Phospholipid profile of developing heart of rats exposed to low-protein diet in pregnancy

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Tappia, Paramjit S., Mohinder S. Nijjar, Aric Mahay, Nina Aroutiounova, and Naranjan S. Dhalla. Phospholipid profile of developing heart of rats exposed to low-protein diet in pregnancy. Am J Physiol Regul Integr Comp Physiol 289: R1400–R1406, 2005. First published July 14, 2005; doi:10.1152/ajpregu.00319.2005—Although the myocardial phospholipid and fatty acid content have profound effects on the heart function, very little information is available on the effects of restricted maternal protein intake during pregnancy on the phospholipid profile and fatty acid content of the developing heart. The present study was therefore undertaken to examine the effect of pregnant dams fed diets containing either 180 (normal) or 90 (low) g/kg casein diet for 2 wk before mating and throughout pregnancy on myocardial phospholipid and fatty acid content of male offspring. Whereas no changes in phosphatidylcholine and phosphatidylethanolamine were detected, increases in lyso phosphatidylcholine, phosphatidyserine, and sphingomyelin were seen in the hearts of offspring in the low-protein (LP) group. Furthermore, assessment of nuclear transcription factors involved in regulation of cardiac metabolism revealed a decrease in the unsaturated fatty acid (linoleate, arachidonate, and decosahexanoate) levels were significantly increased in the developing heart in the LP group. Analysis of cardiac fatty acids revealed that although the saturated fatty acid (myristate, palmitate, and stearate) levels were significantly reduced, the unsaturated fatty acid (linoleate, arachidonate, and decosahexanoate) levels were significantly increased in the developing heart of LP group. Furthermore, assessment of nuclear transcription factors involved in regulation of cardiac metabolism revealed a decrease in myocyte enhancer factor-2C mRNA levels in the LP group, whereas an increase in the mRNA amount of peroxisome proliferator-activated receptor-α was observed in this group. These results demonstrate that maternal LP diet can induce changes in the phospholipid profile and fatty acid content of the developing heart, which may have implications for metabolism of the neonatal heart.

maternal low-protein diet; fatty acids; energy metabolism

THE ENVIRONMENTAL EXPERIENCE OF THE growing fetus influences the development of specific organs, including the heart (6). We have recently reported that a maternal low-protein (LP) diet induces a severe depression of the contractile function of the neonatal heart (6); however, nothing is known about the mechanisms responsible for this effect. Cardiac phospholipids are known to be organized into functionally differentiated domains (32) and provide both structural integrity and a suitable microenvironment for the normal functioning of membrane proteins (32). Furthermore, polyunsaturated fatty acids (PUFAs), which are important structural and functional components of cell membrane phospholipids, are also required to support normal growth and development and are critical for normal cell function (1, 16, 23, 25, 30). The metabolic machinery of the heart is designed to allow many different substrates to be used for ATP synthesis (3), which is required for normal cardiac function. The change in substrate preference during normal maturation from glucose (10, 12, 27–29, 31) and lactate to fatty acids (10, 12, 27–29, 31) is the result of transcriptional regulation of glycolytic and mitochondrial proteins and mitochondrial biogenesis, leading to increased capacity for myocardial oxygen consumption.

Although many aspects of maternal and fetal adaptations during pregnancy have been addressed (14, 41), it is unclear whether feeding a low-maternal-protein diet during pregnancy adversely affects the phospholipid and fatty acid content in the heart of the fetus that persists in the developing neonatal heart. Therefore, by using a well-established rat model of intrauterine growth retardation (6, 24), we undertook the present study to examine whether a maternal LP diet during pregnancy induces changes in the profile of the major phospholipid classes and whether this is associated with changes in the fatty acid content of the developing heart of offspring of preweaning age. In addition, to understand the metabolic consequences of such compositional changes, we also investigated the transcriptional machinery that regulates energy metabolism of the neonatal heart, which could provide novel insights into the mechanisms of the adverse effects of maternal undernutrition and cardiac function.

MATERIALS AND METHODS

Experimental animals. All experimental protocols for animal studies were approved by the Animal Care Committee of the University of Manitoba, following the guidelines established by the Canadian Council on Animal Care. Ten virgin female Sprague-Dawley rats (235–270 g) were housed individually in cages, divided into two equal groups, and maintained at 24°C on a 12:12-h light-dark cycle. The rats were fed ad libitum isocaloric synthetic diets containing 180 g (normal) or 90 (low) g/kg casein diet (6, 24) for 2 wk before mating and throughout pregnancy and had free access to water. The compositions of the synthetic diets used in this study are shown in Table 1. All of the dams in the two groups were successfully mated, and within 12 h of giving birth, the mothers were fed standard laboratory chow and remained on this diet throughout the suckling period. No difference in the maternal behavior between the diets was observed and no stillbirths occurred in the two groups. The litter size was culled to allow a maximum of nine pups across the groups. The body weights of all offspring were recorded at birth, and the biochemical analyses were conducted in 15 randomly selected male offspring (3 pups/dam from the 2 groups). Although the range of birth weights was within ±2%
The migration of these phospholipids was identified after a brief exposure of TLC plates to iodine.

RESULTS

General characteristics of experimental animals. The energy and food intake of the 18 and 9% casein-fed pregnant rats did not differ significantly, although a 54% reduction in the protein intake of the 9% casein-fed group relative to the 18% casein-fed control was calculated (Table 2). Pups born to the dams fed the LP diet were significantly lighter (Table 2).

Age-dependent changes in cardiac membrane phospholipid levels. In a previous study in our laboratory (6), it was reported that exposure of the developing heart to an LP diet induces cardiomyocyte loss due to an increase in apoptosis. The phospholipid profile measured in the hearts of offspring may provide mechanistic insight into cardiomyocyte apoptosis and cardiac functional changes, the phospholipid profile measured in the hearts of offspring may provide mechanistic insight into cardiomyocyte apoptosis and cardiac functional changes.

Table 1. Composition of synthetic diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Control Diet</th>
<th>Low-Protein Diet</th>
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</thead>
<tbody>
<tr>
<td>Casein</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Sucrose</td>
<td>21.3</td>
<td>24.3</td>
</tr>
<tr>
<td>Cellulose fiber</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>42.5</td>
<td>48.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Maize oil</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Composition of diet components is given as g/100 g of diet. The diets were dried for 24 h at 60°C and stored in the dark at −20 °C. The diet was provided to the animals as biscuits (60–80 g dry wt). The compounds were purchased from Harlan Teklad (Madison, WI) with the exception of methionine, which was purchased from Sigma-Aldrich (Oakville, ON, Canada).

Table 2. Maternal body weight, energy, food intake, and birth weight of offspring

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Low-Protein Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>Protein intake, % control</td>
<td>100</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>Maternal body weight, g</td>
<td>238 ± 9</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>264 ± 12</td>
<td>258 ± 11</td>
</tr>
<tr>
<td>Final</td>
<td>382 ± 14</td>
<td>359 ± 25</td>
</tr>
<tr>
<td>Maternal energy intake, kJ/day</td>
<td>543 ± 33</td>
<td>533 ± 28</td>
</tr>
<tr>
<td>Maternal food intake, g/day</td>
<td>30.1 ± 5.8</td>
<td>28.6 ± 4.2</td>
</tr>
<tr>
<td>Offspring birth weight, g</td>
<td>5.23 ± 0.1</td>
<td>4.24 ± 0.08*</td>
</tr>
</tbody>
</table>

Data are means ± SE of animals given control or low-protein diet for 2 wk before mating and then throughout pregnancy. *P < 0.05 vs. control diet.
elevated and progressively declined to basal levels at 21 days of age (Fig. 1A). No differences in myocardial content of phosphatidylcholine (PC) (Fig. 1B) and phosphatidylethanolamine (Fig. 1C) between control and LP diet groups were observed. However, a biphasic change in the sphingomyelin and phosphatidylserine (PS) contents of hearts in the LP group was observed as evidenced by an early accumulation (peak at 3 days of age), declining at 7 days (but remaining significantly higher than the control values), followed by a second peak at 21 days of age (Fig. 1D and E).

Fatty acid content of developing heart. Because exposure of the developing heart to a maternal LP diet induced changes in the cardiac phospholipids, the fatty acid content of these hearts was also investigated. Although no significant changes in the myocardial oleic acid (OA; C18:1) content was seen in the developing heart in both control and LP groups, an age-dependent decrease in OA level was seen in the heart of offspring in the LP group (Fig. 2A), whereas the levels of linoleic acid (LA; C18:2), arachidonic acid (AA; C20:4), and docosahexanoic acid (DHA; C22:6) were significantly elevated (Fig. 2B–D). On the other hand, a progressive decrease in the myristic acid (MA; C14:0), palmitic acid (PA; C16:0), and stearic acid (SA; C18:0) levels in the developing heart in the LP group was detected (Fig. 3A–C). Interestingly, the decline in saturated fatty acids (MA, PA, and SA) in the LP group was accompanied by a parallel increase in unsaturated fatty acids (LA, AA, and DHA) as evidenced by an increase in the ratio of unsaturated to saturated fatty acid (Fig. 3D).

PPAR-α and MEF-2C mRNA expression levels in developing heart. To understand whether the changes in the fatty acid content of hearts of offspring exposed to a maternal LP diet may be a consequence of an alteration in cardiac energy metabolism, we examined in the LP-exposed group the mRNA expression levels of two transcription factors known to regulate cardiac energetics. Figure 4 shows that MEF-2C mRNA levels were decreased in the developing heart of the LP group, whereas there was an age-dependent increase in the cardiac PPAR-α mRNA levels in the LP group (Fig. 5).

DISCUSSION

Although cardiac phospholipids are known to be organized into functionally differentiated domains (32) and provide both structural integrity and a suitable microenvironment for the normal functioning of membrane proteins (32), it is unclear whether feeding low maternal protein during pregnancy adversely affects the phospholipid composition in the fetus that persists in the developing neonatal heart. The present study was therefore conducted to examine the effects of maternal LP diet on the phospholipid profile of the heart of offspring of dams fed a LP diet during the gestational period. Investigators in our group (6) have previously reported that there is an increase in cardiomyocyte loss due to apoptosis and an associated early depression of cardiac function in the LP-exposed group. In the present study, levels of sphingomyelin (the precursor to ceramide) and PS, both of which are involved in

Fig. 1. Phospholipid composition of the developing heart of offspring exposed to a maternal low-protein (LP) diet. Values are means ± SE of 15 animals in each group (3 pups/dam. 5 dams/group). A: lysophosphatidylcholine (LPC). B: phosphatidylcholine (PC). C: phosphatidylethanolamine (PE). D: sphingomyelin (Sph). E: phosphatidylserine (PS). *P < 0.05 vs. control diet.
apoptosis (34, 35, 38), were markedly elevated in the LP group. Furthermore, LPC, which is considered an apoptotic phospholipid (22), was significantly increased in this group. The time course of the changes in these molecules is consistent with the early depression of cardiac function (6) and therefore may contribute to increased apoptosis in the hearts of offspring exposed to a maternal LP diet as reported earlier (6). In fact, a reduction in the number of cardiomyocytes in the hearts of offspring exposed to a maternal LP diet was also recently demonstrated (7). Although the precise functional consequences of such phospholipid compositional changes need to be defined, the net effect could also alter the activities of membrane proteins by affecting membrane fluidity and phospholipid biosynthesis, as well as phospholipid-mediated signal transduction processes (1, 16, 23, 25, 30).

PUFAs are required for normal fetal growth and development and are critical for normal cell function (1, 16, 23, 25, 30). Our studies have revealed that although the control develop-

A

Fig. 2. Age-dependent changes in the unsaturated fatty acid composition of hearts of animals exposed to a LP diet in utero. Values are means ± SE of 15 animals in each group (3 pups/dam, 5 dams/group). A: oleic acid (OA; C18:1, n9). B: linoleic acid (LA; C18:2, n6). C: arachidonic acid (AA; C20:4, n6). D: docosahexanoic acid (DHA; C22:6, n3). *P < 0.05 vs. control diet.

B

C

D

Fig. 3. Saturated fatty acid (FA) composition of the developing heart of animals exposed to a LP diet in utero. Values are means ± SE of 15 animals in each group (3 pups/dam, 5 dams/group). A: myristic acid (MA; C14:0). B: palmitic acid (PA; C16:0). C: stearic acid (SA; C18:0). D: unsaturated-to-saturated fatty acid ratio (U/S). *P < 0.05 vs. control diet.
oping heart contains very low amounts of LA, AA, and DHA, the LP developing heart has significantly high amounts of these PUFAs that increase with age. Although no differences were seen in the OA content of the hearts from both the dietary groups, a progressive decrease in OA level in the developing heart in the LP group is suggestive of a metabolic conversion of OA to PUFAs. Indeed, given the developmental profiles of OA and of LA, AA, and DHA, it is likely that OA was desaturated to LA and that, in turn, further desaturation and elongation of LA to produce AA and DHA may have occurred. Such a precursor (OA) and product (LA, AA, and DHA) relationship indicates that a dynamic transition of monounsaturated fatty acids to PUFAs occurs in the hearts of offspring exposed to a maternal LP diet. A possible mechanism to account for these changes is alterations in desaturase activities. In this regard, a reduced protein intake has been associated with increased Δ6-desaturase activity in nonpregnant adult rats (37) but decreased activities of Δ9-, Δ6- and Δ5-desaturase activities during pregnancy (11). Similarly, feeding a diet containing 80 g/kg protein to rats during pregnancy and lactation resulted in lower Δ5-desaturase activity in the offspring at 3 mo of age (36). Although fatty acid desaturation and elongation have not been determined in the present study, our findings are suggestive of increased desaturase and elongase activities in the hearts of offspring exposed to a maternal LP diet. A possible mechanism to account for these changes is alterations in desaturase activities.

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fatty acid oxidation (8), were measured. Our observation of decreased MEF-2C mRNA in the LP group would imply a reduced capacity for glucose utilization. However, PPAR-α mRNA levels were increased in the LP group, suggesting that fatty acid β-oxidation may be increased in this group. In fact, whereas the unsaturated fatty acid content was significantly elevated in the hearts of the LP group, the saturated fatty acids (MA, PA, and SA) were significantly decreased in the developing LP-exposed heart. Although this could represent an increase in the metabolism of saturated fatty acids to unsaturated fatty acids, an increase in fatty acid β-oxidation of saturated fatty acids could also exist. In this regard, other investigators (15) have reported that maternal protein restriction (8% protein vs. 20% of control) does not affect carnitine palmitoyltransferase activity in hearts of 4-day-old neonatal offspring, suggesting that cardiac ATP supply through fatty acid oxidation is not compromised. In fact, our experiments suggest an early maturation of the fatty acid β-oxidation system and a very rapid compromise in the capacity for glucose oxidation in hearts of the LP group. Apolipoprotein B100 synthesis has been reported to be downregulated by reduced protein consumption in normal healthy humans (17), implying impaired very-low-density lipoprotein (VLDL) secretion. Also, an 80% restriction in dietary protein in adult rats has been reported to decrease plasma triacylglycerol and also to impair VLDL levels (40), lipoprotein levels in pregnant dams fed the same LP diet used in our study were not compromised (5). This suggests that reduced myocardial saturated fatty acid level, observed in the present study, may not be due to a deficiency in the fatty acid supply but may reflect a metabolic shift in an attempt to meet the energy demands of the developing heart. However, caution must be exercised in the interpretation of these data, because a direct assessment of metabolism was not conducted. Furthermore, measurement of MEF-2C and PPAR-α mRNA content is an indirect method of determining glucose and fatty acid oxidation, because the mRNA data are based on semiquantitative measurements rather than quantitative mRNA levels.

Interestingly, growth-restricted offspring that experience a catch-up growth, due to accelerated shortening of chromosomal telomeres as a result of increased cell division, could have increased cell senescence in critical organs (18), including the heart. On the basis of our findings, the changes in the fatty acid profile and PPAR-α may be related to an early aging effect in the heart.

In summary, maternal protein intake during pregnancy alters the phospholipid profile and fatty acid content of hearts of offspring exposed to LP diet in utero. Interestingly, other investigators using the same rat model as ours have shown changes only in the monounsaturated fatty acid content of PC in the heart of pups after weaning age (28 days) (5), indicating that the severity of the membrane compositional changes may be attenuated in free-living offspring. Nonetheless, our findings suggest that poor maternal diet during pregnancy may induce changes in the developmentalfunctional components of the developing heart, including environmental (both phospholipids and fatty acid contents) changes of membrane proteins known to influence cardiac function and metabolic alterations. Although the early depression of cardiac contractility in offspring exposed to a maternal LP diet (6) appears to not be related to energy production but, more likely, to apoptosis, the adaptive nature of the changes reported in the present study may manifest as cardiac abnormalities in later life (6); however, the precise mechanisms by which this occurs remain to be determined.

**GRANTS**

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**REFERENCES**


