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Hepatic and very low-density lipoprotein fatty acids in obese offspring of overfed dams

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Abstract

The combined effects of developmental programming and high-fat feeding at weaning on fatty acid metabolism of the offspring are not well known. In the present study, we aim at characterizing the influence of maternal and offspring's own diets on liver and very low-density lipoprotein (VLDL) lipids; fatty acid profiles of VLDL and liver phospholipids, triglycerides, and cholesteryl esters; and hepatic enzyme activities. Twenty obese male rats born to cafeteria diet–fed dams and 20 control rats born to control diet–fed dams were selected. At weaning, 10 rats of each group were fed control or cafeteria diet. Obese rats had a significant increase in serum glucose, insulin, leptin, VLDL apolipoprotein B100 and lipid levels, and hepatic fatty acid synthase and a reduction in acyl–coenzyme A oxidase and dehydrogenase activities compared with control pups at day 21 and day 90. Hepatic steatosis was apparent only at day 90. The proportions of saturated fatty acids and monounsaturated fatty acids and the oleic to stearic acid ratio were significantly increased, whereas polyunsaturated fatty acids and the arachidonic to linoleic acid ratio were decreased, in liver and VLDL lipids of obese pups compared with controls. The cafeteria diet at weaning induced more severe abnormalities in obese rats. In conclusion, maternal cafeteria diet induced a permanent reduction in hepatic β-oxidation and an increase in hepatic lipogenesis that caused liver steatosis and VLDL and fatty acid alterations in adult offspring. These preexisting alterations in offspring were worsened under a high-fat diet from weaning to adulthood. Nutritional recommendations in obesity must then target maternal and postnatal nutrition, especially fatty acid composition.

1. Introduction

Epidemiologic, clinical, and experimental studies suggested that maternal obesity during pregnancy is an important risk factor for fetal overnutrition, leading to fetal obesity and hyperinsulinemia [1-3]. Maternal obesity gives rise to an offspring phenotype predisposed to development of adulthood obesity and diabetes [2,4,5]. Obesity is associated with glucose and lipid metabolism abnormalities, and increased cardiovascular risk [6,7]. The dyslipidemia typical of the obese state is associated with perturbations in triglyceride (TG)-rich lipoprotein metabolism [8]. An important consequence of insulin resistance is enhanced adipose tissue lipolysis and reduced free fatty acid (FFA) uptake and esterification, leading to an increased flux of FFA into nonadipose tissues such as liver and muscle. Increased FFA flux has been suggested to increase intracellular availability of TG and to indirectly stimulate the assembly and secretion of very low-density lipoprotein (VLDL) particles [9]. In addition, substantial evidence from both humans and animals has indicated that essential fatty acid metabolism is also abnormal in obesity [10,11]. Lower fatty acid oxidation was reported in obesity, leading to increased intracellular long-chain fatty acyl–coenzyme A (CoA) concentrations resulting in TG formation [12]. Liver steatosis is a characteristic in obese patients [13] and is

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closely associated with the insulin resistance syndrome. Recent studies have established that microsomal stearoyl-CoA desaturase is the most highly expressed gene in human and rodent models of obesity and plays a major role in the development of obesity and its associated disorders [14]. Increased delivery of fatty acids, increased de novo lipogenesis, or decreased fatty acid oxidation in the liver could lead to TG accumulation and hepatic steatosis [15,16].

Dietary factors are likely to be of importance in the etiology of obesity in humans. This is consistent with the fact that obesity, dyslipidemia, and insulin resistance can be induced in rodents by the consumption of high-fat diets [17,18]. Reduced fatty acid oxidation has also been observed in high-fat–fed rats [16]. Modifications of fatty acid concentrations and composition in tissue lipids are induced by a high-fat diet and have been associated with alterations in lipid metabolism and insulin sensitivity [19,20].

Experimental obesity can be induced by dietary manipulations. Offering rats a variety of snack-type foods, normally consumed by humans, in a multichoice "cafeteria" arrangement has been shown to produce hyperphagia and obesity [18,21]. Several authors have used animal models of cafeteria-diet–induced obesity and indicated that maternal high-fat feeding during pregnancy and lactation has long-term metabolic consequences in the offspring, similar to that found in the human metabolic syndrome [22-25]. Abnormal glucose homeostasis, abnormal lipid profiles, increased adiposity, proatherogenic lesions, and hyperleptinemia have all been reported [22,25-27]. Maternal fat intake also contributed to the development of hepatic steatosis through up-regulated hepatic lipogenesis in adult offspring [28].

However, few studies have examined the effect of maternal high-fat feeding during pregnancy and lactation on fatty acid profiles and on hepatic key enzymes of the offspring. In addition, the combined effects of developmental programming and high-fat feeding at weaning are not well known.

We have previously used an animal model to examine the effects of maternal obesity induced by energy-dense palatable foods rich in fat and sugar designed for human consumption [22].

We have demonstrated that offspring exposed to maternal cafeteria diet developed exacerbated overeating, increased adiposity, and obesity with metabolic abnormalities such as hyperleptinemia, hyperinsulinemia, dyslipidemia, and oxidative stress [22]. In the present study, we aim at further characterizing the longer-term influence of maternal overnutrition on liver, VLDL lipids, and fatty acid profiles in the offspring. We also analyzed key enzymes in liver fatty acid synthesis and oxidation to determine the mechanisms by which hepatic steatosis is developed in the offspring. In addition, we examined whether there are additive effects of feeding the offspring a highly palatable diet from weaning to adulthood.

2. Material and methods

2.1. Animals and experimental protocol

Adult Wistar rats were obtained from Iffa-Credo (Lyon, France). After mating, the first day of gestation was estimated by the presence of spermatozoids in vaginal smears. Pregnant rats weighing 180 to 200 g were housed individually in wood-chip–bedded plastic cages at a constant temperature (25°C) and humidity (60% ± 5%) with a 12-hour light/dark cycle. The rats had free access to water and were assigned to 2 dietary groups, with one group (control, n = 10) fed a control commercial diet (UAR, Villemoison-sur-Orge, France), whereas the second group (cafeteria group, n = 10) was fed a fat-rich hypercaloric diet throughout gestation and lactation. The control diet (330 kJ/100 g) was 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% C20:4n-6). The components of the cafeteria diet were paté, cheese, bacon, chips, cookies, and chocolate (in a proportion of 2:2:2:1:1:1, by weight); and control diet (mix/control diet, W/W) was given to each rat daily as published previously [22,29]. The composition of the 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). The dams were fed the same diet continuously for the entire gestation and lactation periods. A total of 70 pups from the 10 cafeteria-fed dams and 90 pups from the 10 control dams were delivered spontaneously and weighed within 12 hours. The postnatal litter size was adjusted at 8 pups/dam to maintain a similar postnatal nutritional intake during the suckling period. Weaning occurred on day 21 of lactation.

Offspring were weaned on to the control commercial diet (UAR) or the cafeteria diet. Male rats were housed separately and were followed into adulthood (12 weeks). Four groups were then formed: group 1 consisted of 10 offspring of control dams that were fed control diet at weaning (CC); group 2 consisted of 10 offspring of control dams that were fed cafeteria diet at weaning (CCAF); group 3 consisted of 10 obese offspring of overfed dams that were fed control diet at weaning (OC); and group 4 consisted of 10 obese offspring of overfed dams that were fed cafeteria diet at weaning (OCAF). Food intake and body weights of rats were recorded daily.

The study was conducted in accordance with the national guidelines for the care and use of laboratory animals. All the experimental protocols were approved by the Regional Ethical Committee.

2.2. Blood and liver samples

After overnight fasting, in each diet group and at each experimental point, that is, at weaning (3 weeks of age) and at

adulthood (12 weeks of age), rats from each group were anesthetized with pentobarbital (60 mg/kg body weight) and bled from the abdominal aorta; the liver was dissected out immediately. Serum was obtained by low-speed centrifugation (1000*g* for 20 minutes) and was used for glucose, insulin, leptin, and lipoprotein determinations. The liver was removed, washed with cold saline, quickly blotted, and weighed. One aliquot of the liver was homogenized in chloroform/methanol with an Ultra Turrax homogenizer (Bioblock Scientific, IllKirch, France) for lipid extraction. Another aliquot of the liver was homogenized in 0.25 mol/L sucrose and centrifuged at 500*g* for 10 minutes [30]. The supernatant was recentrifuged at 9000*g* for 10 minutes to isolate mitochondria. The mitochondrial fraction was suspended in the same medium. The supernatant was recentrifuged at 100 000*g* for 1 hour to obtain cytosolic fractions.

2.3. Isolation of the VLDL fraction

Serum lipoproteins of density less than 1.21 kg/L were isolated by single ultracentrifugation flotation (model L8-55 ultracentrifuge, 50 Ti rotor; Beckman Instruments, Palo Alto, CA). The VLDL fraction was isolated from total lipoproteins by a single-spin discontinuous gradient according to the method of Redgrave et al [31].

2.4. Chemical analysis

Serum glucose was determined by the glucose oxidase method using a glucose analyzer (Beckman Instruments). Serum insulin and leptin were analyzed using radioimmunoassay kits with antibodies to authentic rat insulin and leptin, respectively (Linco Research, St. Charles, MO). Serum total FFAs were measured by using the enzymatic NEFA Kit (Biolyon, Dardilly, France). VLDL–apolipoprotein (apo) B100 was measured by Biomerieux kit (Marcy l'Etoile, France) using turbidimetric methods.

Liver and VLDL TG, phospholipid (PL), and total cholesterol (TC) contents were determined using colorimetric enzymatic assays (Sigma, St. Louis, MO). Total lipids of VLDL and liver were extracted according to the method of Folch et al [32]. The VLDL and liver lipid classes (TGs, PLs, cholesteryl esters [CEs]) were isolated by thin-layer chromatography. Each lipid fraction was transmethylated, and fatty acids were analyzed by gas liquid chromatography [33] using a Becker gas chromatograph (Spiral-RD, Couternon, France) equipped with a 50-m capillary glass column packed with Carbowax 20M (Becker Instruments, Downers Grove, IL). Methyl ester (17:0) was used as an internal standard. Identification of different fatty acids was performed by comparison of relative retention times with those of commercial standards. Areas were calculated with an ENICA 21 integrator (DELSI Instrument, Suresnes, France).

2.5. Hepatic enzyme activities

Acyl-CoA oxidase (EC 1.3.3.6) activity was measured in the 500*g* supernatant fraction of liver homogenates as described by Small et al [34]. The spectrophotometric assay of acyl-CoA oxidase was based on the estimation of H_2O_2 production by coupling with the peroxidation of leuko-2′,7′-dichlorofluorescein to 2′,7′-dichlorofluorescein, a green dye. Acyl-CoA dehydrogenase (EC 1.3.99.3) activity was measured in mitochondrial fraction spectrophotometrically using phenazinemethosulfate and 2,6 dichloroindophenol (DCIP) as intermediate and terminal electron acceptors, respectively, with palmitoyl-CoA as substrate according to the method described by Hall [35]. Enzyme activity was expressed as nanomoles of DCIP reduced per minute per milliliter of enzyme solution or per gram liver. The extinction coefficient of DCIP (21 mmol L^{-1} cm^{-1}) at 600 nm was used as the basis for computation of the amount of DCIP reduced. The fatty acid synthase (FAS) activity was measured in cytosolic fraction by measuring malonyl-CoA–dependent oxidation of NADPH at 37°C [36]. One unit of enzyme activity represents 1 mmol of NADPH oxidized per minute at 37°C. Serum aspartate aminotransferase (AST) (EC 2.6.1.1) and alanine aminotransferase (ALT) (EC 2.6.1.2) activities were determined by the colorimetric method using Randox Diagnostic kits (Randox Laboratories Ltd, Co Antrim, UK).

2.6. Statistical analysis

Results are expressed as means ± SD. Significant differences among the groups were analyzed statistically by Student *t* test (day 21) and a 1-way analysis of variance (ANOVA) (day 90). The individual effects of the maternal diets and the offspring's diets were distinguished by 2-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 $P < .05$. These calculations were performed using STATISTICA version 4.1 (STATSOFT, Tulsa, OK).

3. Results

3.1. Body weight, energy intake, liver relative weight, serum glucose, insulin, leptin, and FFA concentrations

At weaning, pups from cafeteria-fed dams had higher weight, glucose, insulin, and leptin concentrations than pups from control dams (Table 1). At this age, no differences in serum FFA concentrations and in relative liver weight were observed between pups (Table 1).

At 12 weeks of age, obese rats from cafeteria diet–fed dams being weaned on the control or cafeteria diet (OC and OCAF) still had significantly higher body weights than control rats from control diet–fed dams (CC and CCAF) (Table 2). In addition, obese rats from the group fed the cafeteria diet (OCAF) showed higher weight gain than obese rats fed the control diet (OC).

Table 1
Physiologic parameters in obese and control offspring at weaning (day 21)

Parameters	Control rats	Obese rats
Body weight (g)	56 ± 2.15	98.75 ± 3.26*
Glucose (mmol/L)	4.98 ± 0.40	7.22 ± 0.35*
Insulin (ng/mL)	1.85 ± 0.20	2.40 ± 0.18*
Leptin (ng/mL)	1.65 ± 0.30	3.58 ± 0.55*
Liver (relative weight)	4.15 ± 0.23	4.22 ± 0.38
FFA (mmol/L)	0.50 ± 0.03	0.55 ± 0.04
VLDL TG (mmol/L)	0.56 ± 0.05	0.88 ± 0.04*
VLDL TC (mmol/L)	0.18 ± 0.02	0.32 ± 0.03*
VLDL PL (mmol/L)	0.22 ± 0.02	0.46 ± 0.04*
VLDL apo B100 (g/L)	0.08 ± 0.01	0.17 ± 0.02*
Liver TG (mg/g liver)	3.80 ± 0.63	4.05 ± 0.55
Liver PL (mg/g liver)	20 ± 3.12	23.10 ± 2.43
Liver TC (mg/g liver)	7.25 ± 0.88	8.36 ± 0.44
ALT (IU/L)	35.66 ± 6.35	38.50 ± 5.25
AST (IU/L)	49.45 ± 4.57	52.50 ± 4.55
Acyl-CoA dehydrogenase (nmol/[min g liver])	85.67 ± 8.33	37.35 ± 5.58*
Acyl-CoA oxidase (nmol/[min g liver])	48.12 ± 3.50	20.50 ± 4.35*
FAS (nmol/[min g liver])	166 ± 26.75	289 ± 22.75*

Values are presented as means ± SD.
* Significant differences between obese and control rats; $P < .05$.

The daily energy intake was increased in obese rats compared with control rats regardless of their diet at weaning (Table 2). The cafeteria diet was associated with increased daily energy intake in both control and obese pups; the highest values were obtained in obese offspring fed cafeteria diet at weaning.

At day 90, the obese pups (OC) had a significant increase in the levels of serum glucose, insulin, leptin, and FFA concentrations compared with control pups (CC) (Table 2). The cafeteria diet significantly increased serum insulin, leptin, and FFA levels in both obese (OCAF) and control rats (CCAF); the highest values were observed in OCAF group. Indeed, the cafeteria diet induced a significant increase in serum glucose levels in obese group (OCAF) but not in control group (CCAF).

To determine the individual effects of the maternal diets and the offspring's own diets, 2-way ANOVA was performed. There were significant effects of maternal diet, the offspring's own diet, and maternal diet × the offspring's own diet on serum glucose ($P = .013$, $P = .025$, and $P = .006$, respectively), insulin ($P = .007$, $P = .005$, and $P = .003$, respectively), leptin ($P = .006$, $P = .005$, and $P = .003$, respectively), and FFA ($P = .04$, $P = .005$, and $P = .003$, respectively) levels.

3.2. Liver and VLDL lipid contents

At weaning, liver TC, TG, and PL contents in offspring of cafeteria-fed dams were similar to those in control rats (Table 1). In contrast, VLDL lipid and apo B100 contents were significantly higher in offspring of the cafeteria-fed dams than in their control counterparts.

At day 90, liver TG and PL contents were significantly increased in obese pups compared with controls regardless of their diet at weaning, whereas liver cholesterol amounts did not vary between the 4 groups of rats (Table 2). The cafeteria diet significantly increased liver TGs and PLs in both obese and control groups; the highest values were obtained in OCAF group. The cafeteria diet did not affect liver cholesterol.

Adult obese offspring in both the control and cafeteria diet groups (OC and OCAF) presented significantly higher VLDL TG, PL, cholesterol, and apo B100 levels than their respective controls (CC and CCAF) (Table 2). The cafeteria diet induced a significant increase in VLDL lipids and apolipoproteins in both CCAF and OCAF groups; the highest values were seen in OCAF pups.

Table 2
Physiologic parameters in obese and control offspring fed control or cafeteria diet at adulthood (day 90)

Parameters	CC	CCAF	OC	OCAF
Body weight (g)	346 ± 43^{a}	478 ± 33^{b}	486 ± 28^{b}	567 ± 47^{c}
Energy intake (kcal/d)	68.45 ± 5.67^{a}	128.34 ± 12.76^{b}	121 ± 15.33^{b}	157.29 ± 14.55^{c}
Glucose (mmol/L)	5.68 ± 0.49^{a}	6.22 ± 0.58^{a}	8.86 ± 0.38^{b}	12.05 ± 0.86^{c}
Insulin (ng/mL)	1.98 ± 0.21^{a}	2.65 ± 0.32^{b}	2.88 ± 0.30^{b}	3.44 ± 0.27^{c}
Leptin (ng/mL)	4.21 ± 0.26^{a}	7.53 ± 0.46^{b}	8.94 ± 0.35^{c}	11.37 ± 0.50^{d}
Liver (RW)	3.80 ± 0.26^{a}	3.98 ± 0.45^{a}	3.87 ± 0.40^{a}	4.02 ± 0.39^{a}
FFA (mmol/L)	0.86 ± 0.05^{a}	1.45 ± 0.17^{b}	1.67 ± 0.11^{c}	1.89 ± 0.15^{d}
VLDL TG (mmol/L)	0.70 ± 0.12^{a}	1.24 ± 0.22^{b}	1.27 ± 0.32^{b}	1.64 ± 0.30^{c}
VLDL TC (mmol/L)	0.23 ± 0.04^{a}	0.42 ± 0.04^{b}	0.46 ± 0.05^{b}	0.62 ± 0.04^{c}
VLDL PL (mmol/L)	0.35 ± 0.07^{a}	0.55 ± 0.06^{b}	0.57 ± 0.05^{b}	0.73 ± 0.05^{c}
VLDL apo B100 (g/L)	0.13 ± 0.03^{a}	0.22 ± 0.04^{b}	0.24 ± 0.03^{b}	0.33 ± 0.04^{c}
Liver TG (mg/g liver)	4.20 ± 0.32^{a}	6.04 ± 0.35^{b}	6.50 ± 0.42^{b}	8.74 ± 0.37^{c}
Liver PL (mg/g liver)	30.05 ± 2.67^{a}	40.25 ± 2.65^{b}	41.05 ± 2.33^{b}	55.92 ± 3.05^{c}
Liver TC (mg/g liver)	10.84 ± 1.49^{a}	9.82 ± 1.18^{a}	10.56 ± 1.78^{a}	11.00 ± 1.84^{a}
ALT (IU/L)	57.37 ± 5.85^{a}	59.50 ± 4.25^{a}	60.26 ± 5.85^{a}	64.50 ± 6.75^{a}
AST (IU/L)	68.55 ± 5.87^{a}	70.40 ± 5.15^{a}	73.28 ± 4.39^{a}	75.50 ± 6.55^{a}
Acyl-CoA dehydrogenase (nmol/[min g liver])	98.58 ± 7.43^{b}	96.50 ± 8.53^{b}	63.70 ± 7.53^{a}	57.35 ± 6.50^{a}
Acyl-CoA oxidase (nmol/[min g liver])	75.56 ± 4.50^{b}	70.50 ± 4.35^{b}	50.12 ± 3.50^{a}	46.50 ± 4.57^{a}
FAS (nmol/[min g liver])	188 ± 20.44^{a}	180 ± 22.35^{a}	310.50 ± 30.15^{b}	317.43 ± 28.35^{b}

Values are presented as means ± SD. RW = relative weight. Values with different superscript letters (a, b, c, d) are significantly different ($P < .05$).

Two-way ANOVA revealed an effect for maternal diet on VLDL TG, PL, cholesterol, and apo B100 levels ($P = .004$, $P = .012$, $P = .024$, and $P = .006$, respectively) and also on liver TG and PL amounts ($P = .012$ and $P = .015$, respectively). There were significant effects (2-way ANOVA) of offspring's diet on VLDL lipids ($P < .01$), apo B100 ($P = .003$), and liver lipids ($P < .01$). All these concentrations were more affected by maternal diet × offspring's diet interaction ($P < .001$) than individual effects.

3.3. Hepatic enzymes activities

Serum AST and ALT activities, hepatocellular markers, did not differ between obese and control rats at days 21 and 90 (Tables 1 and 2). Neither the maternal diet nor the offspring's diet affected AST and ALT activities in the offspring, as revealed by 2-way ANOVA.

At day 21, hepatic acyl-CoA dehydrogenase and acyl-CoA oxidase activities were decreased, whereas FAS activity was increased, in obese offspring of cafeteria-fed dams compared with control rats (Table 1). At day 90, acyl-CoA dehydrogenase and acyl-CoA oxidase activities remained lower and FAS activity higher in obese offspring than in controls whatever their diet (Table 2). The cafeteria diet from weaning to adulthood did not affect hepatic enzyme activities in both obese and control rats. However, there were significant effects (2-way ANOVA) of maternal diet on acyl-CoA dehydrogenase, acyl-CoA oxidase, and FAS activities ($P = .006$, $P = .005$, and $P = .004$, respectively).

3.4. Hepatic and VLDL lipid fatty acid compositions

The fatty acid profile of hepatic and VLDL lipids of weanling rats (day 21) reflected the fatty acid composition of the maternal diet. Afterward, at weaning, pups from cafeteria-fed dams showed a significant increase in lipid saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) and a significant decrease in polyunsaturated fatty acids (PUFA) compared with pups from chow fed dams (results not shown).

3.4.1. Hepatic and VLDL TG fatty acids in adulthood

At day 90, the proportions of hepatic SFA and MUFA were significantly increased, whereas PUFA were decreased, in obese pups compared with controls (Table 3). The hepatic PUFA to SFA ratio (P/S), the arachidonic to linoleic acid ratio, and Δ5 and Δ6 desaturation index were also significantly lower in obese than control rats. In contrast, the oleic to stearic acid ratio and Δ9 desaturation index were higher in obese than control rats. The cafeteria diet induced a significant increase in hepatic SFA, MUFA, and Δ9 desaturation index and a significant decrease in PUFA, P/S, and Δ5-Δ6 desaturation index in both control and obese offspring. The same alterations related to obesity or to cafeteria diet were also observed in VLDL TG fatty acid composition (Table 3). There were significant effects (2-way ANOVA) of maternal diet, the offspring's diet, and maternal diet × the offspring's diet on hepatic and VLDL TG SFA, MUFA, PUFA, P/S, and Δ9 and Δ5-Δ6 desaturation indexes ($P < .001$).

3.4.2. Hepatic and VLDL PL fatty acids in adulthood

At day 90, hepatic and VLDL PL fatty acid composition changes were parallel to those observed with TG fatty acids (Table 4). In fact, in obese rats, SFA, MUFA, and Δ9 desaturation index were increased, whereas PUFA, P/S, and Δ5-Δ6 desaturation index were decreased, in liver and VLDL compared with control values. The cafeteria diet was also associated with a significant increase in hepatic and VLDL SFA, MUFA, and Δ9 desaturation index and a significant decrease in PUFA, P/S, and Δ5-Δ6 desaturation index in both control and obese offspring. There were significant effects (2-way ANOVA) of maternal diet, the

Table 3
Hepatic and VLDL TG fatty acid composition in obese and control offspring fed control or cafeteria diet at adulthood (day 90)

Fatty acids	CC	CCAF	OC	OCAF
Liver				
SFA (g/100 g fatty acids)	42.00 ± 1.68[a]	46.50 ± 1.08[b]	46.00 ± 1.37[b]	50.03 ± 1.11[c]
MUFA (g/100 g fatty acids)	24.27 ± 1.66[a]	28.17 ± 0.89[b]	27.88 ± 1.42[b]	31.00 ± 1.23[c]
PUFA (g/100 g fatty acids)	33.72 ± 1.53[c]	25.33 ± 1.02[b]	26.12 ± 1.33[b]	19.02 ± 1.04[a]
P/S	0.78 ± 0.05[c]	0.54 ± 0.06[b]	0.54 ± 0.04[b]	0.38 ± 0.03[a]
18:1n-9/18:0	0.80 ± 0.04[a]	1.35 ± 0.05[c]	1.26 ± 0.03[b]	2.37 ± 0.22[d]
20:4n-6/18:2n-6	0.53 ± 0.03[c]	0.33 ± 0.02[b]	0.30 ± 0.03[b]	0.22 ± 0.03[a]
VLDL				
SFA (g/100 g fatty acids)	37.32 ± 1.55[a]	40.34 ± 1.04[b]	41.45 ± 1.34[b]	47.68 ± 1.37[c]
MUFA (g/100 g fatty acids)	34.23 ± 1.36[a]	38.25 ± 1.11[b]	38.77 ± 1.26[b]	40.32 ± 1.13[c]
PUFA (g/100 g fatty acids)	28.45 ± 1.04[c]	21.40 ± 1.18[b]	19.77 ± 1.15[b]	13.00 ± 0.87[a]
P/S	0.76 ± 0.06[c]	0.53 ± 0.05[b]	0.48 ± 0.06[b]	0.27 ± 0.02[a]
18:1n-9/18:0	1.20 ± 0.11[a]	1.74 ± 0.07[c]	1.55 ± 0.04[b]	2.22 ± 0.20[d]
20:4n-6/18:2n-6	0.46 ± 0.03[c]	0.30 ± 0.04[b]	0.32 ± 0.04[b]	0.23 ± 0.04[a]

Values are presented as means ± SD. Values with different superscript letters (a, b, c, d) are significantly different ($P < .05$). 18:1n-9/18:0 indicates oleic to stearic acid ratio, Δ9 desaturation index; 20:4n-6/18:2n-6, arachidonic to linoleic acid ratio, Δ6 desaturation index.

Table 4
Hepatic and VLDL PL fatty acid composition in obese and control offspring fed control or cafeteria diet at adulthood (day 90)

Fatty acids	CC	CCAF	OC	OCAF
Liver				
SFA (g/100 g fatty acids)	45.23 ± 1.25[a]	49.37 ± 1.38[b]	48.76 ± 1.59[b]	53.04 ± 1.44[c]
MUFA (g/100 g fatty acids)	15.34 ± 1.04[a]	18.67 ± 1.10[b]	17.93 ± 1.03[b]	22.75 ± 1.21[c]
PUFA (g/100 g fatty acids)	39.43 ± 1.65[c]	31.95 ± 1.55[b]	33.30 ± 1.68[b]	24.22 ± 1.36[a]
P/S	0.87 ± 0.07[c]	0.66 ± 0.05[b]	0.67 ± 0.04[b]	0.45 ± 0.03[a]
18:1n-9/18:0	0.64 ± 0.05[a]	1.62 ± 0.12[c]	1.01 ± 0.09[b]	2.25 ± 0.22[d]
20:4n-6/18:2n-6	1.50 ± 0.13[c]	1.08 ± 0.11[b]	1.04 ± 0.12[b]	0.73 ± 0.05[a]
VLDL				
SFA (g/100 g fatty acids)	46.07 ± 1.11[a]	50.23 ± 1.55[b]	49.99 ± 1.86[b]	55.03 ± 1.34[c]
MUFA (g/100 g fatty acids)	16.11 ± 1.22[a]	20.33 ± 1.39[b]	19.45 ± 1.26[b]	24.35 ± 1.67[c]
PUFA (g/100 g fatty acids)	37.82 ± 1.46[c]	29.44 ± 1.78[b]	30.56 ± 1.32[b]	20.62 ± 1.28[a]
P/S	0.82 ± 0.05[c]	0.58 ± 0.06[b]	0.61 ± 0.03[b]	0.38 ± 0.04[a]
18:1n-9/18:0	0.56 ± 0.04[a]	1.44 ± 0.23[c]	1.18 ± 0.15[b]	2.04 ± 0.23[d]
20:4n-6/18:2n-6	0.96 ± 0.07[c]	0.68 ± 0.04[b]	0.65 ± 0.05[b]	0.43 ± 0.04[a]

Values are presented as means ± SD. Values with different superscript letters (a, b, c, d) are significantly different ($P < .05$).

offspring's diet, and maternal diet × the offspring's diet on hepatic and VLDL PL SFA, MUFA, PUFA, P/S, and Δ9 and Δ5-Δ6 desaturation indexes ($P < .001$).

3.4.3. Hepatic and VLDL CE fatty acids in adulthood

At day 90, hepatic and VLDL CE fatty acid composition changes in obese rats were parallel to those observed with TG and PL fatty acids (Table 5). In obese rats, SFA, MUFA, and Δ9 desaturation index were high, whereas PUFA, P/S, and Δ5-Δ6 desaturation index were low, in liver and VLDL compared with control values. The cafeteria diet induced the same but strong changes in hepatic and VLDL cholesteryl fatty acid composition in obese offspring. However, in control rats, there were no diet-related hepatic and VLDL cholesteryl fatty acid changes because CE fatty acids in control rats fed cafeteria diet (CCAF) were similar to those in control rats fed control diet (CC). There were significant effects (2-way ANOVA) of maternal diet on hepatic and VLDL CE SFA, MUFA, PUFA, P/S, and Δ9 and Δ5-Δ6 desaturation indexes ($P < .01$).

4. Discussion

The present study showed that, in the rat, maternal nutrition in pregnancy and lactation has a long-term programming effect on lipid and fatty acid metabolism in the offspring. Dams given a high-fat diet in both pregnancy and lactation had offspring that in adult life had significant increases in lipoprotein and liver lipids with alterations in hepatic enzyme activities and in fatty acid composition compared with those of controls. High-fat diets commencing from weaning (day 21) also induced lipid metabolism abnormalities that were worsened by the combined effects of maternal and postnatal overnutrition.

In this study, the diet used to induce obesity, called *cafeteria diet*, is rich in fats, particularly SFA and MUFA, and poor in PUFA compared with standard control diet. The experimental protocol permitted to evaluate 3 specific effects: maternal obesity, postnatal diet-induced obesity from weaning, and the combined factors. The effects of maternal obesity, which represents the concept of

Table 5
Hepatic and VLDL CE fatty acid composition in obese and control offspring fed control or cafeteria diet at adulthood (day 90)

Fatty acids	CC	CCAF	OC	OCAF
Liver				
SFA (g/100 g fatty acids)	34.30 ± 1.55[a]	34.09 ± 1.36[a]	38.03 ± 1.33[b]	42.00 ± 1.53[c]
MUFA (g/100 g fatty acids)	29.04 ± 1.68[a]	30.45 ± 1.04[a]	31.65 ± 1.36[b]	36.56 ± 1.75[c]
PUFA (g/100 g fatty acids)	36.66 ± 1.37[c]	35.46 ± 1.07[c]	30.32 ± 1.47[b]	21.45 ± 1.22[a]
P/S	1.10 ± 0.06[c]	1.05 ± 0.05[c]	0.78 ± 0.04[b]	0.50 ± 0.05[a]
18:1n-9/18:0	2.34 ± 0.12[a]	2.48 ± 0.11[a]	2.55 ± 0.09[b]	3.44 ± 0.14[c]
20:4n-6/18:2n-6	0.73 ± 0.06[c]	0.70 ± 0.05[c]	0.50 ± 0.04[b]	0.40 ± 0.03[a]
VLDL				
SFA (g/100 g fatty acids)	31.43 ± 1.27[a]	31.66 ± 1.39[a]	38.00 ± 1.83[b]	42. 74 ± 1.37[c]
MUFA (g/100 g fatty acids)	31.54 ± 1.73[a]	31.63 ± 1.05[a]	35.45 ± 1.43[b]	40.02 ± 1.22[c]
PUFA (g/100 g fatty acids)	37.03 ± 1.29[c]	36.71 ± 1.38[c]	26.55 ± 1.11[b]	17.00 ± 1.04[a]
P/S	1.18 ± 0.16[c]	1.15 ± 0.06[c]	0.69 ± 0.05[b]	0.39 ± 0.03[a]
18:1n-9/18:0	2.05 ± 0.14[a]	2.16 ± 0.17[a]	2.50 ± 0.11[b]	3.23 ± 0.21[c]
20:4n-6/18:2n-6	0.55 ± 0.05[c]	0.52 ± 0.04[c]	0.47 ± 0.04[b]	0.36 ± 0.04[a]

Values are presented as means ± SD. Values with different superscript letters (a, b, c, d) are significantly different ($P < .05$).

developmental programming, were seen in obese rats born to cafeteria diet–fed obese dams and weaned on to the control diet (OC). The effects of the postnatal diet-induced obesity were observed in control rats born to control dams and weaned on the cafeteria diet (CCAF). The combined effects of these 2 factors were reported in obese rats born to cafeteria diet–fed obese dams and weaned on to the cafeteria diet (OCAF).

In comparison with their controls (CC), obese rats (OC) had increased body weight with enhanced energy intake at adulthood despite normal control diet. When fed cafeteria diet (OCAF), their energy intake was further higher than the others groups of rats. We attribute these changes, at least in part, to programmed changes in appetite [37]. The control rats that received the cafeteria diet at weaning (CCAF) presented an increase in energy intake that may explain their higher body weight compared with control rats weaned on the control diet (CC), in agreement with previous studies [21,25,29].

We showed that obese offspring of cafeteria-fed dams had significantly higher fasting insulin and glucose concentrations than offspring of control dams fed normal diet, highly suggestive of insulin resistance, in agreement with previous studies [26,27]. In addition, they had higher fasting leptin levels compared with controls; and this observation corroborated our previous study [22]. Obesity is associated with high circulating leptin concentrations related to increased amount of adipose tissue and leptin resistance [38]. Postnatal cafeteria diet feeding also induced obesity with hyperglycemia, hyperinsulinemia, hyperleptinemia, and hyperlipidemia. It is well known that feeding a high-fat diet to rodents causes insulin resistance, hyperinsulinemia, hyperglycemia, and hyperlipidemia [39,40]. 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, and lipid values compared with the other groups of rats.

Obese rats had high VLDL PL, TG, TC, and apo B100 levels at day 21 and day 90, suggesting increased VLDL synthesis and secretion. These findings correlated with an increase in hepatic TG and PL levels only at day 90. Overproduction of VLDL and the rise in hepatic lipids, a common feature of obesity, are direct consequences of hyperinsulinemia and hepatic hyperlipogenesis [41]. Of particular interest are specific changes in hepatic mitochondrial and peroxisomal oxidative function and hepatic lipogenesis that begin before weaning. In fact, weanling obese rats had increased FAS and decreased acyl-CoA oxidase and dehydrogenase activities. The combination of increased hepatic lipogenesis, reduced β-oxidation, and enhanced VLDL synthesis and secretion was sufficient to maintain normal hepatic lipid levels in obese rats at day 21. These decreased capacity of fat oxidation and increased fatty acid synthesis before weaning were maintained until adulthood whatever the diet fed. These continuing decreased fatty acid oxidation capacity and increased FA synthesis lead to enhanced hepatic lipid levels in adult offspring. In obese rats at day 90, liver steatosis was due to TG and PL accumulation. In our study, adult obese rats might respond to the increase in liver lipogenesis by an increase in VLDL production and secretion. Nevertheless, this process was not sufficient to prevent liver steatosis at day 90. In addition, one cause of increased hepatic lipid stores may be increases in serum flux of FFA to the liver that act as additional substrate. At day 90, obese rats had high FFA levels compared with control rats. Cafeteria diet at weaning also induced VLDL lipid and hepatic TG and PL accumulation in control rats and aggravated this accumulation in obese rats because OCAF rats presented the highest VLDL and liver lipids compared with the other groups of rats. The most commonly used plasma biochemical parameters to detect liver damage are ALT and AST as hepatocellular markers. The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver because these enzymes are cytoplasmically located and released into the blood after cell damage. Obese rats did not present significant alterations of AST and ALT, indicating that maternal and offspring's cafeteria diets did not cause damage in the hepatocytes. Our results are in agreement with those of MacQueen et al [42] who showed that, after long-term ingestion of a cafeteria diet by rats, the livers showed abnormal function without changes in transaminase activities.

In our study, pronounced changes in the fatty acid composition of VLDL and liver lipids were also observed in obese rats. These obese rats presented a significant decrease in liver and VLDL lipid PUFA contents, balanced by increases in SFA and MUFA. These observations are in agreement with data reported in obese patients [10-12]. A low P/S ratio correlated well with increased insulin resistance in obese rats.

High SFA levels were found in VLDL and hepatic TGs, PLs, and CEs in obese rats. Increased fatty acid synthesis and reduced fatty acid oxidation might explain the significant elevation of SFA proportions. In addition, obese rats had lower C20:4n-6 to C18:2n-6 ratio in VLDL and liver lipids resulting from the reduced conversion of C18:2n-6 to C20:4n-6, probably related to lower $\Delta 6$ and $\Delta 5$ desaturases activities. Insulin is known to induce $\Delta 6$ and $\Delta 5$ desaturation activity [43]. Similar changes in the fatty acid composition of plasma and liver lipids have been shown to occur in obesity [10-12]. The high ratio of oleic to stearic acid in obese rats indicated a high stearyl-CoA desaturase activity, which has previously been noted in obesity [44].

Cafeteria diet also altered the composition of VLDL and liver PL and TG by increasing SFA and MUFA contents at the expense of PUFA levels in control rats. These cafeteria diet–induced fatty acid alterations were also found by Darimont et al [29]. Indeed, the increase in MUFA and the reduction of PUFA proportions, measured in TG and PL, reflected the difference in fatty acid composition of the 2 diets. However, fatty acid composition of CEs was not

modified by cafeteria diet in control rats. It is well known that PLs mirror the dietary intake for recent months; TGs mirror the dietary intake of the last day, and CEs are more reflective of long-term intake [45]. Dietary fatty acids are known to influence the composition of stored TG and membrane PL in tissues [46]. Furthermore, the modifications of fatty acid concentrations and composition in tissue lipids induced by a high-fat diet has been associated with alterations in lipid metabolism and insulin sensitivity [17,20,39]. Indeed, enrichment of membrane PL with SFA was able to impair insulin action in skeletal muscle and adipose tissue, whereas a higher proportion of PUFA improved insulin sensitivity in these tissues [47]. In our study, high proportion of SFA and low PUFA in VLDL and hepatic lipids could also reflect membrane fatty acid composition of different tissues and could induce impairment in insulin action in rats fed cafeteria diet. In these cafeteria diet–fed rats, the P/S ratio in VLDL and liver lipids was significantly lower than in control diet–fed rats; and this was associated with a significantly higher Δ9 desaturation index. It is well known that diets rich in SFA induce, whereas dietary PUFA diminish, stearyl-CoA desaturase activity in rat liver [44]. Our results showed that fatty acid alterations were more dramatic in obese rats fed cafeteria diet (OCAF) compared with the other groups. These findings suggested that obese rats have preexisting fatty acid metabolism changes that were accentuated with cafeteria diet. Several recent reviews have examined the evidence that maternal fatty acid nutrition can affect metabolic development of offspring [25]. Our results also supported this notion. In obese rats, alterations in fatty acid metabolism were probably programmed in utero, related to permanent changes in the gene expression and metabolism of offspring [48]. We suggested then that maternal cafeteria diet induced a permanent reduction in hepatic β-oxidation and an increase in hepatic lipogenesis that caused liver steatosis and fatty acid alterations in the offspring. These preexisting alterations were accentuated when these rats were fed cafeteria diet at weaning.

In conclusion, maternal obesity and dietary fatty acid nutrition were deleterious to the offspring, affecting liver lipid and VLDL synthesis and secretion, and fatty acid metabolism. Maternal cafeteria feeding affected enzyme activities regulating hepatic fatty acid metabolism in the offspring. These abnormalities in offspring were worsened under a high-fat diet from weaning to adulthood. Nutritional recommendations in obesity must then target maternal and postnatal nutrition, especially fatty acid composition.

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