# Impaired Lipoprotein Metabolism in Obese Offspring of Streptozotocin-Induced Diabetic Rats

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**ABSTRACT:** The time course of changes in lipoprotein metabolism of obese offspring of mildly diabetic rats was studied with respect to serum lipoprotein composition as well as LCAT and tissue lipoprotein lipase (LPL) activities. Mild hyperglycemia in pregnant rats was induced by intraperitoneal injection of streptozotocin on day 5 of gestation. Control pregnant rats were injected with citrate buffer. At birth, obese pups had higher serum glucose, insulin, and lipoprotein (VLDL, LDL-HDL$_1$, HDL$_{2-3}$) levels than control pups. After 1 mon of life, all of these parameters in obese rats became similar to those of controls. However, LCAT, adipose tissue LPL, and hepatic triacylglycerol lipase activities were high. At 2 mon of age, VLDL-TAG levels were higher in obese females than in controls. By the age of 3 mon, obese offspring had developed insulin resistance with hyperglycemia, hyperinsulinemia, and higher serum lipoprotein concentrations. Indeed, qualitative abnormalities of lipoproteins were seen and were typical of obese and diabetic human beings. Fetal hyperinsulinemia should be considered as a risk factor for later metabolic diseases, including dyslipoproteinemia.

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Considerable interest has been generated over the last decade on the potential role of factors that disturb fetal growth in the development of chronic diseases including obesity, diabetes mellitus, and atherosclerosis in adulthood. Previous studies have found that fetal undernutrition as well as fetal overnutrition was associated with the acquisition of later risk factors for chronic diseases (1–4).

Maternal diabetes during pregnancy is an important risk factor for fetal overnutrition leading to fetal obesity, resulting from the combined effects of excessive transfer of maternal nutrients (glucose, amino acids, nonesterified FA) to fetal hyperinsulinemia (4–6). Fetal hyperinsulinemia was associated with the development of glucose intolerance, obesity, and diabetes during childhood and adulthood (1,3,7).

Obesity and diabetes mellitus are associated with compositional changes in serum lipoproteins (8–10). Plasma lipoprotein concentrations and compositions are determined by many factors. Both secretion by liver and intestine and uptake and degradation by specific or nonspecific pathways are involved. In addition, the combined action of several enzymes, such as lipoprotein lipase (LPL), hepatic triacylglycerol lipase (HTGL) and LCAT, on lipoproteins and movements of lipids and apolipoproteins (apo) in plasma also control the lipoprotein levels. It is of interest to determine if fetal hyperinsulinemia is a risk factor for later dyslipoproteinemia. In humans, follow-up studies are difficult to carryout, in part because of long periods and multiple factors affecting growth performance. Therefore, there is a need to establish an animal model for investigation of this issue. Previous studies reported that induction of hyperglycemia in pregnant rats, by streptozotocin injection on day 5 of gestation, resulted in fetal hyperglycemia, hyperinsulinemia, and fetal obesity (11–13). The obese rat pups of diabetic dams maintained accelerated body weight gain, had increased fat storage, and developed glucose intolerance by 12 wk of age (11–13), which reflected an interesting analogy to human observations. Although most of the authors have focused on altered glucose homeostasis in obese hyperinsulinemic offspring of diabetic mothers, lipoprotein profiles that might be indicative of metabolic diseases remain to be discovered.

The purpose of the present investigation was to determine the time course of changes in serum lipoprotein (VLDL, LDL-HDL$_1$, HDL$_{2-3}$) concentrations and compositions as well as serum LCAT and tissue lipolytic activities (liver, adipose tissue, muscle) in obese offspring of streptozotocin-induced mildly hyperglycemic rats. This study would advance our knowledge on the adverse consequences of maternal diabetes, on the offspring.

## MATERIALS AND METHODS

*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 were housed individually in wood-chip bedded plastic cages at constant temperature (25°C) and humidity (60 ± 5%) with a 12-h light-dark cycle. They had free access to water and a commercial diet (UAR, Villemoison-sur-Orge, France) containing 21% (w/w) protein, 4% (w/w) lipid, 53.5% (w/w) carbohydrate, 4.5% (w/w)

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Abbreviations: Apo, apolipoproteins; EC, esterified cholesterol; HTGL, hepatic triacylglycerol lipase; LPL, lipoprotein lipase; PL, phospholipid; TAG, triacylglycerol; UC, unesterified cholesterol.

fiber, 5% (w/w) minerals, and 2% (w/w) vitamins. A total of 30 pregnant rats were made diabetic by intraperitoneal injection of streptozotocin (40 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5, on the fifth day of gestation. Twelve pregnant dams were injected with citrate buffer alone as a control group. On days 13, 16, 18, and 20 of gestation, maternal blood was collected for plasma glucose concentrations by cutting off the tip of tail and squeezing it gently. Pregnant rats with plasma glucose levels between 5.55 and 16.65 mmol/L (compared with 5 mmol/L in controls) were designated as mildly hyperglycemic (13) and were included in the study. The success rate in obtaining these mildly hyperglycemic dams in the current series was 60%, or 18 of 30 streptozotocin-treated rats. A total of 150 pups from the 18 streptozotocin-treated dams and 110 pups from the 12 control dams were delivered spontaneously and weighed within 12 h. Pups from the streptozotocin-treated dams whose birth weights were 1.7 SD (above the 90th percentile) greater than the mean birth weight of the control pups were classified as obese pups (13) and included in the study. The mean birth weight of the control pups was 5.95 ± 0.45 g. Therefore, experimental pups with birth weights greater than 6.8 g were included as obese in the study. The success rate of obtained obese pups was 60% (90 out of 150). The mean birth weight of the obese pups was 8.10 ± 0.50 g. These obese pups were hyperglycemic and hyperinsulinemic at birth. The normal-sized offspring of diabetic mothers (60 out of 150) were excluded, as maternal diabetes related to fetal obesity was the criterion of our experimental population selection.

Twenty newborn rats of each group (control and experimental) were killed by decapitation, and blood was collected. To obtain sufficient serum samples for lipid and lipoprotein determinations, blood was pooled.

The remaining obese and control pups were left with their own mothers. Litter sizes were kept between six and eight pups per nursing dam to maintain a similar postnatal intake during the suckling period. Pups were weighed weekly up to 12 wk of age. The genders of the pups were identified at 3 wk by examining the external genitalia. Pups were weaned at 4 wk of age. Male and female rats were housed separately, two or three rats per cage, and allowed food (commercial diet, UAR) and water *ad libitum*. The general guidelines for the care and use of laboratory animals recommended by the Council of European Communities were followed.

*Blood and tissue samples*. At 4, 8, and 12 wk of age, after overnight fasting, eight rats from each group were anesthetized with pentobarbital (60 mg/kg body weight) and then bled from the abdominal aorta. Serum was obtained by low-speed centrifugation (1000 × *g*, 20 min) and preserved with 0.26 mmol/L EDTA and 3 mmol/L sodium azide. Liver, gastrocnemius muscle, and fat tissue surrounding the kidney and epididymal areas for the male rats or ovary and fallopian tubes for the female rats were removed, washed with cold saline, quickly blotted, and weighed. For lipolytic activity determination, tissue homogenates in 0.9% NaCl containing heparin (Sigma, St. Louis) were prepared as described by Mathe *et al.* (14) for liver, and by Inadera *et al.* (15) for adipose tissue and muscle.

*Isolation of lipoprotein fractions.* Serum lipoproteins of density <1.21 kg/L were isolated by single ultracentrifugation flotation (Model L8-55 ultracentrifuge, 50 Ti rotor; Beckman Instruments, Palo Alto, CA), according to Havel *et al.* (16). The three fractions (VLDL, LDL-$HDL_1$, $HDL_{2-3}$) were isolated from total lipoproteins by a single-spin discontinuous gradient according to the method of Redgrave *et al.* (17) as modified by Meghelli-Bouchenak *et al.* (18). Purified fractions of VLDL ($d < 1.006$), LDL-$HDL_1$ ($1.006 < d < 1.06$), and $HDL_{2-3}$ ($1.06 < d < 1.21$) were dialyzed against 0.15 mol/L NaCl and 1 mmol/L disodium EDTA, pH 7.4, at 4°C in spectra/por 2 dialysis tubing (Spectrum Laboratories, Rancho Dominguez, CA).

*Apo electrophoresis*. After partial delipidation, VLDL and $HDL_{2-3}$ apo were estimated by using a SDS-PAGE system with 2.5–20% acrylamide at 25 V for 18 h, according to Irwin *et al.* (19). After staining with Coomassie brilliant blue (G-250; Sigma, L'Isle d'Abeau, France), destained gels were scanned at 600 nm with a densitometer (model profil 26; Sébia, Issy les Moulineaux, France). Proportions of the various apo were determined from the densitometry tracings. Concentrations of each apo were calculated, based on the percentage of the area for each apo, relative to the total area for each serum sample. Data are expressed as arbitrary units (AU)/L serum. The apo samples for all groups of the rats were electrophoresed in parallel, but the staining affinity of each peptide was not determined. However, when 50–200 µg total protein was applied, the chromogenicity of each major band was shown to change linearly relative to the total amount of protein applied to the gel. To check the validity of our calculations, immunoelectrophoresis according to Laurell (20) was also used for apoA-I and B-100 quantitation.

*Chemical analysis.* Serum glucose was determined by glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA). Serum insulin was analyzed by radioimmunoassay kit (Serono Diagnostic, Braintree, MA) using rat insulin standards.

Lipoprotein-TAG, phospholipid (PL), total cholesterol, and unesterified cholesterol (UC) contents were determined by Boehringer Mannhein (Mannheim, Germany) kits, using enzymatic methods. Esterified cholesterol (EC) concentrations were obtained as the difference between total cholesterol and UC values. Total apo contents of each lipoprotein fraction were determined according to Lowry *et al.* (21).

*Assay for LCAT activity*. Serum LCAT activity was assayed by conversion of $^3$H-UC to $^3$H-EC, according to the method of Glomset and Wright (22), modified by Knipping (23), as previously described (24). Serum LCAT activity was expressed as nanomoles esterified cholesterol per hour per milliliter of serum.

*Lipolytic activities determination.* Tissue homogenates were centrifuged at 1500 × *g* for 5 min, and the supernatants containing heparin-releasable lipase were assayed for LPL or HTGL activities according to the method of Nilsson-Ehle and

Ekman (25). For HTGL assay, the concentration of NaCl in 100 µL of liver supernatant (the enzyme source) was adjusted to 1 mol/L NaCl, and the solution was incubated at 37°C, for 1 h, with 100 µL of [$^3$H]triolein emulsion substrate (final concentrations: 1.42 mmol/L triolein, 0.1 mmol/L lysophosphatidylcholine, 0.2% (wt/vol) albumin, 0.1 mmol/L Tris-HCl, pH 9, 0.5 mol/L NaCl). For adipose tissue or muscle LPL determinations, the incubation medium contained 1.42 mmol/L triolein, 0.1 mmol/L lysophosphatidylcholine, 0.2% (wt/vol) albumin, 5% (vol/vol) heart-inactived serum (providing apoC-II, an activator of LPL), 0.1 mol/L Tris-HCl (pH 8), 0.15 mol/L NaCl. At the end of the incubation period, the FA released were extracted with chloroform/methanol/heptane (1.25:1.41:1, by vol) followed by 0.1 mol/L potassium carbonate/borate buffer, pH 10.5. $^3$H 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 scintillation counter (Beckman, Palo Alto, CA). Enzyme activity was expressed as nanomoles of FA released per minute per milligram protein.

*Statistical analysis.* Results are expressed as means ± SD. The significance of differences among groups was analyzed statistically by ANOVA, followed by Duncan's multiple-range test (26) for parameter changes with age. The significance of differences between obese and control rats at each age was assessed using Student's *t*-test. These calculations were performed using Statistica, Version 4.1 (Statsoft, Tulsa, OK). Differences were considered statistically significant at $P < 0.05$.

## RESULTS

*Serum glucose and insulin concentrations, and body weight.* At birth, serum glucose and insulin concentrations were higher in obese pups than in controls (1.10 ± 0.20 vs. 0.64 ± 0.11 g/L and 95.3 ± 13.2 vs. 48 ± 10.6 pmol/L, respectively). These values were similar for obese and control rats at 30 and 60 d (results not shown). At day 90, however, obese rats, both males and females, showed higher glucose and insulin levels compared to controls (1.59 ± 0.24 vs. 0.91 ± 0.25 g/L and 284.5 ± 55.7 vs. 187.6 ± 35.6 pmol/L, respectively).

In addition, obese offspring of diabetic dams (both males and females) had significantly higher body weights than controls throughout the first 12 wk of life (results not shown). However, the normal-sized offspring of diabetic mothers (excluded from the present study) were not hyperinsulinemic at birth, had normal growth rates, and showed no significant differences from controls for all parameters studied (results not shown).

*VLDL mass and lipid concentrations.* VLDL mass and lipid contents increased with age in all groups with marked variations during the first month of life. At birth, VLDL mass and VLDL TAG were higher in obese pups than in controls (Table 1). At day 30 and day 60, VLDL mass and lipid contents were similar in control and obese male rats. However, at day 60,

**TABLE 1**
**Postnatal Changes in VLDL Mass and Lipid Contents of Obese and Control Rats[a]**

| | Male rats | | Female rats | |
|---|---|---|---|---|
| | Control | Obese | Control | Obese |
| VLDL mass (g/L) | | | | |
| D0 | 0.26 ± 0.14$^{c}$ | 0.38 ± 0.05$^{d,*}$ | 0.26 ± 0.14$^{c}$ | 0.38 ± 0.05$^{d,*}$ |
| D30 | 1.04 ± 0.42$^{b}$ | 1.14 ± 0.44$^{c}$ | 0.99 ± 0.16$^{b}$ | 1.08 ± 0.33$^{c}$ |
| D60 | 1.26 ± 0.22$^{a}$ | 1.36 ± 0.19$^{b}$ | 1.21 ± 0.36$^{a}$ | 1.68 ± 0.16$^{b,*}$ |
| D90 | 1.28 ± 0.47$^{a}$ | 2.08 ± 0.16$^{a,**}$ | 1.28 ± 0.39$^{a}$ | 2.40 ± 0.16$^{a,**}$ |
| TAG (mmol/L) | | | | |
| D0 | 0.10 ± 0.02$^{c}$ | 0.16 ± 0.05$^{d,*}$ | 0.10 ± 0.02$^{c}$ | 0.16 ± 0.05$^{c,*}$ |
| D30 | 0.60 ± 0.33$^{b}$ | 0.58 ± 0.39$^{c}$ | 0.46 ± 0.16$^{b}$ | 0.44 ± 0.19$^{b}$ |
| D60 | 0.80 ± 0.44$^{a}$ | 0.78 ± 0.19$^{b}$ | 0.64 ± 0.08$^{a}$ | 1.15 ± 0.11$^{a,**}$ |
| D90 | 0.81 ± 0.05$^{a}$ | 1.12 ± 0.11$^{a,*}$ | 0.66 ± 0.33$^{a}$ | 1.17 ± 0.16$^{a,**}$ |
| Phospholipids (mmol/L) | | | | |
| D0 | 0.06 ± 0.05$^{c}$ | 0.09 ± 0.08$^{c}$ | 0.06 ± 0.05$^{c}$ | 0.09 ± 0.08$^{c}$ |
| D30 | 0.27 ± 0.05$^{b}$ | 0.36 ± 0.19$^{b}$ | 0.26 ± 0.08$^{b}$ | 0.40 ± 0.22$^{b}$ |
| D60 | 0.32 ± 0.08$^{a}$ | 0.37 ± 0.11$^{b}$ | 0.31 ± 0.05$^{a}$ | 0.36 ± 0.16$^{b}$ |
| D90 | 0.36 ± 0.11$^{a}$ | 0.52 ± 0.11$^{a,*}$ | 0.34 ± 0.11$^{a}$ | 0.54 ± 0.02$^{a,*}$ |
| Unesterified cholesterol (mmol/L) | | | | |
| D0 | 0.03 ± 0.02$^{b}$ | 0.04 ± 0.02$^{c}$ | 0.03 ± 0.02$^{b}$ | 0.04 ± 0.02$^{c}$ |
| D30 | 0.06 ± 0.02$^{a}$ | 0.08 ± 0.05$^{b}$ | 0.05 ± 0.02$^{a}$ | 0.07 ± 0.02$^{b}$ |
| D60 | 0.07 ± 0.02$^{a}$ | 0.08 ± 0.08$^{b}$ | 0.06 ± 0.02$^{a}$ | 0.10 ± 0.14$^{a}$ |
| D90 | 0.08 ± 0.05a | 0.13 ± 0.02$^{a,*}$ | 0.08 ± 0.08$^{a}$ | 0.14 ± 0.02$^{a,*}$ |
| Esterified cholesterol (mmol/L) | | | | |
| D0 | 0.03 ± 0.02$^{b}$ | 0.03 ± 0.02$^{c}$ | 0.03 ± 0.02$^{c}$ | 0.03 ± 0.02$^{c}$ |
| D30 | 0.12 ± 0.08$^{a}$ | 0.20 ± 0.16$^{b}$ | 0.09 ± 0.14$^{b}$ | 0.18 ± 0.08$^{b}$ |
| D60 | 0.13 ± 0.11$^{a}$ | 0.21 ± 0.16$^{b}$ | 0.11 ± 0.16$^{b}$ | 0.24 ± 0.15$^{b}$ |
| D90 | 0.15 ± 0.05$^{a}$ | 0.30 ± 0.02$^{a,*}$ | 0.20 ± 0.11$^{a}$ | 0.65 ± 0.22$^{a,*}$ |

[a]Values are means ± SD of eight rats from each group. Males and females constitute separate groups. At day 0, the values for both control and obese rats are the same in both males and females, as gender could not be identified at this stage. Significant differences between obese and control rats at each age are indicated as: $*P < 0.05$; $**P < 0.01$. Means within the same group with different superscript letters (a–d) are significantly different according to age for each group ($P < 0.05$).

**TABLE 2**
**Postnatal Changes in LDL-HDL$_1$ Mass and Lipid Contents of Obese and Control Rats[a]**

| | Male rats | | Female rats | |
|---|---|---|---|---|
| | Control | Obese | Control | Obese |
| LDL-HDL$_1$ mass (g/L) | | | | |
| D0 | 0.86 ± 0.08[a] | 1.30 ± 0.11[b,**] | 0.86 ± 0.08[a] | 1.30 ± 0.11[b,**] |
| D30 | 0.68 ± 0.22[b] | 0.74 ± 0.25[c] | 0.74 ± 0.16[b] | 0.73 ± 0.08[d] |
| D60 | 0.66 ± 0.42[b] | 0.83 ± 0.30[c] | 0.75 ± 0.36[b] | 0.98 ± 0.22[c] |
| D90 | 0.69 ± 0.14[b] | 1.54 ± 0.22[a,**] | 0.76 ± 0.11[b] | 1.48 ± 0.16[a,**] |
| TAG (mmol/L) | | | | |
| D0 | 0.13 ± 0.05[a] | 0.23 ± 0.08[a,*] | 0.13 ± 0.05[a] | 0.23 ± 0.08[a,*] |
| D30 | 0.04 ± 0.02[b] | 0.03 ± 0.02[c] | 0.06 ± 0.05[b] | 0.05 ± 0.05[b] |
| D60 | 0.02 ± 0.02[b] | 0.02 ± 0.02[c] | 0.03 ± 0.02[b] | 0.02 ± 0.02[c] |
| D90 | 0.02 ± 0.02[b] | 0.06 ± 0.02[b,*] | 0.02 ± 0.02[b] | 0.06 ± 0.02[b,*] |
| Phospholipids (mmol/L) | | | | |
| D0 | 0.26 ± 0.08 | 0.37 ± 0.11[b,*] | 0.26 ± 0.08 | 0.37 ± 0.11[b,*] |
| D30 | 0.26 ± 0.16 | 0.36 ± 0.28[b] | 0.30 ± 0.22 | 0.31 ± 0.16[b] |
| D60 | 0.28 ± 0.28 | 0.27 ± 0.25[b] | 0.28 ± 0.16 | 0.40 ± 0.14[b] |
| D90 | 0.31 ± 0.11 | 0.78 ± 0.33[a,*] | 0.31 ± 0.28 | 0.60 ± 0.16[a,*] |
| Unesterified cholesterol (mmol/L) | | | | |
| D0 | 0.21 ± 0.02 | 0.30 ± 0.05[b,*] | 0.21 ± 0.02 | 0.30 ± 0.05[b,*] |
| D30 | 0.19 ± 0.16 | 0.24 ± 0.14[b] | 0.18 ± 0.05 | 0.26 ± 0.08[b] |
| D60 | 0.19 ± 0.14 | 0.31 ± 0.16[b] | 0.17 ± 0.22 | 0.32 ± 0.16[b] |
| D90 | 0.16 ± 0.08 | 0.42 ± 0.16[a,*] | 0.20 ± 0.08 | 0.47 ± 0.11[a,*] |
| Esterified cholesterol (mmol/L) | | | | |
| D0 | 0.14 ± 0.05[c] | 0.22 ± 0.05[d,*] | 0.14 ± 0.05[c] | 0.22 ± 0.05[d,*] |
| D30 | 0.30 ± 0.08[b] | 0.32 ± 0.14[c] | 0.28 ± 0.11[b] | 0.34 ± 0.16[c] |
| D60 | 0.41 ± 0.28[a] | 0.49 ± 0.16[b] | 0.55 ± 0.42[a] | 0.59 ± 0.28[b] |
| D90 | 0.36 ± 0.11[a] | 0.60 ± 0.16[a,*] | 0.46 ± 0.22[a] | 0.79 ± 0.16[a,*] |

[a]For footnote see Table 1.

VLDL mass and TAG were significantly higher in obese females than in control females (+40 and +100, respectively). At day 90, VLDL levels and all components of VLDL were significantly greater in obese rats, both males and females, compared to controls. In terms of percentage, the relative composition of VLDL in obese males was close to that of control males, suggesting an increase in VLDL particles at day 90. However, in obese females, the surface to core components [(protein + PL + UC)/(TG + EC)] and EC to triacylglycerols (EC/TG) ratios were higher than control values (1.38 ± 0.05 and 0.42 ± 0.05, respectively, in obese females vs. 1.20 ± 0.04 and 0.22 ± 0.06, respectively in control females). These data suggested an increase in VLDL particles that were enriched in EC in obese females at day 90.

*LDL-HDL$_1$ mass and lipid concentrations.* Serum LDL-HDL$_1$ levels showed a significant decrease in the course of the first month of life in both control and obese rats, accompanied by compositional changes (Table 2). In fact, LDL-HDL$_1$ particles became enriched in EC and depleted in TAG. Afterward, LDL-HDL$_1$ levels and composition remained constant in control males and females. In contrast, a significant increase in all lipid contents of LDL-HDL$_1$ was observed between day 30 and day 90 in obese offspring, both males and females.

At birth, LDL-HDL$_1$ amounts were significantly higher in obese pups compared to control values (+50%), as a result of an increase in all LDL-HDL$_1$ components. At day 30 and day 60, no significant differences in LDL-HDL$_1$ particles were observed among rat groups. At day 90, all lipid contents of LDL-HDL$_1$ were greater in obese rats than in controls. As the relative composition of these fractions was not modified, the results suggested an increase in LDL-HDL$_1$ particles in adult obese rats.

*HDL$_{2-3}$ mass and lipid concentrations.* During the first month of life, HDL$_{2-3}$ levels increased in both obese and control rats due to a rise in PL, UC, and EC contents (Table 3). Afterward, the rise in HDL$_{2-3}$ amounts was reflected mainly by higher PL in control rats, by TAG and PL in obese males, and by TAG, PL, UC, and EC in obese females.

At birth, obese pups had higher HDL$_{2-3}$ amounts compared to controls (+39%). In addition, all apo and lipids of HDL$_{2-3}$ were increased, suggesting an increase in HDL particles in obese pups. At day 30 and day 60, HDL$_{2-3}$ lipid and apo levels became similar in control and obese rats. However, at day 90, HDL$_{2-3}$ amounts were significantly higher in obese males and females than in controls (32 and 33%, respectively). Indeed, HDL$_{2-3}$-PL, TAG, and EC levels were higher in obese males and females than in their respective controls at day 90. The surface to core components ratio was higher in obese than in control males (0.32 ± 0.04 vs. 0.24 ± 0.03) and in obese than in control females (0.35 ± 0.05 vs. 0.26 ± 0.04), suggesting a modification of HDL relative composition in adult obese rats.

*VLDL and HDL$_{2-3}$ apo. (i) VLDL apo.* A significant age-related increase in VLDL-apo concentrations was observed in all groups (Table 4). There were no significant differences in VLDL-apo between obese and control groups at day 0, and

**TABLE 3**
**Postnatal Changes in $HDL_{2-3}$ Mass and Lipid Contents of Obese and Control Rats[a]**

| | Male rats | | Female rats | |
|---|---|---|---|---|
| | Control | Obese | Control | Obese |
| $HDL_{2-3}$ mass (g/L) | | | | |
| D0 | 1.12 ± 0.05[c] | 1.56 ± 0.14[d,**] | 1.12 ± 0.05[c] | 1.56 ± 0.14[d,**] |
| D30 | 2.86 ± 0.44[b] | 3.04 ± 0.42[c] | 2.80 ± 0.53[b] | 2.90 ± 0.72[c] |
| D60 | 3.36 ± 1.09[a] | 3.68 ± 0.67[b] | 3.25 ± 0.95[a] | 3.75 ± 0.42[b] |
| D90 | 3.40 ± 0.44[a] | 4.48 ± 0.25[a,**] | 3.48 ± 0.30[a] | 4.62 ± 0.67[a,**] |
| TAG (mmol/L) | | | | |
| D0 | 0.09 ± 0.02[a] | 0.16 ± 0.05[a,*] | 0.09 ± 0.02[a] | 0.16 ± 0.05[a,*] |
| D30 | 0.06 ± 0.02[b] | 0.06 ± 0.02[b] | 0.05 ± 0.05[b] | 0.08 ± 0.05[b] |
| D60 | 0.05 ± 0.02[b] | 0.08 ± 0.02[b] | 0.05 ± 0.02[b] | 0.07 ± 0.05[b] |
| D90 | 0.05 ± 0.02[b] | 0.13 ± 0.05[a,**] | 0.04 ± 0.05[b] | 0.14 ± 0.08[a,**] |
| Phospholipids (mmol/L) | | | | |
| D0 | 0.39 ± 0.14[c] | 0.50 ± 0.05[d,*] | 0.39 ± 0.14[c] | 0.50 ± 0.05[d,*] |
| D30 | 0.93 ± 0.14[b] | 1.01 ± 0.08[b] | 1.00 ± 0.11[b] | 0.92 ± 0.30[c] |
| D60 | 1.34 ± 0.44[a] | 1.37 ± 0.53[b] | 1.20 ± 0.28[a] | 1.32 ± 0.33[b] |
| D90 | 1.39 ± 0.53[a] | 2.02 ± 0.44[a,*] | 1.40 ± 0.39[a] | 1.82 ± 0.42[a,*] |
| Unesterified cholesterol (mmol/L) | | | | |
| D0 | 0.28 ± 0.05[b] | 0.38 ± 0.05[b,*] | 0.28 ± 0.05[b] | 0.38 ± 0.05[d,*] |
| D30 | 0.53 ± 0.44[a] | 0.68 ± 0.30[a] | 0.50 ± 0.39[a] | 0.48 ± 0.08[c] |
| D60 | 0.63 ± 0.33[a] | 0.83 ± 0.36[a] | 0.65 ± 0.50[a] | 0.67 ± 0.28[b] |
| D90 | 0.71 ± 0.44[a] | 0.87 ± 0.42[a] | 0.80 ± 0.44[a] | 0.91 ± 0.39[a] |
| Esterified cholesterol (mmol/L) | | | | |
| D0 | 0.10 ± 0.05[b] | 0.22 ± 0.02[c,*] | 0.10 ± 0.05[b] | 0.22 ± 0.02[d,*] |
| D30 | 0.93 ± 0.36[a] | 0.96 ± 0.28[b] | 0.75 ± 0.50[a] | 0.70 ± 0.30[c] |
| D60 | 1.18 ± 1.03[a] | 1.22 ± 0.44[a] | 0.96 ± 0.30[a] | 1.27 ± 0.61[b] |
| D90 | 0.95 ± 0.33[a] | 1.42 ± 0.72[a,**] | 1.08 ± 0.25[a] | 1.71 ± 0.39[a,**] |

[a]For footnote see Table 1.

also at day 30 and day 60 (results not shown). At day 90, VLDL-apoB-100, -apoB-48, -apoE, -apoC-II and -apoC-III were markedly increased in both male and female obese rats, compared with controls. In addition, the apoC-II to apoC-III ratio in VLDL was higher in obese rats than in controls (0.50 ± 0.03 vs. 0.37 ± 0.02 in males and 0.52 ± 0.04 vs. 0.38 ± 0.02 in females).

*(ii) $HDL_{2-3}$ apo.* $HDL_{2-3}$ apoA-I, -A-II, -A-IV, -C-II, and -C-III increased with age in both obese and control pups (Table 4). $HDL_{2-3}$ apoE levels did not vary significantly with age in any group. At birth, $HDL_{2-3}$ apoA-I, -A-II, -A-IV, -C-II, -C-III, and -E concentrations were higher in obese pups than in controls. At day 30 and day 60, no significant differences in $HDL_{2-3}$ apo profiles were observed between obese and control rats (results not shown). At day 90, $HDL_{2-3}$ apoC-II concentrations were greater in obese males and females compared to their respective controls (+91 and +90%, respectively). Indeed, the apoC-II/apoC-III ratio in $HDL_{2-3}$ was increased in obese offspring compared with controls (0.74 ± 0.03 vs. 0.39 ± 0.04 in males and 0.65 ± 0.04 vs. 0.42 ± 0.02 in females).

*Serum LCAT activity.* LCAT activity increased gradually with age in both obese and control rats (Fig. 1). At birth, LCAT activity was significantly greater in obese pups than in controls (+57%). At days 30, 60, and 90, this activity was about 1.5-fold higher in both male and female obese rats compared with controls.

*Tissue lipolytic activities.* Because tissue lipolytic activities are low at birth, developmental changes in LPL activities were monitored after 1 mon of life. Adipose tissue LPL, as well as HTGL activities, decreased gradually with age in all groups (Fig. 2). Adipose tissue LPL activity, expressed as nmol FFA·$min^{-1}$·$mg^{-1}$protein), was significantly greater in both male and female obese rats than in their respective controls at day 30 and day 60. However, at day 90, values were similar in all groups. No difference in muscle LPL activity was observed between obese and control rats, whatever the age (results not shown). In contrast, HTGL activity was significantly greater in obese rats at all ages.

## DISCUSSION

Offspring of mildly diabetic rats were obese, hyperinsulinemic, and hyperlipidemic at birth and gained significantly more weight than control offspring (results not shown). The increased body weight was reflected by increased adipose tissue weight in these offspring (both males and females) at all ages (results not shown). Thus, the increase in adiposity seen in either male or female offspring of diabetic mothers was maintained throughout adulthood. However, the posnatal metabolic changes varied according to sex and age.

Our results showed that at birth, obese rats had higher VLDL amounts, which were accompanied by higher VLDL-TAG concentrations than control values, suggesting an

**TABLE 4**
**Postnatal Changes in VLDL and $HDL_{2-3}$ Apolipoproteins of Obese and Control Rats[a,b]**

| | Male rats | | Female rats | |
|---|---|---|---|---|
| | Control | Obese | Control | Obese |
| VLDL apoproteins | | | | |
| ApoB-100 D0 | 40.31 ± 11.48[b] | 48.90 ± 13.96[b] | 40.31 ± 11.48[b] | 48.90 ± 13.96[b] |
| D90 | 90 ± 12.43[a] | 140 ± 11.68[a,**] | 101.60 ± 14.42[a] | 181.12 ± 12.15[a,**] |
| ApoB-48 D0 | 16.40 ± 4.63[b] | 18.90 ± 5.18[b] | 16.40 ± 4.63[b] | 18.90 ± 5.18[b] |
| D90 | 43.12 ± 8.90[a] | 91.92 ± 9.81[a,**] | 48.83 ± 7.53[a] | 95.33 ± 8.93[a,**] |
| ApoE D0 | 21.30 ± 6.77[b] | 26.92 ± 5.63[b] | 21.30 ± 6.77[b] | 26.92 ± 5.63[b] |
| D90 | 62.15 ± 8.15[a] | 108.40 ± 11.53[a,**] | 62.10 ± 6.61[a] | 110.21 ± 9.30[a,**] |
| ApoC-II D0 | 4.80 ± 1.57[b] | 6.70 ± 2.64[b] | 4.80 ± 1.57[b] | 6.70 ± 2.64[b] |
| D90 | 19.01 ± 3.15[a] | 39.42 ± 7.66[a,**] | 18.22 ± 3.08[a] | 36.40 ± 5.94[a,**] |
| ApoC-III D0 | 4.80 ± 1.97[b] | 7.73 ± 2.89[b] | 4.80 ± 1.97[b] | 7.73 ± 2.89[b] |
| D90 | 45.93 ± 6.75[a] | 80.30 ± 9.48[a,**] | 49.27 ± 5.11[a] | 81.01 ± 6.32[a,**] |
| $HDL_{2-3}$ apoproteins | | | | |
| ApoA-I D0 | 181.31 ± 11.42[b] | 246.90 ± 22.64[b,*] | 181.31 ± 11.42[b] | 246.90 ± 22.64[b,*] |
| D90 | 564.81 ± 60.11[a] | 609.88 ± 58.70[a] | 589.70 ± 70.18[a] | 646.87 ± 80.20[a] |
| ApoA-II D0 | 22.40 ± 3.18[b] | 40.61 ± 6.43[b,*] | 22.40 ± 3.18[b] | 40.61 ± 6.43[b,*] |
| D90 | 61.10 ± 21.43[a] | 64.80 ± 19.11[a] | 69.31 ± 24.78[a] | 96.47 ± 35.16[a] |
| ApoA-IV D0 | 63.54 ± 11.14[b] | 117.38 ± 21.38[b,*] | 63.54 ± 11.14[b] | 117.38 ± 21.38[b,*] |
| D90 | 251.80 ± 50.64[a] | 250.55 ± 42.33[a] | 201.10 ± 38.64[a] | 234.81 ± 55.36[a] |
| ApoE D0 | 201.68 ± 8.11 | 237.24 ± 9.48* | 201.68 ± 8.11 | 237.24 ± 9.48* |
| D90 | 244.78 ± 30.14 | 247.70 ± 20.10 | 229.60 ± 30.26 | 269.50 ± 40.50 |
| ApoC-II D0 | 30.20 ± 4.15[b] | 41.76 ± 5.98[b,*] | 30.20 ± 4.15[b] | 41.76 ± 5.98[b,*] |
| D90 | 81.30 ± 10.06[a] | 155.60 ± 14.11[a,**] | 86.70 ± 15.64[a] | 165.31 ± 18.11[a,**] |
| ApoC-III D0 | 50.87 ± 4.14[b] | 61.11 ± 5.73[b,*] | 50.87 ± 4.14[b] | 61.11 ± 5.73[b,*] |
| D90 | 206.20 ± 25.38[a] | 211.40 ± 30.14[a] | 213.58 ± 36.95[a] | 256.90 ± 47.86[a] |

[a]Values are means ± SD of eight rats from each group. Males and females constitute separate groups. At day 0, the values for both control and obese rats are the same in both males and females as gender could not be identified at this stage. Significant differences between obese and control rats at each age are indicated as: *$P < 0.05$; **$P < 0.01$. Means within the same group with different superscript letters (a,b) are significantly different according to age for each group ($P < 0.05$).

[b]Expressed as AU/L (arbitrary units/L serum).

increase in hepatic TAG synthesis and secretion. Combined effects of fetal hyperinsulinemia and an enhancement in glucose and FFA transfer in the diabetic mother could explain elevated fetal hepatic VLDL secretion and hypertriglyceridemia, as reported by previous studies (27–29). At birth, obese offspring of diabetic dams also presented higher LDL-$HDL_1$ and $HDL_{2-3}$ levels, accompanied by higher lipid and apo contents of these fractions, suggesting an increase in the number of LDL-$HDL_1$ and $HDL_{2-3}$ particles, resulting probably from their enhanced synthesis. Since the HDL fraction is primarily responsible for lipid transport during fetal life in humans (30) and in rats (31), elevated HDL contents might reflect an increase in the requirement for cholesterol and PL for plasma membrane, hormone, and surfactant synthesis in obese fetuses. It is well known that high insulin levels lead to enhanced growth of fetal tissues (6). We have previously described similar results in human macrosomic newborns of type 1 diabetic mothers (32,33).

In this study, after 1 mon of life (day 30), which corresponded to the suckling period, serum glucose, insulin, and lipoprotein concentrations and compositions in male and female obese offspring of diabetic dams became similar to those of their respective controls. However, serum LCAT, adipose tissue LPL, and HTGL activities were higher in obese than in control rats. The early elevation of adipose tissue LPL activity associated with normal muscle LPL activity was a contributory factor to the maintenance of obesity in offspring of diabetic rats. High LPL and HTGL activities are normally associated with enhanced lipoprotein and remnant catabolism, resulting especially in low TAG and VLDL levels, and

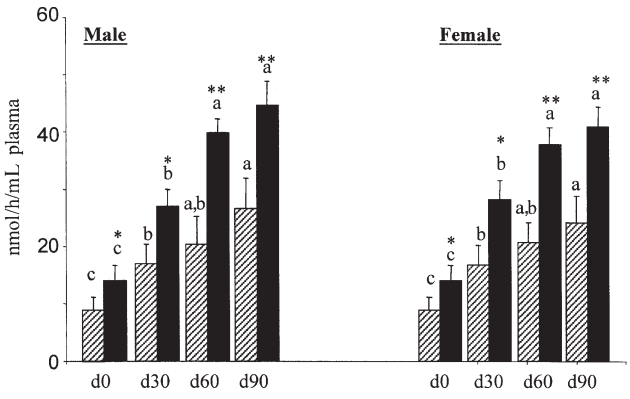


**FIG. 1.** LCAT activity of rat sera. Values are means ± SD of eight rats from each group. Males and females constitute separate groups. At day 0 (d0), the values for both control and obese rats are the same in both males and females as gender could not be identified at this stage. Significant differences between obese and control rats at each age are indicated as: *$P < 0.05$, **$P < 0.01$. Means within the same group with different superscript letters (a–c) are significantly different according to age for each group ($P < 0.05$). Hatched and filled histograms represent, respectively, control and obese rats.

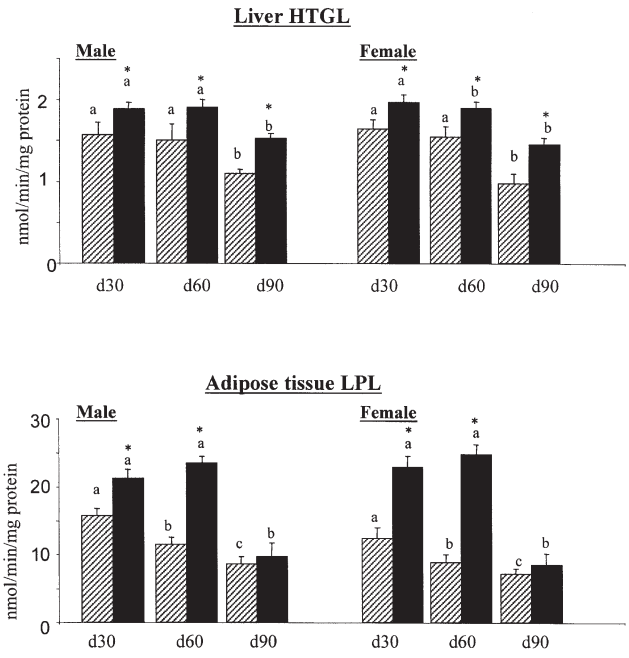


**FIG. 2.** Adipose tissue lipoprotein lipase (LPL) and hepatic TG lipase (HTGL) activities in obese and control rats. Values are means ± SD of eight rats from each group. Males and females constitute separate groups. Significant differences between obese and control rats at each age are indicated as: $*P < 0.05$, $**P < 0.01$. Means within the same group with different superscript letters (a–c) are significantly different according to age for each group ($P < 0.05$). Hatched and filled histograms represent, respectively, control and obese rats.

in high LDL and $HDL_1$ levels in the rats (14). These correlations were not found in our obese rats at day 30. On the other hand, the well-known correlation between LCAT activity and HDL levels also was not seen in these rats. These findings suggest that an increased rate of lipoprotein synthesis combined with an enhanced lipoprotein catabolism in obese offspring of diabetic rats might contribute to maintaining a normal lipoprotein profile at day 30. A higher demand for lipid during the phase of rapid growth could explain high lipoprotein turnover, knowing that most of the obese rats tissues were hypertrophic. Indeed, an increased insulin sensitivity could be envisaged, as suggested by other works (6,7). Therefore, persistence of a higher number of insulin receptors and/or greater insulin-binding affinity in the target tissue may contribute to enhanced anabolic effects despite normal serum insulin levels in these obese rats at day 30.

After 2 mon of life (day 60), which corresponded to 1 mon postweaning period, the obese rats again showed serum glucose, insulin, and lipoprotein levels similar to those in controls, except for VLDL-TAG levels, which were enhanced in obese females compared to control females. This could be the result of increased estrogen levels in obese females, since estrogen enhances hepatic TAG production and secretion (34).

After 3 mon of life (day 90), a period reflecting adulthood, the situation was different. Some metabolic alterations that had disappeared at birth and that had subsequently disappeared at day 30 and day 60 were present and were worsened. Adult obese offspring (both males and females) had elevated glucose, insulin, and lipoprotein levels compared to controls. They also displayed significant increases in LCAT and HTGL activities. In these adult obese rats, adipose tissue LPL activity became similar to that found in adult control rats. Taken together, the results strongly suggested that obese offspring of diabetic dams developed insulin resistance in adulthood, in agreement with other studies (11,13). It is well known that the development of obesity is linked with insulin sensitivity, whereas weight maintenance in the obese state is associated with insulin resistance (35). In several animal models, obesity and adipocyte hypertrophy precede the development of insulin resistance (36,37). At day 90, adult obese offspring of diabetic dams presented both quantitative and qualitative abnormalities of different lipoproteins. Compositional changes were more apparent when composition was expressed as the percentage of total lipoprotein mass rather than absolute concentration of the components. Adult obese rats showed high VLDL concentrations, accompanied by a concomitant increase in all VLDL-apo and lipid components, suggesting elevated VLDL particle numbers. Overproduction of VLDL, a common feature of human and various experimental obesities, is a direct consequence of hyperinsulinemia and hepatic hyperlipogenesis (8,9,36,38). Indeed, in obese females, VLDL particles were enriched in EC at day 90. This compositional abnormality is well documented in human diabetes and results from increased lipid transfer between VLDL and HDL (8,9). However, in the rat, which has low EC transfer protein activity, HDL concentrations would not be directly affected by interactions between HDL and TAG-rich lipoproteins. Thus, the increased cholesterol content in the VLDL fraction could reflect VLDL hepatic synthesis rich in cholesterol. These adult obese rats also presented enhanced $HDL_{2\text{-}3}$ levels. In contrast to reduced HDL concentrations observed in obesity and diabetes in man (9,10,39), a positive correlation between adiposity and HDL levels was seen in several animal models of obesity (40,41). Adult obese offspring of diabetic dams also presented compositional changes of $HDL_{2\text{-}3}$ particles, including enrichment in TAG, cholesterol, and PL.

In our study, adult obese offspring had high HDL-apoC-II contents compared to control values. Indeed, they also had high VLDL-apoC-II and -apoC-III concentrations. The apoC-II to apoC-III ratio was increased in both VLDL and HDL particles of these adult obese rats compared to control values. Since apoC-II is an activator of LPL and apoC-III is an inhibitor of LPL, high proportions of apoC-II compared to apoC-III in obese offspring at day 90 could be a physiological compensatory mechanism to establish TAG homeostasis during a period related to insulin resistance. In conclusion, obese offspring of mildly diabetic rats presented altered lipoprotein metabolism at birth and at adulthood.

Developmental lipoprotein profile changes in offspring of diabetic dams were characterized by two periods. A first period (days 0–60), with increased insulin sensitivity, was

associated with increased fat storage and high lipoprotein turnover leading to a normalization of lipoprotein profiles. A second period (day 90), with decreased insulin sensitivity and the appearance of insulin resistance, was associated with circulating lipoprotein abnormalities. Fetal hyperinsulinemia in diabetic pregnancy should then be considered as one of the potential risk factors for later metabolic diseases, including adult dyslipoproteinemia. Attention needs to be focused on the disordered lipoprotein profile in offspring of diabetic mothers.

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