Dietary isoflavones during pregnancy and lactation provide cardioprotection to offspring rats in adulthood

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Souzeau, Emmanuelle, Sonia Bélanger, Sylvie Picard, and Christian F. Deschepper. Dietary isoflavones during pregnancy and lactation provide cardioprotection to offspring rats in adulthood. Am J Physiol Heart Circ Physiol 289: H715–H721, 2005. First published March 18, 2005; doi:10.1152/ajpheart.00061.2005.—In adult rats, elongation of cardiac myocytes (CMs) correlates with dilatation (and sometimes dysfunction) of cardiac ventricles. Although sex steroids may constitute one possible factor that affects the dimensions of CMs, studies on their effects in rodents is complicated by the fact that most commercial soy-based diets also contain abundant phytoestrogens. We report that feeding Wistar-Kyoto rat dams during gestation and lactation with a phytoestrogen-rich soy-based diet caused the CMs of their adult offspring to be shorter than in counterparts originating from mothers fed with a phytoestrogen-free casein-based diet. The soy-based diet had no such effects when given to rats after 6 wk of age, and its effects were replicated when supplementing the maternal casein-based diet with the isoflavones daidzein and genistein (the most abundant phytoestrogens in soy-based diets). In contrast to rats whose mothers had been fed with a soy-based diet, the hearts of adult rats raised with a casein-based diet only featured dilated eccentric hypertrophy and progressed toward congestive heart failure when further challenged. Thus the presence of isoflavones in the maternal diet provides cardioprotection to the hearts of their offspring during adulthood.

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Dietary isoflavones and cardioprotection

H716

The effects of sex and diet on the dimensions of CMs isolated from adult hearts and neonatal whole body weight

Effects of sex and diet on the dimensions of CMs isolated from adult hearts and neonatal whole body weight

Isolation of CMs and video microscopy. CMs were isolated from the hearts of rats either at 12 wk of age (for unoperated animals) or at 22 wk of age (when surgery was performed). The hearts were rapidly removed from anesthetized animals previously injected intraperitoneally with 500 units heparin sulfate, and [Ca$^{2+}$]-tolerant CMs were isolated by the Langendorff method (cardiac retrograde aortic perfusion), as described previously (9, 40). CMs were separated from non-CMs by sedimentation on a 6% solution of BSA and then fixed for 30 min in 0.08 mol/l phosphate buffer containing 1.5% glutaraldehyde in phosphate buffer at 4°C. Both solutions have been shown to preserve the volume of fixed cells compared with unfixed ones (16). Fixed CMs were allowed to settle in petri dishes containing 0.15 mol/l phosphate buffer and examined with a Zeiss Axiosvert microscope connected to a video camera that allowed capture of the images as electronic files. With the use of the Northern Eclipse version 6.0 software from Empix imaging (Mississauga, Ontario, Canada), ~100 cells from each animal were analyzed for the determination of $L$ (defined as the longest length parallel to the longitudinal axis of the myocyte) and cell surface (calculated on the basis of the manual contour drawn around the myocyte). $W$ was calculated by dividing the value of surface by that of $L$.

Cardiac morphology. The heart from each animal was removed with the ascending aorta attached. A polyethylene-90 catheter was inserted into the aorta above the level of insertion of the coronary arteries, and the heart was infused with a cold isotonic solution containing 100 mM KCl and 50 mM NaCl to arrest the heart in diastole. After diastolic arrest was obtained, the catheter was pushed further into the LV cavity and filled with the same solution to obtain an intracavital pressure of 15 mmHg, as described previously (9). The hearts were fixed in their distended form by immersion in formalin for 24 h, so that subsequent morphological comparisons were performed between hearts that had all been fixed under identical standardized intracavital pressure conditions. Sagittal sections (~2 mm) were cut at the midventricular level of all fixed hearts. Sections were examined on each side with a stereo microscope, and images were captured as electronic files and analyzed using Northern Eclipse version 6.0 software. For each heart section, morphological values were calculated by averaging the values obtained for each side of the section.

For determination of organ weight, lungs were weighed immediately after death, and biventricular cardiac weight was determined after the 24-h fixation period. The organ weight values were normalized by dividing them by the value of tibia length, as calculated by performing direct measurements on X-ray pictures of the hind legs of each animal.

Statistical analyses. Significance levels between groups were assessed by one-way ANOVA followed by Fisher’s least-significant difference post hoc tests.

RESULTS

We first compared the dimensions of CMs isolated from the hearts of either male or female Wistar-Kyoto rats, originating from colonies fed with either regular SBD or CBD for at least two generations (Table 1). In animals fed with SBD, the values for $L$ and $L/W$ of CMs were ~5% higher (and significantly so) in males than females. However, these differences were no longer detectable when the animals were maintained with PE-free CBD. Moreover, the PE content of the diet appeared to affect the dimensions of CMs to a greater extent than the sex of the animals, because CMs of either male or female rats maintained with CBD were ~13% longer than those of their counterparts maintained on SBD. The sizes of the litters originating from dams maintained with CBD [10.7 pups (SD 1.48), $n = 9$] were not different from that of dams maintained with SBD [10.55 pups (SD 1.42), $n = 9$]. Likewise, there were no significant differences between the dimensions of CMs isolated from male or female rats, whether they were fed SBD or CBD (Table 1).

Table 1. Effects of sex and diet on the dimensions of CMs isolated from adult hearts and neonatal whole body weight

<table>
<thead>
<tr>
<th></th>
<th>Dimensions of Cardiomyocytes</th>
<th>Normal Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$L$, $W$, $L/W$</td>
<td>Day 0</td>
</tr>
<tr>
<td>SBD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>125.8 (SD 3.43)$^\dagger\ddagger$</td>
<td>22.4 (SD 0.64)$^\dagger$</td>
</tr>
<tr>
<td>Female</td>
<td>119.5 (SD 1.27)$^\ddagger\varepsilon$</td>
<td>22.3 (SD 0.44)</td>
</tr>
<tr>
<td>CBD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>143.1 (SD 3.27)$^*$</td>
<td>24.5 (SD 0.33)$^*$</td>
</tr>
<tr>
<td>Female</td>
<td>135.4 (SD 2.68)$^\ddagger$</td>
<td>22.7 (SD 0.62)</td>
</tr>
</tbody>
</table>

Values are means (SD); $n = 5–8$ animals/group. Cardiomyocytes (CMs) were isolated from the hearts of adult rats at 12 wk of age. SBD, soy-based diet; CBD, casein-based diet; $L$, length; $W$, width; $^*P \leq 0.01$ vs. SBD-fed male rats; $^\dagger P \leq 0.01$ vs. CBD-fed male rats; $^\ddagger P \leq 0.001$ vs. SBD-fed male rats; $^\varepsilon P \leq 0.01$ vs. CBD-fed male rats; $^\dagger\ddagger P \leq 0.001$ vs. CBD-fed female rats. Significance levels were assessed by one-way ANOVA followed by Fisher’s least-significant difference (LSD) post hoc tests.

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no significant differences in the weights of litters originating from dams maintained with either type of diet (Table 1).

To determine when during development exposure to PE may affect the dimensions of CMs, we used mothers previously maintained on CBD and reintroduced SBD during either gestation (by switching diets just before they were mated), lactation (by switching diets just after pups were born), or the combined two periods. After being weaned (at about 3 wk of age), the progenies from all mothers were maintained solely on CBD, and ventricular CMs were isolated when they reached 12 wk of age. In the adult female progeny, we observed that the \textit{L}/\textit{W} of CMs from rats originating from mothers who had received SBD either before and/or after birth was smaller than that of CMs from rats whose mothers had not received any dietary PE (Fig. 1). The effects of exposure to SBD during gestation and lactation were additive: the greatest effect was observed when SBD was given to mothers during both periods, and the \textit{L}/\textit{W} of corresponding CMs was similar to that of female rats originating from colonies maintained continuously on SBD. We also observed that when rats maintained on the PE-free CBD were ovariectomized before puberty, the dimensions of CMs isolated from adult rats were not different from those of intact female rats maintained on the same diet. Moreover, there was a clear time window for the effect of SBD, because reintroducing it between 6 to 12 wk of age to ovariectomized rats without any prior exposure to PE had no effect on the dimensions of CMs under resting conditions (data not shown).

When using the \textit{L}/\textit{W} of CMs as the sole end point, the effects of dietary PE on the dimensions of male CMs were similar to those observed in females (Fig. 1). However, contrary to female rats, both the \textit{W} and \textit{L} of CMs from adult male rats that had been gonadectomized before puberty were smaller than those of CMs from sham-operated counterparts (Fig. 1). This effect of testicular hormones also differed from that of PE because they affected the \textit{L} and \textit{W} of CMs in a proportional manner, so that the net effect of the \textit{L}/\textit{W} was nil. Consequently, all further experiments were performed using animals from one sex only. By focusing on female progenies, we avoided the possible confounding effects of androgens, because the latter appeared to have effects that were additional to those of dietary PE.

To verify that the effect of SBD may be attributed to PE only, dams that had been maintained before being mated on CBD were gavaged daily during the combined gestational and lactational periods with either vehicle or a suspension of either daidzein or genistein. The latter compounds are two isoflavones that represent the most abundant PEs in rodent SBD (8, 39). We observed that both isoflavones decreased the \textit{L}/\textit{W} significantly compared with animals whose mothers received only vehicle, down to values similar to what we had observed with animals whose mothers had received SBD.
during the same period (Fig. 2). Thus isoflavones can account for the entirety of the effect of SBD on the relative dimensions of CMs, provided that administration of these compounds by gavage impacts on the final concentration of isoflavones in plasma in a manner that is comparable to when they are present in rat chow.

Given the association previously reported between elongated CMs and dilated eccentric hypertrophy (14), we tested whether PE in the maternal diet also had effects on the morphology and function of the hearts of the progeny. Under basal conditions, we observed that, in addition to increased CM $L$, the surfaces defined by the LV parenchyma and LV cavity were both significantly higher in rats whose mothers had been maintained with CBD than in rats whose mothers had received SBD (Fig. 3 and Table 2). Such cardiac features define what is referred to as dilated eccentric ventricular hypertrophy. Blood pressure was not different in animals from both groups (Table 3) and therefore could not account for the differences in cardiac morphology. Functionally, we also observed that rats originating from mothers without PE exposure had features of isolated diastolic dysfunction, because the time constant of LV pressure decay was increased in the absence of alterations of other indexes of contractility (Table 3).

Because eccentric dilated ventricular hypertrophy has been associated with an adverse cardiovascular prognosis (22, 42), we tested whether this condition would predict the ability to resist the transition from compensated to decompensated heart failure. ACF is a maneuver that induces volume overload and cardiac remodeling (4). In Sprague-Dawley rats fed with standard SBD, the overload induced by this intervention has been shown to be accompanied by progressive heart decompensation in males but not in females (13). Surgery was performed at 10 wk of age to induce ACF in rats originating from mothers who had received either type of diet, and rats were killed 12 wk after the surgery. In rats originating from mothers who had received SBD, ACF increased the $L$ and surface of CMs by $\pm 10\%$ and $16\%$, respectively (Table 2), but the surgical maneuver did not lead to changes that were detectable at the level of the whole heart (Table 2 and Fig. 3). In contrast, 12 wk of volume overload did increase the values of the LV parenchyma surface, LV cavity surface, and adjusted biventricular weight in rats whose mothers had not received any dietary PE, because corresponding values were increased by $16\%$, $26\%$, and $37\%$, respectively. These ventricular changes were accompanied by signs of congestive cardiac failure, because there was a $\sim 17\%$ increase in adjusted lung weight. The dimensions of the CMs also changed to a greater extent than in rats whose mothers had received SBD, because their $L$ and surface were increased by $\sim 16\%$ and $31\%$, respectively.

**DISCUSSION**

Isoflavones are compounds that are abundant in most (if not all) commercial standard laboratory rat chows. Our findings show that the presence of isoflavones in the maternal diet has long-term consequences on the morphology and function of the hearts of the adult progeny. In humans, there is evidence that...
isoflavones may have several beneficial effects on cardiovascular health, including effects on plasma lipoprotein concentration and on systemic arterial compliance (an indicator of atherosclerosis extent) (6). However, most of the effects of these compounds have been attributed to exposure during adult life, and they have not previously been shown to have enduring structural effects. Likewise, isoflavones have been reported to inhibit L-type Ca\(^{2+}\) current in cultured cardiomyocytes (27), but such direct actions in vitro are clearly of a different nature than the effects observed in the present study. Of note, genistein (but not daidzein or its metabolites) has anti-tyrosine kinase properties (27), and daidzein (but not genistein) can be metabolized by intestinal flora into the bioactive metabolite equol (2). However, the equivalent potency of both compounds indicates that their effects on the length of CMs cannot be attributed solely to inhibition of tyrosine kinases or intestinal metabolism. When PE was absent from the maternal diet during the combined gestational/lactational period, the increased length of CMs in the adult offspring was accompanied by morphological features of dilated eccentric hypertrophy and isolated diastolic ventricular dysfunction. When these hearts were further challenged by volume overload, they progressed (unlike those of rats whose mothers had been fed with SBD) toward CHF.

Although administration of isoflavones during either gestation or lactation had additive effects, the effects of lactational exposure were most prominent. Of note, transfer of isoflavones from mothers to either fetuses by placental transfer (10) or to lactating pups via maternal milk (11) have been documented previously for rats. However, the efficiency of transfer appears to be higher during lactation than during gestation (10, 11), which may explain in part the relatively stronger effect of lactational exposure. Most strikingly, the effects of perinatal dietary isoflavones persisted in the adult offspring well beyond the time these compounds had been removed from the diet. Interestingly, epidemiological studies in human populations have indicated that poor fetal and infant growth associate with the occurrence of metabolic syndrome later during adult life (17). Similar findings have been reported in rats (23). However, there is no evidence that the effects of PE on CMs may be due to a similar mechanisms, because the nutritional value of CBD and SBD were similar, and there were no differences in the weights of the neonatal litters originating from mothers fed with either type of diet.

The finding that dietary PE affect the morphology of CMs only when present during the perinatal period is somewhat paradoxical given the fact that the growth of these cells is far from being complete at 3 wk of age and that they continue to grow well beyond that age (24). Although one may hypothesize that the perinatal effects are such that later growth can no longer compensate for the early structural changes, this would be surprising in light of the fact that the adult heart retains remodeling capabilities. Alternatively, one may consider the possibility that dietary PE induce epigenetic modifications of genomic DNA within cardiac cells during the perinatal period. Such effects [that are typically accompanied by changes in the acetylation status of histones and in the recruitment of regulators of chromatin (20)] may result in the reprogramming of the level of expression of certain cardiac genes and of regulators of chromatin (20) may result in the reprogramming of the level of expression of certain cardiac genes and, therefore, may persist well after PE is removed from the diet. Accordingly, the incorporation of genistein in the diet has been reported to induce changes in the methylation status of several cytosine guanine dinucleotide islands in tissues other than the heart (7). Moreover, estrogen receptors themselves can recruit coactivators that modify chromatin structure (32), and isoflavones in particular have been reported to increase the histone acetylation activity of estrogen receptors (18). Chromatin-mediated effects may well lead to modifications of the structural properties of CMs, because histone deacetylases are

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**Table 2. Impact of maternal diet on organ features and dimensions of CMs under basal conditions or in the presence of volume overload**

<table>
<thead>
<tr>
<th>Variable</th>
<th>SBD (PE Rich)</th>
<th>CBD (PE Free)</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>ACF</td>
<td>%Change</td>
</tr>
<tr>
<td>LV cavity, mm(^2)</td>
<td>22.4 (SD 2.48)</td>
<td>21.05 (SD 6.87)</td>
<td>↓ 6</td>
</tr>
<tr>
<td>LV surface, mm(^2)</td>
<td>76 (SD 2.45)</td>
<td>74.5 (SD 6.73)</td>
<td>↓ 6</td>
</tr>
<tr>
<td>VW/tibia (g/mm) \times 100</td>
<td>2.45 (SD 0.09)</td>
<td>2.43 (SD 0.16)</td>
<td>⇐</td>
</tr>
<tr>
<td>Lung weight/tibia (g/mm) \times 100</td>
<td>2.77 (SD 0.07)</td>
<td>2.79 (SD 0.08)</td>
<td>⇐</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>229 (SD 5)</td>
<td>244 (12)</td>
<td>↑ 6</td>
</tr>
<tr>
<td>CM L, μm</td>
<td>125.1 (SD 1.74)</td>
<td>137.4 (SD 2.28)</td>
<td>↑ 9.9</td>
</tr>
<tr>
<td>CM surface, μm(^2)</td>
<td>2916 (SD 73)</td>
<td>3381 (SD 80)</td>
<td>↑ 15.9</td>
</tr>
</tbody>
</table>

Values are means (SD); \(n = 5–8\) animals/group. LV, left ventricular; VW, biventricular weight; PE, phytoestrogen; ACF, aortocaval fistula. *\(P \leq 0.05\) vs. SBD; †\(P \leq 0.01\) vs. sham SBD; ‡\(P \leq 0.05\) vs. sham CBD; §\(P \leq 0.01\) vs. sham CBD. Significance levels were assessed by one-way ANOVA followed by Fisher’s LSD post hoc tests.

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**Table 3. Impact of maternal diet on hemodynamic variables of the female progeny**

<table>
<thead>
<tr>
<th>Variable</th>
<th>SBD</th>
<th>CBD</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 wk of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 24-h BP, mmHg</td>
<td>98.2 (SD 7.0)</td>
<td>97.4 (SD 5.2)</td>
<td></td>
</tr>
<tr>
<td>Systolic 24-h BP, mmHg</td>
<td>117.5 (SD 6.5)</td>
<td>118.7 (SD 4.6)</td>
<td></td>
</tr>
<tr>
<td>Diastolic 24-h BP, mmHg</td>
<td>80.3 (SD 8.0)</td>
<td>78.1 (SD 5.7)</td>
<td></td>
</tr>
<tr>
<td>24-h HR, beats/min</td>
<td>342 (SD 11)</td>
<td>344 (SD 8)</td>
<td></td>
</tr>
<tr>
<td>22 wk of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-VEDP, mmHg</td>
<td>1.66 (SD 1.65)</td>
<td>1.35 (SD 0.87)</td>
<td></td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>129.7 (SD 21.2)</td>
<td>118.5 (SD 9.41)</td>
<td></td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>7.503 (SD 992)</td>
<td>6.803 (SD 1068)</td>
<td></td>
</tr>
<tr>
<td>+dP/dt, mmHg</td>
<td>10.672 (SD 1966)</td>
<td>9.621 (SD 1364)</td>
<td></td>
</tr>
<tr>
<td>τ, ms</td>
<td>11.04 (SD 1.12)</td>
<td>15.64 (SD 2.0)(^*)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (SD); \(n = 5–8\) animals/group. BP, blood pressure; HR, heart rate; I-VEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; τ, time constant of LV pressure decay. \(*P \leq 0.05\) by t-test comparison.
potent regulators of the left ventricular hypertrophic transcriptional response (43).

At present, we do not know whether estrogen receptors are required to observe the effects of dietary PE on CMs. Neither endogenous ovarian hormones nor PE had any effects on CMs after puberty. Interestingly, the administration of diethylstilbestrol has been reported to alter the methylation status of the lactoferrin gene when given to neonatal mice from postnatal days 1–5 but not when given after 30 days of age (7). In contrast to either endogenous ovarian hormones or dietary PE, postpubertal testicular hormones (presumably androgens) increased the size of CMs. This effect is in agreement with the previous report (28) showing that CMs contain androgen receptors and that androgens increase protein synthesis in cultured adult CMs. Of note, testicular hormones increased both the W and L of CMs in a proportional manner, so that there was no net effect on the L/W (in contrast to the effect of dietary PE). Changes in the dimensions of CMs are believed to have functional consequences mostly when the relative proportions of L versus W are altered (14, 15).

It is somewhat surprising to observe that minute differences in the composition of the maternal diet have cardiac effects whose magnitude is similar to that observed in many transgenic mouse models of cardiac dysfunction. However striking, it is unclear at present whether the present observations about the effects of PE on the dimensions of CMs can be extended to all mammals. For instance, we observed that the impact of both types of diet on the dimensions of CMs varied greatly in different strains of inbred mice (E. Souzeau and C. F. Deschepper, unpublished observations). On a broader note, several studies support the hypothesis that diets associated to certain cultures have beneficial health effects (35). For the Japanese in particular, some of these effects have been attributed in part to abundance of isoflavones in the diet (21). If the current findings are applicable to humans, that hypothesis might be extended to support the concept that the beneficial effects of certain diets may not be limited to adult consumption but may derive from early exposure during the development period.

GRANTS

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