Developmental programming of lipid metabolism and aortic vascular function in C57BL/6 mice: a novel study suggesting an involvement of LDL-receptor

Kanta Chechi,1 John J. McGuire,2 and Sukhinder K. Cheema1,2

1Department of Biochemistry, and 2Division of BioMedical Sciences, Memorial University, St. John’s, Newfoundland, Canada

Submitted 18 November 2008; accepted in final form 28 January 2009

Chechi K, McGuire JJ, Cheema SK. Developmental programming of lipid metabolism and aortic vascular function in C57BL/6 mice: a novel study suggesting an involvement of LDL-receptor. Am J Physiol Regul Integr Comp Physiol 296: R1029–R1040, 2009. First published February 4, 2009; doi:10.1152/ajpregu.90932.2008.—We have previously shown that a maternal high-fat diet, rich in saturated fatty acids (SFA), alters the lipid metabolism of their adult offspring. The present study was designed to investigate 1) whether alterations in hepatic LDL-receptor (LDL-r) expression may serve as a potential mechanism of developmental programming behind the altered lipid metabolism of the offspring, 2) whether altered lipid metabolism leads to aortic vascular dysfunction in the offspring, 3) whether deleterious effects of SFA exposure preweaning are influenced by postweaning diet, and 4) whether gender-specific programming effects are observed. Female C57BL/6 mice were fed a high-SFA diet or regular chow during gestation and lactation while their pups, both male and female, received either SFA or a chow diet after weaning. Male offspring obtained from mothers fed an SFA diet and those who continued on chow postweaning had higher plasma triglycerides and total cholesterol, whereas female offspring had higher plasma total and LDL cholesterol levels, lower hepatic LDL-r mRNA expression, and reduced aortic contractile responses compared with the offspring that were fed chow throughout the study. A comparison of the postweaning diet revealed significantly lower hepatic LDL-r expression along with significantly higher plasma LDL-cholesterol concentration in the female offspring that were obtained from mothers fed an SFA diet and who continued on an SFA diet postweaning, compared with the female offspring that were obtained from mothers fed an SFA diet but who continued on chow postweaning. In conclusion, we report a novel observation of hepatic LDL-r-mediated programming of altered lipid metabolism, along with aortic vascular dysfunction, in the female offspring of mothers fed a high-SFA diet. Male offspring only exhibited dyslipidemia, suggesting gender-mediated programming. This study further highlighted the role of postweaning diets in overriding the effects of maternal programming.

fetal programming; plasma lipids; hepatic LDL-receptor; dietary fats; cardiovascular disease

RECENT EVIDENCE BASED ON THE developmental origins of health and disease hypothesis suggests that early nutrition can be critical in determining the cardiovascular health of an individual (43, 44). According to this hypothesis, which was originally known as Barker’s hypothesis, an adverse maternal nutrition during gestation can create a stressed environment for the developing fetus. The fetus responds to this nutritional stress by programming its own growth in a way that could increase its risk of developing metabolic disorders in later life (6–8). Considering that a typical Western diet is rich in dietary fat content (17), especially saturated fatty acids (SFA), some studies have investigated the role of high-fat feeding in the concept of developmental origins of health and disease (3–5). These studies indicate that maternal high-SFA consumption during pregnancy can induce features of metabolic syndrome including dyslipidemia (26, 27, 35), insulin resistance (57), and hypertension (36, 35) in the adult offspring. However, the underlying mechanisms of these effects are not known. Moreover, the role of interaction between the prenatal and postnatal diet on the health outcome in the offspring is yet to be determined. It is also important to evaluate whether the postnatal diet can ameliorate or deteriorate the programming effects of the maternal diet.

A diet rich in SFA causes plasma lipid abnormalities (18) and endothelial dysfunction (11), which are considered to be early predictors for the future development of cardiovascular disease (CVD). SFA intake raises plasma LDL levels (54, 60) and inhibits the expression of hepatic LDL-receptor (LDL-r) (29, 31). Both in vitro and in vivo studies have shown that aortic endothelial function may be abnormal within a few hours of exposure to increased levels of LDL cholesterol (32, 40). Furthermore, SFA intake can evoke free radical synthesis that may contribute to vascular dysfunction by decreasing endothelium-derived vasodilator molecules like nitric oxide (NO) (20).

We have previously compared the effects of maternal diet enriched in SFA vs. polyunsaturated fatty acid (PUFA) on the lipid metabolism in the adult female offspring of C57BL/6 mice. A continuous exposure to SFA during gestation, lactation, and postweaning had deleterious effects, whereas similar exposure to PUFA had beneficial effects on the lipid metabolism of the offspring (13). The focus of the present study was to investigate whether the deleterious effects of SFA exposure during gestation and lactation are further deteriorated or ameliorated by the postweaning diet in C57BL/6 mice. We further investigated the expression of hepatic LDL-r in the adult offspring as a potential mechanism behind the developmental programming of altered lipid metabolism, and evaluated whether these alterations lead to aortic vascular dysfunction in the offspring. Both male and female offspring were studied to identify any gender-specific differences.

The offspring obtained from C57BL/6 mothers fed a high-SFA diet during gestation and lactation showed dyslipidemia, vascular dysfunction, and abnormal aortic fatty acid composition. These effects were partly determined by offspring sex and were exaggerated when the offspring continued on the high-SFA diet postweaning. Furthermore, we report for the first time a correlation between maternal intake of a high-SFA diet and...
reduced hepatic LDL-r mRNA expression as a mechanism behind increased plasma LDL-cholesterol levels in the female offspring.

MATERIALS AND METHODS

Animals and diets. Female C57Bl/6 mice (7-wk-old) were obtained from Charles River Laboratories and were kept on a standard rodent Chow diet (Agribrands Purina) for 1 wk prior to being fed the experimental diets. After this acclimatization period, the mice were divided into two groups; one group continued on rodent chow, while the other group was fed a semipurified diet (MP Biochemicals) containing high fat (20% wt/wt) rich in SFA, where animal lard obtained from a local supermarket was used as a source of SFA. A base semipurified diet, in powder form, with fat source omitted, was designed specifically to permit control of the fat content level at 20% wt/wt. Female breeders were fed either SFA or chow for 2 wk ad libitum before mating, while the males were fed chow. The females continued on the same diets during mating, pregnancy, and lactation. At weaning, the offspring obtained from each mother were divided into two balanced groups; one group continued on SFA diet and the other group was fed rodent chow. Thus, we obtained four groups of offspring that were identified by pre-postweaning diet combinations: SFA/SFA (S/S), SFA/chow (S/C), chow/chow (C/C), and chow/SFA (C/S). The offspring were fed their assigned diets ad libitum for another 8 wk. Both male as well as female offspring from each mother were used for the study to identify any gender differences.

All the animals were housed in a single room with a 12:12-h light-dark period cycle. The temperature and humidity were maintained at 21°C and 35 ± 5%, respectively. Body weights and food consumption of the offspring were recorded weekly. At 11 wk of age, mice were fasted for 12 h overnight and then killed by anesthetizing the animals with halothane vapor in a closed chamber. The Institutional Animal Care Committee of Memorial University approved all the experimental procedures that were in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Plasma lipid analysis. Blood was collected using cardiac puncture into tubes containing 4.5 mM EDTA, pH 7.5. Plasma was collected by centrifugation of whole blood at 3,000 × g for 15 min. Plasma triglycerides (TG) and total cholesterol concentrations were determined using TG assay kit model 2150-101 and cholesterol assay kit model 1010-430 (Stanbio Laboratories). HDL was precipitated using HDL reagent model 200-26A (Diagnostics Chemicals) and HDL-cholesterol concentration was determined using cholesterol assay kit model 1010-430 (Stanbio Laboratories). The plasma LDL-cholesterol concentration was calculated from plasma total cholesterol, HDL-cholesterol, and TG concentration according to the method of Friedewald et al. (24).

Plasma glucose and free fatty acid analysis. Glucose concentrations were measured using a commercially available glucometer (LifeScan) in the fasted animals from the tail blood at the time of sacrificing the animals. Free fatty acid content of the plasma was determined using a commercially available kit model 999-34691 (Wako Chemicals).

Quantitative-PCR analysis of hepatic LDL-r. Liver tissues from killed animals were snap frozen in liquid nitrogen and stored at −70°C until further analysis. Total RNA was isolated from the liver samples as previously described (14). Reverse transcription of total RNA into cDNA was performed using the one-step reverse transcription kit from Roche Diagnostics. The mRNA expression levels were determined on a Lightcycler 2.0 Detection System (Roche Diagnostics). An upstream primer 5′-AGGCTGGGGCTCATAGG-3′ with a downstream primer 5′-TGGCCTCAGGTCTCATCT-3′ corresponding to a coding sequence of mouse LDL-r and an upstream primer 5′-TGAAGGACGCTGACAGGG-3′ with a downstream primer 5′-CGAAGGTTGAGGTGGGAG-3′ corresponding to a coding sequence of mouse GAPDH cDNA were used. Briefly, standard curves were generated using the serial dilution of a control sample for both LDL-r and GAPDH genes and the PCR efficiency for each reaction was calculated. No differences were found in the expression of GAPDH among various groups. The LDL-r expression for each sample was then calculated in relation to the expression of GAPDH, thus normalizing and correcting the data for the differences in PCR efficiencies for each set of primers.

Vascular function analysis. Thoracic descending aortae were dissected, and adherent fat was removed while being viewed with the aid of a dissection microscope. Rings of aortae (2-mm lengths) were mounted in separate chambers of a multymograph 610M (Danish Myograph Technology). Aortae were bathed in Krebs bicarbonate buffered (pH 7.4) physiological saline solution composed of 114 mM NaCl, 4.7 mM KCl, 0.8 mM KH2PO4, 1.2 mM MgCl2, 2.5 mM CaCl2, 25 mM NaHCO3, and 11 mM d-glucose at 37°C and bubbled with a gas mixture of 5% CO2-95% O2. Each ring was stretched to 90% of the diameter that was estimated to produce wall stress equivalent to 100 mmHg (48). The resting baseline tension was not found to be different among groups. After a 1-h equilibration period, drug concentration-contractile response curves for KCl (30–120 mM), phenylephrine (PE; 10 nM to 10 μM), and thromboxane A2 mimetic (U46619; 10 nM to 1 μM) were then constructed in separate aortic rings. Endothelium-dependent and-independent relaxation responses were measured using ACh (10 nM to 10 μM) and sodium nitroprusside (SNP; 10 nM to 10 μM), respectively, in vessels contracted submaximally with U46619 (50–75% of Emax). To determine the involvement of NO synthases (NOS), cumulative KCl and PE concentration-contractile responses were repeated after treatment with the NOS inhibitor Nω-nitro-l-arginine methyl ester (lNAME; 300 μM; 10 min). From each aortic ring, E50 values for each drug dose-response relationship were determined by fitting to four parameters logistic dose-response curves (GraphPad Software). Emax represents the maximal response to each drug. All chemicals were purchased from Sigma Aldrich (St. Louis, MO).

Aortic fatty acid analysis. Sections of aorta, cleaned of adherent fat, but which were not utilized for the vascular function analysis, were placed in physiological saline solution and frozen at −20°C until a later date for fatty acid analysis. Aortic pieces of individual mice were pooled together for each of the experimental groups, and lipids were extracted using chloroform and methanol (2:1 vol/vol) (22). Fatty acid methyl esters were derivitized and then separated by gas chromatograph with detection using flame ionization method (34).

Statistical analysis. Data were compared by ANOVA followed by Newman Keuls post hoc test (GraphPad Prism software, version 3.2) and P < 0.05 was considered significant. Different superscript letters in each figure indicate significant differences for specific comparisons between groups as indicated in figure legends. Values, symbols, and bars represent the means ± se for n = 8, in each group. All assay measurements were made in ≥ 2 samples from each mouse.

RESULTS

Effects of pre- and postweaning diets rich in SFA on body weight, food intake, and plasma index of glucose and free fatty acids. Male offspring obtained from mothers fed SFA during gestation and lactation that were fed chow postweaning weighed significantly less than the offspring of mothers fed chow and those who continued on chow postweaning (S/C vs. C/C, P < 0.05) (Table 1). However, the body weight of male offspring fed a high-SFA diet postweaning was similar regardless of their maternal diet (S/S vs. C/S, P > 0.05). Maternal or offspring diet rich in SFA alone did not affect the body weight of the female offspring (S/C vs. C/C, P > 0.05; S/S vs. C/S, P > 0.05). However, female S/S offspring weighed significantly heavier than the S/C and C/C offspring (P < 0.05), suggesting that a continuous exposure to SFA during the
pregnatal and postnatal time period had additive effects on weight gain. Exposure to high-SFA diet, either during the prenatal or postnatal time period, was associated with a reduction in food intake. Both male and female S/S, S/C, and C/S offspring ingested ~30% less food compared with the C/C offspring (P < 0.01), although no changes were observed in the caloric intake across the groups (Table 1).

Fasting plasma glucose concentration (Table 1) was 28% higher in the male S/S offspring than the C/C offspring (P < 0.05), suggesting that continuous exposure to SFA during the prenatal and postnatal time period can have exaggerated effects compared with either prenatal or postnatal time periods alone. On the other hand, female C/S offspring had significantly higher plasma glucose concentration compared with the S/C and C/C offspring, suggesting a negative effect of the postnatal diets rich in SFA on glucose metabolism. No differences were observed in plasma free fatty acid concentration among various groups of male offspring. However, the female S/S group had ~30% higher plasma free fatty acid concentration compared with all other groups (P < 0.05).

Effects of pre- and postweaning diets rich in SFA on plasma lipid levels. Plasma TG concentration of male offspring obtained from mothers fed SFA during gestation and lactation that were continued on regular chow postweaning, was significantly higher than the offspring obtained from mothers fed chow and those who continued on chow postweaning (S/C vs. C/C, P < 0.05) (Fig. 1A), indicating a deleterious effect of the maternal diet rich in SFA. The plasma TG concentration remained higher in S/S, S/C, and C/S offspring compared with the C/C offspring (P < 0.05), indicating deleterious effects of SFA exposure during postnatal time periods. There was no effect of prenatal or postnatal SFA exposure on plasma TG concentration in the female offspring (Fig. 1A).

Plasma total cholesterol concentration was not different between male S/C and C/C offspring (S/C vs. C/C, P > 0.05) and between the male S/S and C/S offspring (S/S vs. C/S, P > 0.05). However, both male S/S and C/S offspring had higher plasma total cholesterol than the S/C (P < 0.01) and C/C offspring (P < 0.001), indicating the importance of SFA intake during postnatal time period. Interestingly, in the case of females, the S/C offspring had 32% higher plasma total cholesterol than the C/C group (P < 0.05), suggesting the importance of maternal diet. Plasma total cholesterol concentration was not different between female S/S and C/S offspring; however, both groups had significantly higher plasma total cholesterol concentration than the S/C (P < 0.01) and C/C offspring (P < 0.001), indicating deleterious effects of postnatal diet rich in SFA (Fig. 1B).

Plasma LDL-cholesterol concentration was significantly higher in male S/S and C/S offspring than the S/C (P < 0.01) and C/C (P < 0.05) offspring (Fig. 1C), an observation similar to the plasma total cholesterol concentration. Maternal diet had a significant effect on the plasma LDL-cholesterol concentration in the case of the females, where the S/C offspring had 58% higher LDL-cholesterol than the C/C offspring (P < 0.05). No differences were observed between plasma LDL-cholesterol concentration of S/S and C/S offspring, while both groups had higher LDL-cholesterol concentration than the S/C (P < 0.05) and the C/C (P < 0.001) offspring, suggesting the importance of postnatal diets.

Plasma HDL-cholesterol concentrations were not different among various groups of male offspring. Plasma HDL-cholesterol concentration was also not different between female S/C and C/C offspring (S/C vs. C/C, P > 0.05); however, the C/S offspring had 40% higher HDL-cholesterol than the S/S offspring (S/S vs. C/C, P < 0.01), indicating that a continuous exposure to SFA during prenatal and postnatal time period was associated with reduced levels of plasma HDL-cholesterol in the offspring.

The ratio of LDL/HDL cholesterol is regarded as one of the markers for establishing the risk of developing CVD. The LDL-to-HDL ratio was not different among various groups of male offspring (Fig. 1E). However, female S/S offspring showed a significantly higher LDL-to-HDL ratio than C/S (40%, P < 0.05), S/C (65%, P < 0.01), and C/C offspring (80%, P < 0.001) (Fig. 1E), indicating that a continuous exposure to SFA during prenatal, as well as postnatal, time period would predict deleterious effects.

Effects of pre- and postweaning diets rich in SFA on hepatic LDL-r mRNA expression. Hepatic LDL-r plays an important role in the removal of LDL-cholesterol from circulation. Offspring exposed to the high-SFA diet during the prenatal time period showed an increase in plasma LDL-cholesterol levels; thus we investigated whether a maternal diet rich in SFA programmed the expression of LDL-r in the offspring. The mRNA expression of hepatic LDL-r was not significantly different among various groups of male offspring (Fig. 2). Interestingly, the female S/C offspring showed 30% lower expression of hepatic LDL-r mRNA compared with the C/C offspring (P < 0.05), suggesting a programming effect of the maternal diet. The decrease in hepatic LDL-r mRNA levels in the S/C offspring was in parallel with their high plasma LDL-cholesterol levels. Both S/S and C/S female offspring also showed significantly reduced hepatic LDL-r mRNA levels compared with the S/C (P < 0.001) and C/C offspring (P <

Table 1. Body weight, food intake, plasma glucose, and free fatty acid concentrations of various offspring groups at the time of sacrifice

<table>
<thead>
<tr>
<th>Preweaning Diet</th>
<th>SFA</th>
<th>Chow</th>
<th>Postweaning Diet</th>
<th>SFA</th>
<th>Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>24.3 ± 1.2b</td>
<td>18.6 ± 0.8b</td>
<td>24.1 ± 0.7a</td>
<td>21.7 ± 0.2a</td>
<td></td>
</tr>
<tr>
<td>Food intake, g/wk</td>
<td>23.5 ± 2.0a</td>
<td>22.4 ± 1.3b</td>
<td>17.3 ± 1.3b</td>
<td>29.2 ± 2.6a</td>
<td></td>
</tr>
<tr>
<td>Caloric intake, kcal/day</td>
<td>17.1 ± 1.5</td>
<td>13.2 ± 0.6</td>
<td>26.2 ± 0.9</td>
<td>17.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>11.7 ± 1.0a</td>
<td>9.4 ± 0.5a</td>
<td>10.4 ± 1.1b</td>
<td>8.5 ± 0.4b</td>
<td></td>
</tr>
<tr>
<td>Plasma free fatty acids, meq/l</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

| Female offspring |     |      |                 |     |      |
| Body weight, g    | 20.3 ± 0.8a | 17.1 ± 0.7b | 18.8 ± 0.8ab | 17.5 ± 0.5b |
| Food intake, g/wk | 21.5 ± 1.8b | 22.1 ± 0.5b | 19.3 ± 1.5b | 28.8 ± 1.7b |
| Caloric intake, kcal/day | 15.7 ± 1.1 | 13.1 ± 0.4 | 14.1 ± 1.3 | 17.0 ± 2.1 |
| Plasma glucose, mM | 9.8 ± 0.8a | 8.9 ± 0.5a | 11.8 ± 0.3a | 8.1 ± 0.4b |
| Plasma free fatty acids, meq/l | 1.7 ± 0.2 | 1.1 ± 0.2 | 0.9 ± 0.1b | 1.0 ± 0.1b |

Values are expressed as means ± SE. SFA, saturated fatty acids. SFA/SFA, SFA/chow, chow/chow, and chow/SFA represent pre-/postweaning diet combinations. a,bSuperscripts represent significant differences (P < 0.05) between various male (n = 8) and female (n = 8) groups, evaluated using 1-way ANOVA and Newman Keuls post hoc test.
0.001), indicating that postnatal diets rich in SFA, also had an impact on the expression of hepatic LDL-r mRNA levels (Fig. 2).

Effects of pre- and postweaning diets rich in SFA on contractile responses of the aorta. Vascular function was assessed in the offspring by comparing the contraction and relaxation responses of isolated aortic rings. E_max of aortic rings in response to depolarization with high concentrations of KCl were ~50% lower in male offspring obtained from mothers fed SFA (S/S and S/C) compared with the offspring obtained from mothers fed chow (C/C, P < 0.05; C/S, P < 0.01), regardless of their postweaning diet, indicating that a maternal diet rich in SFA was associated with a reduction of contractile responses.

Fig. 1. Plasma analysis of various male (n = 8) and female (n = 8) offspring groups for triglycerides (A), total cholesterol (B), LDL-cholesterol (C), HDL-cholesterol (D), and LDL/HDL-to-cholesterol ratio (E). Values are expressed as means ± SE. a,b Superscripts represent significant differences (P < 0.05) among groups evaluated using 1-way ANOVA and Newman Keuls post hoc test. SFA, saturated fatty acids. SFA/SFA (S/S), SFA/chow (S/C), chow/chow (C/C), and chow/SFA (C/S) represent pre-/postweaning diet combinations.
toward KCl (Fig. 3A). On the other hand, maximal contractile responses to KCl in female S/S (P < 0.01), S/C (P < 0.05), and C/S offspring (P < 0.05) were ~45% lower than the C/C offspring, indicating that SFA feeding during both prenatal and postnatal time periods was associated with reduced contractile responses (Fig. 3A).

The contractile responses of the aortae in response to α1-adrenergic receptor agonist PE were not different between male offspring (Fig. 3B). However, female S/S (P < 0.001), S/C (P < 0.01), and C/S offspring (P < 0.01) had ~70% lower maximum contractions in response to PE than the C/C offspring. The sensitivity (EC50 values) to PE was not different among aortae from male (S/S, 7.7 ± 0.2; S/C, 7.6 ± 0.1; C/C, 7.5 ± 0.2; C/S, 7.7 ± 0.2) and female offspring (S/S, 7.7 ± 0.1; S/C, 7.6 ± 0.1; C/C, 7.6 ± 0.2; C/S, 7.3 ± 0.1).

The contractile responses of the aortae, from various offspring groups, were also tested in response to thromboxane A2 receptor agonist U46619 (Fig. 3C). Neither the contractions nor the EC50 values (S/S, 7.2 ± 0.1; S/C, 7.3 ± 0.1; C/C, 7.6 ± 0.2; C/S, 7.6 ± 0.1) of aortae to U46619 were different among males, which was similar to the findings with PE. However, female S/S (P < 0.01), S/C (P < 0.01), and C/S offspring (P < 0.05) had ~40% lower maximum contractions by U46619 than the C/C offspring, suggesting an inhibiting effect of SFA feeding, regardless of the prenatal or postnatal diet. The female S/C aortae (EC50, 7.0 ± 0.1) were significantly less sensitive to U46619 than the C/C offspring aortae (EC50, 7.7 ± 0.1; P < 0.01) (Fig. 3C), also suggesting that maternal high-SFA intake interacted with the thromboxane-mediated contractile responses. The C/S offspring aortae (EC50, 7.0 ± 0.2) were significantly less sensitive to U46619 than the S/S (EC50, 7.5 ± 0.1; P < 0.05) and C/C offspring aortae (EC50, 7.7 ± 0.1; P < 0.01), suggesting that SFA feeding postweaning alone could also cause a reduction in sensitivity toward U46619.

Effects of pre- and postweaning diets rich in SFA on endothelium-dependent and -independent relaxation responses of the aorta. Altered vascular contraction and relaxation responses to agonists may reflect a dysfunction in the ability of the endothelium to produce vasodilator substances; thus we measured endothelium-dependent (ACh) and -independent (SNP) relaxation responses in the aortic rings of all offspring. The maximal relaxations induced by ACh of aortic rings were not different among males (Fig. 4A). However, the sensitivity to ACh in the aortic vessels of male S/S offspring (EC50, 8.1 ± 0.1) was significantly higher than the S/C (EC50, 7.4 ± 0.2; P < 0.05), C/C (EC50, 7.3 ± 0.2; P < 0.05), and C/S offspring (EC50, 7.4 ± 0.2, P < 0.05). In females, neither ACh-induced relaxations nor their EC50 values (S/S, 7.4 ± 0.1; S/C, 7.2 ± 0.2; C/C, 7.1 ± 0.1; C/S, 6.9 ± 0.2) were different among groups (Fig. 4A).

SNP-induced maximal relaxations of aortic rings in various groups of male offspring were not different, whereas the sensitivity to SNP in aortic vessels of the male S/C offspring (EC50, 8.2 ± 0.1) was higher than the S/S offspring (EC50, 7.7 ± 0.1; P < 0.05) but was not different than the C/C (EC50, 7.9 ± 0.1, P > 0.05) or C/S offspring (EC50, 8.0 ± 0.1, P > 0.05) (Fig. 4B), suggesting that a continuous exposure to SFA during prenatal and postnatal diet led to a small reduction in the aortic sensitivity to SNP. Unlike males, no differences were found in either maximal relaxation or EC50 values (S/S, 8.1 ± 0.2; S/C, 8.1 ± 0.1; C/C, 7.7 ± 0.1; C/S, 8.0 ± 0.1) in response to SNP among females (Fig. 4B).

Effects of l-NAME on contractile responses of aorta. We tested the hypothesis that increased basal NOS activity in aortic vessels of various offspring groups was contributing to the mechanisms underlying reduced contractility. Thus, we tested whether l-NAME-mediated inhibition of NOS restored the contractile responses of the aortic vessels. l-NAME treatment significantly increased contractions to KCl in both male and female S/C offspring (54% & 40%, respectively) compared with the untreated S/C group, indicating that maternal dietary intake of SFA was associated with alterations of NOS pathway (Fig. 5A). A significant increase in contractions to KCl in female C/S offspring (44%) in response to l-NAME was also observed, compared with the untreated C/S group, indicating the importance of postnatal diet. l-NAME treatment did not affect the contractile responses to KCl in the case of both male and female C/C offspring reinforcing that high-SFA intake interfered with the NOS pathway.

The contractile response to PE was increased by l-NAME treatment only in male S/C group, whereas no differences were found in the female groups (Fig. 5B). l-NAME treatment had no effect on contractions to U46619 in either males or females (data not shown).

Effects of pre- and postweaning diets rich in SFA on fatty acid composition of the aorta. Fatty acid composition of the aortic vessels can be directly related to their structure and function that is given in Table 2. Palmitic acid (C16:0) and stearic acid (C18:0) content of the aorta was higher in the male and female S/S, S/C, and C/S groups compared with the C/C.
offspring. Male S/S offspring had the highest content of C16:0 and C18:0, whereas the female C/S offspring had the highest C16:0 and C18:0. The amount of oleic acid (C18:1), a monounsaturated fatty acid, was highest for the C/S offspring in both males and females. The amount of α-linolenic acid (C18:3), eicosapentaenoic acid (20:5), and docosahexaenoic acid (DHA; 22:6) that belong to the n-3 PUFA, was lesser for male S/S and C/S offspring compared with the S/C and C/C offspring. On the other hand, the C/S female offspring had the lowest content of α-linolenic acid and eicosapentaenoic acid, while S/S offspring had the lowest content of DHA compared with other groups, suggesting that postnatal diet rich in SFA alters the n-3 content of aortic vessels. Linoleic acid (C18:2) and arachidonic acid (AA; 20:4), which belong to n-6 PUFA, were lowest in male S/S compared with all other groups. However, the female offspring showed the highest content of AA in the S/S group compared with other groups. The sum total of SFA was highest in the male S/S offspring, suggesting exaggerated effects of SFA intake during prenatal and postnatal time periods. On the other hand, the sum total of SFA in female C/S offspring was higher compared with other groups, suggesting an effect for postnatal diet only. The sum total of monounsaturated fatty acid was highest in the case of C/S group compared with all other groups for both male and female offspring, indicating the importance of postnatal diets. The sum total of PUFA was lowest for the S/S group compared with all other groups in the case of male offspring, whereas it was the highest for the S/S group in the case of female offspring, suggesting gender differences.

**DISCUSSION**

This study evaluated the relative effects of high-SFA feeding during prenatal and postnatal time periods on the cardiovascular risk factors in the adult offspring of C57B1/6 mice. We demonstrated that maternal SFA feeding induced sex-selective
features of dyslipidemia and vascular dysfunction in their offspring. Furthermore, this study established that maternal high-SFA intake can program the expression of hepatic LDL-r mRNA in their offspring, thereby causing an increase in plasma LDL-cholesterol levels. The suppression of hepatic LDL-r gene expression may prove to be a key factor in the pathogenesis of cardiovascular abnormalities in these offspring. The present study further highlighted the interaction between pre- and postnatal diets in the manifestation of dyslipidemia and aortic vascular dysfunction in the offspring.

Female offspring of mothers fed SFA and those who continued on the SFA diet postweaning (S/S) were heavier than all other groups. On the other hand, male offspring fed an SFA diet postweaning (S/S and C/S), regardless of their maternal diet, were heavier than other groups. These findings suggest that both pre- and postweaning diets rich in SFA caused an increase in body weight of the offspring. The observed body weight gain in the offspring was not due to increased energy intake, as all groups maintained similar calorific intake for both males and females (Table 1). However, offspring exposed to SFA-rich pre- or postweaning diets (S/S, S/C, and C/S), consumed significantly less food compared with offspring exposed to chow throughout the study (C/C). Previous studies have reported increased adiposity and body weight in male and female offspring of mothers fed an SFA-rich diet during pregnancy and lactation in different rat models (9, 27, 37). It has also been shown earlier that rodents, like humans, can regulate their food intake and energy expenditure to maintain a set body weight (33). This would explain the reduction of food intake in the case of S/S and C/S offspring. Although the calorific intake was similar for all dietary groups, the offspring consuming a high-SFA diet postweaning, obtained more calories from fat compared with the C/C offspring, which could account for their increased body weight. Interestingly, the S/C offspring also consumed significantly less food than the C/C offspring, while maintaining a similar body weight. It appears that the intake of SFA diet by mothers during pregnancy had a programming effect on the developing fetus to lower their appetite after birth. Maternal nutrition during pregnancy has previously been shown to program the appetite-regulation in the offspring (21, 46, 47, 59), which is proposed to involve leptin. Leptin, an adipocyte-derived hormone has been reported to influence the appetite-controlling centers in the brain and hence is proposed to play an important role in the regulation of food intake (47, 58). A diet rich in SFA has been reported to increase plasma leptin concentrations both in mice as well as in humans (15, 23). It is likely that SFA feeding of the mothers during pregnancy may have caused an increase in leptin concentration, which would, in turn, program the developing fetus to consume less food after birth. We were, however, not able to measure the plasma leptin concentration due to a limited amount of samples.

Plasma lipids were higher in the S/C offspring compared with C/C offspring, indicating that a high maternal SFA intake during pregnancy induced features of dyslipidemia in both male and female adult offspring. Plasma lipids were also increased in the S/S and C/S offspring, compared with the S/C and C/C offspring, indicating that postnatal intake of SFA was
equally important in inducing features of dyslipidemia in the offspring. The deleterious effects of SFA intake on the plasma lipid parameters are in line with the proposed effects of SFA intake. It has been established that a high-SFA intake increases the risk of CVD by causing dyslipidemia (45). Some, but not all of the previous studies investigating the role of maternal high-fat feeding in the fetal programming of adult disorders have reported features of dyslipidemia in the offspring (26, 35, 37). We observed that males revealed an increase in TG concentration in response to the prenatal SFA exposure, whereas the females exhibited increased total- and LDL-cholesterol concentrations. Both sexes appeared to respond to changes in the prenatal nutritional environment by exhibiting altered plasma lipids; our data, however, indicated the involvement of gender-associated mechanisms behind programming of dyslipidemia.

The ratio of LDL- to HDL-cholesterol concentration is considered to be one of the markers for the future development of CVD (39, 51). A diet rich in SFA has been shown to increase plasma LDL-cholesterol and decrease the HDL-cholesterol concentrations, resulting in an increased LDL-to-HDL ratio (45). Female S/S offspring had the highest LDL-to-HDL ratio among all other groups, suggesting that a continuous exposure to high-SFA intake during the prenatal and postnatal time periods had additive effects, thus increasing the risk of developing CVD in females.

A possible explanation for the increased circulating levels of plasma LDL-cholesterol can be related to its reduced clearance from the circulation. Liver LDL-r removes LDL-cholesterol from circulation, thereby maintaining cholesterol homeostasis (12). A high-SFA intake during the postnatal time period has been reported to lower the expression of hepatic LDL-r in several animal models (19, 30, 31, 55) and in human studies (29). The LDL-r mRNA expression was significantly reduced in the female S/S and C/S offspring. Moreover, female S/C offspring also had more significantly reduced LDL-r levels than the C/C offspring, which was in parallel with their increased plasma LDL-cholesterol concentrations. Thus, a decrease in hepatic LDL-r levels supported the hypothesis that in female offspring, the increase in LDL-cholesterol was a result of programming effects of maternal diet enriched in SFA. This is the first study to report the programming of hepatic LDL-r mRNA expression in the offspring, by maternal diet rich in SFA, providing a mechanistic link between dyslipidemia and CVD.

Our study design allowed us to investigate whether programming effects of the maternal diet could be reversed by postnatal diets. The offspring obtained from mothers fed SFA during gestation and lactation that continued on chow diet postweaning (S/C) showed an increase in hepatic LDL-r expression compared with S/S offspring. These findings suggest that although maternal SFA feeding programmed the hepatic LDL-r mRNA expression of female offspring, the postnatal chow diet,
Table 2. Total fatty acid composition of aorta from various groups of male and female offspring

<table>
<thead>
<tr>
<th>Postweaning Diet</th>
<th>SFA</th>
<th>Chow</th>
<th>SFA</th>
<th>Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>2.6</td>
<td>2.2</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td>C16:0</td>
<td>40.1</td>
<td>31.2</td>
<td>33</td>
<td>24.1</td>
</tr>
<tr>
<td>C18:0</td>
<td>30.6</td>
<td>21.3</td>
<td>20.3</td>
<td>16.8</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.9</td>
<td>1.2</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>C 18:1</td>
<td>10.9</td>
<td>17.4</td>
<td>26.2</td>
<td>14.8</td>
</tr>
<tr>
<td>C18:2</td>
<td>2.1</td>
<td>9</td>
<td>6.4</td>
<td>6.3</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.6</td>
<td>1.3</td>
<td>0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>C 20:4</td>
<td>1.1</td>
<td>5.4</td>
<td>4.2</td>
<td>5.8</td>
</tr>
<tr>
<td>C20:5</td>
<td>ND</td>
<td>1.7</td>
<td>ND</td>
<td>2.7</td>
</tr>
<tr>
<td>C22:6</td>
<td>ND</td>
<td>1.9</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>73.3</td>
<td>54.6</td>
<td>55.5</td>
<td>43.3</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>11.8</td>
<td>18.6</td>
<td>28.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>3.76</td>
<td>19.24</td>
<td>12.6</td>
<td>18.3</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Female offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>1.7</td>
<td>2.4</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>C16:0</td>
<td>26.9</td>
<td>24.1</td>
<td>31.6</td>
<td>20.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>19.6</td>
<td>16.8</td>
<td>22.8</td>
<td>16.3</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.2</td>
<td>1.5</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>C 18:1</td>
<td>9.7</td>
<td>14.8</td>
<td>24.6</td>
<td>4.7</td>
</tr>
<tr>
<td>C18:2</td>
<td>1.8</td>
<td>6.3</td>
<td>7</td>
<td>1.5</td>
</tr>
<tr>
<td>C18:3</td>
<td>4.7</td>
<td>2</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>C 20:4</td>
<td>11.8</td>
<td>4.3</td>
<td>4.2</td>
<td>2.7</td>
</tr>
<tr>
<td>C20:5</td>
<td>9.2</td>
<td>2.7</td>
<td>ND</td>
<td>1.9</td>
</tr>
<tr>
<td>C22:6</td>
<td>0.6</td>
<td>1.6</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>48.2</td>
<td>43.3</td>
<td>56.5</td>
<td>38.5</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>12.9</td>
<td>16.3</td>
<td>26.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>28</td>
<td>16.9</td>
<td>13.2</td>
<td>8.2</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>1.1</td>
<td>0.6</td>
<td>0.2</td>
<td>1</td>
</tr>
</tbody>
</table>

Aortic tissue composition of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidonic acid (AA, C20:4), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) of various male and female offspring groups. Total fatty acid composition was determined after pooling aortic tissues (n = 8) for each offspring group. Fatty acids are expressed as a percentage of the total extracted fatty acids. MUFA, monounsaturated fatty acid; PUFA, polysaturated fatty acid. SFA/SFA (S/S), SFA/chow (S/C), chow/chow (C/C), and chow/SFA (C/S) represent pre-/postweaning diet combinations.

in part, reversed the programming effects of the maternal SFA feeding.

Vascular dysfunction has been considered to be another marker for the onset of CVD. It has been reported earlier that endothelial function may be abnormal within a few hours of exposure to increased levels of LDL cholesterol (16, 32, 40). Thus, vascular reactivity was expected to change in the presence of high levels of LDL cholesterol, especially in the female offspring. Increased vascular contractility and decreased vasodilator capacity has been observed in adult models of maternal overnutrition and CVD (25, 37, 38). Feeding a high-SFA maternal diet caused a reduction in vascular contractility, whereas vasodilator capacity was unaffected in our study. The male offspring exhibited reduced contractile responses toward depolarizing KCl concentrations in both S/S and S/C offspring compared with the C/C and C/S offspring, indicating that maternal SFA intake may alter the components involved in voltage-gated ion channel activation and/or sensitivity of the contractile apparatus to calcium ions in the offspring (50). In females, the contractile responses to all of the agents i.e., KCl, PE, and U46619, were reduced in S/S, S/C, and C/S offspring compared with the C/C offspring, suggesting that female aortic vessels were more affected by SFA feeding. Ozaki et al. (50) have previously reported a reduction in the maximal contractile responses to PE in the femoral arteries of 20-day-old offspring and an increase in the maximal vasoconstriction to U46619 and sensitivity to KCl in 200-day-old offspring of mothers subjected to 30% caloric food restriction during pregnancy. Although it is difficult to exclude the possibility for specific defects in the sympathetic and thromboxane responsiveness of these vessels, a simultaneous reduction in all components of the contractile apparatus points toward a more general effect of high-SFA feeding, regardless of the prenatal or postnatal nutrition, especially in the female offspring.

The principle vasodilating agent generated in conduit vessels is currently thought of as being NO (41). We proposed that an increased production of basal NO would lower thecontractions to all of the agonists used in this study. This hypothesis was tested by assessing the effects of NOS inhibition on contractile responses of the aortic vessels using a NOS inhibitor, L-NAME caused a small increment in the contractile responses to KCl and PE in S/C males, which would be consistent with our hypothesis. Previous studies dealing with maternal overnutrition during pregnancy have indicated an impairment of ACh-induced vasorelaxation, albeit using smaller-caliber resistance-type arteries, where the endothelial-derived relaxing factors differ from those present in aorta, e.g., endothelium-derived hyperpolarizing factor (26, 35, 36, 38). Female offspring did not show any alterations in the relaxation responses to ACh and SNP. The aortae of male S/S, however, were more sensitive to ACh and yet showed reduced responses to the nitrovasodilator SNP. An increased sensitivity to ACh may be considered consistent with our findings of increased basal NO activity, perhaps linked to an insulin-mediated increase in NO availability in these animals. Insulin has been shown to increase the basal NO availability through the phosphatidylinositol-3 kinase and PKB-mediated phosphorylation and hence activation of endothelial NOS (61). Maternal high-SFA intake during pregnancy has been shown to induce hyperglycemia and hyperinsulinemia (25) and whole body insulin resistance in the 24-wk-old rat offspring (57). The age of the offspring in our study then would be a discriminating factor associated with the observed differences to other studies, such that at later ages these mice may become insulin resistant, and we may then observe the impairment of endothelium-dependent vasodilation associated with worsened CVD status. We have not determined the insulin resistance status of these animals, but fasting glucose concentrations reflected that S/S males were hyperglycemic relative to the C/C offspring, which would be expected to precede insulin resistance. On the other hand, female S/S showed an increase in the plasma free fatty acid concentration that could reflect upon these females being insulin resistant later in life.

Maternal high-fat intake has also been reported to generate reactive oxygen species (ROS) and oxidative stress, which has been linked to vascular disorders in the offspring (25, 38). It has been reported that increased ROS generation can lead to increased production of hydrogen peroxide in vascular cells that can act as an endothelial-derived relaxing factor and can induce the vasodilation of aortic vessels in response to ACh (42), despite the presence of endothelial dysfunction (49).
Although, we could not determine the ROS production in the aortic vessels, it is likely that high-SFA intake during pre- and postnatal life caused an increase in the oxidative stress that could be a factor in generating vascular dysfunction in these animals.

Membrane fatty acids, especially those of the endothelial cells, play a crucial role in the manifestation of the signaling cascades that are responsible behind the agonist-induced contraction and relaxation responses of the vascular tissue (52). Alterations in the aortic fatty acid composition can also affect the fluidity of the membrane and hence its function (10). The total fatty acid composition of the aortic vessels was altered in the case of S/S, S/C, and C/S male and female offspring compared with the C/C offspring, suggesting that dietary SFA intake either during prenatal or postnatal time periods could alter vessel composition. The amount of total SFA was highest in the case of S/S, followed by S/C and C/S for male offspring, whereas, female C/C offspring showed the highest aortic SFA content followed by S/S and S/C. These findings could be a basis for gender-associated differences in the vascular function of the offspring that needs to be explored further. Alterations in the fatty acid pool of aortic vessels can also lead to changes in receptor signaling within the vasculature (10). Palmitic acid, an SFA, was increased in the aortic vessels of female S/S, S/C, and C/S offspring that showed reduced aortic contractile responses. Palmitic acid was recently shown to induce endothelial NO synthesis in conjunction with CD36, a class B scavenger receptor (63). Thus, it is likely that palmitic acid-mediated increase in basal NO levels was responsible for the reduction of contractile responses observed in our study. Palmitic acid, on the other hand, has also been reported to increase ROS generation and increase lipid peroxidation (56) in aortic endothelial cells that can lead to the increased risk of developing atherosclerosis later in life. It is not conclusive, however, from our observations whether increased levels of palmitic acid are directly regulating contractile function through NOS pathway or redox mechanisms.

AA, an n-6 fatty acid, is the precursor of a host of vasoactive substances including vasodilators and vasoconstrictors and has been proposed to cause vasodilation directly by inhibiting delayed rectifier K+ currents (53) and increasing the opening of Ca2+-activated K+ channels (1). Ghosh et al. (26) have previously reported a marked reduction in the aortic content of AA and DHA in the offspring of mothers that were fed lard-rich diets during pregnancy, which is similar to our observations. In our study, the S/S males showed decreased content of AA, whereas it was increased in the case of S/S females, which might partly explain the lowering of the contractile responses in the female offspring. As opposed to n-6 PUFAs, the n-3 PUFAs, such as eicosapentaenoic acid and DHA, have been reported to have cardioprotective (2) and antiarrhythmic effects (62). DHA can profoundly affect the membrane fluidity of vascular endothelial cells, thereby altering the endothelial cell function (28). A reduction in the DHA content was observed in both male and female S/S offspring, which can be associated with their reduced contractile responses. Most of the extracted fatty acids in this study represent a pool from membranous, as well as the cytosolic, fractions of the smooth muscle cells and the endothelial cells of the aortic vessels. The fatty acid composition of polar phospholipids would have been a better representative of the membrane fatty acids of the aortic vessels, which was beyond the scope of present study due to limited sample size.

**Perspectives and Significance**

We demonstrated that a maternal high-fat diet enriched in SFA could alter plasma lipid levels, aortic vascular function, and cause alterations in the fatty acid composition of the aortic vessels in the offspring. The novel observation of reduced LDL-r mRNA expression, in the offspring obtained from mothers fed SFA, not only emphasizes the programming effects of maternal diet, but can further provide a plausible mechanism behind the developmental programming of CVD. We propose that maternal SFA intake-induced reduction in LDL-r leads to an increase in plasma LDL-cholesterol in the offspring. An increase in LDL-cholesterol may be associated with increasing the risk of insulin resistance and vascular dysfunction in these offspring, eventually leading to the development of CVD in later life. In addition, we speculate that maternal high-SFA intake may directly induce oxidative stress, insulin resistance, and alterations in the NOS pathways that may work in concert with the deleterious effects of increased LDL-cholesterol in the offspring, thereby increasing the risk of developing CVD. Our study further suggested that although maternal dietary fat intake plays an important role in determining the future health of the offspring, the postnatal dietary fat intake has the potential to either further exaggerate or to protect the offspring, from the effects of maternal programming.

**GRANTS**

The authors thank the Natural Science and Engineering Research Council of Canada for providing financial support. Infrastructure was supported by the Canada Foundation for Innovation New Opportunities, Industrial Research and Innovation Fund Grant 0405-017, and the Canadian Institutes of Health Research Grants ROP-72465 and RSH-78370.

**REFERENCES**


GESTATIONAL FAT INTAKE AND OFFSPRING HEALTH

R1039

39. Taylor PD, McConnell J, Khan IY, Holeman K, Lawrence KM, Asare-Anane H, Persaud SJ, Jones PM, Petrie L, Hanson MA, Poston L. Impaired glucose homeostasis and mitochondrial abnormalities in...


