Age-related changes in cholesterol metabolism in macrosomic offspring of rats with streptozotocin-induced diabetes

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Abstract **The aim of this study was to determine the impact of diabetic macrosomia on cholesterol and lipoprotein metabolism. Age-related changes in the activities of serum LCAT, hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT, the major enzymes involved in cholesterol metabolism, were determined in macrosomic offspring of streptozotocin-induced diabetic rats. Hepatic, serum, and lipoprotein cholesterol contents were also examined. Mild hyperglycemia in pregnant rats was induced by intraperitoneal injection of streptozotocin (40 mg/kg body weight) on day 5 of gestation. Control pregnant rats were injected with citrate buffer. At birth, macrosomic pups had higher serum, LDL-HDL$_1$, and HDL$_{2\text{-}3}$ cholesterol levels ($P < 0.05$) associated with increased LCAT activity (+57%) compared with control values. At 1 and 2 months of life, serum and lipoprotein cholesterol concentrations in macrosomic rats were similar to those of controls, whereas LCAT activity remained elevated about 1.5-fold. In addition, there was no change in hepatic cholesterol contents but hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT activities were higher in both macrosomic males and females than in their respective controls ($P < 0.01$). By 3 months, macrosomic rats had developed hypercholesterolemia with a rise in all lipoproteins. Enzyme activities were still increased in these mature macrosomic rats, and hepatic cholesteryl esters were higher only in macrosomic females. These data demonstrate an overproduction, combined with overutilization, of cholesterol during the phase of rapid growth in macrosomic rats. However, cholesterol oversynthesis exceeded its removal and was a major contributor to hypercholesterolemia in adult macrosomic rats. In conclusion, macrosomia was associated with alterations in cholesterol metabolism through adulthood.**—Merzouk, H., S. Madani, A. Boualga, J. Prost, M. Bouchenak, and J. Belleville. **Age-related changes in cholesterol metabolism in macrosomic offspring of rats with streptozotocin-induced diabetes. *J. Lipid Res.* 2001.** 42: **1152–1159.**

Supplementary key words maternal diabetes • macrosomia • LCAT • ACAT • cholesterol 7α-hydroxylase • HMG-CoA reductase

Infants of diabetic mothers are at risk for neonatal macrosomia (1). Several alterations in carbohydrate and lipid metabolism are observed in these macrosomic infants and are thought to be a consequence of maternal hyperglycemia leading to fetal hyperglycemia and hyperinsulinemia (2–4). Neonatal macrosomia is associated with increased incidence of obesity during adolescence and diabetes mellitus during adulthood (5, 6). We have previously used an animal model to explore the association between increased birth weight and the predisposition of macrosomic pups of diabetic dams to obesity development and the onset of adult diabetes (7). Induction of mild hyperglycemia in pregnant rats by streptozotocin injection on day 5 of gestation resulted in obese hyperglycemic and hyperinsulinemic offspring (7, 8).

We then produced a situation in which the fetal environment simulated that of a fetus in a poorly controlled human diabetic mother, which resulted in accelerated fetal growth in conjunction with fetal hyperinsulinemia. These macrosomic rats maintained accelerated postnatal growth combined with high adipose tissue weight up to 12 weeks of age (7). They showed several metabolic disturbances that varied according to age and gender. Interestingly, at 3 months of age, compared with controls, macrosomic rats had increased serum cholesterol levels associated with high hepatic cholesterol contents in females (7).

Hypercholesterolemia is considered one of the main causes of atherosclerosis in humans. As such, understanding factors regulating the concentration of cholesterol is of prime importance. Several metabolic factors are implicated in this process, including cholesterol uptake in the intestinal tract, its synthesis, and its esterification in the liver, and lipoprotein turnover. Four key enzymes in cholesterol metabolism, hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, ACAT, and serum LCAT, have been

Abbreviation: SREBP–lc, sterol regulatory element binding protein-1c.

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shown to account for the variability of cholesterol levels (9, 10). HMG-CoA reductase (EC 1.1.1.34) is the rate-limiting enzyme in the biosynthesis of cholesterol. Cholesterol 7α-hydroxylase (EC 1.14.13.17) is the key enzyme that converts cholesterol into bile acids. ACAT (EC 2.3.1.26), an intracellular enzyme, and LCAT (EC 2.3.1.43), located on the surface of serum HDL, are the major enzymes esterifying cholesterol. It is of interest to determine whether macrosomic rats present alterations in cholesterol-metabolizing enzyme activities, and whether these abnormalities contribute to the development of hypercholesterolemia during adulthood.

The present study used a previously established animal model (7) to determine the time course of changes in hepatic, serum, and lipoprotein cholesterol levels, as well as hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, ACAT, and serum LCAT activities, in macrosomic offspring of streptozotocin-induced mildly hyperglycemic rats.

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) fiber, 5% (w/w) minerals, and 2% (w/w) vitamins. Thirty 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 the tail and squeezing it gently. Pregnant rats with plasma glucose levels between 5.55 and 16.65 mM (compared with 5 mM in controls) were designated as mildly hyperglycemic (7, 8) 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 exceeded the mean birth weight of control pups by more than 1.7 SD (above the 90th percentile) were classified as macrosomic (7, 8), and were included in the study. The mean birth weight of control pups was 5.95 ± 0.45 g. Therefore, experimental pups with birth weights greater than 6.8 g were considered macrosomic in the study. The success rate in obtaining macrosomic pups was 60% (90 of 150 pups). The mean birth weight of macrosomic pups was 8.10 ± 0.50 g. These macrosomic pups were hyperglycemic and hyperinsulinemic at birth (7). The nonmacrosomic offspring of diabetic mothers (60 of 150) were excluded, because maternal diabetes related to fetal macrosomia was the criterion for our experimental population selection. However, these normal-sized offspring of diabetic mothers were not hyperinsulinemic at birth, had normal growth rates, and showed no significant differences from controls for serum lipids. Twenty newborn rats of each group (control and experimental) were killed by decapitation, and blood from four animals was collected and pooled to obtain sufficient serum samples for lipid determination.

The remaining macrosomic 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 nutritional intake during the suckling period. Pups were weighed weekly up to 12 weeks of age. The gender of pups was identified at 3 weeks by examining the external genitalia. Pups were weaned at 4 weeks of age. Male and female rats were separately housed, at 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 (11), were followed.

Blood and liver samples

At 4, 8, and 12 weeks 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 (1,000 *g*; 20 min), and preserved with 0.26 mM EDTA and 3 mM sodium azide. Livers were removed, washed with cold saline, quickly blotted, and weighed, and each was cut into three pieces. One aliquot of each liver was homogenized in an Ultraturax homogenizer (Bioblock Scientific, IllKirch, France) for lipid extraction. Another aliquot was used to prepare microsomes for HMG-CoA reductase and cholesterol 7α-hydroxylase activities. The remaining piece of each liver was used to prepare the microsomal fraction for ACAT activity.

Isolation of lipoprotein fractions

Serum lipoproteins of density <1.21 kg/liter were isolated by single ultracentrifugation flotation (model L8-55 ultracentrifuge, 50 Ti rotor; Beckman Instruments, Palo Alto, CA), according to Havel, Eder, and Bragdon (12).

The three fractions (VLDL, LDL-HDL_1, and HDL_{2-3}) were isolated from total lipoproteins by a single-spin discontinuous gradient according to the method of Redgrave, Robert, and West (13) as modified by Meghelli-Bouchenak, Boquillon, and Belleville (14). Purified fractions of VLDL ($d < 1.006$), LDL-HDL_1 ($1.006 < d < 1.06$), and HDL_{2-3} ($1.06 < d < 1.15$) were dialyzed against 0.15 M NaCl and 1 mM disodium EDTA, pH 7.4, at 4°C in Spectra/Por 2 dialysis tubing (Spectrum Medical Industries, Los Angeles, CA).

Chemical analysis

Serum and lipoprotein total cholesterol and unesterified cholesterol contents were determined with Boehringer Mannheim (Germany) kits, using enzymatic methods. Esterified cholesterol concentrations were obtained by determining the difference between total cholesterol and unesterified cholesterol values.

Liver lipids were extracted according to the method of Folch, Lees, and Sloane-Stanley (15). Assays of liver total and unesterified cholesterol were performed by gas-liquid chromatography with a glass capillary column (10 m × 0.3 mm internal diameter) coated with SE30 (nonpolar polydimethyl siloxane; Supelco, St. Germain en Laye, France) as stationary phase (16).

Assay for LCAT activity

Serum LCAT activity was assayed by conversion of unesterified [^{3}H]cholesterol to esterified [^{3}H]cholesterol, according to the method of Glomset and Wright (17), modified by Knipping (18), as previously described (19). Serum LCAT activity was expressed as nanomoles of esterified cholesterol per hour per milliliter of serum.

Microsomal enzyme activities

Microsomes for the determination of HMG-CoA reductase and cholesterol 7α-hydroxylase activities were prepared from liver as described by Al-Shurbaji et al. (20). Liver microsomes for ACAT activity were prepared according to Stahlberg, Angelin, and Einarsson (10). Microsomal protein contents were determined by the method of Lowry et al. (21), using BSA as standard. The activity of HMG-CoA reductase was measured in liver microsomes with [3-^{14}C]HMG-CoA as substrate. Labeled mevanolactone was separated from unreacted HMG-CoA by column chromatography containing AG1-X8 resin (formate 200–400, analytical grade; Bio-Rad, Hercules, CA) (22). HMG-CoA reductase activity was expressed as picomoles of [3-^{14}C]HMG-CoA transformed into [^{14}C]mevanolactone per minute per milligram of microsomal protein, after correcting for recovery of [^{3}H]mevanolactone from the column. The activity of cholesterol 7α-hydroxylase was measured as described by Jelinek et al. (23) with hydroxycholesterol as internal standard. Cholesterol 7α-hydroxylase activity was expressed as picomoles of [^{14}C]cholesterol transformed into [^{14}C]hydroxycholesterol per minute per milligram of microsomal protein. The activity of ACAT in the microsomes was determined by the rate of incorporation of [1-^{14}C]oleate into cholesterol ester fractions as described by Balasubramaniam, Mitropoulos, and Venkatesan (24).

Statistical analysis

Results are expressed as means ± SEM. The significance of differences among groups was analyzed by analysis of variance, followed by Duncan's multiple-range test (25) for parameter changes with age. The significance of differences between macrosomic and control rats at each age was assessed by Student's *t*-test. Males and females were analyzed separately. These calculations were performed with Statistica, version 4.1 (Statsoft, Tulsa, OK). Differences were considered significant at $P < 0.05$.

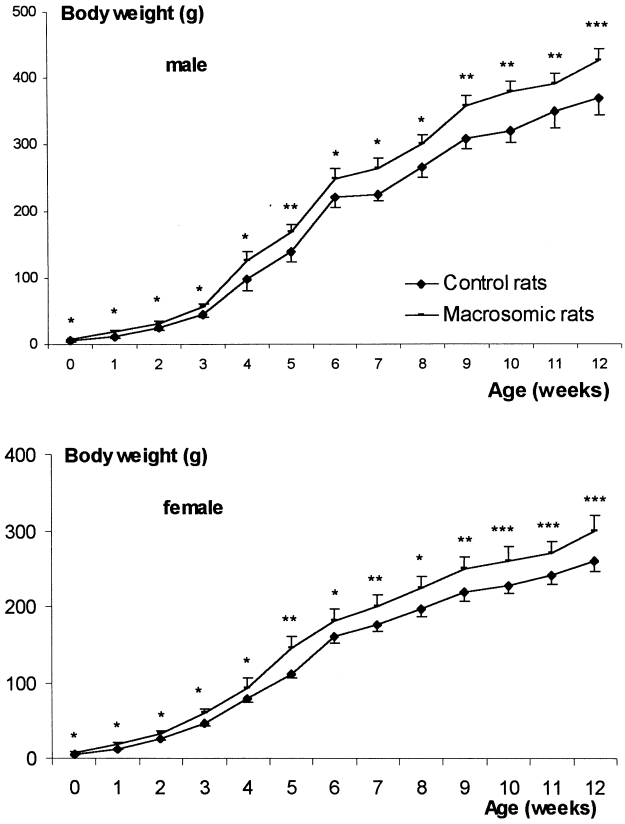

Fig. 1. Changes in body weights in male and female rats during the first 12 weeks of life. Values represent means ± SEM. Males and females constitute separate groups. Significant differences between macrosomic and control rats, at each age, are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

RESULTS

Body weight

Macrosomic rats (both males and females) had significantly higher body weights than controls throughout the first 12 weeks of life (**Fig. 1**).

Liver and serum cholesterol content

Hepatic concentrations of unesterified cholesterol and cholesteryl esters did not change significantly in male rats, but increased gradually with age in both control and macrosomic females (**Table 1**). At 30 and 60 days, hepatic concentrations of unesterified cholesterol and cholesteryl esters were similar in control and macrosomic rats. Yet, at day 90, the content of hepatic cholesteryl ester was significantly higher in macrosomic females than in control females (+100%). This cholesteryl ester accumulation was not observed in macrosomic males.

Serum cholesterol levels increased with age in both control and macrosomic rats (Table 1). At birth, serum unesterified cholesterol and cholesteryl ester concentrations were higher in macrosomic pups than in controls (+46% and +81%, respectively). At day 30 and day 60, serum cholesterol levels were similar in all groups. However, at day 90, serum unesterified cholesterol and cholesteryl ester concentrations were markedly increased in both male and female macrosomic rats, compared with controls ($P < 0.01$).

Lipoprotein cholesterol content

To distinguish which lipoprotein fraction was responsible for hypercholesterolemia, the cholesterol content of each lipoprotein fraction (VLDL, LDL-HDL$_1$, and HDL$_{2\text{-}3}$) was analyzed. Unesterified cholesterol and cholesteryl ester concentrations of VLDL and HDL$_{2\text{-}3}$ fractions were increased in control rats during the first month of life, and remained fairly constant afterward (**Table 2**). However, these values increased gradually with age in macrosomic rats. No significant age-related changes in LDL-HDL$_1$ unesterified cholesterol concentrations were observed in control rats. In contrast, LDL-HDL$_1$ cholesteryl ester levels increased from birth to day 30 in control males, and rose gradually to day 60 in control females. In macrosomic rats, a significant age-related rise was observed in the cholesterol content of LDL-HDL$_1$ fractions, with only a significant fall in unesterified cholesterol values from birth to day 30.

At birth, cholesterol concentrations of LDL-HDL$_1$ and HDL$_{2\text{-}3}$ fractions were higher in macrosomic pups than in controls. At day 30 and day 60, unesterified cholesterol and cholesteryl ester concentrations of all lipoproteins were similar in macrosomic and control rats.

TABLE 1. Postnatal changes in liver and serum cholesterol content of macrosomic and control rats

	Male Rats		Female Rats	
	Control	Macrosomic	Control	Macrosomic
Liver (μmol/g tissue)				
Unesterified cholesterol				
Day 0	ND	ND	ND	ND
Day 30	6.42 ± 0.32	8.14 ± 1.23	4.17 ± 1.49^{b}	4.92 ± 0.69^{b}
Day 60	6.64 ± 0.69	6.85 ± 2.24	7.44 ± 1.45^{a}	7.71 ± 0.96^{a}
Day 90	5.89 ± 0.64	7.92 ± 1.66	7.33 ± 0.91^{a}	8.41 ± 1.44^{a}
Cholesteryl esters				
Day 0	ND	ND	ND	ND
Day 30	6.99 ± 2.74	6.36 ± 1.38	4.82 ± 0.44^{b}	3.96 ± 0.72^{c}
Day 60	6.65 ± 1.98	7.71 ± 1.10	6.24 ± 0.82^{a}	8.47 ± 2.42^{b}
Day 90	7.31 ± 0.35	8.44 ± 2.52	6.02 ± 1.89^{a}	13.10 ± 0.85^{a,**}
Serum (mM)				
Unesterified cholesterol				
Day 0	0.52 ± 0.05^{c}	0.76 ± 0.06^{b,*}	0.52 ± 0.05^{c}	0.76 ± 0.06^{b,c,*}
Day 30	0.78 ± 0.06^{a,b}	0.87 ± 0.12^{b}	0.73 ± 0.08^{b}	0.68 ± 0.19^{c}
Day 60	0.89 ± 0.21^{a}	1.12 ± 0.30^{a}	0.88 ± 0.18^{a,b}	1.01 ± 0.26^{b}
Day 90	0.92 ± 0.11^{a}	1.32 ± 0.14,^{a,**}	1.18 ± 0.10^{a}	1.44 ± 0.11^{a,**}
Cholesteryl esters				
Day 0	0.27 ± 0.03^{c}	0.49 ± 0.03^{d,*}	0.27 ± 0.03^{c}	0.49 ± 0.03^{d,*}
Day 30	1.38 ± 0.12^{b}	1.36 ± 0.16^{c}	1.11 ± 0.21^{b}	1.26 ± 0.18^{c}
Day 60	1.74 ± 0.27^{a}	1.92 ± 0.23^{b}	1.68 ± 0.28^{a}	2.12 ± 0.54^{b}
Day 90	1.74 ± 0.13^{a}	2.43 ± 0.12^{a,**}	1.74 ± 0.11^{a}	3.15 ± 0.15^{a,**}

Values represent means ± SEM. Males and females constitute separate groups. Significant differences between macrosomic and control rats at each age are indicated as follows: * $P < 0.05$; ** $P < 0.01$. Means within the same group with different superscript letters ($a>b>c>d$) are significantly different according to age, for each group ($P < 0.05$). ND, not determined.

At day 90, however, macrosomic rats, both males and females, had higher VLDL and LDL-HDL$_1$ unesterified cholesterol and cholesteryl ester concentrations compared with controls ($P < 0.05$ for unesterified cholesterol and $P < 0.01$ for cholesteryl esters). In the HDL$_{2\text{-}3}$ fraction, only cholesteryl ester values were enhanced in macrosomic rats at day 90 (+50% for males and +58% for females).

Serum LCAT activity

To investigate whether the increased plasma cholesteryl esters observed in macrosomic rats were related to specific impairments of cholesterol esterification, serum LCAT activity was determined. Serum LCAT activity increased during the first month of life in all groups (**Fig. 2**). Afterward, it remained fairly constant in both male and female control rats. In contrast, LCAT activity increased gradually with age in macrosomic rats ($P < 0.05$).

At birth, LCAT activity, expressed as nanomoles of cholesterol esterified per hour per milliliter of serum, was significantly greater in macrosomic pups than in controls (+57%).

At day 30, day 60, and day 90, this activity was about 1.5-fold higher in both male and female macrosomic rats compared with controls.

Hepatic enzyme activities

To test whether hypercholesterolemia occurring in macrosomic animals was associated with possible impairments of hepatic cholesterol metabolism, both activities of the key enzymes responsible for hepatic cholesterol synthesis (HMG-CoA reductase), storage (ACAT), and catabolism (cholesterol 7α-hydroxylase) were evaluated. HMG-CoA reductase activity decreased, while ACAT activity increased gradually with age in all groups (**Fig. 3**). In contrast, no significant age-related changes were observed in cholesterol 7α-hydroxylase activity in any group. At day 30, day 60, and day 90, HMG-CoA reductase activity was significantly high in macrosomic rats. In macrosomic males and females, this activity was about 1.7-fold and 1.5-fold compared with their respective control values at any age. Cholesterol 7α-hydroxylase was also high in macrosomic rats. It was about 2-fold at day 30, and about 2.5-fold at day 60 and day 90 in macrosomic males and females compared with controls. Macrosomic rats also showed increased ACAT activity at all ages studied ($P < 0.01$).

DISCUSSION

To investigate the long-term effects of fetal macrosomia, we used streptozotocin treatment of pregnant Wistar rats to induce mild maternal hyperglycemia resulting in obese, hyperglycemic, and hyperinsulinemic offspring (7).

Indeed, all serum lipid levels were higher in macrosomic than in control newborns. These data show that fat synthesis was increased in the hyperinsulinemic fetus, and are in agreement with earlier reports (3, 5, 26). Insulin stimulates sterol regulatory element binding protein-1c (SREBP-1c) production (27). SREBP-1c is a transcription factor that activates specific genes involved in lipogenesis and cholesterol synthesis (28). This factor is active in fetal tissues and participates in the regulation of lipogenic genes during proliferation (29). SREBP-1c content might be increased in macrosomic newborns. Because the HDL fraction is primarily responsible for lipid transport dur-

TABLE 2. Postnatal changes in cholesterol content of rat serum lipoproteins

	Male Rats		Female Rats	
	Control	Macrosomic	Control	Macrosomic
VLDL (mM)				
Unesterified cholesterol				
Day 0	0.03 ± 0.01^{b}	0.04 ± 0.01^{c}	0.03 ± 0.01^{b}	0.04 ± 0.01^{c}
Day 30	0.06 ± 0.01^{a}	0.08 ± 0.02^{b}	0.05 ± 0.01^{a}	0.07 ± 0.01^{b}
Day 60	0.07 ± 0.01^{a}	0.08 ± 0.03^{b}	0.06 ± 0.01^{a}	$0.10 \pm 0.05^{a,b}$
Day 90	0.08 ± 0.02^{a}	$0.13 \pm 0.01^{a,*}$	0.08 ± 0.03^{a}	$0.14 \pm 0.01^{a,*}$
Cholesteryl esters				
Day 0	0.03 ± 0.01^{b}	0.03 ± 0.01^{c}	0.03 ± 0.01^{b}	0.03 ± 0.01^{c}
Day 30	0.14 ± 0.08^{a}	0.20 ± 0.06^{b}	0.10 ± 0.05^{a}	0.21 ± 0.08^{b}
Day 60	0.15 ± 0.04^{a}	0.21 ± 0.06^{b}	0.12 ± 0.06^{a}	0.14 ± 0.05^{b}
Day 90	0.12 ± 0.02^{a}	$0.30 \pm 0.01^{a,**}$	0.18 ± 0.06^{a}	$0.65 \pm 0.08^{a,**}$
LDL-HDL$_1$ (mM)				
Unesterified cholesterol				
Day 0	0.21 ± 0.01	$0.31 \pm 0.02^{a,*}$	0.21 ± 0.01	$0.31 \pm 0.02^{a,*}$
Day 30	0.19 ± 0.06	0.14 ± 0.05^{b}	0.18 ± 0.04	0.16 ± 0.03^{b}
Day 60	0.19 ± 0.05	0.21 ± 0.06^{b}	0.18 ± 0.08	0.22 ± 0.06^{b}
Day 90	0.16 ± 0.03	$0.32 \pm 0.06^{a,*}$	0.22 ± 0.03	$0.37 \pm 0.04^{a,*}$
Cholesteryl esters				
Day 0	0.14 ± 0.02^{b}	$0.22 \pm 0.02^{c,*}$	0.14 ± 0.02^{c}	$0.22 \pm 0.02^{c,*}$
Day 30	0.30 ± 0.08^{a}	0.32 ± 0.05^{b}	0.29 ± 0.04^{b}	0.34 ± 0.06^{b}
Day 60	0.41 ± 0.10^{a}	0.49 ± 0.06^{a}	0.44 ± 0.15^{a}	0.59 ± 0.16^{a}
Day 90	0.36 ± 0.04^{a}	$0.60 \pm 0.06^{a,**}$	0.48 ± 0.05^{a}	$0.79 \pm 0.06^{a,**}$
HDL$_{2-3}$ (mM)				
Unesterified cholesterol				
Day 0	0.28 ± 0.02^{b}	$0.38 \pm 0.02^{b,*}$	0.28 ± 0.02^{b}	$0.38 \pm 0.02^{c,*}$
Day 30	0.53 ± 0.16^{a}	0.65 ± 0.11^{a}	0.50 ± 0.14^{a}	0.48 ± 0.03^{b}
Day 60	0.63 ± 0.12^{a}	0.83 ± 0.13^{a}	0.65 ± 0.18^{a}	0.67 ± 0.14^{a}
Day 90	0.71 ± 0.16^{a}	0.87 ± 0.15^{a}	0.88 ± 0.08^{a}	0.91 ± 0.14^{a}
Cholesteryl esters				
Day 0	0.10 ± 0.02^{b}	$0.22 \pm 0.01^{c,*}$	0.10 ± 0.02^{b}	$0.22 \pm 0.01^{d,*}$
Day 30	0.93 ± 0.13^{a}	0.83 ± 0.12^{b}	0.73 ± 0.18^{a}	0.70 ± 0.11^{c}
Day 60	1.18 ± 0.37^{a}	1.22 ± 0.16^{a}	0.96 ± 0.11^{a}	1.27 ± 0.22^{b}
Day 90	0.95 ± 0.12^{a}	$1.42 \pm 0.16^{a,**}$	1.08 ± 0.05^{a}	$1.71 \pm 0.14^{a,**}$

Values represent means ± SEM. Males and females constitute separate groups. Significant differences between macrosomic and control rats at each age are indicated as follows: * $P < 0.05$; ** $P < 0.01$. Means within the same group with different superscript letters ($a>b>c>d$) are significantly different according to age, for each group ($P < 0.05$).

ing fetal life in humans (30) and in rats (31), elevated LDL-HDL$_1$ and HDL$_{2-3}$ cholesterol concentrations might reflect an increase in required cholesterol for membrane, hormone, and surfactant synthesis in macrosomic newborns.

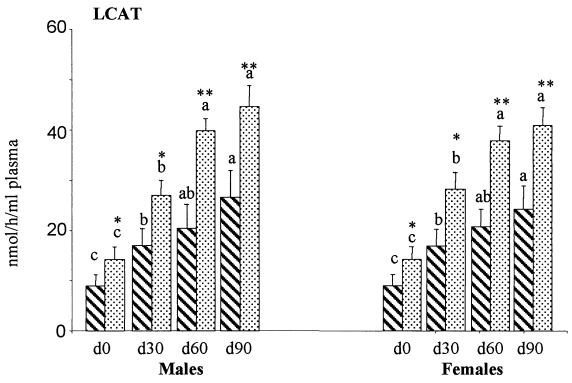

Fig. 2. LCAT activity of rat sera. Values represent means ± SEM. Males and females constitute separate groups. Significant differences between macrosomic rats (dotted columns) and control rats (hatched columns) at each age are indicated as follows: * $P < 0.05$; ** $P < 0.01$. Means within the same group with different superscript letters (a>b>c>d) are significantly different according to age, for each group ($P < 0.05$).

High insulin levels are known to lead to enhanced growth of fetal tissues (3). We have previously shown that serum and lipoprotein cholesterol levels were increased in human macrosomic newborns of diabetic mothers compared with controls (32, 33). Indeed, in the case of relatively poor maternal glycemic control, venous cord blood cholesterol concentrations were also found to be increased as compared with levels in the fetus of nondiabetic mothers (34). Considering the role of LCAT in the esterification of plasma cholesterol, increased LCAT activity in macrosomic newborns accounted for elevated cholesteryl ester levels. High LCAT activity was most likely due to an increase in enzyme mass, secondary to enhanced synthesis in macrosomic pups. Moreover, insulin stimulates liver protein synthesis in utero (3). These findings are in agreement with our results on human macrosomic newborns, who showed greater LCAT activity than did controls (33).

Consistent with other previous studies (8), our macrosomic rats maintained an accelerated weight gain through to 12 weeks of age. Therefore the present study is consistent with observations of humans (5, 35) that show a high incidence of obesity during adolescence and early adulthood for macrosomic infants of diabetic mothers. Serum

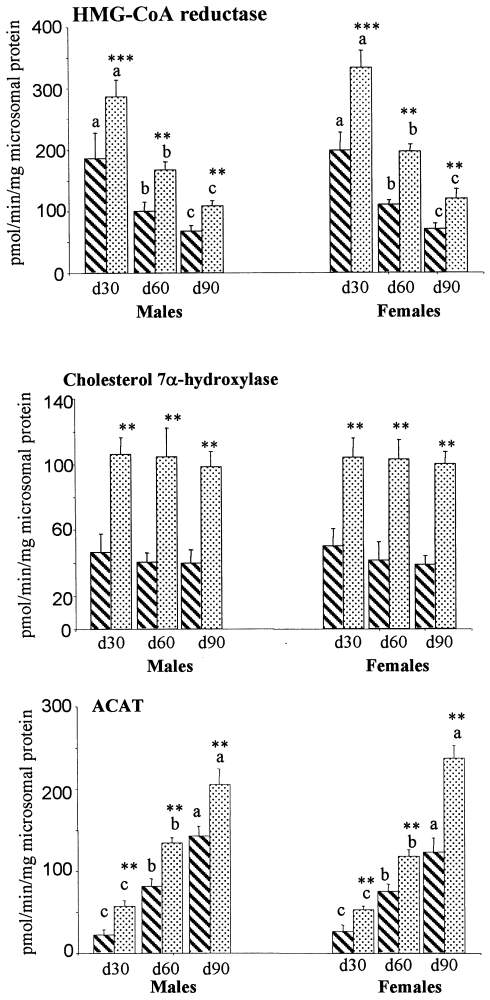

Fig. 3. HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT activities in hepatic microsomes from rats 1, 2, and 3 months old. Values represent means ± SEM. Males and females constitute separate groups. Significant differences between macrosomic rats (dotted columns) and control rats (hatched columns) at each age are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Means within the same group with different superscript letters (a>b>c) are significantly different according to age, for each group ($P < 0.05$).

cholesterol concentrations increased with age in control and macrosomic rats (both male and female), but this was not due to increased cholesterol synthesis. Indeed, liver HMG-CoA reductase activity, the rate-limiting enzyme in cholesterol biosynthesis, decreased gradually with age in all groups, in agreement with other studies (10, 36). In the present work, the rise in serum and lipoprotein cholesterol concentrations observed with age might be due to increased hepatic cholesterol storage for further excretion in lipoprotein. Liver microsomal ACAT activity, an enzyme that esterifies cholesterol, increased throughout development in control and macrosomic rats (both male and female). Increased liver ACAT activity with advancing age has already been reported in control rats (37).

At 1 and 2 months of life (day 30 and day 60), serum and lipoprotein cholesterol concentrations in male and female macrosomic rats became similar to those of their respective controls. Liver cholesterol levels in these obese rats were also compared with control values. However, serum LCAT, hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT activities were higher in macrosomic than in control rats.

Interestingly, insulin is one of the few hormones that has been shown to have a critical role in the regulation of cholesterol synthesis through HMG-CoA reductase by modulating gene expression at the level of its mRNA, and enzyme protein synthesis (38). Therefore the first 2 months of life, a period related to an increase in tissue insulin sensitivity, was also characterized by an increase in hepatic HMG-CoA reductase and cholesterol synthesis. Overexpression of the mature form of SREBP-1c in macrosomic rats might explain these findings. In addition, the increase in LCAT activity in these macrosomic rats could also be related to hyperresponsiveness of protein synthesis to insulin in hepatocytes. Another explanation for the increased synthesis of cholesterol in macrosomic rats at day 30 and day 60 would be a higher demand for cholesterol during the rapid growth phase, knowing that most obese rat tissues were hypertrophic (3, 5, 7).

Despite enhanced HMG-CoA reductase activity, a concomitant rise in hepatic and plasma cholesterol concentrations was not observed in macrosomic rats at day 30 and day 60. The amount of cholesterol in blood depends on the balance between removal from plasma, endogenous neosynthesis, and excretion from the body. When abnormal cholesterol synthesis occurs in the liver, neosynthesized cholesterol may enter three metabolic fates: storage in the liver in the esterified form, release into bile as free cholesterol, or catabolism to bile acids, to be excreted as plasma lipoprotein cholesterol. Macrosomic rats seemed to respond to the increased cholesterol synthesis with a higher rate of bile acid synthesis as shown by increased cholesterol 7α-hydroxylase activity. Similar results were shown in Yoshida rats and in Wistar fatty rats (39, 40). Indeed, enhanced ACAT activity reflected higher esterification in macrosomic compared with control rats. However, despite high ACAT activity, hepatic cholesteryl ester content was not increased in these obese rats at day 30 and day 60. Cianflone et al. (41) have suggested that cholesteryl ester formation plays an important role in hepatic VLDL synthesis and secretion. Increased hepatic cholesteryl ester incorporation into lipoprotein particles, overproduction, and secretion of lipoproteins could occur in macrosomic rats, and could be responsible for nonaccumulation of liver cholesterol at day 30 and day 60. However, VLDL, LDL-HDL_1, and $HDL_{2\text{-}3}$ cholesterol were not increased in these macrosomic rats. Increased expression of lipoprotein receptors suggesting enhanced lipoprotein catabolism in macrosomic rats might contribute to maintain normal lipoprotein cholesterol levels. All these results suggested a high cholesterol turnover in macrosomic rats. Macrosomia led to an increased rate of cholesterol synthesis combined with an enhanced rate of cholesterol utilization. These data were consistent with increased cholesterol turnover rates observed in obese humans (42).

In addition, in species without CETP activity, for example, the rat, HDL cholesteryl ester levels are totally derived from LCAT reaction. Thus, the rate of production of cholesteryl esters by LCAT should reflect the HDL cholesteryl ester turnover rate rather than HDL cholesterol concentrations. We then hypothesized that high LCAT activity was associated with a high HDL cholesteryl ester turnover rate in both male and female macrosomic rats at day 30 and day 60. LCAT activity is also elevated in obese rats (9).

At day 90, the situation was somewhat different. Macrosomic rats (both males and females) were hypercholesterolemic with high lipoprotein cholesterol concentrations. The male macrosomic rats showed liver cholesterol values similar to those of male controls. However, in macrosomic females, liver cholesteryl esters were increased, compared with female controls of a comparable age (day 90). In addition to these abnormalities, macrosomic males and females regained high serum LCAT, hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT activities. These data suggest that at day 90, cholesterol production was more enhanced than its removal. Our report and other earlier data showed that macrosomic rats were hyperinsulinemic and developed insulin resistance in adulthood (7, 8, 43). This led to the hypothesis that hyperinsulinemia caused peripheral but not hepatic insulin resistance in macrosomic rats. At day 90, hyperinsulinemia induced increased HMG-CoA reductase and hepatic cholesterol synthesis. Enhanced liver cholesterol availability might increase cholesterol 7α-hydroxylase and ACAT activities, with enhanced lipoprotein secretion. This process was sufficient to prevent liver accumulation in macrosomic males, but not in females.

In conclusion, fetal macrosomia in the offspring of streptozotocin-induced mildly diabetic rats influences cholesterol metabolism at birth and through adulthood. The main metabolic impairment appeared to relate to an overproduction of liver cholesterol. During the rapid growth phase, a combined overutilization of cholesterol by tissues contributed to maintain normal cholesterol levels. However, during adulthood, cholesterol oversynthesis exceeded its removal and was a major contributor to hypercholesterolemia in mature macrosomic rats.

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