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The impact of maternal protein restriction during perinatal life on the response to a septic insult in adult rats

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Abstract

Although abundant evidence exists that adverse events during pregnancy lead to chronic conditions, there is limited information on the impact of acute insults such as sepsis. This study tested the hypothesis that impaired fetal development leads to altered organ responses to a septic insult in both male and female adult offspring. Fetal growth restricted (FGR) rats were generated using a maternal protein-restricted diet. Male and female FGR and control diet rats were housed until 150–160 d of age when they were exposed either a saline (control) or a fecal slurry intraperitoneal (Sepsis) injection. After 6 h, livers and lungs were analyzed for inflammation and, additionally, the amounts and function of pulmonary surfactant were measured. The results showed increases in the steady-state mRNA levels of inflammatory cytokines in the liver in response to the septic insult in both males and females; these responses were not different between FGR and control diet groups. In the lungs, cytokines were not detectable in any of the experimental groups. A significant decrease in the relative amount of surfactant was observed in male FGR offspring, but this was not observed in control males or in female animals. Overall, it is concluded that FGR induced by maternal protein restriction does not impact liver and lung inflammatory response to sepsis in either male or female adult rats. An altered septic response in male FGR offspring with respect to surfactant may imply a contribution to lung dysfunction.

Original Article

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Introduction

Characterized by low birth weight, fetal growth restriction (FGR) occurs in 8% of the population.[1,2] There are strong epidemiological data indicating that impaired fetal growth contributes to the development of diseases in adulthood in both men and women.[1–4] The most common clinical manifestations include chronic conditions such as diabetes, hypertension, dyslipidemia, fatty liver disease, and asthma.[1–4] Animal studies have explored the sexual dimorphisms in these adult-onset diseases and have been crucial in dissecting some of the underlying transcriptional and epigenetic mechanisms.[5–8] Interestingly, many of these mechanistic pathways, including aberrant immune responses, that are also highly relevant in the responses to acute insults.[9]

One of the most clinically relevant acute insults, in terms of patient numbers, mortality, and health care costs, is sepsis.[10,11] Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[12] At the core of the pathophysiology of sepsis, the inflammatory response is initiated by bacterial products, including lipopolysaccharide (LPS), damage-associated molecular patterns, and bacterial toxins.[13,14] Although activation of pro-inflammatory processes is required to combat the infection, the dysregulation of these inflammatory conditions (*e.g.* sepsis) leads to adverse outcomes. As implied by the definition, these maladaptive processes occur in several organs resulting in functional impairments, including the lungs and liver.[13,14] Within the lungs, sepsis manifests itself by upregulated inflammatory responses and notable alterations to the pulmonary surfactant system.[14,15] Septic livers are also characterized by marked inflammation.[13,16]

An important component of the above definition of sepsis is the contributing role of the dysregulated host response to infection, as it implies that variations in host defense systems and inflammatory processes among individuals will ultimately impact the development and severity of sepsis. Considering the potential altered immune function due to FGR, this concept led to the hypothesis that impaired fetal development leads to altered organ responses to a septic insult in both male and female adult offspring. To address this hypothesis, we utilized the well-characterized maternal protein dietary restriction in rats to induce FGR in the offspring.[6,17,18]

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The adult maternal protein-restricted and control-diet animals were then exposed to a similar acute septic insult via the injection of a fecal slurry,[19] and both lungs and livers were examined.

Methods

Animal procedures

A total of 15 female and 5 male Wistar rats at breeding age (250 g) were purchased from Charles River (La Salle, St-Constant, Quebec, Canada). Rats were housed in individual cages and allowed to acclimatize for a minimum of 3 d on a 12 h: 12 h light:dark cycle with free access to water and standard chow. After the acclimatization period, female rats were housed with stud males. The following morning, impregnation was confirmed by the presence of sperm in the vaginal smear. Upon confirmation of impregnation, pregnant rats were housed individually and randomized to one of two dietary conditions: a 20% protein (Control, $n = 7$ litters) or an 8% low protein diet (LP, $n = 9$ litters).[6,18] The LP diet contained equal fat content and was made isocaloric by the addition of carbohydrates (Bio-Serv, Frenchtown, NJ). At birth (d1), offspring weights were recorded and the litter size was culled to 10 animals. After birth, lactating mothers were kept on the same dietary regimes until weaning at postnatal d21. Following weaning, male and female animals were housed separately and received a control diet and water *ad libitum*.

At d130–150 weights were recorded and rats from each individual litter were randomized into sham or septic groups with a maximum of two animals/litter being allocated to a specific experimental group. Animals allocated to the septic condition were subjected to fecal-induced peritonitis (FIP) via an intraperitoneal (IP) injection of a fecal slurry. Briefly, equal amounts of fresh rat feces were collected from animals of both sexes and both dietary groups approximately 12 h before the IP injection. The collected feces were weighed and mixed with saline solution to a concentration of 0.5 g/ml. This fecal slurry was homogenized by vortexing the fecal solution for 2 min. The collected homogenate was filtered through 4-ply gauze to remove particulate matter and the fecal slurry was kept overnight at 4 °C. The next morning, 15 min prior to the IP injection, analgesia was provided by a subcutaneous injection of 0.65 ml buprenorphine (0.03 mg/ml). Subsequently, rats randomized to the experimental group were given an IP injection of the fecal slurry solution (1.0 ml/100 g of body weight [BW]) using a syringe and 21G needle. Sham rats received an injection of sterile saline (1.0 ml/100gBW).

Following the IP injection, animals were monitored using a rodent sepsis score (MSS)[20] at 15 min post-injection and 30-min intervals thereafter. Variables included respiratory quality (i.e. frequency/labored breathing or gasping), appearance (i.e. the degree of piloerection and/or changes in the eye color), responding to auditory stimuli (i.e. fast or slow), and spontaneous activity (i.e. continuous/lack of investigating movements) of the animal. Each variable was scored between 0 and 4. Rats were euthanized if the total MSS at any given time point was greater than 9, or if any of the individual scores increased by more than 3. In addition, at each timepoint, the respiratory rate was measured. Following the completion of 6 h monitoring, animals were euthanized with an overdose of IP injection of sodium pentobarbital (110 mg/kgBW).

Sample collection and analysis

Immediately after euthanasia, blood samples were taken from the abdominal aorta to evaluate systemic infection (bacteremia) and inflammatory responses. Columbia blood agar plates (BD Bioscience, Mississauga, ON) inoculated with 0.1 ml blood samples were incubated overnight in an aerobic chamber at 37°C. Bacterial growth was evaluated the following morning. The remaining volume of blood was centrifuged to collect the serum, which was stored at −80 °C for cytokine analysis.

Table 1. Forward and reverse primer sequences used for quantitative real-time PCR

GENE	Primer sequence	GenBank/ Reference
IL-1α	Forward: AGACAAGCCTGTGTTGCTGA Reverse: AGGTCGGTCTCACTACCTGT	NM_017019.1
TNFα	Forward: CCGGGCAGGTCTACTTTGGA Reverse: AGGCCACTACTTCAGCGTCTCG	NM_012675.3
IL-6	Forward: CTTCCAGCCAGTTGCCTTGTTG Reverse: TGGTCTGTGTTGTGGGTGGTATCC	NM_012589.2
IL-8	Forward: ACTGGGGCTCTTGGAAACTACAC Reverse: CCTTGGTTTTGTTCGGGTTCA	NM_001031638.1
β-Actin	Forward: CACAGCTGAGAGGGAAAT Reverse: TCAGCAATGCCTGGGTAC	NM_031144

For all experimental groups, the liver was dissected following euthanasia and flash-frozen in liquid nitrogen for molecular analysis. RNA was isolated from male and female frozen liver tissue using TRIzol® (Life Technologies, Grand Island, NY) extraction combined with the RNeasy® mini kit (Qiagen, Hilden, Germany). Tissue homogenization and lysis in TRIzol reagent (ThermoFisher Scientific, Mississauga, ON) were followed by the addition of chloroform (ThermoFisher Scientific, Mississauga, ON) and vigorous mixing and centrifugation at 12,000 RPM at 4°C for 15 min. The clear supernatant layer containing RNA was collected and used subsequently. RNA was precipitated with isopropanol (ThermoFisher Scientific, Mississauga, ON), and the pellet was washed with 70% ethanol (ThermoFisher Scientific, Mississauga, ON) in DEPC-treated water (ThermoFisher Scientific, Mississauga, ON). RNA was solubilized with MilliQ-processed water (Millipore-Sigma, Etobicoke, ON). Sample yield and purity were quantified by absorbance at 260 and 280 nm using NanoDrop 2000c Spectrophotometer (ThermoFisher Scientific, Mississauga, ON).

Quantitative Reverse transcription polymerase chain reaction (RT-PCR) was employed to determine the steady-state mRNA expression of inflammatory cytokines in the liver including tumor necrosis factor 1α, interleukin-1α (IL-1α), interleukin-8 (IL-8), and interleukin-6 (IL-6) (Table 1). Complementary DNA (cDNA) was synthesized from 2 µg of RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) as per the manufacturer's instructions using PerfeCta® SYBR® green FastMix® (Quanta Biosciences, Gaithersburg, MD) on the LightCycler480 (Roche, Mississauga, Ontario, CA). The cycling conditions included polymerase activation (95°C for 10 min), followed by 40 cycles of denaturing (95°C for 15 s) and annealing/elongation (60°C for 1 min). Values obtained for all gene targets of interest were normalized to beta-actin (β-Actin), which as determined to be a suitable housekeeping gene by using both the comparative ΔCt method and algorithms from geNorm, Normfinder, and BestKeeper.[21,22] For each sex, relative transcript abundance was calculated for each primer set as determined by the comparative ΔCt method and expressed as fold increase over control.

The lung samples for surfactant and cytokine analysis were obtained by lung lavage as previously described.[23] The recovered

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lavage volume was measured and underwent differential centrifugation to remove cellular debris and isolate surfactant subfractions, large and small aggregates (LA and SA). A 1 ml volume of the lavage material was stored in aliquots at −80 °C for cytokine analysis. Other samples were stored at −20 °C for further analysis of total surfactant phospholipid content as well as the different subfractions.[24,25] Due to significant differences in BWs between different experimental groups, values for BAL surfactant phospholipid levels were reported as the amount of phospholipid corrected for BW (mg of the total phospholipid in BAL/kgBW).[23] In addition, total protein levels were measured in the lavage fluid using a Micro BCA protein assay kit (Pierce, Rockford, Ill., USA) according to the manufacturer's instructions.

Concentrations of four cytokines (IL-6, G-CSF, TNF-α, and IFN-γ) and two chemokines (KC and MCP-1) were measured in BAL fluids using multiplexed immunoassay kits according to the manufacturers' instructions (R&D Systems, Minneapolis, MN). A Bio-Plex 200 readout system was used (Bio-Rad), which utilizes Luminex® xMAP fluorescent bead-based technology (Luminex Corporation, Austin, TX). Cytokine levels (pg/ml) were automatically calculated from standard curves using Bio-Plex Manager software (v. 4.1.1, Bio-Rad).

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using the GraphPad Prism statistical software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical comparisons between two groups were performed using an unpaired, two-way student's t-test. All other comparisons involved a two-way analysis of variance (ANOVA) followed by a one-way ANOVA with a Tukey's post hoc test. Probability (P) values of less than 0.05 were considered statistically significant.

Results

Model verification

The current study utilized a LP-diet model of FGR to study the responses to sepsis later in life. The outcomes of the maternal low protein diet on BWs at birth and at the time of the randomization for the septic component of the study are shown in Table 2. At birth, both male and female pups in the low protein diet cohort had significantly lower BW as compared to pups in the control diet group. A significant difference in BW was also observed at 130–150 d in the male animals; however, there was no significant difference in the female animals.

Six hours following exposure to either saline or FIP injection, a variety of measures were obtained to verify a septic response in animals randomized to receiving FIP. The results shown in Tables 3 (males) and 4 (females) indicate a significant increase in the sepsis score, blood cultures, and a variety of inflammatory mediators in all animals receiving FIP compared to their respective controls. There were no significant differences between the two diet groups and responses were similar in the male and female cohorts.

Liver outcomes

Since the liver is a primary target for the septic inflammation,[13] we initially measured liver weight in all experimental groups. These weights were not significantly different among the groups for either males or females (Table 5). Subsequently, the steady-state mRNA levels of inflammatory mediators were analyzed in the liver of rats receiving a saline injection or a FIP injection. As shown in Fig. 1, the mRNA levels of all four inflammatory mediators, TNF-α, IL-1β, IL-6, and IL-8, were significantly higher following the injection of FIP as compared to the respective control groups receiving saline. There were no significant differences between male animals from the maternal control diet versus those from the maternal LP diet. A similar pattern was observed for the mRNA expression of female hepatic inflammatory mediators, which significantly increased due to FIP injection but were not affected by maternal diet (Fig. 2).

Table 2. Animal weights at birth and at time of randomization to FIP or saline injection

	Male		Female	
Diet	Control ($n = 27$)	LP ($n = 29$)	Control ($n = 23$)	LP ($n = 23$)
Day 1 weight (g)	7.0 ± 0.3	6.0 ± 0.2[#]	6.5 ± 0.2	5.7 ± 0.2[#]
Day 130–150 (g)	661 ± 12	591 ± 11[#]	379 ± 7	361 ± 8

[#]= $P < 0.05$ vs control diet.

Pulmonary outcomes

Since pulmonary surfactant plays a crucial role in lung function[26] and is altered in sepsis-induced lung injury,[15,27] the surfactant system from animals in all groups was analyzed. The data for male animals are shown in Fig. 3. In male animals receiving saline, the total amount of surfactant, as well as the amount of LA recovered from the lung, were not significantly different between rats from the control diet group compared to those from the LP diet (Fig. 3a and b). However, in response to sepsis, male animals in the LP group had a significantly lower amount of surfactant and LA compared to the saline-injected animals (Fig. 3a and b). There were no significant differences in the amount of SA (Fig. 3c), or surface tension reducing activity (Fig. 3d) attributed to either the maternal diet or in response to sepsis. In female animals, there were no significant differences in amounts of total surfactant, surfactant subfractions, or surface tension reducing activity among the four experimental groups (Fig. 4).

Finally, lung lavage fluid was utilized for a multiplex analysis of six inflammatory mediators, similar to those analyzed in serum and reported in Tables 2 and 3. There were no detectable levels of any of the six mediators analyzed regardless of diet, sex of FIP-insult (data not shown).

Discussion

Given the strong epidemiological links between adverse events in utero and postnatal disease,[1,2] our study tested the hypothesis that impaired fetal development via maternal protein restriction leads to altered organ responses to a septic insult in male and female offspring. Using a well-characterized sepsis model, the results revealed inflammatory responses in the liver but limited indices of inflammation in the lung. Moreover, these responses were similar in males and females from both dietary groups. The only differential effect of FGR observed was a decrease in the relative amount of surfactant and surfactant LA in male septic animals from the maternal LP diet group; a change not observed in males from the control group or in the female animals. Overall, it is

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Table 3. Septic responses in male animals

	Male – Saline injection		Male – FIP injection	
	Control ($n = 13$)	LP ($n = 14$)	Control ($n = 10$)	LP ($n = 10$)
Sepsis score	1.5 ± 0.3	0.9 ± 0.2	5.3 ± 0.2*	6.3 ± 0.4*
Blood cultures	Negative	Negative	Positive	Positive
Resp rate (breath/min)	120 ± 5	117 ± 4	99 ± 4*	85 ± 4*
Mediator analysis	Control ($n = 4$)	LP ($n = 4$)	Control ($n = 5$)	LP ($n = 5$)
Serum IL-6 (pg/ml)	nd	nd	113,471 ± 30,740*	131,357 ± 29,317*
Serum TNFα (pg/ml)	nd	nd	89 ± 12*	78 ± 17*
Serum G-CSF (pg/ml)	nd	nd	34 ± 11*	19 ± 4*
Serum IFN-γ (pg/ml)	nd	nd	106 ± 43*	163 ± 82*
Serum KC (pg/ml)	nd	nd	20,938 ± 4604*	42,376 ± 22,261*
Serum MCP-1 (pg/ml)	784 ± 84	692 ± 110	44,496 ± 21,319*	16,498 ± 1069*

*= $P < 0.05$ vs saline injection, nd = non detectable.

Table 4. Septic responses in female animals

	Female – Saline injection		Female – FIP injection	
	Control ($n = 10$)	LP ($n = 11$)	Control ($n = 10$)	LP ($n = 10$)
Sepsis score	0.9 ± 0.2	1.2 ± 0.4	5.2 ± 0.4*	6.3 ± 0.4*
Blood cultures	Negative	Negative	Positive	Positive
Resp rate (breath/min)	126 ± 7	128 ± 7	95 ± 4*	87 ± 5*
Mediator analysis	Control ($n = 4$)	LP ($n = 4$)	Control ($n = 5$)	LP ($n = 4$)
Serum IL-6 (pg/ml)	nd	nd	133,889 ± 39,028*	117,288 ± 80,060*
Serum TNFα (pg/ml)	nd	nd	82 ± 24*	91 ± 20
Serum G-CSF (pg/ml)	nd	nd	46 ± 20*	20 ± 6*
Serum IFN-γ (pg/ml)	nd	nd	311 ± 92*	84 ± 55*
Serum KC (pg/ml)	nd	nd	50,848 ± 21,357*	27,323 ± 14,558*
Serum MCP-1 (pg/ml)	1653 ± 831	727 ± 69	17,546 ± 4674*	25,400 ± 7925*

*= $P < 0.05$ vs saline injection, nd = non detectable.

Table 5. Liver weights

	Saline injection		FIP injection	
	Control	LP	Control	LP
Male liver weights (g)	18.96 ± 0.83 ($n = 7$)	18.81 ± 0.76 ($n = 8$)	16.53 ± 1.06 ($n = 8$)	16.67 ± 1.28 ($n = 6$)
Female liver weight (g)	10.35 ± 0.50 ($n = 6$)	9.52 ± 0.67 ($n = 6$)	11.35 ± 0.39 ($n = 6$)	10.40 ± 0.56 ($n = 7$)

concluded that, in this experimental model, FGR does not impact septic responses during adulthood in either males or females.

An important component of our experimental approach was to use the well-established maternal protein restriction model of FGR.[7,28] This is a relevant model to study FGR given amino acids are critical for fetal growth[29] and that placental deficiency in humans results in fetal protein deficiency.[30] In addition, this maternal low protein model reciprocates human FGR in that it results in adverse health consequences later in life.[7,28] Furthermore, large human cohort studies have implicated both lung and liver dysfunction in adulthood. For example, FGR has been implicated in the development of asthma and other chronic lung diseases.[31–33] With respect to the liver, human FGR also increases the risk for dyslipidemia[34,35] and fatty liver disease[36,37] in postnatal life. Despite the fact that (1) these disease processes are linked to inflammation and (2) FGR infants are more susceptible to infection in early life, to date, the impact of FGR on adult sepsis has not been studied. In this study, we verified that this model of maternal protein restriction, previously linked to postnatal pulmonary and hepatic complications,[5,17,18] resulted in FGR. It should be noted that this FGR model does not impact on maternal physiology (*e.g.* weight gain or food intake) or postnatal food intake.[7,17,38] Thus, the use of this established model allowed us to effectively address our hypothesis.

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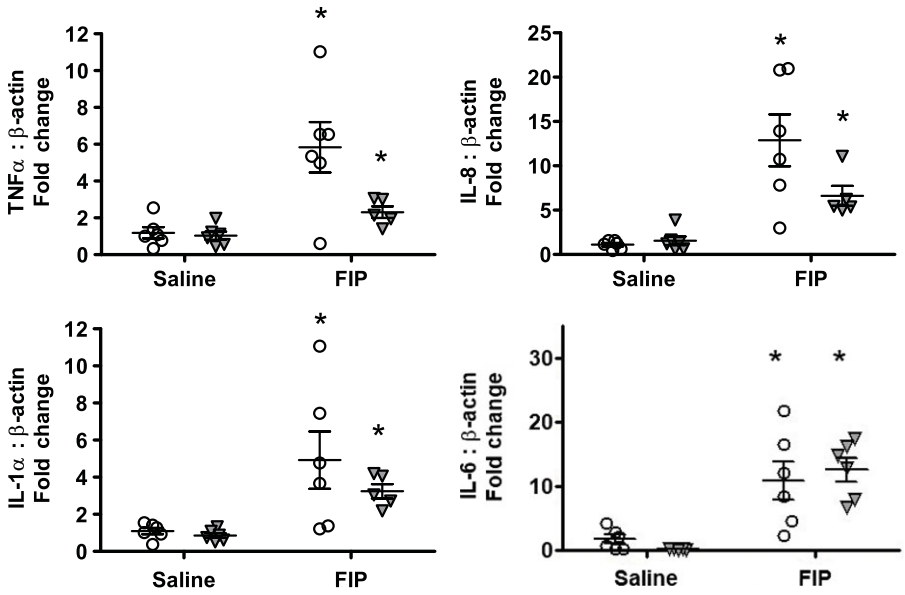

Fig. 1. Liver mRNA values at 6 h following saline or FIP injection in adult male rats from either a maternal control diet (open circles) or a low protein diet (shaded triangles). Data from individual animals are shown. Lines represent mean +/- standard error. * = $P < 0.05$ compared to saline group with same diet.

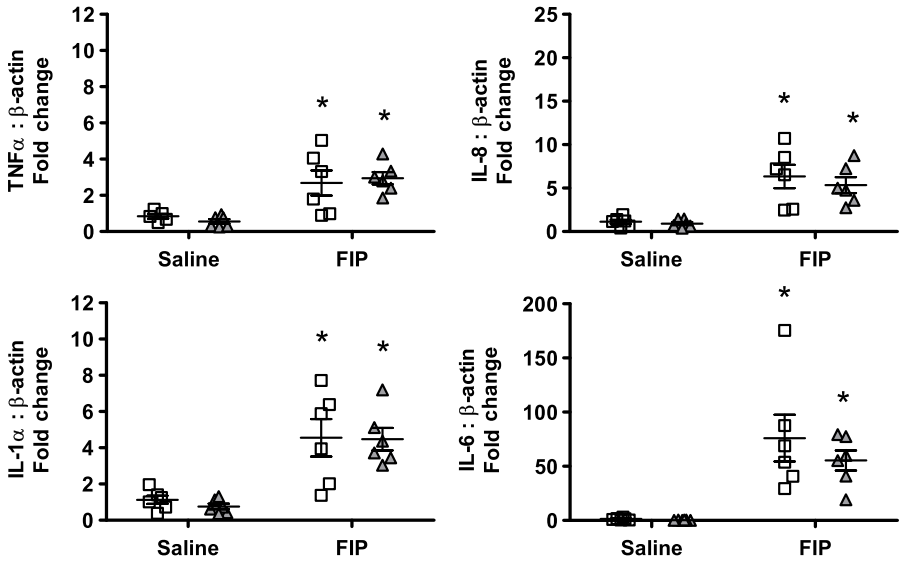

Fig. 2. Liver mRNA values at 6 h following saline or FIP injection in adult female rats from either a maternal control diet (open squares) or a low protein diet (shaded triangles). Data from individual animals are shown. Lines represent mean +/- standard error. * = $P < 0.05$ compared to saline group with same diet.

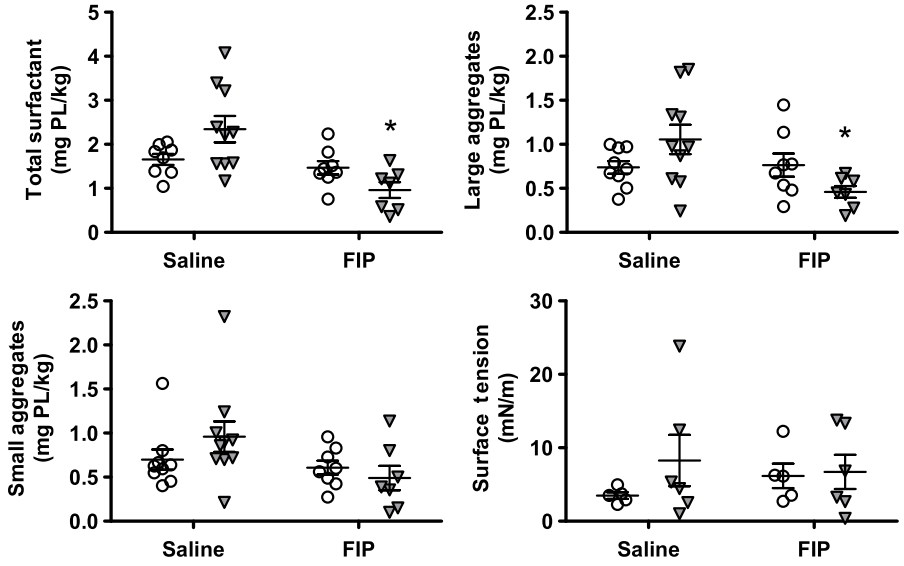

Fig. 3. Surfactant amounts and activity at 6 h following saline or FIP injection in adult male rats bred from either a maternal control diet (open circles) or a low protein diet (shaded triangles). Data from individual animals are shown. Lines represent mean +/- standard error. * = $P < 0.05$ compared to saline group with same diet.

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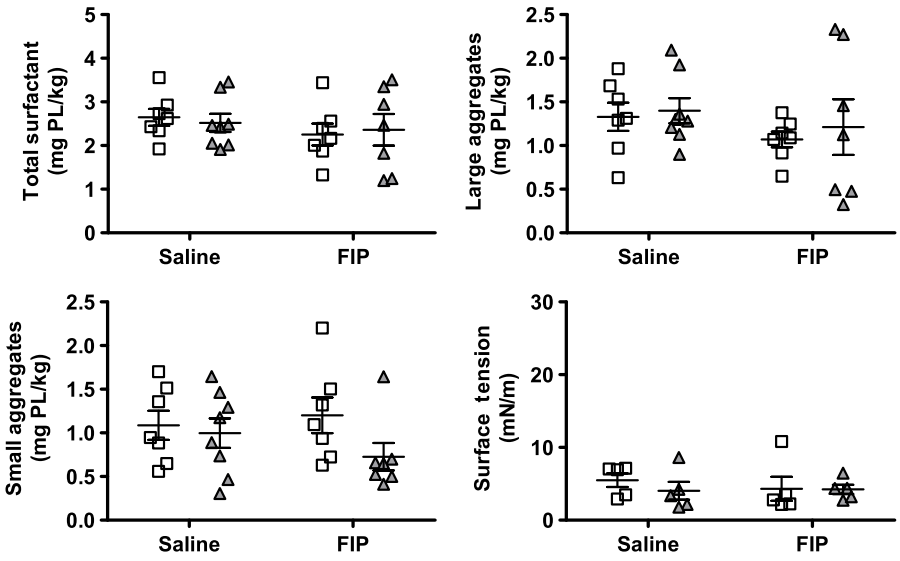

Fig. 4. Surfactant amounts and activity at 6 h following saline or FIP injection in female rats from either a maternal control diet (open squares) or a low protein diet (shaded triangles). Data from individual animals are shown. Lines represent mean +/- standard error.

A second major consideration for addressing this hypothesis was to evaluate the results is the specific model of sepsis utilized. Numerous animal models of sepsis exist, ranging from LPS injection to the gold standard model of cecal ligation and perforation (CLP), each with distinct advantages and disadvantages.[39,40] In the current study, we employed the FIP model, in which a slurry of fecal material was injected i.p. to induce the septic response.[19] Like CLP, this model provides a robust, clinically relevant septic insult but has the advantage of not requiring a surgical procedure. More importantly, in our FIP model, the septic response is induced by fecal matter which was prepared using equal amount of material from males and females from both diet groups, resulting in all animals receiving equivalent septic insults. An approach in which animals would receive fecal matter only from its own cecum, such as CLP, may have resulted in different septic insults related to FGR-induced variability in gut microbiomes.[41,42] Although such an approach would also be of value, this current FIP model in which septic responses were verified through measurements of positive blood cultures and inflammatory mediators allowed for a specific focus on the host response to the septic insult.

After establishing the FGR and sepsis model in our rodent studies, we conclude that there is minimal influence of FGR on septic responses in adulthood. This is strengthened by the fact that outcome measurements were evaluated in two different organs variably affected by sepsis, the liver and the lung. Whereas the liver showed a marked inflammatory response to the septic insult, analysis of BAL mediators indicated lung inflammation was not concomitantly detectible. The mild level of inflammation in the lung was unanticipated considering previous observations in mice utilizing the FIP model, as well as the fact that sepsis is the main cause of acute respiratory distress syndrome which is characterized by pulmonary inflammation. It is feasible that a higher dose of FIP or analysis after a prolonged period of time would have resulted in a more substantial pulmonary inflammation. However, neither organ demonstrated marked differences in inflammation between the two maternal dietary groups.

Among the variety of outcomes measured, one specific interesting observation was that the levels of surfactant and surfactant large aggregates were altered in male FGR offspring in response to the septic insult. These changes were not observed in the control diet group or in female animals. While previous studies have reported alterations to surfactant due to sepsis,[15,27] this is the first report of a sex-specific change to surfactant in an injury model. Considering that the impact of our specific septic insult on the lung was relatively mild, this result was surprising. In previous studies, alterations of surfactant due to sepsis in spontaneously breathing rats were enhanced when the animals were mechanically ventilated.[43,44] As such, it would be of interest to determine if the underlying changes in surfactant system in septic male FGR animals would result in more severe mechanical ventilation induced lung injury as compared to septic control male and septic female FGR animals.

An additional interesting outcome, and a potential area for future studies, relates to the inflammatory mediators measured in the serum, specifically IFN-γ. Although the objective of these measurements was to verify sepsis in a subset of our animals, it was interesting to note that despite the low n-value, serum mediators in female animals tended to be lower in FIP exposed LP animals as compared to FIP exposed control animals. Similarly, steady-state mRNA expression of inflammatory cytokines in the liver following FIP tended to be lower in male FGR animals compared to control diet animals. Together, these trends suggest that it is feasible that FGR has affected aspects of the innate immune system to alter the response to a septic insult. For example, it is feasible that natural killer cells, which are known to be involved in septic responses,[45–47] may have contributed to altered mediator levels in the female serum and male livers. This speculation needs to be confirmed experimentally but would provide interesting insight into sexual dimorphisms in the septic responses, beyond the organs and outcomes evaluated in the current study.

Although we utilized established models of sepsis and FGR in testing our hypothesis, there are several limitations with our experimental approach. First, we limited our study to measurements 6 h after the septic insult. This only reflects the acute response and further research would be required to examine if FGR affects sepsis progression over prolonged time periods. Second, the maternal low protein model represents only one cause of FGR. Several other models are available, each reflecting different aspects of the impaired fetal environment in humans that can lead to FGR and lifetime consequences. For example, the uterine ligation model leading to FGR leads to decreases in both fetal nutrients and oxygenation; however, the impact of a decrease in both maternal and

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placental weight during pregnancy must also be considered.[48,49] Third, following gestation and prenatal period within their specific diet groups, our animals were maintained in a relatively clean animal care facility until adulthood (130–150 d). This relatively unchanging environment may be markedly different from humans with FGR whose response to sepsis may be further influenced by prior exposure to infections and diseases, as well as other lifestyle factors such as exercise.[1,50–52] Thus, although our overall conclusion is that in this particular model of experimental sepsis, lung and liver responses during adulthood are not affected by FGR in either males or females, further research is needed to fully explore the influence of a poor in utero environment on a variety of other acute and chronic inflammatory insults later in life.

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the Canadian Council of Animal Care guidelines on the care and use of laboratory animals and has been approved by Animal Care Committee at the University of Western Ontario.

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