Developmental regulation of cardiovascular function is dependent on both genotype and environment

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A RELATIONSHIP between suboptimal intrauterine and postnatal environments and the development of the metabolic syndrome (type II diabetes, obesity, insulin resistance, hypertension, stroke, dyslipidemia, and cardiovascular disease) has been reported (2, 3, 13). Human cohorts have shown an association with impaired nutrition in mothers and the development of heart disease in their offspring in later life (1, 31). A similar relationship exists between maternal undernutrition and the development of hypertension in the offspring of rats (22), guinea pigs (19), and sheep (27). Although adverse antenatal and postnatal environments increase the risk of particular adult diseases, not all individuals exposed to adverse environments develop these diseases, suggesting that an individual’s genotype may impact the eventual outcome (10, 11, 29, 41).

The laboratory mouse has been used extensively to analyze complex genetic traits. Indeed, these traits can be dissected in well-defined inbred or recombinant inbred strains of mice in which genetic factors have segregated and become fixed during inbreeding and where environmental effects can be controlled (32). Results from genetic studies of mouse models may provide target chromosomal regions and/or candidate genes that can subsequently be tested in population or family studies in humans. Chromosomal maps of single nucleotide polymorphisms have shown that the A/J (AJ) and C57BL/6J (B6) mouse strains are phylogenetically distant as indicated by major bifurcations in their phylogetic hierarchical trees (6). These strains are divergent in many physiological characteristics (Mouse Phenome Database; www.jax.org). Chromosome substitution strains of AJ and B6 mice have been developed to facilitate the determination of the genetic basis of disease (26, 33). With relevance to the current study, B6 are more susceptible than AJ mice to the development of the metabolic syndrome when exposed to a high-fat diet postweaning (33, 34), and B6 mice develop hypertension when fed a high-fat, high simple carbohydrate diet, whereas AJ mice do not (25).

We have recently used these strains to develop a murine model of the developmental origins of health and disease (DOHaD) in which an adverse intrauterine environment (modeled through a 30% prenatal dietary restriction) led to remarkable strain differences in maternal plasma glucocorticoid levels, term fetal organ weights, and gestation length (21). Furthermore, we have also observed that the offspring of prenatal nutritionally restricted B6 mothers displayed a greater incidence of metrics of the metabolic syndrome than similarly treated AJ mice. B6 males exhibited delayed growth trajectory, increased obesity, and impaired glucose tolerance in response to prenatal nutrient restriction, relative to AJ mice (20). Prior work demonstrated that B6 mice develop postnatal hypertension in response to prenatal nutrient restriction, relative to AJ mice (18). This difference in response to prenatal nutrient restriction suggests that B6 mice may be more susceptible to the development of postnatal cardiovascular dysfunction caused by prenatal nutrient restriction.

Cardiac impairments are evident after exposure to an adverse early environment (1, 31), but the mechanisms responsible are unclear. While it may be secondary to hypertension, it may also be caused by a direct effect on cardiac function. Therefore, our current objectives were to 1) characterize postnatal cardiovascular function of offspring exposed to prenatal...
nutrient restriction, 2) determine whether a postnatal high-fat diet modifies this relationship, and 3) investigate the impact of the genetic background (i.e., AJ and B6 strain differences) on these relationships.

**MATERIALS AND METHODS**

All experiments were approved by the Samuel Lunenfeld Research Institute Animal Care Committee in accordance with the guidelines of the Canadian Council for Animal Care. Inbred female AJ and B6 mice (6–12 wk of age, Jackson, Bar Harbor, ME) were fed sterile 1-g Dustless Precision pellets (S0173, Bio-Serv, Frenchtown, NJ) and sterile water ad libitum under standard environmental conditions as previously described (20, 21). Primiparous female AJ and B6 mice were mated with an experienced male of the same strain, and following visualization of a vaginal plug, female mice were housed individually and fed ad libitum. Pregnant mice were randomly chosen to be treated as either controls and fed ad libitum for the duration of pregnancy or subjected to prenatal nutrient restriction involving a 30% reduction in maternal caloric intake as described previously (21). Maternal nutrient restriction was imposed from day 6.5 to day 17.5 of pregnancy (full term is ~19 days), after which the mice were fed ad libitum. Postpartum mothers were fed ad libitum, and offspring remained with their mothers until weaning at 21.5 days of age. A maximum of five and minimum of four pups remained with mother postpartum. The effect of diet did not significantly alter the litter size or the male-to-female ratio as we previously reported (20). At weaning, the pups were randomly separated into groups of two to four same-sex siblings and were fed ad libitum either standard diet or a high-fat diet (S3282, Bio-Serv). The high-fat diet, while matched in essential nutrients, contained 58.7% kcal/g from fat compared with 10% kcal/g from fat in the standard diet.

**Tail-cuff measurements.** Arterial blood pressure (systolic) and heart rate were measured between 10:00 AM and 12:00 PM in conscious mice at 9 and 25 wk of age using an automated tail-cuff system (BP-2000, Visitech Systems, Apex, NC). Daily measurements obtained over 4 days were averaged. Our research group has previously showed that tail-cuff blood pressure measurements are highly correlated with, and nearly equal to, carotid artery mean arterial pressure measured using a chronic arterial catheter in mice (40).

**Echocardiography measurements.** An echocardiographic exam at 26 wk of age was performed using microultrasound (30 MHz; VisalSonics, Toronto, Canada) using published methods (12, 42) on mice anesthetized with 1 to 2% isoflurane in oxygen. A heat lamp and heated mouse pad were used to maintain physiological body temperature between 36° and 38°C. The blood velocity of flow in the ascending aorta and in the mitral inflow to the left ventricular (LV) chamber was measured using pulsed-Doppler ultrasound. Peak velocity of E wave (early passive filling), peak velocity of A wave (atrial contraction), isovolumetric relaxation (IRT) and contraction (ICT) times, and ejection time (ET) were measured, and the E-wave-to-A-wave ratio (E/A) and the myocardial performance index [Tei = (IRT + ICT/ET)] were calculated (4, 37). The myocardial performance index, also known as the Tei index, is a measure of global ventricular function, which quantifies the combined systolic and diastolic function (37). In the event that the E- and A-wave peaks were fused so that the peak velocities could not be accurately visualized, the ultrasound exam was repeated within 7 days. If still fused, then this measurement was not obtained, resulting in lower N values (reported in figures), because of missing data. M-mode was used to measure LV inner chamber dimensions (ID) at end systole (s) and end diastole (d), and the percent fractional shortening [(IDd − IDd)/IDd × 100] was calculated.

**Statistical analysis.** Data are presented as means ± SE. Multivariate regression analyses were used to evaluate experimental outcomes. Variable selection in multivariate modeling was based on stepwise regression procedures (forward and backward selection), including the assessment of interaction terms to arrive at the most parsimonious model. Variables considered (as appropriate) in the multivariate models included strain, maternal diet, sex of offspring, maternal weight at term, the number of offspring in the litter, the proportion of males in each litter, body weight at time of test, and biologically relevant interactions. The final models used to assess cardiovascular function included strain, maternal diet, and offspring postnatal diet modeled as fixed effects. Unexpectedly, the sex of the offspring did not significantly alter the relationship between strain and maternal diet; thus data from males and females were combined. A summary of the covariates used for each measurement is included in the figure and table legends. SAS statistical software (Statistical Analysis Systems Institute 1999, Version 9.1, Cary, NC) was used for data analysis. P values < 0.05 were considered statistically significant.

**RESULTS**

In B6 mice, prenatal nutrient restriction led to a significantly elevated arterial blood pressure at 9 wk compared with same-aged B6 controls (Fig. 1A; P = 0.001). Although arterial blood pressure did not change significantly with postnatal age for either prenatal diet, the elevation in arterial pressure was no longer statistically significant between groups by 25 wk of age (Fig. 1A). Similar results were obtained on a postnatal high-fat diet (Fig. 1B). In AJ mice, arterial pressure significantly decreased between 9 and 25 wk of age in controls (P = 0.01; Fig. 1A). This decrease was absent in AJ mice following prenatal nutrient restriction (Fig. 1A). Arterial blood pressure in AJ mice did not significantly differ between restricted and control groups at either 9 or 25 wk, although it tended to be higher at 25 wk (Fig. 1A). Similar results were obtained on the postnatal high-fat diet, except that in this case, hypertension was statistically significant at 25 wk in the prenatally restricted group. In control mice, there was no significant difference in arterial pressure in B6 versus AJ mice at 9 wk of age on either postnatal diet, but by 25 wk, arterial pressure in AJ mice was significantly lower than that in B6 mice on both postnatal diets (P = 0.01; Fig. 1, A and B).

In B6 mice, prenatal nutrient restriction led to a significantly elevated heart rate at 9 wk in mice on the postnatal control diet (Fig. 1C; P = 0.01). Although the heart rate was similarly elevated on the postnatal high-fat diet, it was not significantly higher than in controls (Fig. 1D). Interestingly, this meant that the heart rate at 9 wk was already at a level similar to that of B6 control mice at 25 wk of age (Fig. 1C; P = 0.01). Thus heart rate in the B6 nutrient-restricted group failed to show the increase in heart rate with age observed on both the control and high-fat diets in control B6 mice (Fig. 1, C and D). Heart rate responses were markedly different in the AJ strain. Heart rate decreased in control AJ mice from 9 to 25 wk on both postnatal diets (P = 0.01; Fig. 1, C and D). However, in nutrient-restricted AJ mice, this decrease was already apparent at 9 wk at which time heart rate was significantly lower than that in control mice on both postnatal diets (Fig. 1, C and D; both, P = 0.01). In controls, heart rate was significantly higher in AJ than in B6 mice at 9 wk and was significantly lower at 25 wk on both postnatal diets (P = 0.001; Fig. 1, C and D).

Cardiac function was assessed using microultrasound echocardiography at 26 wk of age in isoflurane-anesthetized mice. Prenatal nutrient restriction did not significantly alter heart rate in either strain of mice at 26 wk of age on either the postnatal control or high-fat diet (Table 1 and 2), and this result was
controls (Fig. 2, A and C). However, the decrease in the Tei index was achieved in a strain-dependent manner, with ET being increased in B6 controls, whereas it decreased with age in AJ mice (Fig. 3, B and C). While a postnatal high-fat diet did not alter this relationship in B6 mice, it abolished the increase in B6 mice (Fig. 3, A and D).}

**Table 1. Hemodynamics after postnatal control diet**

<table>
<thead>
<tr>
<th></th>
<th>AJ</th>
<th>C57Bl/6j</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-C</td>
<td>R-C</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>502±10 (12)</td>
<td>497±11 (15)</td>
</tr>
<tr>
<td>Aortic blood velocity, mm/s</td>
<td>1.32±0.78 (12)</td>
<td>1.26±0.64 (15)</td>
</tr>
<tr>
<td>Aortic diastolic diameter, mm</td>
<td>1.18±0.02 (12)</td>
<td>1.13±0.04 (15)</td>
</tr>
<tr>
<td>Aortic systolic diameter, mm</td>
<td>1.33±0.02 (12)</td>
<td>1.30±0.04 (15)</td>
</tr>
<tr>
<td>LVIdd, mm</td>
<td>3.1±0.05 (12)</td>
<td>3.12±0.09 (15)</td>
</tr>
<tr>
<td>LVIdd/body wt0.33, mm/g0.33</td>
<td>1.03±0.02 (12)</td>
<td>1.07±0.03 (15)</td>
</tr>
<tr>
<td>body weight, g</td>
<td>27.5±1.2 (12)</td>
<td>25.0±0.7 (15)</td>
</tr>
</tbody>
</table>

Values are means ± SE (number of mice). Column labels: first letter indicates prenatal diet [control (C) or restriction (R)] and second letter indicates postnatal diet [C or high fat (HF)]. LVIdd, left ventricular inner chamber dimension at end diastole. *P < 0.05 within strains (C-C < R-C); †P < 0.05 between strains (C57Bl/6j C-C < AJ C-C).
nutrient restriction-associated improvement in the Tei index in AJ mice (Fig. 3B). In control mice, there was no significant strain difference in the Tei index on either postnatal diet.

Strain differences were seen in peak blood velocity in the ascending aorta following prenatal nutrient restriction; peak velocity increased in B6 (P = 0.03) but not in AJ mice (Table 1). The increase in velocity in B6 mice was not apparent on the control diet (Table 1B). There were differences in peak velocity, aortic diameter, and heart rate were unchanged). Interestingly, whereas the diameter of the aorta and heart rate were unchanged, the peak blood velocity increased by ~15% in the aorta of B6-restricted compared with B6 control mice (Table 1), suggesting that cardiac output was ~15% higher. This is consistent with the trend toward a larger LVID in diastole in B6-restricted compared with B6 control mice (Table 1). In control mice, the peak velocity of blood within the aorta in the B6 strain was lower than in the AJ strain (P = 0.05) (Table 1).

DISCUSSION

We observed strain-dependent alterations in cardiovascular function in later life after prenatal nutrient restriction. Prenatal restriction increased arterial pressure and heart rate in B6 offspring, whereas it prematurely decreased heart rate in AJ offspring. Whereas no strain differences were observed in cardiac systolic or diastolic function in response to prenatal restriction, there was an increase in peak aortic blood velocity following prenatal nutrient restriction in B6 mice, with no change in aortic diameter or heart rate. Taken together, these findings suggest that at 26 wk of age, cardiac output was higher in B6 mice that were subjected to prenatal nutrient restriction; in contrast, AJ mice apparently showed no change in cardiac output in response to prenatal nutrient restriction (i.e., peak velocity, aortic diameter, and heart rate were unchanged).

Interestingly, both strains showed an improvement in global myocardial performance with a decrease in the Tei index in response to prenatal nutrient restriction. These results add to our prior work using this model, in which the 30% dietary restriction during pregnancy caused similar reductions in fetal body
weight in both strains but a larger reduction in maternal weight gain during pregnancy and higher maternal corticosterone and cortisol levels in B6 than in AJ mice (21). Postnatally, prenatally restricted B6 males developed glucose intolerance and higher percent body fat than dietary controls, whereas B6 females and AJ mice were not affected by this intervention (20). In contrast to this prior work, responses in the current study were not significantly influenced by the sex of the offspring, and both B6 and AJ strains exhibited significant alterations in postnatal function in response to prenatal dietary restriction.

It has been hypothesized that prenatal nutrient restriction alters development so that the offspring is preadapted to better survive poor postnatal conditions. When there is a mismatch between the anticipated and the actual postnatal environment, these adaptations can lead to disease (14). Thus we anticipated that we would exacerbate the postnatal effects by giving a high-fat diet postnatally to the offspring of nutrient-restricted pregnancies because this would augment the degree of mismatch between the prenatal and postnatal nutrient environments. However, contrary to our expectations, we observed beneficial or only modest detrimental effects of a postnatal high-fat diet on later cardiovascular function in the offspring of nutrient-restricted pregnancies because this would augment the degree of mismatch between the prenatal and postnatal nutrient environments. However, contrary to our expectations, we observed beneficial or only modest detrimental effects of a postnatal high-fat diet on later cardiovascular function in the offspring of nutrient-restricted pregnancies, and these responses were strain dependent. Following prenatal nutrient restriction, the high-fat diet resulted in an increase in E/A, suggesting it had improved diastolic function in B6 mice. In the AJ strain, however, this diet abolished the decrease in the Tei index caused by prenatal nutrient restriction, suggesting that it reversed this apparent improvement in cardiac function. We did not observe a dramatic worsening of arterial pressure or cardiovascular function in control B6 mice fed a high-fat diet. Possibly this is because the high-fat diet used also did not result in dramatic obesity in this mixed population of males and females.

Striking differences between B6 and AJ mice were observed in the developmental changes in arterial pressure and heart rate with age between 9 and 25 wk as assessed in awake mice by tail-cuff plethysmography. These differences in development interacted with diet to result in strain-dependent responses in arterial pressure and heart rate caused by prenatal nutrient restriction. We and others (Mouse Phenome Database) found that arterial pressure in AJ and B6 mice was similar at 9 wk under control conditions. Our arterial pressure values for B6 control mice (~120 mmHg) were comparable with those previously reported in ad libitum fed B6 mice whether measured awake by tail cuff (~115–130 mmHg) (Mouse Phenome Database) or as mean pressure in the carotid artery by catheter (~110 mmHg) (23) or by telemetry (~113 mmHg) (5). This is in accord with our prior work showing a strong correspondence between tail-cuff arterial and mean carotid artery blood pressure measurements in awake mice (40). In B6 control mice, arterial pressure did not change significantly with age between 9 and 25 wk, and this is in agreement with the results for carotid arterial pressures measured under light enflurane anesthesia in prior work in this strain (38). However, arterial pressure significantly decreased with age from 9 to 25 wk in AJ mice. Interestingly, this age-related decrease in arterial pressure in AJ mice was not observed following prenatal nutrient restriction in both the postnatal control and high-fat diet groups, resulting in significant hypertension in AJ mice at 25 wk (+29 mmHg) on the high-fat diet and a trend toward higher pressures on the control diet (+11 mmHg). Prenatal restriction also caused significant hypertension in B6 mice at 9 wk of age on both the postnatal control and high-fat diets (+14 and +17 mmHg, respectively). Although the pressures remained higher at 25 wk (+9 and +12 mmHg, respectively), these increases were no longer significant. Our results are in accord with prior work showing similar increases in arterial pressure (~10 mmHg; P < 0.05) in B6 mice at 8 and 16 wk of age following prenatal nutrient restriction (18). Thus the current study shows that adult hypertension is influenced by pre- and postnatal diet, postnatal age, and genetic differences between the B6 and AJ strains.

Differences in developmental changes in heart rate in B6 versus AJ mice were also observed, as were strain-dependent...
differences in the heart rate response to prenatal nutrient restriction in awake mice. Results showed that the age-related decrease in heart rate in AJ mice and the age-related increase in heart rate in B6 mice occurred earlier (i.e., at 9 wk of age) in mice subjected to prenatal nutrient restriction. These data are the first to suggest that there is an early maturation of heart rate in mice exposed to an adverse intrauterine environment. Interestingly, no significant age-related changes in heart rate were observed over this age interval in a prior report in B6 mice measured acutely under light enflurane anesthesia using a carotid catheter (~550–580 beats/min from 7 to 83 wk) (38). Given the important role of sympathetic tone in determining heart rate in adult mice (16) and the suppression of sympathetic activity caused by anesthesia (36), divergent heart rate responses to age and to prenatal dietary restriction in AJ and B6 mice may be due to divergent maturational changes in sympathetic tone. Differences in physiological responses to restraint and/or heat applied during tail-cuff blood pressure measurements may also contribute. Prior work has shown that prenatal stress can cause long-term changes in sympathetic activity and/or stress responses. For instance, human young adults with low birth weights had lower muscle sympathetic nerve activity and heart rates than those with birth weights within the normal range (39), and the adult offspring of pregnant rats made hypoxic during gestation had lowered autonomic nervous activity in cardiac-related structures, i.e., the stellate ganglion, heart, and adrenals (28). Other work in human adolescents concluded that low birth weight is associated with increased sympathetic activity and that this association is dependent on genetic factors (15). Alternatively, differences in thermogenic and/or endocrine responses in response to prenatal nutrient restriction and/or age may result in changes in the intrinsic heart rate of mice given the important roles of endocrine/paracrine signals in intrinsic heart rate control (35). Thus future studies are required to investigate the mechanisms causing the differences in heart rate observed with age, between strains, and as a consequence of prenatal diet in the current study. Confirmation of these differences by telemetry is required. The contrasting responses of these two strains could be used to explore their genetic basis using chromosome substitution strains and QTL analysis (26, 33).

The current study suggests that cardiac output following nutrient restriction is slightly higher in B6 mice compared with same-aged controls. This premise is based on a higher peak aortic blood velocity, whereas aortic diameter and heart rate were unchanged. This inference is supported by published methods for cardiac output measurements using echocardiography (8, 30). Increased cardiac output in response to an adverse intrauterine environment has been previously observed in the offspring of ewes exposed to high levels of dexamethasone during pregnancy (9). As with our own data, the increased cardiac output in these sheep was not due to increased heart rate or associated with changes in fractional shortening. Transgenic ablation of brown adipose tissue in mice was also associated with increased cardiac output without altering heart rate or fractional shortening (7). The consequences of an increased cardiac output in these B6 mice remains to be determined, but we speculate that it might lead to adverse cardiovascular function as the mice age.

Our rationale for using the Tei index, also known as the myocardial performance index, is that it provides a measure of global ventricular function that quantifies combined systolic and diastolic function (37). It has been shown to strongly correlate with an invasive measure of contractility, intraventricular maximum first derivative of LV pressure, over a range of hemodynamic conditions in mice (4) and to provide an indicator of changes in cardiac function following myocardial infarction in rats (17). In the current study, we found that the Tei index was lower following nutrient restriction in both AJ and B6 mice, suggesting that prenatal nutrient restriction resulted in an improved cardiac performance at 26 wk. It is interesting to note that while both strains demonstrated an improvement in their global myocardial performance index, it was accomplished in very different ways. B6 mice increased their ET, whereas AJ mice decreased their ICT and IRT in response to prenatal nutrient restriction, implying that cardiac function was altered by different mechanisms. In both strains, tail-cuff blood pressure measurements suggest that central arterial pressure at 26 wk may be slightly elevated relative to that of the prenatal dietary control groups. An increase in afterload would be predicted to increase, rather than decrease, the Tei index (4). The observed decrease in the Tei index suggests either an increase in cardiac contractility and/or an increase in cardiac preload (4). Although body temperature influences this variable (4), it was monitored and controlled during microultrasound exams and so is unlikely to be a factor. Interestingly, no change in either cardiac systolic (fractional shortening) or diastolic function (E/A) was observed. The results suggest that prenatal dietary restriction results in direct or indirect alterations in cardiac function in both strains of mice and that this effect is altered by a postnatal high-fat diet in a strain-dependent manner. While further studies are required to establish the mechanisms mediating these responses, these results show that these differences in cardiac function can be detected noninvasively using the Tei index and so would be amenable to high-throughput genetic analysis.

The advantage of using inbred mice strains to evaluate relative contributions of genetics, in gene-environment interaction studies, is highlighted by our observed strain differences in cardiovascular function following identical environmental manipulations between strains. Our results add to the known physiological differences between B6 and AJ mice, differences that may be exploited to elucidate their genetic basis using chromosomal substitution strains (26, 33). We observed differences in age-related changes in arterial pressure and heart rate and differences in baseline fractional shortening between the two strains, as well as differences in their responses to prenatal dietary restriction and a postnatal high-fat diet. These strains may therefore be used in the future to study the genetic mechanisms regulating normal developmental changes in cardiovascular function and how these changes are altered by prenatal nutrient restriction. The critical importance of genetics in determining the postnatal response to prenatal environment was seen in a twin cohort study where low birth weight was associated with altered postnatal blood pressure and sympathetic activation only within dizygotic twin pairs (i.e., genotypes differ) but not within monozygotic twin pairs (i.e., genotypes the same) (15). Together, these studies suggest that genetic factors play a role in the association between altered early life environment and the development of cardiovascular risk factors.
DOHaD AND CARDIOVASCULAR FUNCTION

In summary, we have developed a murine model of DOHaD that has the potential to better define the genetic factors that contribute to cardiovascular outcome. The results support prior work showing that prenatal nutrient restriction results in significant changes to the heart and/or vasculature which leaves the offspring more susceptible to cardiovascular disease in its adult life (24). The strain-dependent alteration in cardiovascular function in response to prenatal restriction observed in this study supports a genetic component to this developmental program. The strain-dependent differences identified in the current study provide an entry point for future studies investigating the genetic and physiological mechanisms that underlie DOHaD.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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