Statin Treatment in Hypercholesterolemic Pregnant Mice Reduces Cardiovascular Risk Factors in Their Offspring

Maqsood M. Elahi, Felino R. Cagampang, Frederick W. Anthony, Nick Curzen, Sunil K. Ohri, Mark A. Hanson

Abstract—Increasing evidence suggests that hypercholesterolemia during pregnancy initiates pathogenic events in the fetus leading to increased risk of cardiovascular disease in the adult offspring. In this study we examined in mice whether pharmacological intervention using statins in late pregnancy could alleviate the detrimental effects of a high-fat, high-cholesterol (45% fat) maternal diet on the health of the dams and their offspring. Pregnant C57 mice on high-fat, high-cholesterol diet were given the 3hydroxy3methylglutaryl-coenzyme A reductase inhibitor pravastatin in the drinking water (5 mg/kg of body weight per day) in the second half of pregnancy and during lactation to lower cholesterol and improve postweaning maternal blood pressure. Weaned offspring were then fed the high-fat, high-cholesterol diet until adulthood (generating dam/offspring dietary groups high-fat, high-cholesterol/high-fat, high-cholesterol and high-fat, high-cholesterol plus pravastatin during the second half of pregnancy and lactation/high-fat, high-cholesterol). These groups were compared with offspring from mothers fed standard chow (control), which were then fed control diet to adulthood (control/control). Compared with high-fat, high-cholesterol, high-fat, high-cholesterol plus pravastatin during second half of pregnancy and lactation dams showed significantly reduced total cholesterol concentrations and reduced systolic blood pressure. The high-fat, high-cholesterol plus pravastatin during second half of pregnancy and lactation/high-fat, high-cholesterol offspring were significantly lighter, less hypertensive, and more active compared with the high-fat, high-cholesterol/high-fat, high-cholesterol group. Total serum and low-density lipoprotein cholesterol concentrations were significantly lower, and high-density lipoprotein cholesterol concentrations were raised in high-fat, high-cholesterol plus pravastatin during the second half of pregnancy and lactation/high-fat, high-cholesterol offspring, compared with the high-fat, high-cholesterol/high-fat, high-cholesterol group. The control/control offspring showed the lowest blood pressure and cholesterol levels. These findings indicate that the cholesterol-lowering effect of statins in pregnant dams consuming a high-fat, high-cholesterol diet leads to reduced cardiovascular risk factors in offspring that are sustained into adulthood. (Hypertension. 2008;51:939-944.)

Key Words: statins ■ diet ■ hypercholesterolemia ■ hypertension ■ pregnancy

Increasing evidence suggests that hypercholesterolemia during pregnancy initiates pathogenic events in the fetus and, thus, increases the risk of cardiovascular disease in the offspring.12 Atherosclerosis progresses faster in offspring of hypercholesterolemic mothers than those of mothers with normal cholesterol levels.3 In animals, we and others have shown that feeding a high-fat, high-cholesterol diet (HF) during pregnancy and/or lactation induces obesity, vascular dysfunction, impaired skeletal muscle development, sedentary behavior, and gender-specific hypertension in the offspring.5–10 Thus, early interventions may be very effective as shown in studies where the introduction of a low saturated fat diet in infancy and maintaining it during the first decade of life is associated with a reduction in serum cholesterol concentration and enhanced endothelial function in children.11

In humans, the influence of an HF diet is unlikely to commence during pregnancy. The existing data thus raise the important question of whether lipid-lowering interventions during pregnancy in mothers already consuming an HF diet could provide long-lasting benefits to their offspring. Palinski et al7 demonstrated a reduction of atherosclerosis in offspring of rabbits treated with cholestyramine or vitamin E, as well as those receiving combined treatments. Prevailing practice advocates that interruption of total cholesterol synthesis during the first trimester is potentially hazardous to the growing embryo. The cholesterol-lowering “statin” drugs are, therefore, clinically contraindicated in pregnancy, and initial animal studies have shown that they are potentially teratogenic.12,13 Although a recent study reported no evidence of an increase in congenital anomalies in humans compared within the general population after maternal exposure to simvastatin.

Received September 5, 2007; first decision September 25, 2007; revision accepted January 26, 2008.

From the Institute of Developmental Sciences, Developmental Origins of Health and Disease Division (M.M.E., F.R.C., F.W.A., M.A.H.), and Wessex Cardiothoracic Centre (N.C., S.K.O.), University of Southampton School of Medicine, Southampton General Hospital, Southampton, United Kingdom. Correspondence to Mark A. Hanson, Institute of Developmental Sciences, Developmental Origins of Health and Disease Division, University of Southampton School of Medicine, Southampton General Hospital, Mailpoint 887, Southampton SO16 6YD, United Kingdom. E-mail m.hanson@soton.ac.uk

© 2008 American Heart Association, Inc.

Hypertension is available at http://hypertension.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.107.100982

939
or lovastatin, these statins are still highly lipophilic and can result in embryonic-placental concentrations similar to those in maternal plasma. Pravastatin, on the other hand, is the most hydrophilic statin and has not been reported to induce abnormal pregnancy outcomes, even in animals. We, therefore, chose to study the consequences of lowering maternal cholesterol with pravastatin treatment in late pregnancy and lactation with the view of testing the hypothesis that this could also reduce cardiovascular risk factors in the adult offspring. We used the C57BL/6 hypercholesterolemic mouse model, giving the HF diet from the time the dams were weaned, then throughout pregnancy and lactation. We measured blood pressure, body weight, and physical activity in female offspring, because a previous study revealed more pronounced effects on blood pressure in the female offspring of lard-fed pregnant rats compared with males. In addition, we compared plasma cholesterol concentrations in the dams with that of their offspring.

Methods

Experimental Protocol
All of the animal procedures were in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986. Female C57 mice (Charles River Laboratories United Kingdom) were maintained under a 12-hour light-dark cycle at constant temperature (25±2°C) with food and water available ad libitum. At 4 weeks old, the females were randomly allocated to either a control diet of standard laboratory chow (C; 5.3% fat [corn oil], 21.2% protein, 49.2% carbohydrate; Special Diet Services United Kingdom) or an HF experimental diet supplemented 18% weight/weight with animal lard with additional vitamins and minerals, protein, and choline to correct for the dilution (final composition in percentage of grams [weight/weight]; lard 17.8; casein 26.5; choline chloride 0.3; l-cystine 0.4; rice starch 28.3; cellulose 6.1; soya oil 4.3; sucrose 10.4; minerals 4.3; vitamins 1.2; Special Diet Services diet 824053). This HF diet has been used in previous studies. At 10 weeks old, the females were time mated and after confirmation of mating (ie, presence of vaginal plug), were individually housed. From the second half of the pregnancy and throughout lactation, half of the pregnant females on the HF diet were given a water-soluble 3hydroxy3methylglutaryl-coenzyme A reductase inhibitor (pravastatin, Sigma United Kingdom) in their drinking water (HF-S). Pravastatin was dissolved at a concentration that gave a daily dose of 5 mg/kg per day, based on the daily water consumption of pregnant and lactating mice, which we had determined in a preliminary study. The pregnant dams were allowed to give birth, pups were weighed, and litter size was standardized to 8 pups with equal numbers of males and females if possible. From weaning (3 weeks postpartum), offspring from the C dams were fed the same C diet as their mothers. The offspring of HF and HF-S dams were fed ad libitum with the HF diet to generate the HF/HF and HF-S/HF groups. Body weights of the offspring (from 1 week of age to avoid maternal rejection of the pups) and food intake (from weaning) were monitored until 27 weeks of age. We took the average body weights and food intake from each of the 8 litters in each treatment group.

Blood Pressure Measurements
Systolic arterial pressure was measured by tail cuff plethesmography, as described previously by Krege et al. Measurements were conducted in a heated room (≈34°C) to get optimal blood pressure (BP) readings and were conducted at the same time during the day (afternoon). All of the animals were accustomed to the procedure for 7 days before each BP measurement session. At least 5 readings were taken from each animal per session with the highest and lowest readings discarded, and the remaining readings were averaged to get a single session value. BP was measured at 13, 18, 23, and 27 weeks of age. At each time point, we took the average BP values from 8 female offspring picked randomly from each of the 8 litters in each treatment group.

Measurement of Locomotor Activity
Locomotor activity was measured by placing individual animals in automated activity cages equipped with infrared photocells interfaced with a computer, as described previously. Recorded beam breaks were used to automatically calculate the total distance traveled. Measurements were taken at 13, 18, 23 and 27 weeks of age. At each time point, we took the average measurements from 8 female offspring picked randomly from each of the 8 litters in each treatment group.

Measurement of Serum Lipid Profile
A blood sample was drawn by direct heart puncture after anesthetizing the animal with isoflurane and cervical dislocation. Blood samples were taken from a subgroup of females at the time of mating (14 weeks of age) and in dams after weaning their pups. Blood samples were also taken from offspring at 13, 18, 23, and 27 weeks of age. At each time point, we sampled 8 female offspring picked randomly from each of the 8 litters in each treatment group. Total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol in the serum were measured with commercially available kits (Vitros Products) using enzymatic methods and measured by reflectance spectrophotometry, as reported previously.

Data Analysis
The biochemical and biophysical parameters in dams were analyzed using 1-way ANOVA followed by the Tukey-Kramer comparisons test. All of the data are expressed as means±SEM. In the female offspring, effect estimates are from a mixed model analysis that considers all of the time points through the study, controlling for the set of dam-pup relationships. A P<0.05 was considered to be statistically significant. All of the statistical analysis was calculated with SPSS 14.0 (SPSS Inc).

Results

Statin Treatment in Hypercholesterolemic Dams Ameliorates Their BP and Alters Total Cholesterol, LDL Cholesterol, and HDL Cholesterol Profiles
Before becoming pregnant, 14-week-old HF females (fed the HF diet for 10 weeks) showed significantly raised total cholesterol (4.08±0.4 versus 1.99±0.2 mmol/L; P<0.001) and LDL cholesterol (2.1±0.2 versus 0.8±0.2 mmol/L; P<0.001) relative to their C counterparts. HF dams were heavier than C dams during midpregnancy (body weight: 67.4±2.4 versus 41.2±1.8 g; P<0.001) and at the time of weaning their offspring (57.4±3.4 versus 30.7±1.3 g; P<0.001). At weaning of their offspring, HF dams were more hypertensive with raised total cholesterol and LDL cholesterol compared with C dams (Table 1). Statin treatment in HF dams reduced systolic BP, total cholesterol, and LDL cholesterol levels at weaning of their offspring. In addition, administering statin to HF dams increased HDL cholesterol concentrations compared with the untreated HF mothers.

Statin Treatment in Hypercholesterolemic Dams Has Beneficial Effects on Female Offspring BP, Lipid Profiles, and Locomotor Activity
HF/HF offspring were of similar weight as the C/C offspring 1 week postpartum (HF/HF, 3.2±0.1 versus C/C, 2.9±0.2).
However, their overall body weight gain was significantly greater compared with the C/C offspring (Figure, part A and Table 2). The HF-S/HF offspring showed a smaller increase in body weight gain compared with the HF/HF offspring. Systolic BP was significantly lower at 13 to 27 weeks in HF-S/HF compared with HF/HF offspring (Figure, part B and Table 2). As expected, systolic BP for the C/C group was lower at all of the time points examined. We also found that systolic BP in the HF-fed female offspring at 27 weeks of age was much more elevated compared with their HF-fed mothers (151.6±3.6 versus 136.2±1.4 mm Hg, respectively; \(P<0.01\)). Offspring from HF-S mothers were significantly more active at 13 to 27 weeks of age than HF/HF offspring, although not as much as the C/C animals (Figure, part C and Table 2). Total serum and LDL cholesterol concentrations for offspring on HF or C diets followed a similar pattern to dams on HF or C, respectively, and previous exposure of their dams to pravastatin resulted in significantly lower total and LDL cholesterol levels, similar to its effect in the dams themselves (Figure, part D and part E, respectively, and Table 2). The elevated levels of total cholesterol observed in HF/HF offspring at 27 weeks were similar to levels found in the HF dams. It is interesting to note that total serum and LDL cholesterol concentrations in HF-S/HF offspring become progressively closer together over time to levels found in the HF dams. The HDL cholesterol concentration for offspring on HF or C diets also showed a similar pattern to dams on HF or C, respectively, and previous exposure of their dams to pravastatin resulted in significantly higher HDL cholesterol concentration, similar to its effect in the dams themselves (Figure, part F and Table 2).

### Table 1. Statistical Analyses of Biochemical and Biophysical Parameters in Dams at Weaning

<table>
<thead>
<tr>
<th>Variables</th>
<th>Dietary Groups, Mean±SEM</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF</td>
<td>HF-S</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.02±0.20</td>
<td>3.45±0.24†</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>136.2±1.4</td>
<td>128.6±1.1†</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.50±0.09</td>
<td>1.13±0.09†</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.12±0.17</td>
<td>2.60±0.05†</td>
</tr>
</tbody>
</table>

Statistical test in the right column is the ANOVA result for all of the groups. Statistical result displayed in each column is for the dietary group comparison with the others group using the Tukey-Kramer test; \(n=8\) for all of the groups.

*\(P<0.01\) and †\(P<0.001\) for HF-S or C against HF.
‡\(P<0.001\) for C against HF-S.

**Figure.** Statin treatment in hypercholesterolemic mothers during late pregnancy and lactation has beneficial effects on the cholesterol profile, blood pressure, and locomotor behavior in their female offspring. A, Body weight gain; B, systolic blood pressure; C, locomotor activity; D, serum cholesterol; E, serum LDL; and F, serum HDL in offspring from C mothers, HF mothers, or HF-S mothers. Weaned offspring were then fed either HF or C diets to adulthood, thus generating the dam/offspring dietary groups: HF/HF, HF-S/HF, and C/C (\(n=8\) per group). See Table 2 for results of data analysis.
present study, we gave future mothers an HF diet very early such as during pregnancy and/or lactation periods only. In the confined to short-term modifications in the maternal diet, over, previous studies have shown that there is intrauterine an early rate-limiting step in cholesterol biosynthesis.25 More-
the importance of the timing and duration of the nutri-
Altering the maternal diet during critical periods of gesta-
and development of the central nervous system,31 the effects these effects are not known. Although inadequate cholesterol behavior in the offspring. Moreover, statin treatment of the high-fat diet during pregnancy can also result in sedentary development of obesity. This would almost certainly be before and during pregnancy but can also result in the not only lead to hypercholesterolemia and hypertension been reported.
Table 2. Estimate of Mean Difference and 95% CI of Biochemical and Biophysical Parameters in Female Offspring

<table>
<thead>
<tr>
<th>Variables</th>
<th>HF/HF vs HF-S/HF</th>
<th>HF/HF vs C/C</th>
<th>HF-S/HF vs C/C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 95% CI P</td>
<td>Mean 95% CI P</td>
<td>Mean 95% CI P</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>2.9 2.2 to 3.6 &lt;0.001</td>
<td>4.7 4.0 to 5.4 &lt;0.001</td>
<td>1.8 1.1 to 2.6 &lt;0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>20 15 to 25 &lt;0.001</td>
<td>45 40 to 50 &lt;0.001</td>
<td>26 21 to 31 &lt;0.001</td>
</tr>
<tr>
<td>Locomotor activity, cm/3 min</td>
<td>112 85 to 140 &lt;0.001</td>
<td>235 207 to 263 &lt;0.001</td>
<td>122 95 to 150 &lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>0.88 0.60 to 1.16 &lt;0.001</td>
<td>3.68 3.39 to 3.96 &lt;0.001</td>
<td>2.79 2.51 to 3.08 &lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>0.87 0.56 to 1.17 &lt;0.001</td>
<td>3.73 3.43 to 4.04 &lt;0.001</td>
<td>2.87 2.56 to 3.17 &lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.17 0.13 to 0.20 &lt;0.001</td>
<td>0.26 0.22 to 0.29 &lt;0.001</td>
<td>0.09 0.05 to 0.12 &lt;0.001</td>
</tr>
</tbody>
</table>

Effect estimates (n=8 for all groups) are from a mixed-model analysis that considers all of the time points through the study, controlling for the set of dam-pup relationships.

Discussion

The present study demonstrates that treating pregnant animals that are hypercholesterolemic and hypertensive with pravastatin not only improves their health but can also have long-lasting beneficial effects on BP and activity and induces a transient reduction of cholesterol in their offspring even if they consume a similar high-fat diet. This work has underscored the importance of maternal nutrition even before pregnancy on metabolic and cardiovascular outcome of the future offspring. Furthermore, the present findings provide the first indication that cholesterol lowering, or other effects of statins, benefits postnatal BP.

Several studies, including ours, have shown that a maternal diet rich in fat and cholesterol during pregnancy can induce obesity, vascular dysfunction, impaired skeletal muscle development, sedentary behavior, and gender-specific hypertension in the offspring.24–26 However, these studies have been confined to short-term modifications in the maternal diet, such as during pregnancy and/or lactation periods only. In the present study, we gave future mothers an HF diet very early in life to produce an effect on offspring health. Thus, our experimental approach is more representative of the human condition.

Altering the maternal diet during critical periods of gestation22,23 or throughout gestation and/or the suckling period6 results in a varying degree of phenotypic outcomes, suggesting the importance of the timing and duration of the nutritional insult. This is emphasized by one of the novel findings using our animal model, namely that BP in the HF/HF offspring was much greater than in their HF-fed mothers despite having lower cholesterol levels and the mother weighing more than their offspring at the time of BP measurement. Such effects are fundamental to the concept of the developmental origins of disease24 and also indicate that intervention in early life may be particularly important in reducing later risk of disease in the face of lifestyle factors such as a high-fat diet.

It remains to be investigated what mechanisms may have changed the phenotype of the offspring through maternal pravastatin treatment. Statins are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, an early rate-limiting step in cholesterol biosynthesis.25 Moreover, previous studies have shown that there is intrauterine transfer of maternal cholesterol to the embryo, as well as the fetus.26,27 Thus, statin treatment may indirectly affect patterns of growth and development of organs and tissues within the fetus by influencing maternal-fetal cholesterol transfer across the placenta and preventing changes in liver, kidney, and vascular function in the fetus because of the detrimental effects of the hypercholesterolemic condition. Aside from the lipid-lowering effects of statins, they have also been found to upregulate endothelial NO synthase, increase NO bioavailability, and decrease oxidative stress.28 It is, therefore, possible that statins could blunt the deleterious effects of an imbalanced maternal diet by a range of mechanisms.

We also observed that offspring from hypercholesterol-
emic dams are less active, providing another aspect of the model that mimics the early origins of the “couch-potato” syndrome in humans.29 Although this has been observed previously when dams were undernourished during pregnancy,29,30 the present study is the first to show that a maternal high-fat diet during pregnancy can also result in sedentary behavior in the offspring. Moreover, statin treatment of the dams ameliorates this effect. The mechanisms underlying these effects are not known. Although inadequate cholesterol provision to the developing fetus is deleterious to patterning and development of the central nervous system,31 the effects of hypercholesterolemic condition during pregnancy have not been reported.

We recognize that prolonged exposure to the HF diet can not only lead to hypercholesterolemia and hypertension before and during pregnancy but can also result in the development of obesity. This would almost certainly be associated with insulin resistance, increased inflammation, and concomitant immune responses, which also affect developmental programming of cardiovascular disease.32,33 It is, therefore, not possible at this time to attribute unequivocally the changes in offspring BP and activity level to maternal hypercholesterolemia. It also remains to be determined whether the protective effects of statin treatment are because of cholesterol lowering, per se, during pregnancy or because of the reduced obesity, hypertension, or insulin resistance in mothers in late pregnancy, ie, whether statins prevent pathogenic programming by improving maternal health or whether they interfere with in utero programming mechanisms. It is more likely that the protective effect is because of cholesterol lowering, because the statin that we have used, pravastatin, is hydrophilic and does not cross the placental barrier. How-
ever, we cannot discount the possibility that there may be other effects of the drug (eg, on endothelial cells).

It remains to be determined how the cholesterol-lowering effect of statin in the pregnant mothers affects postnatal BP or activity levels in the offspring. To resolve these questions, it would be necessary to compare the effect of statin with that of other hypocholesterolemic drugs and to use an experimental design that minimizes differences in body weight and other parameters induced by the diet before pregnancy. This was beyond the scope of the present study but should be considered as a limitation in interpreting the data. It is also possible that the beneficial effect of statin occurs during the early postnatal period, because we continued giving it in the dam’s drinking water during the lactation period. Studies have shown excretion of statin into the milk in rat dams that were treated postpartum with the statin atorvastatin.34 Statin could, at this point, reverse the programming effect of the childbearing age.

Composition and diet in young women even before they reach childbearing age.

Perspectives
The present findings indicate that statin administration to HF-fed pregnant dams not only improves their cardiovascular and metabolic health but also gives some postweaning protection to their offspring. This might allow time for other intervention strategies to be put in place to protect the offspring, which are also likely to consume a poor postweaning diet. This new evidence to suggest that maternal hypercholesterolemia has a detrimental effect on the next generation may necessitate a reconsideration of present recommendations against the use of statins during pregnancy.

Acknowledgments
We thank Clive Osmond for advice on statistical analysis of the data.

Sources of Funding
This work was supported by the British Heart Foundation. M.M.E. is supported by a BUPA/HOPE research fellowship.

Disclosures
None.

References
14. Pollack FS, Shields KE, Burnett DM, Osborne MJ, Cunningham ML, Stepanavage ME. Pregnancy outcomes after maternal exposure to sim-


Statin Treatment in Hypercholesterolemic Pregnant Mice Reduces Cardiovascular Risk Factors in Their Offspring
Maqsood M. Elahi, Felino R. Cagampang, Frederick W. Anthony, Nick Curzen, Sunil K. Ohri and Mark A. Hanson

Hypertension. 2008;51:939-944; originally published online February 19, 2008; doi: 10.1161/HYPERTENSIONAHA.107.100982
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/4/939

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/