Adverse perinatal environment contributes to altered cardiac development and function

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Velten M, Gorr MW, Youtz DJ, Velten C, Rogers LK, Wold LE. Adverse perinatal environment contributes to altered cardiac development and function. Am J Physiol Heart Circ Physiol 306: H1334–H1340, 2014. First published March 7, 2014; doi:10.1152/ajpheart.00056.2014.—Epidemiological observations report an association between intrauterine growth restriction (IUGR) and cardiovascular diseases. Systemic maternal inflammation is the most common stress during pregnancy, leading to IUGR. We hypothesized that perinatal inflammation and hyperoxygenation induce discernible alterations in cardiomyocyte contractility and calcium signaling, causing early cardiac dysfunction. Pregnant C3H/HeN mice were injected with LPS or saline on embryonic day 16. Newborn mice were placed in 85% O2 or room air (RA) for 14 days. Pups born to LPS-injected dams had reduced birth weight. Echocardiographic measurements revealed that in vivo LV function was compromised in LPS/O2 mice as early as 3 days of life. Isolated cardiomyocytes from LPS/O2 mice at day 14 exhibited decreased sarcomere fractional shortening, along with decreased time-to-90% peak shortening. Calcium transient amplitude was greatest in LPS/O2 mice. SERCA2a mRNA and protein levels were increased and phospholamban mRNA levels were decreased in LPS/O2 mice. Phosphorylation of phospholamban was increased, along with Sorcin mRNA levels in LPS/O2 mice. Combined exposure to perinatal inflammation and hypoxia resulted in growth restriction, in vivo and in vitro cardiac dysfunction, coinciding with humans and animal models of cardiac dysfunction. Expression of calcium handling proteins during the neonatal period was similar to that observed during fetal stages of development. Our data suggest that perinatal inflammation and hypoxia exposure alter fetal development, resulting in early cardiac dysfunction.

fetal echocardiography; myocyte; calcium signaling; in utero; hypoxia; inflammation

Epidemiological observations during the past three decades have reported a greater risk for the development of adulthood hypertension, metabolic, pulmonary, and cardiovascular diseases in children born with low birth weight (1–3). Coinciding with our hypothesis, clinical data indicate low birth weight adjusted for gestational duration is associated with the onset of cardiovascular dysfunction to a greater extent than absolute birth weight or gestational duration (10). Furthermore, intrauterine growth restriction (IUGR) compounded by preterm birth imposes the greatest risk (15). However, the responsible mechanisms and causal relationships that affect the development and onset of cardiovascular dysfunction have not been examined.

Clinical studies and animal models focusing on low birth weight as the primary outcome have not been helpful in elucidating the underlying pathogenic mechanisms or causal relationships for the development of cardiovascular dysfunction in adults (5). Therefore, the focus should shift from examining major outcome parameters to identifying causal risk factors that occur during perinatal development and contribute to IUGR, but most importantly, lead to the early onset of cardiovascular dysfunction (6, 7).

An extensive array of maternal conditions and stressors including malnutrition, metabolic diseases, and various sources of inflammation can result in IUGR (5). Elucidating causal risk factors for cardiovascular disease development in the offspring of animal models is progressing. Maternal oxidative stress and hypercholesterolemia have been shown to accelerate atherosclerosis and to affect early predictors of cardiovascular dysfunction as vascular and endothelial dysfunction in the offspring. Immune mechanisms induced by a mild inflammatory stress in the mother or the fetus alter cardiac development and function and are of central importance in developing cardiovascular disease in the offspring. However, mechanisms that induce the pathophysiological changes responsible for cardiovascular dysfunction remain unknown.

Systemic maternal inflammation is the most common and clinically relevant perturbation occurring during pregnancy, causing a stressful intrauterine environment (4, 8, 9). This maternal condition adversely affects the developing fetus, leading to impaired intrauterine growth and a greater risk for the development of persistent cardiovascular dysfunction in the offspring. Following birth, these babies are commonly born with premature organs, including the lungs, and therefore receive various medical interventions including oxygen therapy. Prior studies revealed that hypoxia exposure induces more severe alterations in pulmonary structure and function in mice exposed to maternal inflammation during fetal development, resulting in pulmonary fibrosis (20, 21).

The present study was designed to examine the effects of perinatal inflammation and hypoxia on cardiac function during the early stages of life. We hypothesized that perinatal inflammation and hypoxia exposure would induce discernible alterations in cardiac physiology causing cardiac dysfunction early in life in the absence of additional risk factors.
MATERIALS AND METHODS

Animals and exposure. All animals were handled in a pathogen-free facility according to National Institutes of Health guidelines. Protocols for animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at The Research Institute at Nationwide Children’s Hospital, Columbus, OH. C3H/HeN males and females were housed for at least 1 wk in our facility before breeding. Pregnancy was confirmed and time-dated when a vaginal plug was present. On E16, pregnant C3H/HeN mice were injected with 80 μg/kg LPS ip (No. 437627; Calbiochem, Gibbstown, NY) or saline. LPS dose was determined by preliminary studies where we tested the highest dose that consistently produced equal-sized, viable litters. Each newborn litter was matched with another litter from a dam that received the same treatment on E16. These two litters were combined, and the pups were randomly redistributed to the dams as described previously (21). One of the litters was exposed to 85% O2 for 2 wk (saline/O2, LPS/O2x2), whereas the other litter from the pair remained in room air (RA, LPS/RA, saline/RA). To prevent oxygen toxicity and nutritional differences, dams were alternated between their corresponding O2 and RA litters every 24 h. Day 1 was designated as 24 h of O2 or RA exposure. Mice were euthanized at 14 days of age, and one pup from each litter was analyzed for each experiment.

In vivo cardiac assessment. Cardiac function was assessed at day 3 using echocardiography (40 MHz transducer, VisualSonics Vevo 2100; Toronto, Ontario). Mice were continuously sedated with 1.5% isoflurane (delivered in 100% O2) and temperature was maintained at 37°C. Prewarmed ultrasound gel (Aquasonic, Parker Labs, Fairfield, NJ) was used on the chest with a 15 MHz (optimized for rodent studies) probe placed in the parasternal, short axis orientation. Three loops from each mouse were captured, and data were averaged from 5 beats. LV dimensions in systole and diastole as well as posterior wall thickness in systole and diastole were assessed. Parameters were measured using the leading-edge technique from the American Society for Echocardiography. Fractional shortening was calculated using the equation: % fractional shortening (%FS) = [(LV dimension in diastole – PV dimension in systole)/left ventricular internal dimension in diastole * 100].

In vitro cardiomyocyte function. Hearts were harvested from 14-day-old mice, and myocytes were isolated using liberase (Roche, Basel, Switzerland) and were subsequently synthesized using a Maxima First Strand cDNA Synthesis Kit for RT-quantitative PCR (K1641; Thermo Scientific Fermentas, Glen Burnie, MD). A Mastercycler Realplex RT-PCR Detection System (Eppendorf, Hamburg, Germany) was used for quantitative real-time PCR analyses with Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific Fermentas, K0222; Glen Burnie, MD).

Quantitative real-time PCR. An RNeasy Mini kit (Qiagen, Valencia, CA) was used to isolate RNA from cardiac tissues at embryonic days (E) 17, 18, and 19 and at 14 days of life (d). cDNA was subsequently synthesized using a Maxima First Strand cDNA Synthesis Kit for RT-quantitative PCR (K1641; Thermo Scientific Fermentas, Glen Burnie, MD).

Statistical analyses. Statistical analyses were performed with GraphPad PRISM 5 (La Jolla, CA) using a two-way ANOVA and Tukey’s post hoc with the variables saline or LPS as injection and RA or O2 as exposures. Data are expressed as means ± SE with P < 0.05 considered statistically significant.

RESULTS

Maternal LPS injection results in increased fetal cytokine levels and significantly lower birth weights. To evaluate consequences of maternal LPS injection on fetal (pup) inflammation, we measured TNF-α, IL-1β, and IL-6 mRNA expression using RT-PCR. In the fetal heart, inflammatory cytokines peaked at E19, 72 h following maternal LPS injection (Fig. 1, A–C). Maternal inflammatory responses have been reported previously (22). To evaluate the effects of maternal LPS injection on birth weights, the pups born to maternal LPS and saline injected dams were weighed after birth. There was no difference in the number of pups born to saline or LPS-injected dams. However, pups born to LPS-injected dams showed a significantly lower birth weight compared with pups born to saline-injected dams (1.41 ± 0.02 [g] LPS, vs. 1.59 ± 0.02 [g] saline). TNF-α mRNA was increased in mice that were born to LPS-injected dams and subsequently hyperoxia exposed until day 14 (Fig. 1D).

Maternal LPS injection and neonatal hyperoxia exposure alter LV dimensions and lead to LV dysfunction. We performed echocardiographic analyses to assess LV dimension and function in 3-day-old pups following systemic maternal saline or LPS injection and neonatal hyperoxia or RA exposure. Left ventricular end diastolic volume was not different in any of the groups (Fig. 2A). Furthermore, left ventricular end systolic volume was significantly increased in pups born to LPS-injected dams and hyperoxia exposed (LPS/O2) for 3 days compared with pups born to saline-injected dams that were RA or O2 exposed (Fig. 2B). Statistical analyses indicated a significant interaction between hyperoxia exposure and LPS in-
Injection and significant effects of LPS injection. %FS was significantly lower in LPS/O$_2$ exposed mice compared with all other groups. Furthermore, FS was significantly greater in saline/O$_2$ exposed mice compared with LPS/RA and saline/RA exposed mice (Fig. 2C). Statistical analyses indicated an interaction between LPS injection and hyperoxia exposure and effects of LPS injection.

**Maternal LPS injection in addition to neonatal hyperoxia exposure alters cardiomyocyte function in vitro.** Functional assessment of isolated cardiomyocytes was performed as an evaluation of the cellular effects of systemic maternal LPS injection and neonatal hyperoxia exposure. Isolated cardiomyocytes from maternal saline-injected and neonatal hyperoxia exposed (saline/O$_2$) and myocytes isolated from maternal LPS-injected and neonatal RA exposed (LPS/RA) mice did not display alterations in in vitro cardiomyocyte function compared with maternal saline-injected and neonatal RA exposed (saline/RA) controls. However, isolated cardiomyocytes from maternal LPS-injected and neonatal hyperoxia exposed (LPS/O$_2$) mice had significantly decreased percent peak shortening (percent FS; Fig. 3A), along with decreased time-to-90% peak shortening (Fig. 3B; myocyte systolic dysfunction), but time-to-90% relengthening was not affected in LPS/O$_2$ exposed mice (Fig. 3C; myocyte diastolic function). Statistical analyses indicated independent effects of hyperoxia exposure and LPS injection for %FS and effects of LPS injection, hyperoxia exposure, and an interaction of LPS and hyperoxia for time-to-90% peak shortening. Therefore, cardiac dysfunction is evident at the cardiomyocyte level only in mice receiving both maternal LPS injection during fetal development and subsequent hyperoxia exposure during the neonatal period.

**Systemic maternal LPS injection and neonatal hyperoxia exposure increase calcium transient amplitude.** There was no difference in calcium transient amplitude in myocytes isolated from maternal saline-injected and neonatal hyperoxia exposed (saline/O$_2$) mice compared with maternal saline-injected and neonatal RA exposed (saline/RA) controls. However, cardiomyocytes isolated from maternal LPS-injected and neonatal RA exposed (LPS/RA) mice showed increased calcium transient amplitude compared with saline/RA exposed mice. Furthermore, the calcium transient amplitude was significantly increased in mice from LPS-injected dams that were also exposed to hyperoxia (LPS/O$_2$) compared with saline/RA and saline/O$_2$ groups. Statistical analyses indicated an effect of LPS injection. These results suggest that maternal LPS injection increases calcium transient amplitude, and subsequent hyperoxia exposure further increases the amplitude of calcium transients (Fig. 4).

Systemic maternal LPS injection in combination with neonatal hyperoxia exposure induces alterations in calcium handling proteins. There was no difference in SERCA2a mRNA or protein levels in heart tissue isolated from maternal saline-injected and neonatal hyperoxia exposed (saline/O$_2$) or maternal LPS-injected and neonatal RA exposed (LPS/RA) mice compared with maternal saline-injected and neonatal RA exposed (saline/RA) controls. However, SERCA2a mRNA or protein levels were significantly increased in heart tissue isolated from maternal LPS-injected and neonatal hyperoxia exposed (LPS/O$_2$) mice compared with maternal LPS and neonatal RA exposed (saline/O$_2$) mice (Figs. 5A and 6A). Statistical analyses indicated effects of LPS injection and an interaction of LPS and hyperoxia exposure on SERCA2a mRNA expression and protein levels. There were no differences in phospholamban mRNA levels in maternal saline and neonatal hyperoxia (saline/O$_2$) or maternal LPS and neonatal RA (LPS/RA) exposed mice compared with maternal saline and neonatal RA (saline/RA) exposed control mice. Ryanodine receptor levels were not different between groups (data not available for maternal saline and neonatal hyperoxia (saline/O$_2$) mice).
shown). However, phospholamban mRNA levels were significantly lower in heart tissue isolated from maternal LPS- and neonatal hyperoxia (LPS/O2)-exposed mice compared with maternal saline- or LPS-injected and neonatal RA exposed mice (saline/RA, LPS/RA) (Fig. 5B), a pattern observed during fetal development. Phosphorylation of phospholamban was increased (Fig. 6B), along with Sorcin mRNA levels in maternal LPS and neonatal hyperoxia (LPS/O2) exposed mice compared with maternal LPS and neonatal RA exposed and maternal saline and neonatal RA (saline/RA) or hyperoxia (saline/O2) exposed mice (Fig. 5C). Statistical analyses indicated an effect of hyperoxia exposure on phospholamban mRNA expression and effects of LPS, hyperoxia exposure, and an interaction of LPS and hyperoxia exposure on phosphorylation of phospholamban. Effects of LPS injection and an interaction between LPS injection and hyperoxia exposure were observed for Sorcin mRNA expression.

DISCUSSION

In the present study, IUGR, cardiac dysfunction, and pathophysiological adaptations consistent with myocardial dysfunction were observed in mouse pups that were born to LPS-injected dams and subsequently exposed to hyperoxia. An increase in calcium transient amplitude and upregulation of Ca\(^{2+}\)/H11001 reuptake mechanisms reinforced these myocardial adaptations.

Epidemiological data have confirmed an association between IUGR and cardiovascular morbidity and mortality during adulthood (2, 3, 10). We have observed reduced birth weight in response to maternal LPS injection, suggesting that maternal inflammation leading to IUGR. Infants suffering from IUGR are frequently born with immature lungs and therefore require medical interventions including oxygen therapy. The combination of inflammation with hyperoxia exposure during
the postnatal period is associated with early-onset cardiovascular disease. In our model, cardiac LV dimensions were altered as early as 3 days of life in LPS/O2 exposed pups. Furthermore, LV alterations were evident in LPS/O2 exposed pups, suggesting that perinatal inflammation and hyperoxia exposure alters cardiac dimensions leading to cardiac dysfunction. These data are consistent with human data reporting cardiac abnormalities in infants born small for gestational age (11, 14, 26). The fact that neonatal hyperoxia exposure in the absence of maternal inflammation results in increased cardiac contractility is complex. We speculate that in these mice, cardiac function is increased in response to hyperoxia-induced stress, whereas mice born to LPS-injected dams are unable to increase cardiac contractility in response to hyperoxia exposure.

Previous studies have demonstrated cardiac inflammation in a sheep model of fetal endotoxin infusion and dysfunction in adult rodents in response to maternal dysnutrition, inflammation, or hypoxia (17–19, 25). The observed increase in cardiac mRNA expression of inflammatory mediators 14 days after birth in response to maternal LPS injection is consistent with previous studies using direct injections of endotoxin to fetal sheep and reported an increase in inflammatory markers within the fetal heart. At the cellular level, abnormal cardiomyocyte function was present at 14 days of life, confirming early systolic changes in LV cardiomyocytes. To our knowledge, this is the first study to determine cardiac dysfunction within the first days of life in offspring following maternal/fetal exposure, suggesting that perinatal inflammation and hyperoxia exposure result in cardiac dysfunction at the cellular level.

Calcium transient amplitude and calcium transport protein composition are known to be altered in humans with and animal models of cardiac disease. Furthermore, alterations in calcium signaling and calcium handling proteins can each affect cardiac function, potentially leading to impaired myocardial contractility. The upregulation of SERCA2a and increased phosphorylation of phospholamban in LPS/O2 exposed mice is indicative of increased calcium cycling. During fetal development, SERCA2a protein levels increase before birth. SERCA2a levels in LPS/O2 exposed mice within our study are similar to the levels observed during the embryonic stages. Our data suggest that perinatal inflammation and hyperoxia prevent fetal cardiac protein composition from changing from the fetal to the postnatal stage. Increases in calcium transient amplitude indicate an increase in intracellular calcium levels during myocyte contraction. We speculate that increased calcium signaling is an attempt to sustain cardiac function. However, calcium increases in LPS/O2 exposed mice are insufficient to sustain cardiac function.
In previous studies, we reported a pulmonary inflammatory response to maternal LPS injection and neonatal hyperoxia exposure resulting in matrix remodeling, collagen deposition, and pulmonary fibrosis (20, 21). Matrix remodeling and fibrosis are hallmarks of cardiac disease development in humans and animals with heart failure. Increased expression of inflammatory mediators is also occurring in the heart in our model (Fig. 1).

In summary, perinatal inflammation and hyperoxia are associated with IUGR and result in reduced birth weight and in vivo cardiac and in vitro cardiomyocyte dysfunction, consistent with animal models and humans with heart failure. Calcium signaling and calcium handling protein levels during the neonatal period were similar to what is normally observed during the fetal stages of development. Our data have implications for perinatal inflammation and hyperoxia as risk factors for alterations in fetal development, resulting in postnatal cardiac dysfunction.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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REFERENCES


