the long-term effects of maternal dietary obesity on adipocyte metabolism of the offspring by analysing the activities of adipocyte enzymes related to lipid metabolism, fatty acid composition of adipocyte TAG and leptin, and adiponectin levels. We also examined whether there are additive effects of feeding the offspring a high-fat hypercaloric diet from weaning to adulthood.

**MATERIALS AND METHODS**

**Animals and experimental protocol**

All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of experimental animal care at Tlemcen University. Adult Wistar rats were obtained from Iffa-Credo. Female rats were housed at 20 ± 2°C with two to three animals in each cage and maintained on a 12 h:12 h light/dark cycle. Rats were assigned to two groups of equal average
body weight. The control group (*n = 10*) was exposed to standard laboratory chow (330 kJ/100 g) composed of 25% of energy as protein, 65% of energy as carbohydrate and 10% of energy as lipids which contain 4% C14:0, 18% C16:0, 2% C16:1n-7, 5% C18:0, 22% C18:1n-9, 43% C18:2n-6, 5% C18:3n-3 and 1% C20:0, 6%). While the experimental group (*n = 10*) was presented with a palatable fat-rich hypercaloric diet composed of pâté, cheese, bacon, potato chips, biscuits and chocolate (in a proportion of 2:2:2:1:1:1, by weight) mixed with standard chow. The composition of this cafeteria diet (420 kJ/100 g) was 23% of energy as protein, 35% of energy as carbohydrates and 42% of energy as lipids (which contain 7% C14:0, 25% C16:0, 1% C16:1n-7, 9% C18:0, 31% C18:1n-9, 24% C18:2n-6, 1% C18:3n-3, 1% C20:0 and 1% C22:0). Females rats were exposed to chow or the cafeteria diet for 4 weeks before mating with male rats. At mating, cafeteria-fed rats were significantly heavier than control-fed rats. Female rats were monitored throughout the 3-week gestation period and housed individually before delivery. The dams were fed the same diet continuously for the entire gestation and lactation periods. A total of 60 survival pups from the ten cafeteria-fed dams and 85 survival pups from the ten control dams were delivered spontaneously (the pup mortality rate was between 5% and 10% in the first day of tissue for 1 min in 4 ml of 50 mM NH₄Cl/aq. NH₃ was homogenized in ice-cold buffer A supplemented by Kabbaj et al. [22]. For LPL measurement, tissue homogenates were prepared by homogenizing 100 mg of tissue for 1 min in 4 ml of 50 mM NH₄Cl/aq. NH₃ (pH 8.1) containing heparin (4 units/ml), as described by Thompson et al. [23].

### Chemical analysis
Serum glucose was measured using the Trinder glucose kit (Sigma). Serum and tissue TAGs were measured using colorimetric enzymatic kits (Roche Diagnostics). Serum NEFAs were measured by using the enzymatic NEFA Kit (Biolyon). For these enzymatic methods, the interassay CV (coefficient of variance) was in the range 1.7–3.6%. Serum leptin, insulin and adiponectin concentrations were determined using commercial RIA kits (Linco), according to the manufacturer’s instructions with an interassay CV (coefficient of variation) of <8%. The insulin resistance index was estimated by HOMA (homeostasis model assessment): HOMA value = fasting plasma insulin (ng/ml) × fasting plasma glucose (mM)/(22.5 × 0.0417). The greater HOMA values corresponded to the greater insulin resistance level [24].

Total lipids of adipose tissue were extracted by chloroform/methanol (2:1, v/v). Adipose TAGs were isolated by TLC and were transmethylated, and fatty acids were analysed by gas–liquid chromatography as previously described [25].

### Adipose tissue enzyme activities
FAS (EC 2.3.1.85) activity was assayed spectrophotometrically in cytosolic extracts of adipose tissue by measuring the oxidation rate of NADPH, as described previously [26], with an interassay CV of 6%. Results are expressed as nmol of NADPH oxidized · min⁻¹ · mg⁻¹ of cytosolic protein.

GPDH activity (EC 1.1.1.8) was determined using the method described by Wise and Green [27], with an interassay CV of 7%. The assay buffer contained 100 mM triethanolamine (pH 7.5), 2.6 mM EDTA, 0.1 mM 2-mercaptoethanol, 0.120 mM NADH and 0.2 mM dihydroxyacetone phosphate. Reactions were started by adding cytosolic extracts, and the change in absorbance at 340 nm was monitored at room temperature (25°C) with a spectrophotometer. One unit of enzyme activity corresponds to the oxidation of 1 nmol of NADH/min.

To estimate HSL (EC 3.1.1.3) activity, a spectrophotometric esterase assay based on the hydrolysis of PNPB ([p-nitrophenyl butyrate]) was used as described

---

**Body weight**: The control group (*n = 10*) was exposed to standard laboratory chow (330 kJ/100 g) composed of 25% of energy as protein, 65% of energy as carbohydrate and 10% of energy as lipids which contain 4% C14:0, 18% C16:0, 2% C16:1n-7, 5% C18:0, 22% C18:1n-9, 43% C18:2n-6, 5% C18:3n-3 and 1% C20:0, 6%). While the experimental group (*n = 10*) was presented with a palatable fat-rich hypercaloric diet composed of pâté, cheese, bacon, potato chips, biscuits and chocolate (in a proportion of 2:2:2:1:1:1, by weight) mixed with standard chow. The composition of this cafeteria diet (420 kJ/100 g) was 23% of energy as protein, 35% of energy as carbohydrates and 42% of energy as lipids (which contain 7% C14:0, 25% C16:0, 1% C16:1n-7, 9% C18:0, 31% C18:1n-9, 24% C18:2n-6, 1% C18:3n-3, 1% C20:0 and 1% C22:0). Females rats were exposed to chow or the cafeteria diet for 4 weeks before mating with male rats. At mating, cafeteria-fed rats were significantly heavier than control-fed rats. Female rats were monitored throughout the 3-week gestation period and housed individually before delivery. The dams were fed the same diet continuously for the entire gestation and lactation periods. A total of 60 survival pups from the ten cafeteria-fed dams and 85 survival pups from the ten control dams were delivered spontaneously (the pup mortality rate was between 5% and 10% in the first day of tissue for 1 min in 4 ml of 50 mM NH₄Cl/aq. NH₃ was homogenized in ice-cold buffer A supplemented by Kabbaj et al. [22]. For LPL measurement, tissue homogenates were prepared by homogenizing 100 mg of tissue for 1 min in 4 ml of 50 mM NH₄Cl/aq. NH₃ (pH 8.1) containing heparin (4 units/ml), as described by Thompson et al. [23].

**Chemical analysis**: Serum glucose was measured using the Trinder glucose kit (Sigma). Serum and tissue TAGs were measured using colorimetric enzymatic kits (Roche Diagnostics). Serum NEFAs were measured by using the enzymatic NEFA Kit (Biolyon). For these enzymatic methods, the interassay CV (coefficient of variance) was in the range 1.7–3.6%. Serum leptin, insulin and adiponectin concentrations were determined using commercial RIA kits (Linco), according to the manufacturer’s instructions with an interassay CV (coefficient of variation) of <8%. The insulin resistance index was estimated by HOMA (homeostasis model assessment): HOMA value = fasting plasma insulin (ng/ml) × fasting plasma glucose (mM)/(22.5 × 0.0417). The greater HOMA values corresponded to the greater insulin resistance level [24].

Total lipids of adipose tissue were extracted by chloroform/methanol (2:1, v/v). Adipose TAGs were isolated by TLC and were transmethylated, and fatty acids were analysed by gas–liquid chromatography as previously described [25].

**Adipose tissue enzyme activities**: FAS (EC 2.3.1.85) activity was assayed spectrophotometrically in cytosolic extracts of adipose tissue by measuring the oxidation rate of NADPH, as described previously [26], with an interassay CV of 6%. Results are expressed as nmol of NADPH oxidized · min⁻¹ · mg⁻¹ of cytosolic protein.

GPDH activity (EC 1.1.1.8) was determined using the method described by Wise and Green [27], with an interassay CV of 7%. The assay buffer contained 100 mM triethanolamine (pH 7.5), 2.6 mM EDTA, 0.1 mM 2-mercaptoethanol, 0.120 mM NADH and 0.2 mM dihydroxyacetone phosphate. Reactions were started by adding cytosolic extracts, and the change in absorbance at 340 nm was monitored at room temperature (25°C) with a spectrophotometer. One unit of enzyme activity corresponds to the oxidation of 1 nmol of NADH/min.

To estimate HSL (EC 3.1.1.3) activity, a spectrophotometric esterase assay based on the hydrolysis of PNPB (p-nitrophenyl butyrate) was used as described

---

© The Authors Journal compilation © 2011 Biochemical Society
by Kabbaj et al. [22], with an interassay CV of 6.5 %. Adipose tissue homogenates were incubated with PNPB and buffer (0.1 M NaH2PO4/Na2HPO4, pH 7.25, 0.9 % NaCl and 1 mM dithiothreitol) at 37 °C for 10 min. The reaction was stopped by addition of 3.25 ml of methanol/chloroform/heptane (10:9:7, by vol.). After centrifugation at 800 g for 20 min, solutions were incubated for 3 min at 42 °C, and the absorbance of the supernatant was measured at 400 nm in a UV spectrophotometer. The enzymatic activity is expressed as μmol of p-nitrophenol released · min⁻¹ · mg⁻¹ of protein.

For the LPL (EC 3.1.1.34) assay, tissue homogenate (the enzyme source) was incubated at 37 °C for 1 h with [3H]triolein (trioleoylglycerol) emulsion substrate [final concentrations: 1.42 mmol/l triolein, 0.1 mmol/l lysophosphatidylcholine, 0.2 % (w/v) albumin, 5 % (v/v) heat-inactivated serum (providing apolipoprotein C-II, an activator of LPL), 0.1 mmol/l Tris/HCl, pH 8.0 and 0.15 mol/l NaCl], as described by Nilsson–Ehle and Ekman [28], with an interassay CV of 5.5 %. At the end of the incubation period, the fatty acids released were extracted with chloroform/methanol/heptane (1.25:1.41:1, by vol), followed by 0.1 mol/l potassium carbonate/borate buffer (0.1 M NaH2PO4/Na2HPO4, pH 10.5. 3H radioactivity in 1.5 ml aliquots of the methanol/water upper phase was measured in 10 ml of scintillation liquid (Ready Solv HP/6; Beckman) in a 7500 LS liquid-scintillation counter (Beckman). Enzyme activity is expressed as nmol of fatty acids released · min⁻¹ · mg⁻¹ of protein.

Statistical analysis
Results are expressed as means ± S.D. The results were tested for normal distribution using the Shapiro–Wilk test. Data not normally distributed were logarithmically transformed. Significant differences among the groups were analysed statistically by Student’s t test (day 21) and a one-way ANOVA (day 90). The individual effects of the maternal diets and the offspring’s diets were distinguished by two-way ANOVA. When significant changes were observed in ANOVA tests, Fisher least significant difference tests were applied to locate the source of significant difference. The significance level was set at P < 0.05. These calculations were performed using STATISTICA version 4.1 (STATSOFT).

RESULTS

Body and adipose tissue weights
Body weight was significantly increased (P < 0.01) in obese offspring of cafeteria-fed dams at weaning (day 21). Furthermore, the enlargement of the visceral fat depots was also statistically significant in these rats compared with the control group (Table 1).

Table I Body and adipose tissue weights in obese and control dam offspring

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Relative adipose tissue weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>60 ± 2.54</td>
<td>0.74 ± 0.10</td>
</tr>
<tr>
<td>Obese rats</td>
<td>106.50 ± 4.50a</td>
<td>1.39 ± 0.07b</td>
</tr>
<tr>
<td>Day 90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>376 ± 53c</td>
<td>0.94 ± 0.12d</td>
</tr>
<tr>
<td>CCAF</td>
<td>486 ± 40e</td>
<td>1.36 ± 0.10f</td>
</tr>
<tr>
<td>OC</td>
<td>592 ± 32d</td>
<td>1.50 ± 0.11h</td>
</tr>
<tr>
<td>OCAF</td>
<td>638 ± 37f</td>
<td>1.85 ± 0.09g</td>
</tr>
</tbody>
</table>

At adulthood (day 90), obese rats from cafeteria-diet-fed dams being weaned on the control or cafeteria diet (OC and OCAF) still had significantly higher body and adipose tissue weights than control rats from control diet-fed dams (CC and CCAF) (Table 1). In addition, obese rats from the group fed the cafeteria diet (OCAF) showed higher weight gain and adiposity than obese rats fed the control diet (OC).

Serum glucose and hormone levels
At weaning, obese pups from cafeteria-fed dams had higher serum glucose, insulin, leptin and adiponectin concentrations than pups from control dams (Table 2). The HOMA index was increased in obese pups compared with controls (P < 0.01)

At day 90, the obese pups (OC) still had a significant increase in the levels of serum glucose, insulin, leptin and adiponectin concentrations and in HOMA index compared with control pups (CC) (Table 2).

The cafeteria diet significantly increased serum glucose, insulin and leptin levels and HOMA index in both obese (OCAF) and control rats (CCAF); the highest values were observed in OCAF group. However, the cafeteria diet had no effect on adiponectin levels in either obese and control groups.

To determine the individual effects of the maternal diets and the offspring’s own diets, two-way ANOVA was performed. There were significant effects of maternal diet, the offspring’s own diet and maternal diet–offspring’s own diet interaction on serum glucose (P = 0.015, P = 0.030, and P = 0.001 respectively), insulin (P = 0.004, P = 0.010 and P = 0.001 respectively), leptin (P = 0.003, P = 0.004 and P = 0.001 respectively) levels...
Programming adipose tissue metabolism

Table 2  Serum glucose and hormone levels in obese and control dam offspring
Values are presented as means ± S.D. for ten rats in each group at weaning (day 21) and at adulthood (day 90). Significant differences between obese and control rats at day 21 are indicated as *P < 0.01. At day 90, values with different superscript letters (a, b, c, d) are significantly different (P < 0.05). CC, offspring of Chow-fed dams weaned on to standard diet; CCAF, offspring of Chow-fed dams weaned on to cafeteria diet; OC, offspring of Chow-fed dams weaned on to standard Chow; OCAF, offspring of obese cafeteria-fed dams weaned on to cafeteria diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mmol/l)</th>
<th>Insulin (ng/ml)</th>
<th>HOMA</th>
<th>Leptin (ng/ml)</th>
<th>Adiponectin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.85 ± 0.33</td>
<td>1.67 ± 0.25</td>
<td>8.63 ± 0.53</td>
<td>1.73 ± 0.26</td>
<td>2.25 ± 0.31</td>
</tr>
<tr>
<td>Obese</td>
<td>6.62 ± 0.27 *</td>
<td>2.53 ± 0.20 a</td>
<td>17.82 ± 1.2 *</td>
<td>4.05 ± 0.42 a</td>
<td>3.32 ± 0.26 a</td>
</tr>
<tr>
<td>Day 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>5.25 ± 0.67 a</td>
<td>1.89 ± 0.33 a</td>
<td>10.52 ± 0.66 a</td>
<td>4.98 ± 0.31 a</td>
<td>3.83 ± 0.18 a</td>
</tr>
<tr>
<td>CCAF</td>
<td>6.34 ± 0.75 a</td>
<td>2.52 ± 0.20 b</td>
<td>16.92 ± 1.02 a</td>
<td>7.65 ± 0.34 b</td>
<td>3.74 ± 0.26 b</td>
</tr>
<tr>
<td>OC</td>
<td>8.76 ± 0.33 c</td>
<td>2.84 ± 0.22 c</td>
<td>26.56 ± 1.43 c</td>
<td>8.88 ± 0.40 c</td>
<td>4.64 ± 0.25 c</td>
</tr>
<tr>
<td>OCAF</td>
<td>10.29 ± 0.65 d</td>
<td>3.45 ± 0.30 d</td>
<td>37.66 ± 1.50 d</td>
<td>12.47 ± 0.37 d</td>
<td>4.47 ± 0.30 d</td>
</tr>
</tbody>
</table>

Table 3  Serum and adipose tissue lipid levels in obese and control dam offspring
Values are presented as means ± S.D. for ten rats in each group at weaning (day 21) and at adulthood (day 90). Significant differences between obese and control rats at day 21 are indicated as *P < 0.01. At day 90, values with different superscript letters (a, b, c, d) are significantly different (P < 0.05). CC, offspring of Chow-fed dams weaned on to standard diet; CCAF, offspring of Chow-fed dams weaned on to cafeteria diet; OC, offspring of Chow-fed dams weaned on to standard Chow; OCAF, offspring of obese cafeteria-fed dams weaned on to cafeteria diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum NEFA (mmol/l)</th>
<th>Serum TAG (mmol/l)</th>
<th>Adipose TAG (mg/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.44 ± 0.04</td>
<td>0.66 ± 0.08</td>
<td>60.53 ± 6.07</td>
</tr>
<tr>
<td>Obese</td>
<td>0.50 ± 0.05</td>
<td>0.99 ± 0.05 *</td>
<td>85.50 ± 5.13</td>
</tr>
<tr>
<td>Day 90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.77 ± 0.31 a</td>
<td>0.96 ± 0.04 a</td>
<td>71.36 ± 3.04 a</td>
</tr>
<tr>
<td>CCAF</td>
<td>1.40 ± 0.20 a</td>
<td>1.42 ± 0.06 a</td>
<td>92.33 ± 3.14 a</td>
</tr>
<tr>
<td>OC</td>
<td>1.86 ± 0.33 c</td>
<td>1.69 ± 0.05 c</td>
<td>99.53 ± 2.05 c</td>
</tr>
<tr>
<td>OCAF</td>
<td>2.25 ± 0.34 d</td>
<td>1.94 ± 0.08 d</td>
<td>118.62 ± 5.11 d</td>
</tr>
</tbody>
</table>

and on HOMA index (P = 0.005, P = 0.003 and P = 0.001 respectively). For adiponectin, there was only a significant effect of maternal diet (P = 0.001).

Serum and adipose tissue lipid levels
At weaning, serum NEFA contents in offspring of cafeteria-diet-fed dams were similar to those in control rats (Table 3). In contrast, serum and adipose tissue TAG contents were significantly higher in offspring of the cafeteria-diet-fed dams than in their control counterparts.

At day 90, serum NEFA and TAG concentrations and adipose tissue TAG contents were significantly increased in obese pups compared with controls regardless of their diet at weaning (Table 3). The cafeteria diet significantly increased serum and adipose lipids in both obese and control groups; the highest values were obtained in OCAF group.

Two-way ANOVA revealed an effect for maternal diet on serum NEFA and TAG, and adipose tissue TAG levels (P = 0.010, P = 0.001 and P = 0.004, respectively). There were also significant effects (two-way ANOVA) of offspring’s diet on serum and adipose tissue lipids (P < 0.01). All these concentrations were more affected by maternal diet–offspring’s diet interaction (P < 0.001) than individual effects.

Adipose tissue enzymes activities
Adipose tissue FAS activity did not differ between obese and control rats at days 21 and 90 (Figure 1). Neither the maternal diet nor the offspring’s diet affected adipose FAS activity in the offspring, as revealed by two-way ANOVA.

At day 21, adipose tissue GPDH, LPL and HSL activities were significantly increased in obese pups compared with controls, whereas adipose HSL activity did not vary between the two groups of rats (Figure 1).

At day 90, adipose GPDH, LPL and HSL activities were significantly increased in obese offspring compared with controls regardless of their diet at weaning. The cafeteria diet significantly increased these enzyme activities in both obese and control groups; the highest values were obtained in OCAF group. There were significant effects (two-way ANOVA) of maternal diet, the offspring’s diet and maternal diet–offspring’s diet on adipose GPDH (P = 0.006, P = 0.010 and P = 0.003 respectively), LPL (P = 0.004, P = 0.006 and P = 0.002 respectively) and HSL (P = 0.030, P = 0.010 and P = 0.007 respectively) activities.

Serum and adipose tissue TAG fatty acid composition
The fatty acid profile of serum and adipose tissue TAG of weanling rats (day 21) reflected the fatty acid composition
of the maternal diet. Afterward, at weaning, offspring of cafeteria-diet-fed dams showed a significant increase in SFAs (saturated fatty acids) and MUFAs (monounsaturated fatty acids) and a significant decrease in PUFAs (polyunsaturated fatty acids) compared with offspring of chow-fed dams (results not shown).

At day 90, the proportions of SFAs and MUFAs were still significantly increased, whereas PUFAs were decreased, in obese offspring compared with controls regardless of their diet at weaning (Table 4). The P/S (PUFA/SFA ratio) was significantly lower, while the oleic to stearic acid ratio, which represents the Δ9-desaturation index was higher in obese than in control rats. The cafeteria diet induced a significant increase in SFAs, MUFAs and Δ9-desaturation index and a significant decrease in PUFAs and P/S in serum and adipose tissue TAGs of both control and obese offspring.

There were significant effects (two-way ANOVA) of maternal diet and the offspring’s diet on serum and adipose tissue TAG SFAs, MUFAs, PUFAs, P/S and Δ9-desaturation index (P < 0.01). All these parameters were more affected by maternal diet-offspring’s diet interaction (P < 0.001) than individual effects.

**DISCUSSION**

In the present study, we explored the long-term effects of obesity during pregnancy and lactation on adipose tissue metabolic function of the offspring. Although the detrimental consequences of maternal overnutrition have been studied, little attention has been directed towards the consequences of maternal obesity on offspring adipose tissue. In our previous studies, a cafeteria diet was given to the dams at mating; the dams have generally not become overtly obese [2]. In the present study, a palatable cafeteria diet was given to dams 4 weeks before mating to induce long-term dietary obesity in breeders. Offspring of these dams were heavier than offspring from dams fed control standard diet, and they remained obese throughout adulthood, in agreement with previous studies [1–3]. They displayed increased adiposity and an adult metabolic-syndrome-like phenotype characterized by hyperglycaemia, hyperinsulinaemia, hyperleptinaemia and hyperlipidaemia, as reported previously [1–3,20]. The programming effects of maternal obesity are remarkably similar to those noted with the thrifty phenotype hypothesis, which proposed that maternal undernutrition...
during pregnancy exerts permanently long-term changes in the offspring [29]. Indeed, in our present study, offspring of obese dams presented significant increases in adipose tissue TAGs with alterations in adipose enzyme activities, such as an increase in enzymes involved in lipogenesis, and abnormalities in fatty acid composition such as an increase in SFAs and Δ^9-desaturation index and a decrease in PUFAs and P/S ratio compared with those of controls. Cafeteria diet commencing from weaning (day 21) also induced similar adipose tissue metabolism abnormalities that were worsened by the combined effects of maternal and postnatal overnutrition; the highest adipose enzyme activities, SFAs and Δ^9-desaturation index values and the lowest PUFAs and P/S ratio were obtained in obese offspring weaned on cafeteria diet at adulthood (day 90).

The major function of adipose tissue is uptake of glucose and NEFAs followed by storage as TAGs in postprandial state and supplement of NEFAs and glycerol by lipolysis of stored TAG to other tissues. Thus the regulation of TAG synthesis and lipolysis in adipose tissue is very important for maintaining the normal glucose, lipid and energy homeostasis. The activity of LPL is an important first step in plasma TAG clearance and NEFA delivery to the adipocyte. Insulin and glucose have been shown to stimulate adipose tissue LPL activity, implying a preferential partitioning of lipoprotein-derived fatty acids towards adipose tissue [30]. Insulin-induced increase of glucose and NEFA uptake is also important as substrate supply in efficient TAG synthesis in adipocytes. The enhanced activity of adipose tissue LPL activity is significantly correlated with enhanced TAG uptake by adipose tissue [10].

In offspring of cafeteria dams, increased adipose tissue weight and TAG contents are concomitant with the increase in enzyme activities involved in lipid storage such as LPL and GPDH [30,31]. This could be due to high insulin levels in these obese rats at weaning and at adulthood. The contribution of hyperinsulinaemia to the maintenance of high adipose tissue LPL activity in the obese Zucker fa/fa rat is well known [32]. High glucose levels are consistent with high GPDH activity since glucose can be utilized for de novo lipogenesis after esterification of fatty acids with glycerol 3-phosphate [33]. The early elevation of adipose tissue LPL and GPDH activities was a contributory factor to the maintenance of obesity in offspring of cafeteria-diet-fed rats.

At days 21 and 90, obese offspring had higher adiponectin levels whatever their diets were at weaning. Produced predominantly in adipocytes, circulating adiponectin levels in humans and rodent models are paradoxically inversely related to adiposity, but directly with insulin sensitivity [34,35]. Other actions of adiponectin to affect insulin sensitivity have been proposed, including enhancement of glucose uptake through AMPK (AMP-activated protein kinase) activation [36]. Increased adiponectin in obese rats could explain high GPDH activity whatever their diets were at weaning.

It has been reported that exposure to metabolic and hormonal signals of increased nutrition before birth results in an increase in the expression of the adipogenic factor PPARγ and in lipoprotein lipase, adiponectin and leptin mRNA expression in fetal perirenal fat [19]. An

### Table 4  Serum and adipose tissue TAG fatty acid composition in obese and control dam offspring fed control or cafeteria diet at adulthood (day 90)

Values are presented as means ± S.D. for ten rats in each group. Values with different superscript letters (a, b, c, d) are significantly different (P < 0.05). CC, offspring of chow-fed dams weaned on to standard diet; CCAF, offspring of chow-fed dams weaned on to cafeteria diet; OC, offspring of obese cafeteria-fed dams weaned on to standard chow; OCAF, offspring of obese cafeteria-fed dams weaned on to cafeteria diet; C18:1n-9/C18:0 (oleic acid/stearic acid ratio), Δ^9-desaturation index.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fatty acids</th>
<th>CC</th>
<th>CCAF</th>
<th>OC</th>
<th>OCAF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SFAs (g/100 g of fatty acids)</td>
<td>35.60 ± 1.77a</td>
<td>40.03 ± 1.11b</td>
<td>40.23 ± 1.53b</td>
<td>45.63 ± 1.02c</td>
</tr>
<tr>
<td></td>
<td>MUFA (g/100 g of fatty acids)</td>
<td>18.50 ± 1.36c</td>
<td>23.32 ± 1.04d</td>
<td>24.10 ± 1.02d</td>
<td>28.55 ± 1.07e</td>
</tr>
<tr>
<td></td>
<td>PUFA (g/100 g of fatty acids)</td>
<td>45.85 ± 1.33c</td>
<td>36.53 ± 1.24d</td>
<td>35.28 ± 1.50d</td>
<td>25.67 ± 1.46c</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>1.28 ± 0.10c</td>
<td>0.92 ± 0.05d</td>
<td>0.88 ± 0.05c</td>
<td>0.56 ± 0.04c</td>
</tr>
<tr>
<td></td>
<td>C18:1n-9/C18:0</td>
<td>0.75 ± 0.10c</td>
<td>0.78 ± 0.07d</td>
<td>0.96 ± 0.06c</td>
<td>1.13 ± 0.12b</td>
</tr>
<tr>
<td></td>
<td>Adipose tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SFAs (g/100 g of fatty acids)</td>
<td>27.52 ± 1.02c</td>
<td>26.40 ± 1.14d</td>
<td>30.25 ± 1.01c</td>
<td>37.23 ± 1.41d</td>
</tr>
<tr>
<td></td>
<td>MUFA (g/100 g of fatty acids)</td>
<td>28.58 ± 1.18c</td>
<td>47.48 ± 1.22d</td>
<td>48.36 ± 1.11c</td>
<td>50.07 ± 1.22c</td>
</tr>
<tr>
<td></td>
<td>PUFA (g/100 g of fatty acids)</td>
<td>43.51 ± 1.33c</td>
<td>26.05 ± 1.38c</td>
<td>21.24 ± 1.02d</td>
<td>12.50 ± 1.03b</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>1.56 ± 0.10b</td>
<td>0.98 ± 0.07b</td>
<td>0.70 ± 0.05b</td>
<td>0.33 ± 0.02b</td>
</tr>
<tr>
<td></td>
<td>C18:1n-9/C18:0</td>
<td>5.06 ± 0.43c</td>
<td>7.02 ± 0.37b</td>
<td>7.05 ± 0.54a</td>
<td>7.22 ± 0.60a</td>
</tr>
</tbody>
</table>
increase in maternal, and hence fetal, nutrition results in a precocious increase in adipogenic, lipogenic and adipokine gene expression in adipose tissue and that these changes may be important in the development of obesity in later life. These previous findings are consistent with our results since obese offspring showed high leptin and adiponectin levels and adipose tissue LPL and GPDH activities. In our present study, metabolic changes observed in obese rats seemed to be acquired through permanent modulation of adipose metabolism in early development (fetal life).

Several reports have suggested that adiponectin correlates negatively with serum TAG and positively with adipose LPL activity [37]. High adiponectin could explain high LPL activity and adipose tissue TAG accumulation in obese rats. In mice overexpressing adiponectin and in severe forms of childhood obesity, it has been suggested that adiponectin serves as a starvation signal released by the adipocyte, indicating a local need to further expand TAG stores in adipose tissue, promoting the storage of TAGs [38,39]. However, the inverse relationship with serum TAG and adiponectin was not observed in obese offspring. Indeed, high LPL activity is normally associated with enhanced lipoprotein catabolism, resulting especially in low serum TAG levels. This inverse correlation between lipolytic activities and circulating lipids was also not found in our obese rats in which TAG production could be more enhanced than their removal. In fact, we have previously reported high hepatic lipogenesis in the obese offspring of cafeteria-diet-fed dams [25].

In our present study, postnatal cafeteria-diet feeding also induced obesity with hyperglycaemia, hyperinsulinemia, hyperleptinemia and hypertriglyceridaemia. It is well known that feeding a high-fat diet to rodents causes insulin resistance, hyperinsulinemia, hyperglycaemia and hyperlipidaemia [40]. Increased adipose tissue weight, serum NEFAs and HOMA index in cafeteria-diet-fed offspring probably predict an increase in adipose tissue and skeletal muscle insulin resistance, as reported previously [24]. Indeed, the cafeteria diet stimulates adipose tissue LPL and GPDH in both control and obese rats. Other reports showed that high-fat diets induce increased glucose uptake, high GPDH and LPL activities but low FAS activity in adipose tissue [41].

All these alterations were worsened by the combined effects of maternal and postnatal cafeteria-diet-induced obesity because OCAF rats presented the highest glucose, insulin, leptin, lipid concentrations, adipose LPL and GPDH activities compared with the other groups of rats. However, in our present study, adipose tissue FAS activity was not affected by maternal or offspring diets. Adipose HSL activity was normal at day 21 but high at day 90 in obese rats. Indeed, postnatal cafeteria feeding induced an increase in adipose HSL activity in both obese and control rats. The increase in HSL activity was accompanied by an increase in serum NEFA levels. Indirect evidence has suggested that HSL is the rate-limiting enzyme in intracellular lipolysis; overexpression of HSL prevents differentiated adipocytes from accumulating TAG [42]. A positive relationship between fat cell size and HSL activity was also seen with high-fat feeding in rats [43]. It can be suggested that high adipose tissue HSL activity and lipolysis may prevent worsening of the TAG accumulation related to high LPL activity in adult obese rats. In addition, increased adipose HSL activity and lipolysis with increased amounts of NEFA released from enlarged adipocytes might also be a ‘spillover’ effect resulting from saturation of the capacity for TAG storage, as suggested previously [44].

In our present study, pronounced changes in the fatty acid composition of serum and adipose tissue TAGs were also observed in obese rats. These obese rats presented a significant decrease in TAG PUFA contents, balanced by increases in SFAs and MUFAs. These observations are in agreement with data reported in obese patients [45]. A low P/S ratio correlated well with increased insulin resistance in obese rats. The high ratio of oleic to stearic acid in obese rats indicated a high stearyl-CoA desaturase activity. The effects of cafeteria feeding on the composition of fatty acids in retroperitoneal fat pad have been previously studied [46], and the findings were similar to our present results. Adipose tissue stearoyl-CoA desaturase, an enzyme that catalyses the synthesis of MUFAs, is increased in obese rats and humans [47].

Our present results showed that fatty acid alterations were more dramatic in obese rats fed on a cafeteria diet (OCAF) compared with the other groups. These findings suggested that obese rats have pre-existing fatty acid metabolism changes that were accentuated with cafeteria diet.

The PARs (predictive adaptive responses) proposed by Gluckman et al. [48] would suggest that offspring of cafeteria-diet-fed mothers are adaptively more suited to a postnatal cafeteria diet. However, our present results did not support these PARs because when obese offspring were fed on a postnatal cafeteria diet, they demonstrated a greater increase in obesogenic responses compared with controls, suggesting that these offspring are not better able to metabolically handle a postnatal cafeteria diet.

In conclusion, maternal cafeteria feeding affected adipose tissue metabolism leading to permanent changes in adipose tissue adipokines, enzyme activities and fatty acid composition in the offspring. These abnormalities in offspring were worsened under a high-fat diet from weaning to adulthood. Maternal overnutrition induces permanently programmed adipose tissue metabolism that contribute to the maintenance of obesity in the offspring. Adipose adipokines and enzymes could provide a potential therapeutic target, and work in this field could constitute strategies to improve the impact of early-life overnutrition.
AUTHOR CONTRIBUTION

Nassira Batoul Benkalfat performed the in vivo rat experiments, contributed to all the measurements, collected and analysed the data and discussed the experimental protocols. She participated actively in the literature search and in drafting of the manuscript. Hafida Merzouk designed the study, co-ordinated all steps of the study, supervised all of the experiments, collected and analysed the data and wrote the paper. She performed the role of collecting funds. Samira Bouanane conducted some laboratory experiments, especially enzyme activities, and participated in data collection and analysis. Sid-Ahmed Merzouk performed statistical analysis. Jérôme Bellenger took active part in data interpretation and analysis, the literature search and revision of the manuscript. Joseph Guichard, C., Dugail, I., Le liepvre, X. and Lavau, M. (1992) Adipose tissue as an endocrine organ. Trends Endocrinol. Metab. 11, 327–332

REFERENCES


