Chronic hyperleptinemia results in the development of hypertension in pregnant rats

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Running head: Hyperleptinemia increases blood pressure in pregnant rats

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ABSTRACT

Despite the fact that obesity is a major risk factor for preeclampsia (PE), the pathophysiological mechanisms whereby obesity and metabolic factors such as leptin increase this risk are unclear. While human data have shown that hyperleptinemia is associated with PE, the long-term effect of hyperleptinemia on blood pressure during pregnancy is unknown. Thus we tested the hypothesis whether chronic circulating leptin elevations in pregnant rats increase blood pressure and placental factors known to play a role in PE. On gestational day (GD)14, rats were assigned to the normal pregnant group with food intake ad libitum (control), leptin-treated (0.5 µg/Kg/min i.p.) pregnant group with food intake ad libitum (pregnant+LEP), and normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR). On GD19, mean arterial pressure (MAP) was assessed and tissues were collected. Serum leptin concentration was elevated in pregnant+LEP compared to control and pregnant-FR (18.0±2.8 vs 0.8±0.1 vs 0.3±0.1 ng/mL; P<0.05), which was associated with increased MAP (121.3±8.1 vs 102.4±2.4 vs 101.3±1.8 mmHg; P<0.05). Food intake and body weight were reduced in pregnant+LEP and pregnant-FR by the end of gestation. Additionally, placentas and fetuses of these groups were lighter than those of control. However, placental expression of TNF-α was significantly greater in pregnant+LEP compared to controls (1.6±0.1 vs 1.1±0.1 pg/mg; P<0.05).

In conclusion, leptin increases blood pressure and placental TNF-α during pregnancy despite its effect of reducing food intake and body weight, and represents a mechanism whereby obesity can promote the development of hypertension in PE.

Keywords: hyperleptinemia, blood pressure, pregnancy, hypertension, preeclampsia.
INTRODUCTION

Preeclampsia is a serious pregnancy disorder characterized by hypertension and proteinuria, which affects between 3-5% of pregnancies worldwide (21). Besides being a major cause of maternal and perinatal mortality and morbidity (16, 24), preeclamptic women and their offspring are at increased risk for developing cardiovascular diseases later in life (13, 35). In addition, there are several recognized risk factors for preeclampsia, including preexisting chronic diseases such as hypertension, diabetes, and obesity (14). Indeed, epidemiological studies indicate that obesity (defined as a body mass index ≥ 30 kg/m²) is associated with an up to 5 fold increase in the rate of preeclampsia (34).

Many metabolic adaptations occur during pregnancy in order to provide proper nutrient supply to the growing fetus. At first and second trimesters, maternal metabolism is predominantly anabolic targeting the storage of a large amount of nutrients, as evidenced by an accumulation of depots of body fat. At the third trimester, however, maternal metabolism is catabolic directing the transfer of nutrients to the fetus through the placenta, as verified by increases in circulating levels of cholesterol, triglycerides, and free fatty acids during this last stage of pregnancy (56). The adipose tissue also exerts endocrine and paracrine functions in gestation as it produces and secretes hormones such as leptin. (55). Leptin is the peptide product of the obese (ob) gene, and it has been identified as a modulator of numerous physiological processes including food intake, adipose storage, and reproduction (19, 58). Indeed, circulating leptin levels are greater in pregnant women than their non-pregnant counterparts. However, hyperleptinemia over the levels seen in normal pregnancy has been associated with preeclampsia (19, 47). This is important because leptin has been shown to have direct effects on blood pressure regulation in male rats (51). Clinical studies have also shown that increased circulating leptin levels are associated with increased risk for the development of preeclampsia (43, 50), and the degree of hyperleptinemia in preeclamptic women is correlated
with disease severity (1). Therefore, because hyperleptinemia is consistently observed in obese humans and animal models (58), this is a potential mechanism that links obesity with the development of hypertension in preeclampsia.

Growing evidence supports that incomplete remodeling of uterine spiral arteries results in reduced placental perfusion. The hypoxic/ischemic placenta, in turn, releases multiple mediators into the maternal circulation, such as the anti-angiogenic factor soluble fms-like tyrosine kinase (sFlt)-1 and the inflammatory cytokine tumor necrosis factor (TNF)-α, which leads to widespread maternal endothelial dysfunction and then the clinical symptoms of preeclampsia (45). Recent clinical studies have reported a positive association of circulating leptin with sFlt-1 (15) or TNF-α (7) in preeclampsia. However, the direct effect of hyperleptinemia on these placental factors and blood pressure regulation during pregnancy are unknown. Therefore, the purpose of the present study was to test the hypothesis that chronic exposure to hyperleptinemia increases blood pressure and placental sFlt-1 and TNF-α levels in pregnant rats.

METHODS

**Animals.** All protocols were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee, and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Age-matched, timed-pregnant Sprague Dawley rats (Harlan, Indianapolis, IN) were received on gestational day (GD) 11. They were maintained on a 12:12 h light:dark cycle at 23 °C. On GD 14, rats were randomly assigned to the normal pregnant group with food intake *ad libitum* (pregnant, n=8-12), leptin-treated pregnant group with food intake *ad libitum* (pregnant+LEP, n=8-12), or normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR, n=11). Water was provided *ad libitum* to all groups. From GD 13 to GD 19, body weight (BW), food intake (FI),
and water intake (WI) were recorded daily. We did not consider FI and WI on GD 15 and 19 because animals were still recovering from the surgeries performed on GD 14 (minipump placement, see below) and 18 (carotid catheter placement, see below), respectively.

**Chronic leptin treatment.** On GD 14, under isoflurane anesthesia (Butler Schein Animal Health, Dublin, OH), which was delivered by an anesthetic vaporizer (Ohmeda, BOC Health Care, Steeton, WY, England), an osmotic minipump (model 2ML1, Alzet, Cupertino, CA) was placed intraperitoneally in the pregnant+LEP group to deliver leptin (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) at a dose of 0.5 μg/Kg/min (25) for 5 days.

**Blood pressure measurement in conscious rats.** On GD 18, rats were anesthetized with isoflurane as described above and implanted with indwelling carotid catheters consisting of V-1 tubing attached to V-3 tubing (Scientific Commodities Inc., Lake Havasu City, AZ). Catheters were tunneled under the skin and externalized at the scapular region. On GD 19, rats were placed in individual restraining cages and catheters connected to pressure transducers (MLT0699, ADInstruments, Colorado Springs, CO) coupled to a computerized data acquisition system (PowerLab and Lab Chart Pro V7 software, ADInstruments). Animals were allowed to acclimate to restraint for approximately 1 hour. Once hemodynamic readings stabilized, mean arterial blood pressure was recorded for at least another 1 hour.

**Tissue harvest.** On GD 19, rats were anesthetized with isoflurane as mentioned before and a ventral midline incision was made to externalize the uterus. Blood was collected into Corvac separator tubes (for collection of serum; Tyco Healthcare Kendall, Mansfield, MA) and Vacutainer K2EDTA tubes (for collection of plasma; BD, Franklin Lakes, NJ) by punching the abdominal aorta. The number of viable and reabsorbed fetuses in each animal was recorded and individual fetuses and placentas were weighed. Representative placentas from each horn
were flash frozen in liquid nitrogen and stored at -80ºC until processed. Serum and plasma samples were obtained by centrifugation of whole blood at 2000 g for 12 min at 4 ºC and stored at -20 ºC until assayed.

**Serum and plasma measurements.** Serum leptin and insulin concentrations were quantified by ELISA (both from R&D Systems, Minneapolis, MN) following the manufacturer’s instructions. Plasma total cholesterol (Cayman Chemical, Ann Arbor, MI) was quantified by fluorimetric assay, whereas glucose (Cayman Chemical), triglycerides (Cayman Chemical), and free fatty acids (Zen-bio, Durham, NC) were quantified by colorimetric assays.

**Placental measurements.** Frozen placentas were crunched with mortar and pestle in liquid nitrogen, and tissue fragments were suspended in radioimmunoprecipitation (RIPA) lysis buffer with a protease inhibitor cocktail containing phenylmethyl-sulfonyl fluoride and sodium orthovanadate (all from Santa Cruz Biotechnology, Santa Cruz, CA). After homogenization with a glass dounce tissue grinder, homogenates were centrifuged at 14,000 g for 25 min at 4 ºC, and supernatants were used to quantify sFlt-1 and TNF-α by ELISA (both from R&D Systems). Total protein concentration in samples was measured using the bicinchoninic acid (BCA) method (Thermo Scientific, Rockford, IL).

**Statistical analysis.** Graphs and statistical analysis were prepared using GraphPad Prism 5.0 software (San Diego, CA). Comparisons among groups were performed using ANOVA followed by Tukey’s multiple comparison test. Values are shown as mean ± SEM. A value of P<0.05 was considered statistically significant.

**RESULTS**

**Maternal circulating leptin and blood pressure.** At GD 19, chronic leptin treatment produced a significant increase in serum leptin concentration compared to those observed in
pregnant and pregnant-FR groups (Figure 1A; P<0.0001). Mean arterial pressure was also significantly increased by approximately 20 mmHg in pregnant+LEP rats compared with pregnant and pregnant-FR rats as measured at GD 19 (Figure 1B; P<0.05).

**Placental anti-angiogenic and inflammatory factors.** At GD 19, placental TNF-α concentration was increased in pregnant+LEP rats compared with pregnant and pregnant-FR rats (Figure 2A; P<0.01), but pregnant-FR rats presented similar TNF-α levels compared to pregnant and pregnant+LEP rats (Figure 2A; both P>0.05). There was no difference in placental sFlt-1 concentration among groups (Figure 2B; P>0.05).

**Fetal and placental weights.** As shown in Figure 3, placental and fetal weights from pregnant-FR and pregnant+LEP groups were significantly reduced compared with those from the pregnant group as measured at GD 19 (Figure 3B and 3C; P<0.001 and P<0.0001, respectively). However, there was no difference in the ratio fetal weight/maternal body weight among normal pregnant fed *ad libitum*, normal pregnant with reduced food intake, and leptin-treated pregnant rats (0.0070 ± 0.0003 vs. 0.0073 ± 0.0001 vs. 0.0069 ± 0.0003, respectively; P>0.05). In addition, litter size was not statistically difference among groups (Figure 3A; P>0.05).

**Maternal body weight and food intake.** Initial BW at GD 14 was similar among rats designated for the pregnant group (257.0 ± 4.7 g), the pregnant-FR group (264.4 ± 2.1 g), and the pregnant+LEP group (263.4 ± 2.9 g) (Figure 4A; P>0.05). Baseline FI and WI were assessed from GD 13 to 14, and no differences in FI (15.8 ± 1.5 vs. 17.3 ± 0.5 vs. 17.0 ± 1.0 g/day; Figure 1B; P>0.05) or WI (38.0 ± 4.6 vs. 37.3 ± 4.7 vs. 36.1 ± 3.0 mL/day; P>0.05) were found among pregnant, pregnant-FR, and pregnant+LEP groups, respectively.

By the end of the study, at GD 18, BW in pregnant-FR and pregnant+LEP rats (280.1 ± 2.6 and 284.1 ± 3.9 g, respectively) were decreased compared to pregnant rats (310.3 ± 6.6 g).
From GD 16 to 18, pregnant+LEP rats had lower FI than pregnant rats (GD 16: 6.8 ± 0.8 vs. 21.5 ± 0.4 g/day, GD 17: 9.3 ± 1.1 vs. 23.8 ± 0.7 g/day, and GD 18: 11.3 ± 1.2 vs. 25.2 ± 1.1 g/day; Figure 4B; all P<0.0001). Since the mean GD 16-18 FI of pregnant+LEP rat was 8.6 ± 0.8 g/day, we fed pregnant-FR rat 9.0 g/day of chow from GD 14 to 19. WI was lower in pregnant-FR and pregnant+LEP dams than pregnant dams on GD 16-18 (GD 16: 37.8 ± 2.0 vs. 23.5 ± 0.9 vs. 49.9 ± 1.7 mL/day, GD 17: 37.5 ± 2.9 vs. 29.5 ± 1.9 vs. 58.9 ± 2.2 mL/day, and GD 18 (33.7 ± 2.3 vs. 38.3 ± 2.6 vs. 60.0 ± 2.1 mL/day; all P<0.0001), respectively.

**Maternal circulating metabolic factors.** As shown in Table 1, plasma glucose and serum insulin were not statistically different between pregnant and pregnant+LEP groups as measured at GD 19 (both P>0.05). However, the pregnant-FR group presented lower circulating glucose levels compared with both pregnant and pregnant+LEP groups (P<0.0001), and higher insulin levels compared with the pregnant+LEP group (P<0.05). In addition, plasma cholesterol and triglyceride concentrations were decreased in pregnant-FR and pregnant+LEP rats compared to their normal pregnant counterparts (P<0.01 and P<0.001, respectively). Moreover, plasma free fatty acid was reduced in pregnant+LEP rats compared with pregnant rats (P<0.01), but these levels were similar to the free fatty acid level observed in the pregnant-FR group.

**DISCUSSION**

The main finding reported here is that chronic hyperleptinemia increases mean arterial pressure in pregnant rats. This alteration during chronic leptin excess was associated with increased placental TNF-α. In parallel to decreases in food intake and body weight, circulating cholesterol, triglyceride, and free fatty acid levels were reduced in leptin-treated pregnant rats. These data suggest that the hyperleptinemia encountered in obesity is itself, in the absence of additional metabolic disturbances, an important link between obesity and the development of hypertension during pregnancy.
In support of the involvement of leptin in the pathophysiology of preeclampsia are several reports showing that preeclamptic women have increased circulating leptin concentrations compared to normal pregnant women (1, 6, 18, 20, 36, 38, 40, 41, 47). Importantly, some reports have suggested that serum leptin can be used as a predictive marker for this syndrome. Hyperleptinemia appears to develop in preeclamptic women during first and second trimester (5, 11, 36, 43, 46, 50), i.e. before the manifestation of the clinical symptoms. Moreover, positive correlations between circulating levels of leptin and sFlt-1 (15) and between leptin and TNF-α (7) have been noted in preeclamptic women. Although these association studies did not show a cause or effect relationship between hyperleptinemia and preeclampsia, collectively these findings prompted us to examine whether hyperleptinemia alters blood pressure and placental anti-angiogenic and inflammatory factors during pregnancy.

Our study clearly demonstrated that chronic leptin treatment increases mean arterial pressure in pregnant rats. One pathway by which hyperleptinemia may elicit hypertension during pregnancy is through its role in regulating angiogenic and inflammatory processes (8, 44), that are known to be implicated in preeclampsia. Interestingly, the increased blood pressure in our leptin-treated pregnant rats was accompanied by an elevation in placental TNF-α; however, sustained hyperleptinemia did not alter placental sFlt-1. As demonstrated previously, leptin can stimulate the release of TNF-α from placental tissue explants (29). In addition, leptin in doses comparable to those achieved during first and third trimester of gestation can act as a proinflammatory cytokine upregulating the production of TNF-α by mononuclear leucocytes (44). Moreover, serum leptin (39) and serum and placental TNF-α (26, 27) are elevated in the reduced uterine perfusion pressure (RUPP) model of preeclampsia in rats. Furthermore, chronic TNF-α infusion also increases mean arterial pressure in normal pregnant rats, and inhibition of TNF-α with entanercept attenuates the blood pressure response in RUPP rats (3, 26). TNF-α-induced hypertension in pregnant rats is mainly due to activation of the endothelin (ET) system...
Nonetheless, leptin is also able to increase ET-1 production and ET-1 receptors in endothelial cells (23). Thus, another mechanism whereby hyperleptinemia may increase blood pressure is through exacerbation of ET-1 synthesis directly or indirectly via TNF-α. Since we observed a decrease in circulating cholesterol, triglyceride, and free fatty acid levels in leptin-treated pregnant rats, a role for hyperlipidemia in mediating the blood pressure response to chronic hyperleptinemia can be disregarded.

An alternative mechanism that may play a role in how hyperleptinemia elicits increases in blood pressure during pregnancy is by stimulation of the sympathetic nervous system. Earlier studies in male rats by Hall and colleagues have found that chronic leptin infusion increases mean arterial pressure and heart rate (10, 25, 51). They showed that pharmacological antagonism of α1- and β-adrenergic receptors prevented the blood pressure and cardiac responses to hyperleptinemia (10). Additional evidence for the hypertensive effect of leptin derives from studies with transgenic mice overexpressing leptin in the liver, which presented higher plasma leptin levels and mean arterial pressure than non-transgenic control mice. The increased blood pressure in these animals was also normalized after adrenergic or ganglionic blockade (2). The proposed pathway for leptin-induced hypertension involves activation of neurons in the brain which stimulate the renal sympathetic nerves, leading ultimately to an increase in blood pressure (12). However, blood pressure regulation is different in male and female, especially during gestation (32, 57). Indeed, the blood pressure response to leptin observed in our pregnant rats (ΔMAP ~ 20 mmHg) is higher than that documented in male rats (ΔMAP ~ 6-8 mmHg) (10, 25, 51). Interestingly, a recent report found that acute intracerebroventricular infusion of leptin enhances renal sympathetic activity, but not MAP, in non-pregnant female rats (52). This effect of leptin on stimulating renal nerves was observed only during the estrous phase, suggesting that high estrogen levels are necessary to increase MAP in response to chronic leptin infusions. Therefore, while increased renal sympathetic
activity has been reported to play a role in leptin-induced hypertension in males, the
involvement of the sympathetic nervous system in modulating the blood pressure response to
leptin during pregnancy remains to be determined.

Although human obesity is associated with hyperleptinemia, prior studies indicate that
obese subjects are resistant to the anorexic effects but remain sensitive to the hypertensive
actions of leptin. Most rat and mouse models of obesity also develop hyperleptinemia and
increased caloric intake with preserved blood pressure responsiveness to leptin. Distinct
intracellular signaling pathways of the leptin receptors allow leptin to control separately these
physiological processes (17, 58). This pattern of selective leptin resistance seems also to occur
in preeclampsia. Obesity markedly increases the risk for developing preeclampsia (49) and
preeclamptic women have exacerbated hyperleptinemia and hypertension. Therefore, it was not
surprising that using lean Sprague Dawley normal pregnant rats, we noted both metabolic and
cardiovascular effects of leptin. In addition, although caloric intake was similar between normal
pregnant rats with food restriction and leptin-treated pregnant rats, leptin interestingly abolished
the effects of food restriction on decreasing plasma glucose and increasing serum insulin. All
these results, i.e. reduced food intake and body weight, no difference in circulating glucose but
reduced insulin levels, and elevated blood pressure, are in line with previous findings in lean
Sprague Dawley male rats (25).

Intriguingly, both obese (20, 36, 43) and non-obese (5, 18, 50) preeclamptic women
present with exacerbated hyperleptinemia, suggesting that another source of leptin besides the
adipose tissue contributes to the increased circulating leptin levels seen in preeclampsia. Both
human (9, 33) and rat (4, 53) placentas have been shown to produce leptin as well express
leptin receptors. Indeed, placental leptin gene and protein expressions are elevated in
preeclampsia (18, 22, 31, 48). However, it is unknown to what extent leptin derived from
adipose and/or placental tissues are involved in pregnancy-induced hypertension.
Regarding fetal outcome, we observed a decrease in fetal and placental weights in leptin-treated pregnant rats. An inverse correlation between maternal circulating leptin and birth weight has been noted in humans (6, 37, 38, 46), suggesting that hyperleptinemia can lead to decreased fetal growth. Indeed, pregnant women with intra-uterine growth restriction (IUGR) exhibit exacerbated hyperleptinemia (30, 37). Likewise, preeclamptic women with IUGR have higher circulating leptin levels than preeclamptic women with normal fetal growth (40). However, since fetal and placental weights were comparable in normal pregnant rats with food restriction and leptin-treated pregnant rats, we conclude that the decrease in fetal and placental weights following leptin infusion resulted from the reduced food intake of dams instead of a direct effect of leptin. Previous reports have already addressed whether maternal hyperleptinemia over the levels found in normal pregnancy affects fetal programming, and they found that in utero exposure to high leptin levels modifies the development of energy balance regulatory systems (54) and leads to lean offspring with reduced skeletal growth (42).

**Perspectives**

In this study, we tested the hypothesis that chronic hyperleptinemia increases blood pressure and placental factors in pregnant rats. Our results show that sustained hyperleptinemia does raise blood pressure and increase placental TNF-α. Understanding the molecular pathways that regulate the actions of leptin in the cardiovascular system during pregnancy may offer novel pharmacological agents for the treatment of preeclampsia.

Obesity is a major risk factor for the onset of preeclampsia. Recent epidemiological data suggest that the rate of preeclampsia has increased largely due to a significant increase in the incidence of metabolic diseases such as obesity, but the mechanisms explaining this relationship are still unclear. Preeclamptic pregnancies are associated with exacerbated hyperleptinemia. Importantly, we have direct evidence that chronic hyperleptinemia in pregnant
rats increases blood pressure despite decreasing body weight and numerous metabolic factors commonly associated with the obese milieu. Therefore, our data implicate hyperleptinemia in itself as an important link the between obesity and the development of hypertension during preeclampsia.

ACKNOWLEDGEMENTS
The authors would like to thank Marietta Arany, Kathy Cockrell, and Haiyan Zhang for their technical expertise.

GRANTS
Research reported in this publication was supported by the American Heart Association under the award number 14POST18970005 and by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award numbers P01HL051971 and 1T32HL105324.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


FIGURE LEGENDS

Figure 1. Effects of chronic leptin infusion and food restriction in pregnant rats on circulating leptin (panel A) and mean arterial pressure (panel B) at gestational day 19. Normal pregnant group with food intake ad libitum (pregnant, n=8); leptin (0.5 µg/kg/min i.p.)-treated pregnant group with food intake ad libitum (pregnant+LEP, n=8); normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR, n=11). *P<0.05 vs. pregnant. #P<0.05 vs. pregnant-FR.

Figure 2. Effects of chronic leptin infusion and food restriction in pregnant rats on placental tumor necrosis factor (TNF)-α (panel A) and soluble fms-like tyrosine kinase (sFlt)-1 (panel B) levels at gestational day 19. Normal pregnant group with food intake ad libitum (pregnant, n=8); leptin (0.5 µg/kg/min i.p.)-treated pregnant group with food intake ad libitum (pregnant+LEP, n=8); normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR, n=11). *P<0.05 vs. pregnant.

Figure 3. Effects of chronic leptin infusion and food restriction in pregnant rats on litter size (panel A), placental weight (panel B), and fetal weight (panel C) at gestational day 19. Normal pregnant group with food intake ad libitum (pregnant, n=12); leptin (0.5 µg/kg/min i.p.)-treated pregnant group with food intake ad libitum (pregnant+LEP, n=12); normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR, n=11). *P<0.05 vs. pregnant.

Figure 4. Effects of chronic leptin infusion and food restriction in pregnant rats on body weight (panel A) and food intake (panel B) from gestational day 13 to 18. Normal pregnant group with food intake ad libitum (pregnant, n=12); leptin (0.5 µg/kg/min i.p.)-treated pregnant group with food intake ad libitum (pregnant+LEP, n=12); normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR, n=11). *P<0.05 vs. pregnant.
food intake *ad libitum* (pregnant+LEP, n=12); normal pregnant group with food intake adjusted
to the food intake of pregnant+LEP rats (pregnant-FR, n=11). *P<0.05 vs. pregnant. **P<0.05 vs.
pregnant-FR.
Figure 1.

A) Serum Leptin Concentration (ng/mL)

B) Mean Arterial Pressure (mmHg)
Figure 2.

A) Placental TNF Concentration (pg/mg)

B) Placental sFlt-1 Concentration (pg/mg)
Figure 3.

A) 

![Graph A: Number of Fetuses vs. Pregnant, Pregnant - FR, Pregnant + LEP](image)

B) 

![Graph B: Placental Weight (g) vs. Pregnant, Pregnant - FR, Pregnant + LEP](image)

C) 

![Graph C: Fetal Weight (g) vs. Pregnant, Pregnant - FR, Pregnant + LEP](image)
Figure 4.

A)

B)
Table 1. Effects of chronic leptin infusion and food restriction in pregnant rats on circulating metabolic measurements at GD 19.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Pregnant - FR</th>
<th>Pregnant + LEP</th>
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<tbody>
<tr>
<td><strong>Plasma Glucose Concentration (mg/dL)</strong></td>
<td>161.8 ± 14.2</td>
<td>104.6 ± 3.1*</td>
<td>160.5 ± 5.8#</td>
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<td><strong>Serum Insulin Concentration (µU/mL)</strong></td>
<td>9.6 ± 0.8</td>
<td>14.4 ± 2.4</td>
<td>7.7 ± 2.3#</td>
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<td><strong>Plasma Cholesterol Concentration (mg/dL)</strong></td>
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<td>105.4 ± 6.9*</td>
<td>111.8 ± 27.7*</td>
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<td><strong>Plasma Triglyceride Concentration (mg/dL)</strong></td>
<td>494.5 ± 92.1</td>
<td>160.1 ± 23.1*</td>
<td>91.1 ± 33.7*</td>
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<tr>
<td><strong>Plasma FFA Concentration (mg/dL)</strong></td>
<td>105.1 ± 27.9</td>
<td>53.0 ± 7.7</td>
<td>33.7 ± 11.8*</td>
</tr>
</tbody>
</table>

Pregnant, normal pregnant group with food intake ad libitum (n=8); pregnant + LEP, leptin (0.5 µg/kg/min i.p.)-treated pregnant group with food intake ad libitum (n=8); pregnant - FR, normal pregnant group with food intake adjusted to the food intake of pregnant + LEP rats (n=11); FFA, free fatty acids. *P<0.05 vs. pregnant. #P<0.05 vs. pregnant - FR.