Restricted fetal growth and the response to dietary cholesterol in the guinea pig

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Restricted fetal growth and the response to dietary cholesterol in the guinea pig. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1675–R1682, 1999.—Epidemiological studies suggest that retarded growth before birth is associated with increased plasma total and low-density lipoprotein (LDL) cholesterol concentrations in adult life. Thus perturbations of prenatal growth may permanently alter cholesterol metabolism. To determine directly whether restriction of prenatal nutrition and growth alters postnatal cholesterol homeostasis, the plasma cholesterol response to cholesterol feeding (0.25% cholesterol) was examined in adult guinea pig offspring of ad libitum-fed or moderately undernourished mothers. Maternal undernutrition (85% ad libitum intake throughout pregnancy) reduced birth weight (~13%). Plasma total cholesterol was higher prior to and following 6 wk cholesterol feeding in male offspring of undernourished mothers compared with male offspring of ad libitum-fed mothers (P < 0.05). The influence of birth weight on cholesterol metabolism was examined by dividing the offspring into those whose birth weight was above (high) or below (low) the median birth weight. Plasma total cholesterol concentrations prior to cholesterol feeding did not differ with size at birth, but plasma total and LDL cholesterol were 31 and 34% higher, respectively, following cholesterol feeding in low- compared with high-birth weight males (P < 0.02). The response to cholesterol feeding in female offspring was not altered by variable maternal nutrition or size at birth. Covariate analysis showed that the effect of maternal undernutrition on adult cholesterol metabolism could be partly accounted for by alterations in prenatal growth. In conclusion, maternal undernutrition and small size at birth permanently alter postnatal cholesterol homeostasis in the male guinea pig.

maternal undernutrition; pregnancy; birth weight; plasma cholesterol; low-density lipoprotein cholesterol

Epidemiological studies in England have shown that retarded or perturbed growth before birth, as indicated by reduced weight, length, or abdominal circumference at birth, is associated with increased rates of atherosclerosis (23), coronary heart disease (1), and an increased incidence of major risk factors for heart disease, including hypertension, insulin resistance, and non-insulin-dependent diabetes (1, 3, 4). In addition, small size at birth has been associated with elevated blood cholesterol concentrations in adult life (5). This series of findings led to the formulation of the “fetal origins of adult disease” hypothesis, which suggests that restriction of growth before birth may “program” increased risk of disease in adult life (1, 2). In particular, it has been suggested that exposure to an adverse environment in utero may permanently alter the structure, function, or metabolism of major tissues or organs, thus increasing susceptibility to disease in later life (1, 2). Fetal growth is dependent on an adequate supply of oxygen and nutrients, crossing the placenta from the mother (27). Thus factors that perturb fetal substrate supply, such as maternal undernutrition and placental insufficiency, have been implicated in the long-term programming of adult dysfunction and disease (1, 2).

Many subsequent studies in different countries and communities have now detected associations of size at birth with postnatal blood pressure, insulin sensitivity, glucose intolerance, and risk of cardiovascular disease in humans (2, 19, 20). Several studies have examined the link between cholesterol homeostasis and birth size. In 50- to 53-year-old English men and women, serum concentrations of total and low-density lipoprotein (LDL) cholesterol and apolipoprotein B were inversely related to abdominal circumference at birth (5). In another English cohort, concentrations of high-density lipoprotein (HDL) cholesterol were found to decrease with decreasing birth weight in elderly women (11). In Jamaican school children, an inverse association was observed between serum cholesterol concentrations and length at birth (14). In 8-yr-old Australian children, thinness at birth is associated with higher levels of total and LDL cholesterol (26). The latter association is also evident at 20 yr of age in the same cohort, but only in males (26). These studies therefore support the suggestion that growth before birth can influence postnatal cholesterol metabolism. Nevertheless, other studies have failed to detect an association of postnatal cholesterol concentration with birth size (6, 9, 17). In young adult men and women in Denmark and Croatia, no association was detected between concentrations of total, LDL, or HDL cholesterol and weight or thinness at birth (9, 17). In addition, no difference was found in blood lipid levels in middle-aged men and women exposed to undernutrition in utero during the Leningrad siege compared with those born outside the siege area (31). Whether growth was altered in this study is unknown however, because birth measurements were not available (31).

In rats, protein deficiency throughout pregnancy and lactation reduces plasma levels of total cholesterol,

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HDL cholesterol, and triacylglycerol in adult offspring at 6 mo postnatal age (22). Maternal protein restriction throughout pregnancy only does not significantly alter adult total or HDL cholesterol concentrations in this species (22). Thus the perinatal, but not the prenatal environment alone, can program postnatal lipid metabolism in the rat (22), although the observed effect is opposite to that reported in human epidemiological studies (5). The lipoprotein profile of the rat contrasts with that of the human, however, in that the majority of blood cholesterol in the rat is transported in HDL (8). In addition, the timing of critical periods of development for various systems differs between these species. The effects of specific protein deficiency may also differ from the effects of a reduction in total caloric intake, which accounts for a significant proportion of human intrauterine growth retardation (18). Furthermore, the impact of prenatal perturbations on the ability to maintain plasma cholesterol levels in adult life may become evident only when postnatal dietary intake is varied. In humans, variation is seen between individuals in the ability to regulate serum cholesterol levels in response to changes in dietary fat or cholesterol levels (25). Some of this variation may be due to genetic factors (10), but the prenatal environment may also contribute. In the current study, we have investigated the effect of moderate maternal undernutrition and size at birth on cholesterol homeostasis and the response to a dietary cholesterol challenge in guinea pig offspring. The guinea pig is a precocial species and has higher plasma concentrations of LDL than of HDL in contrast to the rat and responds to dietary cholesterol and fat in a manner similar to humans (8, 21, 30). Thus the effect of 6 wk of cholesterol feeding on plasma lipoprotein levels was examined in adult guinea pig offspring of normally fed and moderately undernourished mothers.

**MATERIALS AND METHODS**

**Animals**

Nulliparous, 3- to 4-mo-old female guinea pigs (IMVS colored, Gillies Plains Animal Resource Center, Gillies Plains, South Australia, Australia) were housed in individual wire-bottomed cages under 12:12-h light-dark conditions. The animals were fed a guinea pig/rabbit ration (Milling Industries Stockfeeds, Murray Bridge, South Australia, Australia) with an increased content of vitamin E (165 mg/kg) and had free access to tap water supplemented with vitamin C (400 mg/l). Control animals \( n = 13 \) were fed ad libitum. Food-restricted animals \( n = 15 \) were given 85% of the average daily ad libitum food intake per kilogram of body weight. Normal ad libitum food intake before and during pregnancy was previously determined for 34 nonpregnant and pregnant guinea pigs of the same strain and similar age, weight, and stage of pregnancy (29). Body weights were measured three times per week for calculation of feeding rates. Food-restricted animals were fed between 0800 and 0930 each morning. Food intake and body weights of the ad libitum-fed animals were also monitored three times per week. After 4–6 wk of controlled feeding, guinea pigs were mated. Females in estrus were placed with a male overnight, and pregnancy was detected by the presence of a vaginal copulatory plug the following morning and a failure to return to estrus in the subsequent cycle.

The feeding schedule was maintained throughout pregnancy. One week before term, pregnant animals were transferred to tubs containing paper bedding. Weight, length, abdominal circumference, and head width and length were measured for each pup at birth. All mothers and their litters were then transferred to plastic tubs with lucerne bedding and allowed ad libitum access to guinea pig chow. A total of 40 pups was born to the 13 ad libitum-fed mothers (mean litter size, 3.08 ± 0.8 pups). Five pups died at birth due to birthing difficulties. Of the 35 ad libitum-fed pups (17 male, 18 female), 14 male and 14 female offspring were randomly assigned to the current study. A total of 31 pups (21 male, 10 female) was born to 11 undernourished mothers (mean litter size, 2.8 ± 0.6 pups). Four undernourished females failed to become pregnant. Fifteen males and five females were selected for the current study. The remaining offspring were used for another study.

Pup weights were measured daily from birth to 40 days of age. Pups were weaned at 28 days of age onto normal guinea pig chow. Thus, where reference is made to “ad libitum-fed” or “undernourished” offspring, the feeding regimen was applied to the mothers only. All offspring were fed ad libitum. At 32 days of age, pups were separated into single-sex groups and were then moved into individual wire cages at 42 days of age. Food intake and body weight were monitored three times per week.

At 100 ± 5 days of age, catheters were inserted into the right jugular vein (Silastic, 0.51 mm ID, 0.94 mm OD) and carotid artery (polyvinyl, 0.4 mm ID, 0.8 mm OD) under general anesthesia induced by ketamine (75 mg/kg body wt ip) and xylazine (6 mg/kg body wt im). Atropine (0.05 mg/kg body wt sc) was given before surgery. Catheters were tunneled under the skin to the back of the neck. Catheter patency was maintained by flushing daily with enough heparinized saline to fill the catheter (250 U/ml).

**Cholesterol Feeding**

Feeding of a cholesterol-loaded diet commenced 7 ± 2 days after surgery (117 ± 2 days of age). The normal guinea pig/rabbit diet was supplemented with 0.25% cholesterol ( wt/wt; Ajax Laboratory Chemicals, Auburn, New South Wales, Australia). Cholesterol feeding continued for 6 wk. Blood samples (300–500 µl) were collected in the fed state on 0900 days 0, 7, 21, and 35 of cholesterol feeding. Animals were fasted overnight before the collection of blood samples on days 14 and 28 of cholesterol feeding. On day 42 of feeding, the guinea pigs were fasted from 0800. The animals were then killed by intravenous overdose of sodium pentobarbitone between 1400 and 1600, and blood was collected by cardiac puncture. Blood was centrifuged at 3,000 rpm for 15 min, and plasma was removed and stored at −20°C. The aortic arch was dissected out, and excess fat and connective tissue were removed from the adventitial surface. The aorta was opened longitudinally and fixed in 10% Formalin containing 0.1 M calcium acetate. Tissue was collected from 13 of the male (5 ad libitum fed, 8 undernourished) and 14 of the female offspring (10 ad libitum fed, 4 undernourished). All animal studies were approved by the Animal Ethics Committee of the University of Adelaide, where the animal work was conducted.

Plasma total cholesterol was measured by colorimetric enzymatic analysis on a COBAS Mira automated centrifugal analyzer using a Roche Unimate 7, Cholesterol kit, and control sera (F. Hoffmann-La Roche, Basel, Switzerland). Plasma HDL cholesterol was determined after precipitation of other lipoproteins with polyethylene glycol 6000. Plasma
Lipophilic Staining in the Aortic Arch

After overnight fixation, aortic arch was rinsed in 70% ethanol and stained for 15 min in 0.1% Oil Red O in ethanol (35%) and acetone (50% vol/vol). Tissues were washed with 80% ethanol followed by running water and stored in 10% Formalin containing 0.1 M calcium acetate until analysis. The area of lipophilic staining was assessed by image analysis (TM/TC Image Analysis Systems, Dighurst, UK and MicroScale Software) and expressed as the percentage of the total area of the aortic arch sample that exhibited lipid staining.

Statistical Analysis

All statistical analyses were carried out using BMDP Statistical Software (Los Angeles, CA). Comparisons between groups were made by one- or two-way ANOVA. Bonferroni post hoc tests were used. Repeated-measures ANOVA was used to examine the effect of maternal undernutrition on maternal weight gain and food intake throughout pregnancy and on the response of plasma cholesterol to cholesterol feeding in the offspring. The influence of weight or abdominal circumference at birth, litter size, or adult body weight on the effect of maternal undernutrition on cholesterol metabolism in offspring was examined by inclusion of these variables as covariates in the repeated-measures ANOVA. Relationships between variables were examined by correlation analysis. Significance was accepted at P < 0.05. All results are expressed as means ± SE.

To examine the effect of size at birth on postnatal outcomes, offspring were divided into two groups, according to birth weight rather than maternal feeding group. All pups were divided into groups, with birth weights greater than or less than the median birth weight. Separate analyses were also performed with males and females divided into birth weight groups, using the median birth weight for each sex.

RESULTS

Pregnancy

Maternal weight at mating was not different between groups (ad libitum fed, 643 ± 17 g, n = 12; undernourished, 623 ± 16 g, n = 10). Maternal undernutrition reduced total maternal weight gain throughout pregnancy (P < 0.004), with ad libitum-fed mothers having gained 449 ± 23 g by day 65 of pregnancy (term, 67–72 days) compared with 313 ± 21 g in the undernourished group. Maternal weight was lower in the undernourished mothers at day 65 of pregnancy (ad libitum fed, 1,085 ± 20 g, n = 13; undernourished, 936 ± 35 g, n = 10, P < 0.001). This was due, in part, to a reduction in total litter weight (at term, ad libitum fed, 302.4 ± 15.9 g; undernourished, 244.7 ± 15.8 g, P < 0.02) but also to a reduction in maternal body weight (at term, ad libitum fed, 774 ± 12 g; undernourished, 720 ± 21 g, P < 0.03). All mothers were fed ad libitum after birth. At weaning, maternal weights were not different (ad libitum fed, 818 ± 11 g; undernourished, 832 ± 17 g). Maternal undernutrition did not alter length of gestation (ad libitum fed, 69.7 ± 0.4 days; undernourished, 70.1 ± 0.3 days).

Birth Size

The effect of maternal undernutrition on size at birth is shown in Table 1. Maternal undernutrition reduced weight (P < 0.002), length (P < 0.005), abdominal circumference (P < 0.001), and head width (P < 0.002) at birth. No differences in any of the measures of size at birth were observed when male offspring of ad libitum-fed mothers were compared with female ad libitum-fed offspring or when male offspring of undernourished mothers were compared with female offspring of undernourished mothers. Birth weight (P < 0.01), abdominal circumference (P < 0.006), and head width (P < 0.005) were reduced in male offspring of undernourished mothers compared with male ad libitum-fed offspring. In female offspring, only birth length was reduced (P < 0.02) in the undernourished group compared with female ad libitum-fed offspring; however, smaller numbers of undernourished females were studied. Birth weight ranged from 127.5 to 780.0 g (males, 124.0–78.0 g; females, 127.5–83.4 g) in the offspring of ad libitum-fed mothers and from 107.1 to 49.5 g (males, 107.1–49.5 g, females, 97.3–78.8 g) in the undernourished group. Mean birth weight for all offspring combined was 93.6 ± 2.1 g (males, 92.9 ± 3.0 g, females, 94.7 ± 2.7 g), whereas the median birth weight was 92.2 g.

Table 1. Effect of moderate maternal undernutrition on birth size and adult body size in guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>All (n = 28)</th>
<th>Male (n = 14)</th>
<th>Female (n = 15)</th>
<th>All (n = 20)</th>
<th>Male (n = 13)</th>
<th>Female (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>98.93 ± 2.50</td>
<td>100.41 ± 3.99</td>
<td>97.45 ± 3.18†</td>
<td>98.64 ± 2.76†</td>
<td>100.14 ± 3.99</td>
<td>97.88 ± 3.06*</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td>15.81 ± 0.18</td>
<td>15.69 ± 0.29</td>
<td>15.93 ± 0.24†</td>
<td>15.95 ± 0.22†</td>
<td>15.69 ± 0.29</td>
<td>15.74 ± 0.30*</td>
</tr>
<tr>
<td>Abdominal circumference at birth, cm</td>
<td>10.36 ± 0.15</td>
<td>10.22 ± 0.17</td>
<td>10.20 ± 0.17</td>
<td>10.36 ± 0.15</td>
<td>10.22 ± 0.17</td>
<td>10.20 ± 0.17</td>
</tr>
<tr>
<td>Head width at birth, cm</td>
<td>2.22 ± 0.023</td>
<td>2.23 ± 0.035</td>
<td>2.22 ± 0.031§</td>
<td>2.21 ± 0.024†</td>
<td>2.19 ± 0.027†</td>
<td>2.22 ± 0.031§</td>
</tr>
<tr>
<td>Adult body weight, g</td>
<td>922.0 ± 20.8</td>
<td>922.0 ± 20.8</td>
<td>712.5 ± 12.2§</td>
<td>848.4 ± 17.4*</td>
<td>712.5 ± 12.2§</td>
<td>695.3 ± 26.0§</td>
</tr>
<tr>
<td>Adult body length, cm</td>
<td>36.0 ± 0.3</td>
<td>36.0 ± 0.3</td>
<td>32.7 ± 0.3§</td>
<td>35.2 ± 0.3*</td>
<td>32.7 ± 0.3§</td>
<td>32.8 ± 0.4§</td>
</tr>
<tr>
<td>Adult liver weight, g</td>
<td>39.1 ± 1.2</td>
<td>39.1 ± 1.2</td>
<td>34.4 ± 1.0†</td>
<td>36.9 ± 1.4</td>
<td>34.4 ± 1.0†</td>
<td>32.2 ± 1.3‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.005 compared with ad libitum-fed offspring of the same sex. ‡P < 0.05 for female offspring compared with ad libitum-fed males; §P < 0.05 for female offspring compared with undernourished males. The smaller number of females in the undernourished group influences the adult body size results when all offspring are combined (these results are not shown).
Table 2. Birth size and adult body size following division of offspring into groups according to weight at birth

<table>
<thead>
<tr>
<th>Birth parameter</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher birth weight (≥90.6 g; n = 15)</td>
<td>Lower birth weight (&lt;90.6 g; n = 14)</td>
<td>Higher birth weight (≥92.5 g; n = 10)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>104.3 ± 2.8</td>
<td>80.7 ± 2.9†</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td>16.1 ± 0.2</td>
<td>14.6 ± 0.2‡</td>
</tr>
<tr>
<td>Abdominal circumference at birth, cm</td>
<td>10.1 ± 0.2</td>
<td>9.5 ± 0.2‡</td>
</tr>
<tr>
<td>Adult body weight, g</td>
<td>917 ± 19</td>
<td>848 ± 20*</td>
</tr>
<tr>
<td>Adult body length, cm</td>
<td>36.0 ± 0.3</td>
<td>35.2 ± 0.2</td>
</tr>
<tr>
<td>Adult liver weight, g</td>
<td>39.7 ± 1.0</td>
<td>36.2 ± 1.5</td>
</tr>
<tr>
<td>Offspring from ad libitum-fed mothers</td>
<td>n = 9</td>
<td>n = 5</td>
</tr>
<tr>
<td>Offspring from undernourished mothers</td>
<td>n = 6</td>
<td>n = 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.005 compared with higher birth weight offspring of the same sex. ‡P < 0.05 for female offspring compared with higher birthweight male. §P < 0.05 for female offspring compared with lower birthweight male.

(males, 90.6 g, females, 92.5 g). Offspring were divided into groups with birth weights above (high birth weight) and below (low birth weight) the median for their sex. Birth parameters for these groups are shown in Table 2.

**Adult Body Size**

At postmortem, adult body weight (P < 0.01) and body length (P < 0.04) were reduced in male offspring of undernourished mothers compared with ad libitum-fed male offspring (Table 1). Maternal undernutrition did not alter adult size in females (Table 1). Adult liver weight (Table 1) and relative liver weight (g/kg body wt; data not shown) were not altered by maternal undernutrition in male or female offspring. When offspring were divided into groups according to birth weight, adult body weight was reduced in the low-birth weight males (P < 0.02, Table 2). Adult liver weight tended to be reduced in low-birth weight male and female offspring (P = 0.06, Table 2) compared with the high-birth weight offspring of the same sex. Relative liver weight (g/kg body wt) was reduced in the low-birth weight females compared with the high-birth weight females (high birth weight, 0.050 ± 0.001 g/kg, n = 10; low birth weight, 0.045 ± 0.001 g/kg, n = 8, P < 0.01). Birth weight was associated with adult body weight (r = 0.57, P < 0.001, n = 29) in male offspring only. Abdominal circumference at birth also correlated with adult body weight (r = 0.44, P < 0.02) and with adult liver weight (r = 0.47, P < 0.01) in males.

**Food Intake**

There was no difference between groups in the amount of the cholesterol-loaded diet eaten as adults (males, ad libitum fed, 39.9 ± 1.4 g/day; maternal undernourished, 39.6 ± 1.5 g/day; females, ad libitum fed, 29.6 ± 0.9 g/day; maternal undernourished, 31.3 ± 0.6 g/day). On a per-kilogram-body weight basis, the intake in males was not different between maternal feeding groups (ad libitum fed, 44.3 ± 1.3 g·kg⁻¹·day⁻¹; maternal undernourished, 47.9 ± 1.5 g·kg⁻¹·day⁻¹), whereas in females, intake was higher in the undernourished group (ad libitum fed, 42.0 ± 1.0 g·kg⁻¹·day⁻¹; maternal undernourished, 46.5 ± 1.8 g·kg⁻¹·day⁻¹, P < 0.04). No differences in food intake were observed when animals were classified according to size at birth.

**Plasma Cholesterol**

Effect of maternal undernutrition. Plasma cholesterol levels were higher prior to cholesterol feeding in male offspring of undernourished mothers compared with control male offspring (P < 0.05, Table 3). Cholesterol concentrations in ad libitum-fed male offspring prior to cholesterol feeding were lower than those of female ad libitum-fed offspring (Table 3, P < 0.03). Maternal undernutrition did not alter basal plasma cholesterol concentrations in female offspring (Table 3); however, this may be due, in part, to the smaller numbers of undernourished female offspring.

Plasma cholesterol concentrations were increased by 6-wk dietary cholesterol loading in both male and female offspring of undernourished and ad libitum-fed mothers (P < 0.001, Table 3). In male offspring, maternal undernutrition did increase plasma cholesterol levels overall, with cholesterol concentrations being higher prior to and following cholesterol feeding in the undernourished offspring (P < 0.05). Inclusion of birth

Table 3. Plasma cholesterol concentrations in offspring of ad libitum-fed or undernourished mothers

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad libitum fed (n = 14)</td>
<td>Undernourished (n = 15)</td>
</tr>
<tr>
<td>Total cholesterol precholesterol feeding, mM</td>
<td>0.70 ± 0.05</td>
<td>0.92 ± 0.10*</td>
</tr>
<tr>
<td>Total cholesterol postcholesterol feeding, mM</td>
<td>4.30 ± 0.30</td>
<td>5.34 ± 0.47*</td>
</tr>
<tr>
<td>LDL cholesterol, mM</td>
<td>3.87 ± 0.29</td>
<td>4.94 ± 0.46</td>
</tr>
<tr>
<td>HDL cholesterol, mM</td>
<td>0.30 ± 0.03</td>
<td>0.28 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with ad libitum-fed offspring of the same sex. †P < 0.05 female offspring compared with male ad libitum-fed offspring. Low-density lipoprotein (LDL) cholesterol was calculated as the difference between total cholesterol and the sum of high-density lipoprotein (HDL) cholesterol and the density < 1.019 lipoprotein fraction cholesterol.
weight or abdominal circumference as a covariate in the analysis removed the effect of maternal undernutrition on plasma cholesterol before and after cholesterol feeding (P = 0.11), suggesting that the impact of maternal undernutrition may be accounted for, at least in part, through effects on fetal growth. Exposure to maternal undernutrition did not alter cholesterol concentrations following cholesterol feeding in female offspring. Controlling for litter size did not alter any of these outcomes.

Seven male ad libitum-fed offspring and six male undernourished offspring had patent vascular catheters that allowed collection of blood samples throughout the 6 wk of cholesterol feeding. Plasma cholesterol was elevated above baseline levels by day 7 of feeding in these males (P < 0.001, Fig. 1). Maternal undernutrition increased the plasma cholesterol response to feeding (interaction of maternal feeding group and dietary cholesterol loading, P < 0.004) and was associated with increased plasma cholesterol concentrations at day 42 of feeding (P < 0.02, Fig. 1). The inclusion of either birth weight or abdominal circumference as a covariate in the repeated-measures ANOVA abolished the effect of maternal undernutrition on plasma cholesterol overall but did not alter the effect of maternal undernutrition on the plasma cholesterol response to dietary cholesterol loading in these males (P < 0.004), suggesting that some impairment of cholesterol homeostasis may occur without variations in prenatal growth.

Concentrations of LDL and HDL, measured after 6 wk of cholesterol feeding, were not altered by maternal undernutrition (Table 3). However, in male offspring of undernourished mothers, there was a tendency for LDL concentrations to be higher than those of ad libitum-fed offspring following cholesterol feeding (Table 3, P = 0.06).

Effect of size at birth. Cholesterol concentrations prior to cholesterol feeding were not different in high- or low-birth weight pups, compared with low-birth weight pups. Cholesterol feeding increased plasma cholesterol concentrations in both birth weight groups (P < 0.0001). In low-birth weight males, cholesterol concentrations were higher at 6 wk of cholesterol feeding (P < 0.02) and the change in cholesterol concentration from baseline was greater (P < 0.008) than in the higher birth weight males (Table 4). Eight high-birth weight male offspring and five low-birth weight males had samples collected throughout the 6 wk of cholesterol feeding. The plasma cholesterol response to feeding was also increased in low-birth weight male offspring compared with high-birth weight offspring in this subgroup (interaction of birth weight group and dietary cholesterol loading, P < 0.002).

No differences between birth weight groups in total cholesterol levels following cholesterol feeding were evident in female offspring, whether they were divided according to the median for the whole group (92.2 g) or according to the median for females (92.5 g; Table 4). The median female birth weight was higher than that for males, because less undernourished females were studied; therefore, females were also divided using 90.6 g (male median birth weight) as the cutoff. No differences between birth weight groups in cholesterol concentration pre- or postcholesterol feeding were evident using this division (data not shown).

LDL cholesterol concentrations, measured after 6 wk of cholesterol loading, were increased in low-birth

<table>
<thead>
<tr>
<th>Table 4. Plasma cholesterol concentrations in offspring divided according to weight at birth</th>
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<table>
<thead>
<tr>
<th></th>
<th>Higher birth weight</th>
<th>Lower birth weight</th>
<th>Higher birth weight</th>
<th>Lower birth weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 14)</td>
<td>(n = 10)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precholesterol feeding,</td>
<td>0.89 ± 0.09</td>
<td>0.74 ± 0.08*</td>
<td>0.91 ± 0.08</td>
<td>1.01 ± 0.14</td>
</tr>
<tr>
<td>mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postcholesterol feeding,</td>
<td>4.20 ± 0.31</td>
<td>5.52 ± 0.45*</td>
<td>4.87 ± 0.39</td>
<td>4.87 ± 0.42</td>
</tr>
<tr>
<td>mM</td>
<td></td>
<td></td>
<td>4.47 ± 0.39</td>
<td>4.55 ± 0.41</td>
</tr>
<tr>
<td>LDL cholesterol, mM</td>
<td>3.79 ± 0.29</td>
<td>5.09 ± 0.46*</td>
<td>3.96 ± 0.39</td>
<td>3.80 ± 0.40</td>
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<tr>
<td>HDL cholesterol, mM</td>
<td>0.27 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.35 ± 0.05</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>3.31 ± 0.32</td>
<td>4.79 ± 0.42*†</td>
<td>3.96 ± 0.39</td>
<td>3.80 ± 0.40</td>
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<td>change from baseline, mM</td>
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Values are means ± SE. *P < 0.05, †P < 0.01 compared with higher birth weight offspring of the same sex. LDL cholesterol was calculated as the difference between total cholesterol and the sum of HDL cholesterol and the density < 1.019 lipoprotein fraction cholesterol.
weight males, compared with high-birth weight males (Table 4, P < 0.02). LDL concentrations were not different between birth weight groups in female offspring (Table 4). HDL concentrations were not different between birth size groups (Table 4).

Relationship to birth size and adult body size. Total cholesterol levels prior to cholesterol feeding did not correlate with birth weight or birth length. However, cholesterol concentrations prior to cholesterol feeding tended to relate negatively to abdominal circumference at birth (r = −0.29, P = 0.055, n = 45). Total and LDL cholesterol levels following cholesterol feeding were not related to any birth size parameters. HDL cholesterol levels, following cholesterol feeding, were positively correlated with abdominal circumference at birth (r = 0.40, P < 0.01, n = 46) but were not related to other birth size measures.

Plasma cholesterol concentration after cholesterol feeding correlated negatively with adult body length (r = −0.29, P < 0.045, n = 47). In males, adult body weight correlated negatively with total cholesterol (r = −0.55, P < 0.002, n = 29) and LDL cholesterol (r = −0.56, P < 0.002) concentrations after cholesterol loading. Similarly, in males, adult body length was negatively related to total (r = −0.67, P < 0.0001, n = 29) and LDL cholesterol (r = −0.66, P < 0.0001) after feeding. Inclusion of body weight and body length as covariates in the ANOVA analyses removed the influence of birth size group on the plasma total and LDL cholesterol response to cholesterol feeding and the effect of maternal undernutrition on plasma cholesterol levels in male offspring. Plasma cholesterol concentrations were not related to adult body size in females.

Adult liver size correlated positively with HDL cholesterol concentrations after cholesterol feeding (r = 0.37, P < 0.01, n = 48). This association was also evident when male offspring were examined alone (r = 0.49, P < 0.01, n = 29) but was not significant in female offspring (r = 0.41, P = 0.08, n = 19). In male offspring, LDL cholesterol concentration was negatively associated with adult liver size (r = −0.37, P < 0.05, n = 29).

Lipophilic staining of aortic arch. Evidence of lipid accumulation in the aortic arch intima was obtained by assessing the percentage area of lipophilic staining with Oil Red O. Maternal undernutrition did not alter the percentage area of lipid staining in the aortic arch in male (ad libitum fed, 2.1 ± 1.2% staining, n = 5; undernourished, 4.6 ± 2.3% staining, n = 8) or female offspring (ad libitum fed, 0.9 ± 0.4% staining, n = 10; undernourished, 2.5 ± 2.1% staining, n = 4). No differences were observed in the area of lipophilic staining when the offspring were divided according to birth size groups (high birth weight, 1.8 ± 0.7%, n = 14; low birth weight, 3.3 ± 1.5%, n = 13). In male offspring, plasma total (r = 0.60, P < 0.03, n = 13) and LDL (r = 0.62, P < 0.02, n = 13) cholesterol concentrations following cholesterol feeding were positively associated with the percent area of lipophilic staining. No associations were observed between the extent of lipid staining and plasma total cholesterol prior to cholesterol feeding or total, LDL, or HDL cholesterol after cholesterol feeding in female offspring.

DISCUSSION

In the current study, maternal undernutrition restricted fetal growth, as indicated by reduced size at birth and increased plasma total cholesterol concentrations prior to and following 6 wk cholesterol feeding in adult male guinea pig offspring. Furthermore, a greater rise in plasma total cholesterol concentrations in response to cholesterol feeding occurred in those adult male offspring of undernourished mothers studied throughout the dietary cholesterol challenge. When offspring were divided into groups with birth weights above or below the median birth weight, plasma total and LDL cholesterol and the change in total cholesterol from baseline following cholesterol feeding were higher in lower birth weight male offspring compared with those males in the upper birth weight range. Furthermore, the effects of maternal undernutrition on cholesterol metabolism could be partly, but not completely, accounted for by alterations in prenatal growth. Thus these findings support the hypothesis that restriction or perturbation of maternal nutrition and fetal growth can have a long-term influence on postnatal cholesterol metabolism.

Low birth weight in male guinea pigs was associated with increased plasma total and LDL cholesterol following dietary cholesterol challenge but not altered basal plasma cholesterol concentrations. This may be due, in part, to some heterogeneity in the low-birth weight animals, some arising from natural variation in fetal growth in the ad libitum-fed group, whereas restricted fetal growth in others was due to maternal undernutrition. Spontaneous variation in fetal growth in the guinea pig has been related to a reduction in substrate supply due to factors such as reduced placental size or function and variations in litter size (28). Therefore, some low-birth weight offspring of ad libitum-fed mothers may also have experienced a spontaneous variation in competition for and placental delivery of maternal substrates.

In humans, adult serum cholesterol concentrations, particularly those of LDL cholesterol, were inversely related to abdominal circumference at birth (5). Abdominal circumference closely reflects liver size at birth in humans (32) and in late gestation in fetal guinea pigs (J. A. Owens, A. Sohlstrom, A. I. Katsman, K. L. Kind, J. S. Robinson). Liver has a major role in the regulation of cholesterol homeostasis (30), and liver size is disproportionately reduced in many forms of intrauterine growth retardation (27). Thus Barker et al. (5) have hypothesized that impaired hepatic growth before birth may lead to permanent changes in postnatal cholesterol metabolism. Abdominal circumference at birth was reduced in the current study, and plasma total cholesterol concentrations prior to cholesterol feeding did tend to relate inversely to abdominal circumference at birth, as observed in human studies (5). In addition, plasma HDL cholesterol concentrations in the cholesterol-fed adult guinea pig were positively correlated
with abdominal circumference at birth. Plasma HDL concentrations were unrelated to abdominal circumference or birth weight in other human studies (5, 9) but did decrease with decreasing birth weight in elderly English women (11). Adult liver size was not significantly altered by maternal undernutrition. In male offspring, however, adult liver size was related to weight and abdominal circumference at birth. Adult liver size was also positively related to HDL cholesterol concentrations and negatively associated with LDL cholesterol concentrations in males. Thus smaller adult liver weight was associated with lower HDL and higher LDL cholesterol concentrations in plasma following cholesterol feeding in male guinea pigs.

The mechanisms through which altered growth before birth may influence postnatal cholesterol metabolism are uncertain. Barker et al. (5) have suggested that altered prenatal hepatic growth may result in permanent changes in hepatic LDL receptor activity. This has not been investigated as yet. In the rat, manipulation of maternal or early perinatal nutrition can have a long-term effect on the hepatic activity of key enzymes of cholesterol metabolism, HMG CoA reductase and cholesterol 7α-hydroxylase (16). Thus other important determinants of cholesterol homeostasis may be influenced by prenatal restriction. Further analyses are required to identify possible mechanisms underlying the impaired ability of low-birth weight male offspring to control plasma cholesterol levels.

An increased risk of carotid stenosis has been reported in elderly men and women that had low weights at birth (23). In the current study, the extent of lipid staining in the aortic arch following cholesterol feeding was not altered by maternal undernutrition or small size at birth. Plasma total and LDL cholesterol concentrations in male offspring were, however, positively associated with the extent of aortic lipid staining. Thus low-birth weight male offspring, who had elevated total and LDL cholesterol levels following cholesterol feeding, may have been at an increased risk of lipid accumulation in the aortic intima.

In contrast to male offspring, the response to cholesterol feeding was not altered in female guinea pigs that were subjected to maternal undernutrition or were small at birth. Smaller numbers of females were studied, however. Gender differences in the response to dietary cholesterol have been reported in guinea pigs (13). Plasma cholesterol levels were higher in female guinea pigs following 4 wk feeding of a 0.25% cholesterol-enriched diet compared with males (13). In the current study, basal plasma total cholesterol concentrations were higher in female, compared with male, offspring of ad libitum-fed mothers. However, plasma cholesterol concentrations after cholesterol feeding and the change in plasma cholesterol from baseline were not different between male and female offspring of ad libitum-fed mothers or undernourished mothers. In rats, limited maternal protein intake throughout pregnancy and lactation reduces basal serum cholesterol levels and reduces lifespan in adult male offspring only (15, 22). Thus male rat offspring also appear more susceptible than females to the effects of early undernutrition on later outcomes.

Early postnatal nutrition can influence the development of cholesterol metabolism (16, 24). Maternal protein restriction during the suckling period reduces plasma cholesterol concentrations in adult rat offspring (22). In human epidemiological studies, elderly men that were exclusively bottle fed or breast fed, but not weaned at one year of age, had higher total and LDL cholesterol levels (12). Juvenile and adult baboons that were breast fed have lower HDL cholesterol concentrations compared with those that were formula fed (24). However, some of the long-term effects of breast vs. formula feeding on lipoprotein metabolism may be mediated by hormones and growth factors within milk, rather than by differences in the quantity and quality of nutrients (24). In the current study, feed-restricted mothers were transferred to ad libitum feeding immediately after giving birth and all pups were breast fed. The effect of moderate maternal undernutrition on milk quality was not assessed. Nevertheless, guinea pigs commence eating solid food from ~3 days of age and thus are not as dependent on milk quality and quantity as species such as the rat. Furthermore, birth size was related to postnatal cholesterol metabolism in this study, suggesting that prenatal influences were important. Cross fostering of undernourished pups to ad libitum-fed mothers is required to determine directly whether early postnatal events were involved.

In summary, this study has shown that maternal undernutrition impairs the cholesterol homeostatic response to dietary cholesterol challenge in adult male guinea pigs and that this can be accounted for, at least in part, by altered prenatal growth.

Perspectives

The findings of this study support the hypothesis, based on epidemiological studies in humans, that restricted growth before birth can have long-term deleterious effects on cholesterol homeostasis (5). Thus a moderate reduction of maternal feed intake was associated with increased plasma total cholesterol concentrations, before and following dietary cholesterol challenge, in the male guinea pig. In low-birth weight male guinea pigs, basal total plasma cholesterol was not changed, but plasma total and LDL cholesterol were increased after cholesterol challenge. In addition, variations in fetal growth and, in particular, in growth of the liver could partly account for the impact of maternal undernutrition on adult cholesterol homeostasis, suggesting that the effect of prenatal perturbation on hepatic cholesterol metabolism should be specifically investigated. Finally, these findings suggest that some postnatal dietary challenge may be necessary for full expression of the prenatally induced impairment in cholesterol metabolism. This emphasizes the need for further study of the interaction between prenatal and postnatal factors in determining the relative importance of environment at different stages of development for homeostasis.
REFERENCES


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