Maternal low-protein diet programs cardiac β-adrenergic response and signaling in 3-mo-old male offspring

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Fernandez-Twinn, Denise S., Sofia Ekizoglou, Adrian Wayman, Clive J. Petry, and Susan E. Ozanne. Maternal low-protein diet programs cardiac β-adrenergic response and signaling in 3-mo-old male offspring. Am J Physiol Regul Integr Comp Physiol 291: R429–R436, 2006. First published February 16, 2006; doi:10.1152/ajpregu.00608.2005.—Low birth weight in humans is associated with an increased risk of cardiovascular disease. Humans with heart failure have a reduced β-adrenergic response. The aim of this study was to investigate the hemodynamic response to the β-adrenergic agonist isoproterenol and to identify molecular deficiencies that may be predictive of cardiac failure in a low-birth weight rodent model that develops insulin resistance and type 2 diabetes in adulthood. Wistar rats were fed a control or a low-protein (LP) diet throughout pregnancy and lactation. The resting heart rate and blood pressure of the 3-mo-old male offspring of these dams, termed “control” and “LP” groups, respectively, and their responses to isoproterenol (ISO) infusion were monitored by radio telemetry. The protein expression of β-adrenergic signaling components was also measured by Western blot analysis. Basal heart rate was increased in LP offspring (P < 0.04), although mean arterial pressure was comparable with controls. Chronotropic effects of ISO were blunted in LP offspring with significant delays to maximal response (P = 0.01), a shorter duration of response (P = 0.03), and a delayed return to baseline (P = 0.01) at the lower dose (0.1 µg·kg⁻¹·min⁻¹). At the higher dose (1.0 µg·kg⁻¹·min⁻¹ ISO), inotropic response was blunted (P = 0.03) but quicker (P = 0.001). In heart tissue of LP offspring, β₁-adrenergic receptor expression was reduced (P < 0.03). β₁-Adrenergic receptor kinase and both stimulatory and inhibitory G protein levels remained unchanged, whereas β₂-receptor levels were higher (P < 0.03). Finally, insulin receptor-β expression was reduced in LP offspring (P < 0.012). LP offspring have reduced β-adrenergic responsiveness and attenuated adenocrine and insulin signaling, suggesting that intrauterine undernutrition alters heart failure risk.

β-adrenergic receptor; insulin receptor; β₂-arrestin

LOW BIRTH WEIGHT in humans is associated with a range of adult diseases, including type 2 diabetes, abnormal lipid metabolism and hypertension (4), and increased risk of death from cardiovascular disease (5, 24, 28) and ischemic heart disease in the adult (19, 22, 39, 55). It has been suggested that this may be a consequence of fetal undernutrition during gestation (4), which induces physiological and/or metabolic adaptations to ensure nutrient supply to vital organs (such as the brain) at the expense of other organs (such as the pancreas; see Ref. 26). Various other adverse fetal environments have been shown to associate with low birth weight and increased risk of similar diseases. These include fetal hypoxia (3), maternal anemia (53), maternal smoking (25, 46), maternal periodontal disease (44), placental villous inflammation (6), and maternal asthma (12).

The underlying mechanisms of these human observations are poorly understood; however, several experimental animal models of maternal dietary manipulation have provided insight into the causal links between poor fetal growth and subsequent disease (43). The low-protein (LP) model is one of the most extensively studied of these and has been used to identify key molecular pathways involved in the development of insulin resistance and type 2 diabetes (47). Other studies in this model showed that these LP male offspring subsequently develop insulin resistance and diabetes in old age (50). Previous studies in this laboratory showed that male offspring of LP-fed rat dams (LP offspring) demonstrate raised epinephrine and norepinephrine concentrations in the fed state at 12 wk of age (51). After an overnight fast, however, these parameters were increased in the control group but not in LP offspring. In addition, α₂-AR-adrenoreceptor levels in adipocytes isolated from epididymal, subcutaneous, and intra-abdominal fat stores were lower in LP offspring, whereas, on the contrary, their β₁- and β₂-adrenoreceptors were higher than controls.

Prenatal hypoxia in rats also results in low birth weight (32, 41) and has been shown to increase the susceptibility of the adult to ischemia-reperfusion injury (41). The potential mechanisms suggested included increased β₂-adrenoreceptor and the Gα-α-Giα ratio and a decrease in heat shock protein 70 and endothelial nitric oxide synthase in the left ventricle (41). In another study, prenatal hypoxia induced increases in ventricular weights and impaired cardiopulmonary vasconstriction (32).

These observations led us to hypothesize that intrauterine growth restriction might program long-term alterations in catecholamine sensitivity in the adult, with potential adverse effects on cardiac function. The aim of this study was therefore to investigate the possible causes of raised catecholamines in LP male rats and the potential effects this might have on their cardiovascular system.

In light of the data from the fetal hypoxia model, we also investigated the expression of cardiac β₁-, β₂-, and β₃-adrenergic receptors (ARs), as well as downstream signaling components of the β-adrenergic signaling pathway, i.e., adenylate cyclase (AC) IV, V, and VI, β-adrenergic receptor kinase (β-ARK)-1/G protein-coupled receptor kinase 2, inhibitory G protein (Gᵢₒ), and β₂-arrestin, to determine whether these mechanisms were involved with in vivo cardiac β-AR function.

METHODS

All biochemicals were purchased from Sigma Chemical (Poole, Dorset, UK) unless otherwise stated. Female Wistar rats were bred...
locally at a designated animal unit of the University of Cambridge. Adult females weighing between 235 and 250 g were mated and assumed to be pregnant when a vaginal plug was expelled. They were then fed ad libitum either a control diet [containing 20% (wt/vol) protein] or an isocaloric LP (8% protein) diet (Hope Farms, Arie Blok, Woerden, Netherlands) during gestation and lactation (see Table 1 for composition of the diets). After birth (2 days), litter sizes were randomly standardized to four males and four females. At 21 days of age, the male offspring were weaned onto a standard rat diet (LAD1; Special Diet Services, Witham, UK) and remained on the LAD1 diet for the remainder of the study. One male each from eight control and eight LP litters were included in this study. All animal procedures were approved by the Local Animal Ethical Review Committee and were carried out under compliance with the United Kingdom Animal (Scientific Procedures) Act 1986. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Surgery. Male rats (3 mo old) were anesthetized with halothane (4% halothane in oxygen for inducing and 2% for maintaining anesthesia; Fluothane; Zeneca, Macclesfield, UK). Sterile catheters (Esco Rubber, 0.5 mm bore; Bibby Sterilin, Stone, UK) were placed bilaterally in the jugular veins. The distal ends of the catheters were tunneled subcutaneously and exteriorized at the nape of the neck. Each catheter was backfilled with heparinized saline (20 U/ml) and then plugged. To maintain patency, the heparin block was first aspirated off, the line was flushed with saline, and then the heparin block was reinstalled daily. A radiotelemetry device was then implanted for the measurement of mean arterial pressure. The probe was inserted in the descending aorta via a small incision in the femoral artery, and the probe and catheter were secured with sutures and tissue adhesive. Correct placement of the probe within the aorta was verified by the reception of an intermittent signal corresponding to the animal’s pulse rate when the device was switched on with a magnet. The body of the transmitter was then placed in the peritoneal cavity and secured with sutures in the body wall. Finally, when hemostasis was ensured, the two skin incisions were closed with interrupted stitches.

The animals received postoperative analgesia (buprenorphine) for 1 wk and were allowed to recover until they appeared to have normal grooming behavior. Stimulation with the ADRENERGIC AGONIST ISOPROTERENOL was performed between 10 and 14 days after surgery between 1100 and 1400 for all rats.

Table 1. Composition of gestational and lactational diets shown as percentage by dry weight of constituents

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (20% casein)</th>
<th>Low Protein (8% casein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard vitamin premix</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>ST.SPOR.PRE Mix</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>CaHPO4·2H2O (low F)</td>
<td>0.65</td>
<td>1.15</td>
</tr>
<tr>
<td>CaCO3 “pure”</td>
<td>1.10</td>
<td>1.10</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>KCl</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>MgSO4·7H2O</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>MgO “very pure”</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Choline CL</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Protein (casein)</td>
<td>22.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Soya oil</td>
<td>4.30</td>
<td>4.30</td>
</tr>
<tr>
<td>Cerealose/dextrose</td>
<td>55.15</td>
<td>68.17</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

ST.SPOR.PRE Mix, standard mineral/trace elements mixture. Units are %.

Rats remained in their individual cages without restraint throughout the duration of the infusion process, and only one rat was infused at a time. After moving to a quiet room, the infusion lines were attached, and then the rat was placed on a platform receiver and allowed to acclimate to the environment for 1 h. From this point onward, heart rate and blood pressures, both systolic and diastolic, were sampled continuously. Baseline measurements were established during this quiet time. Saline was then infused for 30 min to get the animal used to the infusion process, by the end of which heart rate and blood pressure fluctuations had settled. Isoproterenol in saline was then administered initially at a dose of 0.1 μg·kg⁻¹·min⁻¹ for 10 min via the jugular catheter using a syringe infusion pump (Razel model A-99) with an adjustable flow rate. This was followed by an infusion of saline at the same rate for 30 min before the next higher dose (1.0 μg·kg⁻¹·min⁻¹) was applied also for 10 min. This was followed by a final saline flush for 30 min. Recording was then continued for 1 h afterward before removal of infusion lines and return to normal housing. After the experiment, the rats were allowed to recover for 1 wk and then killed by decapitation, and trunk blood and tissue were collected for further analysis.

Telemetry and data acquisition. The radiotelemetry system and software used in this study to measure mean arterial pressure and heart rate were obtained from Data Sciences International (St. Paul, MN). It is comprised of the implantable transmitter (TA11PA-C40); a receiver (RPC-1) on which the animal, which remains in its own cage throughout, is placed; the multiplexer, which consolidates the signals received; and a computer loaded with the software for acquisition and analysis of the data received (Dataquest ART 2.2). For each animal, individual and direct numeric outputs of 10-s intervals were obtained for the parameters of heart rate, mean arterial pressure, and systolic and diastolic pressures. From these outputs, various parameters, including “maximal change in heart rate or mean arterial pressure,” “delay to maximal response,” and “duration of maximal response” subsequent to each dose of isoproterenol, were calculated. “Delay to basal heart rate or mean arterial pressure” was calculated as the time taken by each individual animal to return to its own basal pressure after each dose of isoproterenol treatment was removed.

Western blot analysis. Whole heart lysates were prepared from frozen tissue, and protein content was determined as described previously (21). Cleared protein lysates were standardized to a final concentration of 2 mg/ml in Laemmli’s sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.02% bromphenol blue, and 150 mM dithiothreitol). For preparation of cardiac membranes, frozen heart tissue was ground to a powder in a mortar on dry ice and then mixed with fresh ice-cold buffer containing 5 mM Tris, pH 7.4, 2 mM EDTA, and protease inhibitors (21). Samples were homogenized with 20 passes before centrifugation at 1,000 g for 4°C for 15 min to pellet nuclear material. The supernatants were then centrifuged for 1 h at 100,000 g to pellet cardiac membranes, which were subsequently resuspended in lysis buffer (21), and the protein samples were standardized to 2 mg/ml in Laemmli’s sample buffer. Samples were boiled for 5 min and then separated by SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore), and Western blotting was carried out as previously described (48). Cardiac membranes were probed with antibodies to AC IV (Santa Cruz sc-20763, a rabbit polyclonal antibody raised against a recombinant protein corresponding to amino acids 631–800 mapping at the COOH terminus of human AC IV and cross-reactive with rat and mouse AC IV); AC V and VI (Santa Cruz sc-590, a rabbit polyclonal antibody raised against a peptide mapping to the COOH terminus of human AC V, which is identical to the COOH-terminus sequence of rat AC VI, and cross-reacts with rat and mouse AC V and VI); Goα1 (Upstate, Lake Placid, NY; no. 05–465, a rabbit polyclonal antibody raised against a 10-residue synthetic peptide corresponding to the COOH-terminal region shared by Goα1 and Goα2, which recognizes both rat and mouse Goα1 and -2), and stimulatory G protein (Gαs; an affinity purified rabbit polyclonal antibody raised against a peptide...
mapping within the NH$_2$ terminus of G$_{\alpha}$(b) of human origin, which cross-reacts with mouse, rat, and bovine G$_{\alpha}$(b). Whole lysates were probed with antibodies to $\beta_1$-AR (Affinity Bioreagents; a rabbit polyclonal antibody raised against a synthetic peptide mapping to residues 394–408 of mouse, which is identical to the rat sequence and recognizes mouse and rat $\beta_1$-AR; Applied Bioreagents PA1–730, a rabbit polyclonal antibody raised against residues 384–397 of human $\beta_1$-AR; $\beta_3$-AR (Alpha Diagnostics International; a rabbit polyclonal antibody raised against a 20-amino acid mouse $\beta_3$-AR peptide that is 85% homologous to the rat sequence and recognizes mouse and rat $\beta_3$-AR; $\beta_2$-arrestin (Applied Bioreagents PA1–730, a rabbit polyclonal antibody raised against residues 384–397 of human $\beta_2$-arrestin 2 and cross-reactive with rat $\beta_2$-arrestin and $\beta_3$-arrestin 2); insulin receptor $\beta$-subunit (IR-$\beta$; Santa Cruz sc-711; Autogen Bioclear; a rabbit polyclonal antibody raised against the COOH terminus of IR-$\beta$ of human origin and cross-reactive with mouse and rat IR-$\beta$). A mouse monoclonal antibody to $\beta$-actin with cross-reactivity to rat (ab-6276; Abcam, Cambridge, UK) was used as a loading control. Horseradish peroxidase-conjugated secondary antibodies to mouse and rabbit were obtained from Amersham AAbbott Biocare. Antibody binding was detected using the ECL kit from Amersham. Optical density of immunodetected bands was measured using AlphaEase gs 3.3b. Western blots were image analyzed, and the immunopositive bands were measured by spot densitometry. The arbitrary value obtained (integrated density value or IDV) was then corrected by subtracting the background. The corrected IDVs obtained from both the control and LP samples were then normalized to actin, detected by an actin antibody on either the lower half of the same blot or the same blot stripped and reprobed with actin antibody. Inter- and intra-group coefficients of variation of actin levels were analyzed and found to be within 5%. These normalized values then undergo statistical tests. Control means are then set at 100% ± SE, and then the LP values are calculated as a percentage of the controls.

**Adrenal gland morphometric measurements.** Adrenal glands from control and LP groups were removed at postmortem and fixed in 4% formalin for 16 h before moving to 70% ethanol and then processed to wax. Sections (4 µm) were cut to include the largest cut surface along the longest plane and stained with hematoxylin and eosin. Medullary areas from each section were quantified with the image analysis program analySIS (Olympus), and the largest measurement from each animal was included for statistical analysis.

**Plasma analysis.** Animals were killed by CO$_2$ asphyxiation between 0900 and 1100. ACTH and corticosterone levels of these animals (see RESULTS) were comparable to the least stressful method of killing, as described in Vahl et al. (57). Fasting blood was collected by decapitation, and EDTA plasma was stored at −80°C until used. Plasma insulin concentrations were measured using a rat insulin ELISA kit (Mecordia Ultra-sensitive Rat Insulin ELISA; Mecordia Uppsala, Sweden). Plasma corticosterone and ACTH were measured with ELISA kits from Immunodiagnostic Systems (IDS, Tyne & Wear, UK). All samples were assayed in duplicate, and an intra-assay coefficient of variation of up to 5% was accepted.

**Statistical analysis.** Data are presented as means ± SE, and comparisons between groups were assessed by unpaired two-tailed t-tests using GraphPad Instat (Statistical Solutions), unless stated otherwise. P values of <0.05 were considered statistically significant.

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**RESULTS**

**In vivo hemodynamic measurements.** The basal heart rate of the LP group was increased compared with controls (Table 2). The increases in heart rate after isoproterenol infusion were comparable in LP and control rats for each of the doses of 0.1 and 1.0 µg·kg$^{-1}$·min$^{-1}$ (Table 2). There was, however, a significant delay before the maximal response in the LP group compared with controls, with controls taking less time to reach a maximal response than LPs at the lowest dose (Fig. 1A). The duration of maximal response was also reduced for LPs at this dose. In addition, the recovery time to basal heart rate was extended significantly in LPs. With the higher dose, the LP group reached maximal stimulation quicker, although the amplitude of the response (Table 2) and the duration of the response at this dose were not different between the two groups (Fig. 1A).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Low Protein (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal HR, beats/min</td>
<td>359.8±3.4</td>
<td>386.3±15.1*</td>
<td>0.04</td>
</tr>
<tr>
<td>Iso 1</td>
<td>138.6±7.8</td>
<td>119.1±9.9</td>
<td>0.21</td>
</tr>
<tr>
<td>Iso 2</td>
<td>169.5±10.8</td>
<td>142.7±8.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Iso 1, isoproterenol at 0.1 µg·kg$^{-1}$·min$^{-1}$; Iso 2, isoproterenol at 1.0 µg·kg$^{-1}$·min$^{-1}$; HR, heart rate.

### Table 2. Magnitude of heart rate responses to isoproterenol infusion

*Mann-Whitney nonparametric tests were applied because of the dissimilar variances.
Basal mean arterial pressures, as well as diastolic and systolic pressures, were comparable between the two groups (Table 3). The attenuation in mean arterial pressure was greater at the higher dose of isoproterenol; however, the magnitude of the responses was similar between the two groups (Table 3). The response times were also similar for the lower dose (Fig. 1B). At the higher dose, however, the LPs achieved a maximal response in significantly less time than the controls (Fig. 1B), although the duration of their response was reduced.

β-AR expression. β1-AR expression in LP hearts was reduced compared with controls (P = 0.038; Fig. 2A). β2- and β3-AR expression, however, was comparable in both groups (Fig. 2, B and C). There were no differences in the protein levels of β1- and β3-AR in aorta of controls and LP rats (data not shown). β2 Expression was undetectable in this tissue.

Expression of downstream signaling molecules. Expression of AC IV and V/VI protein in LP heart membranes was found to be comparable to controls (Fig. 3). β1-ARK levels were also comparable (Fig. 3; Table 4). The adrenal gland weights from both groups were comparable (Table 4); however, the percentage adrenal-to-body weight ratio of the LP group was significantly higher than in the control group (P = 0.017; Table 4). LP medullary area was also higher than controls (P = 0.005; Table 4).

Insulin signaling. The protein levels of IR-β were reduced by one-half in the LP heart tissue compared with the control group (P = 0.013; Fig. 4).

Body weights and morphometry of adrenal glands. Birth weights and weights at weaning and at the time of the experiment were found to be significantly lower in the LP group (P < 0.001 at birth and at weaning and P < 0.05 at 3 mo of age; Table 4). The adrenal gland weights from both groups were comparable (Table 4); however, the percentage adrenal-to-body weight ratio of the LP group was significantly higher than in the control group (P = 0.017; Table 4). LP medullary area was also higher than controls (P = 0.005; Table 4).

Plasma data. Plasma glucose in LP offspring was lower than that of controls. Insulin levels also tended to be reduced in the LP group. ACTH levels were comparable between the two groups, as were corticosterone levels (Table 5). ACTH and corticosterone levels in both groups are comparable to the least stressful method of killing and exsanguinations.

Table 3. Magnitude of mean arterial pressure responses to isoproterenol infusion

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Low Protein (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>111.2 ± 3.1</td>
<td>115.7 ± 5.3</td>
<td>0.53</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>99.1 ± 7.1</td>
<td>98.1 ± 3.9</td>
<td>0.90</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>133.4 ± 4.7</td>
<td>132.5 ± 3.9</td>
<td>0.90</td>
</tr>
<tr>
<td>Iso 1 Maximum change in MAP</td>
<td>12.8 ± 2.0</td>
<td>15.2 ± 1.9</td>
<td>0.46</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>14.2 (12.5–15.7)</td>
<td>15.0 (11.8–16.8)</td>
<td>0.80</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>15.2 (12.1–16.5)</td>
<td>14.8 (11.4–17.9)</td>
<td>0.94</td>
</tr>
<tr>
<td>Iso 2 Maximum change in MAP</td>
<td>26.8 ± 1.4</td>
<td>26 ± 7.8</td>
<td>0.84</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>25.5 (23.5–27.6)</td>
<td>24.7 (19.9–33.3)</td>
<td>0.94</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>25.9 (22.3–27.2)</td>
<td>25.6 (18.5–37.5)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Values are means ± SE except for those analyzed by nonparametric tests where values are presented as medians with interquartile ranges in parentheses; n, no. of rats. Units are mmHg. MAP, mean arterial pressure.

Fig. 2. β-Adrenergic receptor (β-AR) expression in heart. The expression of β1-AR (A), β2-AR (B), and β3-AR (C) in whole heart lysates of 3-mo-old male control and LP rats was determined by Western blot analysis of heart samples taken from control and LP offspring rats, as described in METHODS. Open bars, control groups; gray bars, LP groups. Results are expressed as means ± SE. *P < 0.05.

DISCUSSION

Epidemiological studies have shown low birth weight to be related to the development of age-related diseases, including type 2 diabetes (27), hypertension, cardiovascular disease (17, 23), and obesity (60). The rat LP model is well documented in its applicability to the study of metabolic disease pathologies. It results in low-birth-weight offspring and the development of insulin resistance and frank diabetes in older male offspring (50) and an insulin-resistant state in female offspring (21). Nutrition during the suckling period also has a huge developmental impact, as shown by the epidemiological observations...
of Eriksson et al. (18), which suggest that low weight gain during the first year of infancy is also crucial in the development of coronary heart disease. In this model, the LP mother’s diet is maintained at 8% protein during lactation also, which reduced weight gain during this period.

The present study has identified that the increased peripheral epinephrine levels reported previously for LP male offspring rats (51) is likely the result of an increased adrenal-to-body weight ratio and an increase in medullary area. We also found their resting heart rate to be raised, which is consistent with the raised epinephrine.

The overall delay in the response and the reduced duration and magnitude of response imply a reduced cardiac and arterial β-adrenergic responsiveness. Upon activation by the lowest dose, the LP offspring were unable to sustain their maximal response, and heart rate started to decline even before the end of the infusion. Interestingly, however, they took longer to return to the basal heart rate. During the higher dose, however,

Fig. 3. Expression of β-adrenergic downstream signaling molecules in heart. The expression of adenylyl cyclase (AC) IV (A), AC V and VI (B), stimulatory G protein (Gα; C), inhibitory G protein (Gi; D), β1-AR kinase (β1-ARK; E), and β-arrestin (F) in cardiac membranes (A–D) and whole heart lysates (E–F) of 3-mo-old male control and LP rats was determined by Western blot analysis of heart samples taken from control and LP offspring rats, as described in METHODS. Open bars, control groups; gray bars, LP groups. Results are expressed as means ± SE. *P < 0.05.

Fig. 4. Expression of insulin receptor in heart. The expression of insulin receptor (IR) β-subunit in whole heart lysates of 3-mo-old male control and LP rats was determined by Western blot analysis of heart samples taken from control and LP offspring rats as described in METHODS. Open bars, control groups; gray bars, LP groups. Results are expressed as means ± SE. *P < 0.05.
LP offspring responded more quickly, but less strongly, compared with the controls. This led us to investigate whether components of β-adrenergic signaling were altered in LP heart tissue.

β-AR belong to the larger family of G protein-coupled receptors that modulate cardiac function by controlling the inotropic and chronotropic response to catecholamines. β1-AR is coupled to Gs, and its phosphorylation by the β2-AR leads to a blockade of downstream signaling and desensitization of the receptor to further catecholamine stimuli. Chronic overstimulation of the cardiac β-adrenergic system is toxic to the heart and may contribute to the pathogenesis of congestive heart failure (52). Numerous studies have shown a decrease of the cardiac β-ARs in failing hearts (8–10, 16, 34), specifically a reduction of the β1-subtype protein levels and up to 50% reduction in its mRNA, which correlated to disease severity. An accompanying increase in Gα (15) has been shown to reduce the responsiveness of Gs-coupled receptor systems such as the β1-AR in diseased human myocardium (7) and in overexpression systems (14, 31, 54). We observed that the β1-AR, which is the predominant cardiac subtype and provides the strongest stimulus for cardiac function (35), was reduced in the LPs. This is consistent with their reduced responsiveness to isoproterenol. We propose that the increased basal epinephrine previously observed for LP male offspring of a similar age (51) isoproterenol. We propose that the increased basal epinephrine overexpression systems (14, 31, 54). We observed that the β1-ARK expression was not altered in LPs, which mirrors the observations of Leineweber et al. (38) for the aging heart, and neither was Gi expression. Experimental agonist stimulation coupled to the reduced number of β1-AR in the heart led to a progressive desensitization of the available receptors, as evidenced by the shortened duration of activity and prolonged delay to basal heart rate and pressure after removal of the stimulus. β1-ARK expression was not altered in LPs, which mirrors the observations of Leineweber et al. (38) for the aging heart, and neither was Gi expression. However, LP β-arrestin protein levels were raised, which agrees with observations of reduced β-adrenergic signaling also in aging (13) and failing (58) hearts. This suggests that the hearts of LPs might be aging more quickly. Recent studies have implicated a role for β-arrestin in the regulation of β-AR desensitization after agonist binding (2, 49), by acting to slow the rate of cAMP production and increasing the rate of its degradation at the membrane by its association with phosphodiesterase enzymes (1).

Unlike some LP diets, the one used in the current study did not affect blood pressure, which is consistent with the findings of others using the same diet (36). This may be because of some differences in diet compositions.

This study provides evidence showing that maternal undernutrition programs adrenal growth and adrenomedullary hormone secretion. One possibility is that this may be a direct response to the young LP offspring’s mild hypoglycemic state, which would cause levels of epinephrine, a short-term glucose counterregulatory hormone, to rise (33). Persistent adrenergic stimulation might then lead to the observed downregulation of β1-AR levels in the heart as an adaptive response, which could be protective against heart failure, since this is effectively a β-block response. On the other hand, the increased level of β-arrestin is consistent with a requirement for rapid desensitization of the reduced receptor numbers to allow rapid recycling to the plasma membrane. The overall likely effect is one of reduced initial response because of the reduced receptor density but a more rapid response at a higher stimulatory dose, since more arrestin would allow faster uncoupling of the receptor from Gi and return of the available receptors to the plasma membrane. This adaptive response is beneficial in young animals; however, with age, LPs develop a worsening of their glucose tolerance (50), leading to hyperinsulinemia. It has been shown that cross talk between β-adrenergic and insulin receptors in neonatal rat cardiomyocytes exists and that β-AR stimulation has a biphasic effect on insulin-stimulated glucose uptake. Although short-term stimulation induces an additive effect on insulin-induced glucose uptake, long-term stimulation inhibits both insulin-stimulated glucose uptake and insulin-induced autophosphorylation of the insulin receptor (45). Thus the increased peripheral epinephrine and downregulation of insulin receptor density in the LPs suggest they may develop cardiac insulin resistance with age. This is supported by human studies that demonstrate a role for diminished insulin signaling and insulin resistance in the development of cardiomyopathy (29) and heart failure (20) with age.

Various other animal models used to study the effects of human fetal growth restriction support the link between intrauterine growth restriction and cardiovascular disease. In sheep (59), fetuses of ewes nutrient restricted during days 28–78 of gestation showed compensatory left ventricular growth that was associated with increased transcription of genes related to cardiac hypertrophy, compensatory growth, or remodeling.

Table 4. Anatomical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 8)</th>
<th>Low Protein (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth wt, g</td>
<td>6.56±0.19</td>
<td>5.55±0.09</td>
<td>0.0003</td>
</tr>
<tr>
<td>Body wt at weaning, g</td>
<td>49.4±1.11</td>
<td>29.74±1.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body wt at 3 mo, g</td>
<td>416±12</td>
<td>376±12</td>
<td>0.029</td>
</tr>
<tr>
<td>Adrenal wt, mg</td>
<td>73±6</td>
<td>80±4</td>
<td>0.4</td>
</tr>
<tr>
<td>%Adrenal wt/body wt</td>
<td>0.018±0.001</td>
<td>0.022±0.001</td>
<td>0.017</td>
</tr>
<tr>
<td>Medullary area, mm²</td>
<td>1.58±0.25</td>
<td>2.12±0.13</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats.
and the Parthenon Trust.  

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REFERENCES


β-ADRENERGIC RESPONSE IN LOW-PROTEIN RAT OFFSPRING