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Folate Treatment Partially Reverses Gestational Low Protein Diet Induced Glucose Intolerance and the Magnitude of Reversal is Age and Sex Dependent

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Abstract

Introduction: Gestational low protein (LP) programming causes glucose intolerance (GI) and insulin resistance (IR) in adult offspring. Folate supplementation has been shown to rescue the offspring from various programming effects. Our objective was to investigate if folate supplementation during pregnancy will reverse LP induced GI and IR.

Methods: Pregnant rats were fed control (20% protein), isocaloric low protein (LP, 6%) or LPF (LP with 5mg/kg Folate) diet from gestational day4 until delivery. Control diet was given during lactation and to pups after weaning. Glucose tolerance test (GTT) was done at 1, 2 and 3 months of age followed by euglycemic-hyperinsulinemic clamp at 4 months. Rats were sacrificed at 4 months and their gonadal, renal, inguinal, brown fat and pancreas were weighed and expressed relative to their body weight.

Results: LP and LPF fed dams showed similar weight loss during late pregnancy following decreased feed intake. Both LP and LPF pups were smaller at birth but their weights caught up like that of controls by 3 months. In males, folate supplementation reduced LP induced GI at 2 months (Glucose AUC: 1940 mmol/l*180min in LP, 1629 mmol/l*180 min in LPF and 1653 mmol/l*180 min in controls; $p<0.05$, LP vs. Control and $p<0.01$, LP vs. LPF) but the effect diminished at 3 months. In females, folate reduced GI at 1 month (Glucose AUC: 1406 mmol/l*180min in LP, 1264 mmol/l*180min in LPF and 1281 mmol/l*180min in controls; $p<0.05$, LP vs. Control and LP vs. LPF) but had no effect at 2 and 3 months. Interestingly, LPF group had higher pancreatic weights than other groups suggesting that folate helps in pancreatic development enabling the LPF rats to produce/secrete more insulin to maintain euglycemia. Euglycemic hyperinsulinemic clamp shows, both LP and LPF are insulin resistant when compared to controls by 4 months with LPF more severe than LP in males. Interestingly, females were more insulin resistant than males.

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Conclusions: Folate treatment partially reverses LP induced GI and the magnitude of reversal is age and sex dependent. Further, folate treatment does not reverse IR in both sexes but makes it worse in males at 4 months. Our study shows that folate treatment is not sufficient to rescue the LP programming effects.

Keywords

Developmental programming; Glucose intolerance; Insulin resistance; Folate; protein restriction

Introduction

The adverse effect of gestational programming has been implicated in various metabolic diseases [1–7]. Gestational protein deficiency causes intra-uterine growth restriction leading to glucose intolerance and insulin resistance in adult life [2, 7, 8]. This increased susceptibility is due to fetal adaptations in utero in response to altered nutrient availability [9–11]. Various experimental and epidemiological studies have shown that there is a strong association between in utero growth retardation and the development of type II diabetes [2, 4].

Folate is a key nutrient for a developing embryo and poor maternal folate level is often associated with a decrease in infant birth weight [12]. Folate treatment during gestation has shown promise in various studies to neutralize the effect of diet induced gestational programming. In an earlier study, folate supplementation prevented epigenetic modifications involving PPAR gene methylation in hepatic gene expression in pups 6 days post-weaning [10, 11]. Further several studies showed that folate treatment ameliorated various programming effects such as feeding behavior, adiposity and cardiovascular functions [13–15].

We have recently developed and characterized a novel lean type 2 diabetes rat model and have shown that gestational low protein (LP) diet causes impairments in insulin signaling cascade in both male and female offspring leading to progressively worsening glucose intolerance and insulin resistance [16–18]. Our current aim was to investigate if prenatal folate supplementation will reverse LP induced glucose intolerance and insulin resistance in lean T2D rat model and if there are any sex differences.

Methods

Animals

Timed pregnant (Day 4) Wistar rats were purchased from Harlan Sprague Dawley, Indianapolis, IN. Pregnant rats were randomly divided into three groups and were fed with control diet (20% protein, n=7), low protein diet (6% protein, n=7) and low protein diet supplemented with 5mg/kg of folate (6% protein, n=8) from day 4 of pregnancy until delivery. All the diets were isocaloric and were custom made from Harlan Teklad, TX, USA. Rats were given unlimited access to food and water and were housed in a temperature-controlled room (23°C) with a 14:10-hour light/dark cycle. Standard diet was given to dams after delivery until weaning and pups were given standard diet after weaning. Dams were

euthanized after weaning. Pups with extreme weights were culled on day1 after birth to make the litter size to 8 pups per mother ensuring equal nutrient access for all offspring. All rats were euthanized at 4 months to collect fat pads and pancreas, and their weights were recorded. All experimental procedures involving rats were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine.

Intraperitoneal Glucose Tolerance Test and Insulin Assay

Rats were fasted for 6h (6.00 AM-12.00 noon) and were given glucose intraperitoneally (1g/kg BW,i.p.). Blood was collected by saphenous vein prick at 0, 15, 30, 60,120 and 180 minutes. Glucose levels were measured using ACCU-CHEK Nano™ blood glucose test strips (Roche Inc. Branford, CT). Blood was also collected using heparinized tubes for insulin assay. Plasma was isolated from the blood by centrifugation and stored at -80°C until analysis. Insulin was measured using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden) following the manufacturer's instruction as reported earlier [17]. Absorbance was read at 450 nm using a BMG CLARIOstar plate reader (BMG Labtech GmbH, Ortenberg, Germany) and the results were calculated with cubic spline regression fit using omega CLARIOstar data analysis software. Overall glucose levels and insulin secretion were assessed by calculating the area under the curve (AUC) using the trapezoidal method.

Homeostatic Model Assessment (HOMA)

HOMA-Insulin resistance (HOMA-IR) and HOMA-Insulin sensitivity (HOMA-IS) were calculated to assess insulin resistance and insulin sensitivity of control and LP rats using the following equations [19].

$$\text{HOMA-IR} = (\text{Fasting Glucose (mg/dL)} \times \text{Fasting Insulin (mU/l)}) \div 405$$

$$\text{HOMA-IS} = 10000 \div (\text{Fasting Glucose (mg/dL)} \times \text{Fasting Insulin (mU/l)})$$

Euglycemic-hyperinsulinemic Clamp

Euglycemic-hyperinsulinemic clamp was performed on conscious rats using tail vein catheters. Fasting rats (6 hours fasting) were placed in appropriately sized restrainer (Kent Scientific, Torrington, CT, USA) with its tail protruding out. The restrainer was tightened in such a way that the animals had some room to move but cannot turn around. Tail was wiped cleaned and two catheters (BD Insyte Autoguard Catheter, 22 or 24 gauges depending upon the size of the animal) were placed, one catheter (Infusion Catheter) at the proximal end on the dorsal tail vein and another on lateral vein distally (Sampling Catheter). The catheters were secured with a drop of tissue glue at the insertion site and wrapped with perforated tape. A bifusate tube was then connected to the catheter which was connected to insulin in one arm and glucose in the other. Insulin was infused at a constant rate of 240mUnits/kg/h with a flow rate of 200 μ l/h. A 50% dextrose solution was infused with a starting flowrate of 300 μ l/h. Samples were taken at various time points at 0, 5, 10, 20, 30, 40, 50, 60 mins followed by every 15 min for up to 3 hours. Infusion of dextrose was adjusted at every time point to maintain a blood glucose level of 90–110 mg/dL. Steady state values were taken when at least three concordant blood glucose values with in the acceptable range. Once the procedure was completed, the catheters were removed, tail was wiped clean with alcohol

pads and silver sulfamide ointments was applied and the animals were allowed to recover at least for a week.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism™ (La Jolla, CA) software. Data are presented as Mean $\pm$ SEM. Comparison between three groups was performed using one-way ANOVA. When comparisons between groups with two factors were done, statistics was performed with two-way ANOVA followed by Bonferroni test. Differences were considered significant when $p < 0.05$.

Results

Feed Intake by Pregnant Dams

Daily feed intake of pregnant dams was measured from day 4 of pregnancy until delivery. Our results show that LP and LPF fed dams ate more than the control dams during the early pregnancy on day 8 ($p < 0.01$, Control vs. LP and $p < 0.05$, Control vs. LPF) and day 9 ($p < 0.05$, Control vs. LPF) of pregnancy. This tendency continued until day 15 of pregnancy. Interestingly, from days 19 to 21 of pregnancy, their feed intake was significantly lower (Day 19: $p < 0.001$, Control vs. LP; Days 20 and 21: $p < 0.0001$, Control vs. LP and LPF) when compared to that of the controls (Figure 1A).

Folate treatment did not increase maternal weight gain during late pregnancy

Maternal weight gain was monitored from day 4 of pregnancy until delivery. Dams from all the three groups showed similar weight gain trajectories from day 4 until day 18 of pregnancy. However, dams fed with LP and LPF diet did not gain weight like that of the control group during late pregnancy (Days 19–21). Maternal body weights of LP and LPF dams were significantly lower (Day 19: $p < 0.0001$, Control vs. LP and $p < 0.01$, Control vs. LPF; Days 20 and 21: $p < 0.0001$, Control vs LP and LPF) than that of controls (Figure 1B).

LP and LPF pups were small at birth but showed catch up growth

Pups born to LP (Males: 4.6 ± 0.08 g; Females: 4.3 ± 0.1 g) and LPF (Males: 4.5 ± 0.08 g; Females: 4.4 ± 0.08 g) dams were significantly smaller (Males and Females: $p < 0.0001$ Control vs. LP and LPF) at birth when compared to their respective controls (Males: 6.0 ± 0.08 g; Females: 5.8 ± 0.1 g) (Figures 2A and B). Even though the growth velocities were different, both LP and LPF pups showed catchup growth and eventually their weights were similar to that of the controls in both sexes. However, the catchup growth was faster in LPF (Males: 3 months; Females: 2 months) pups than LP (Males: 4 months; Females: 3 months) pups in both males and females. BMI also showed similar overall tendency with LP and LPF groups reaching control values by 3 months (Figures 2C and D).

Fat pad and pancreatic weights at 4 months

Body fat contents were measured by weighing the fat pads (Peri-renal, inguinal, perigonadal and brown adipose tissue) of rats after euthanasia at 4 months. Data from raw weights and percentage body weight did not show any significant differences between the

three study groups in both sexes (Data not shown). Pancreas from LPF group weighed more than that of controls and LP group in both sexes in general (Figure 3). In males, pancreas from LPF offspring weighed significantly more than that of the controls (Control 0.9 ± 0.04 g, LP 0.9 ± 0.04 g and LPF 1.0 ± 0.02 g; $p < 0.05$ Control vs. LPF) (Figures 3A and C). In females, offspring from LP groups had a significantly lower ($p < 0.05$) weight when compared to the control offspring. Interestingly, folate supplementation rescued the pancreatic weight loss in the LPF offspring and were significantly higher than the LP group and was similar to the controls (Control 0.9 ± 0.03 g, LP 0.7 ± 0.03 g and LPF 0.9 ± 0.05 g; $p < 0.001$ LP vs. LPF). The ratio of pancreatic weights to that of the total body weights also showed similar differences like the raw weights in both males and females (Figures 3B and D).

Folate treatment regulates hyperglycemia by increased insulin production

Glucose tolerance tests were performed on control, LP and LPF offspring at 1, 2 and 3 months to identify the progression of glucose intolerance (Fig. 4 and 5). The blood glucose levels peaked at 15 min after the bolus intraperitoneal glucose administration and returned to basal levels by 180 min.

One month old males (Fig. 4A–D) did not show any overall difference in glucose tolerance or insulin response between the control and the treatment groups even though glucose levels were higher in the controls ($p < 0.05$, Control vs. LP) at 15 minutes. Two-month old LP males (Fig. 4E–H) showed significant increase in overall glucose intolerance (AUC, mmol/L*180min) when compared to control (LP-1940 $\pm$ 54 vs Control-1653 $\pm$ 43, $p < 0.05$) and LPF (1629 $\pm$ 75, $p < 0.01$) groups. Fasting blood glucose levels in LP offspring (7.7 ± 0.2 mmol/L) were similar to controls (7.0 ± 0.2 mmol/L) and LPF (7.2 ± 0.2 mmol/L), however after administration of glucose during glucose tolerance test, blood glucose levels in LP offspring were significantly elevated at 15 min (17.5 ± 0.6 mmol/L in LP vs. 13.8 ± 0.5 mmol/L in control, $p < 0.0001$ and 13.8 ± 0.6 mmol/L in LPF, $p < 0.0001$), 30 min (15.4 ± 0.5 mmol/L in LP vs. 12.3 ± 0.8 mmol/L in control, $p < 0.001$ and 11.4 ± 0.8 mmol/L in LPF, $p < 0.0001$), and 60 min (11.6 ± 0.6 mmol/L in LP vs. 10.0 ± 0.6 mmol/L in control, and 9.1 ± 0.6 mmol/L in LPF, $p < 0.01$) and the levels declined and were similar to that of the controls at 120 min (8.7 ± 0.3 mmol/L in LP vs. 7.5 ± 0.1 mmol/L in control, and 8.1 ± 0.3 mmol/L in LPF) and 180 min (7.5 ± 0.3 mmol/L in LP vs. 7.2 ± 0.2 mmol/L in control, and 7.1 ± 0.3 mmol/L in LPF). The overall plasma insulin responses, expressed as Insulin AUC (pmol/L*180 min) after intraperitoneal glucose administration showed an increasing tendency in the LP (54023 $\pm$ 7229 and LPF group (69444 $\pm$ 4984) when compared to controls (43516 $\pm$ 7508), the values however did not reach statistical significance except at 15 min post bolus (674 ± 45 pmol/L in LPF vs. 368 ± 89 pmol/L in controls ($p < 0.01$) vs. 478 ± 95 pmol/L in LP ($p > 0.05$)). At 3 months LP males (Fig. 4I–L) showed an increase in overall glucose intolerance when compared to control ($p < 0.05$) but showed an increasing tendency when compared to LPF group and did not reach statistical significance. Fasting blood glucose levels in LP offspring at 3 months (8.3 ± 0.2 mmol/L) were higher than that of the controls (6.8 ± 0.4 mmol/L) and LPF (7.3 ± 0.3 mmol/L) but did not reach statistical significance. After glucose administration during glucose tolerance test, when compared to controls, blood glucose levels in LP offspring were significantly elevated at 15 min (18.5

± 0.6 mmol/L in LP vs. 15.7 ± 0.6 mmol/L in control, $p < 0.01$ and 17.8 ± 0.5 mmol/L in LPF, $p > 0.05$) and 30 min (16.2 ± 0.6 mmol/L in LP vs. 14.0 ± 0.7 mmol/L in control, $p < 0.05$ and 14.4 ± 0.7 mmol/L in LPF, $p > 0.05$). No differences were observed at 60 min (11.6 ± 0.5 mmol/L in LP vs. 10.0 ± 0.7 mmol/L in control, and 10.7 ± 0.6 mmol/L in LPF), 120 min (8.0 ± 0.2 mmol/L in LP vs. 8.6 ± 0.6 mmol/L in control, and 7.9 ± 0.6 mmol/L in LPF) and 180 min (7.3 ± 0.2 mmol/L in LP vs. 7.2 ± 0.3 mmol/L in control, and 7.2 ± 0.3 mmol/L in LPF). Interestingly, the overall plasma insulin responses, expressed as Insulin AUC (pmol/L*180 min) after intraperitoneal glucose administration showed an increasing tendency in the LP (76774 ± 4535) and significant increase in LPF group (81397 ± 7478 , $p < 0.05$) when compared to controls (54210 ± 6884). Further, LPF group had increased plasma insulin levels at 15 min (642 ± 123 in LPF vs. 502 ± 72 in controls, $p < 0.05$) and 30 min (581 ± 63 in LPF vs. 317 ± 48 in controls, $p < 0.05$) when compared to controls.

In one month old females (Fig. 5A–D), LP pups showed a significant increase in the overall AUC of glucose levels when compared to controls (Glucose AUC: 1406 mmol/l*180min in LP, 1264 mmol/l*180min in LPF and 1281 mmol/l*180min in controls, $p < 0.05$, Control vs. LP) and interestingly folate treatment reversed this increase and the AUC was comparable to that of the controls and was lower than LP group ($p < 0.05$, LP vs. LPF). Fasting blood glucose levels in LP females (7.4 ± 0.2 mmol/L) were comparable to controls (7.4 ± 0.3 mmol/L) and LPF (7.2 ± 0.3 mmol/L), however, after glucose bolus, blood glucose levels in LP offspring were significantly increased at 15 min (13.7 ± 0.7 mmol/L in LP vs. 11.5 ± 1.0 mmol/L in control, $p < 0.05$ and 10.6 ± 0.7 mmol/L in LPF, $p < 0.001$ vs. LP). No differences were observed between the groups at 30 min (9.6 ± 0.8 mmol/L in LP, 8.4 ± 0.7 mmol/L in control and 8.3 ± 0.4 mmol/L in LPF), 60 min (8.9 ± 0.3 mmol/L in LP, 8.2 ± 0.2 mmol/L in control and 8.1 ± 0.3 mmol/L in LPF), 120 min (7.8 ± 0.2 mmol/L in LP, 7.5 ± 0.1 mmol/L in control, and 7.2 ± 0.1 mmol/L in LPF) and 180 min (7.3 ± 0.2 mmol/L in LP, 7.4 ± 0.1 mmol/L in control, and 7.2 ± 0.1 mmol/L in LPF). The overall plasma insulin responses, expressed as insulin AUC (pmol/L*180 min) was after glucose administration showed increase in the LP (27675 ± 1202 , $p < 0.05$) when compared to LPF (21089 ± 2137). The control levels were 22405 ± 1806 . The insulin levels at different time points during GTT did not reach statistical significance except at 15 min post bolus (487 ± 47 pmol/L in LP vs. 218 ± 31 pmol/L in LPF ($p < 0.05$)). No differences were observed in the overall glucose and insulin profiles at two months of age (Fig. 5E–H). However, during GTT, there was a decrease in the blood glucose levels in LP offspring at 15 min (11.0 ± 0.9 mmol/L in LP vs. 14 ± 0.5 mmol/L in control, $p < 0.01$ and 13.0 ± 0.6 mmol/L in LPF, $p < 0.01$).

At 3 months of age (Fig. 5I–L), no differences were observed in the overall glucose levels or at any time points during GTT between the three groups. Insulin levels showed a significant increase in the LP and LPF groups when compared to the controls (513 ± 87 pmol/L in LP and 551 ± 44 pmol/L in LPF, vs. 295 ± 31 pmol/L in control, $p < 0.01$ vs. LP and LPF). Further, LPF group (61381 ± 4747) also showed substantial increase in the overall insulin levels when compared to controls (36000 ± 4669 , $p < 0.01$ vs. LPF) and LP group (43679 ± 2985 , $p < 0.05$ vs. LPF).

Fasting Insulin and HOMA-IR was higher in LP and LPF males

In males, at one (Fig. 6A, B and C) and two (Fig. 6D, E and F) months of age, there were no differences in the fasting insulin concentrations or HOMA-IR/IS between the three groups. However, at two months, LPF showed an increasing tendency when compared to controls and LP. At 3 months (Fig. 6G, H and I), insulin concentrations were higher in LPF group when compared to controls ($p<0.001$) and LP ($p<0.01$). HOMA-IR and HOMA-IS for LPF group showed an increase ($p<0.01$) and decrease ($p<0.05$) respectively when compared to controls. In females, there were no differences between the groups in the fasting insulin levels and consequently no differences were observed in HOMA-IR and HOMA-IS analysis in any of the time points observed (Data not shown).

Euglycemic hyperinsulinemic clamp showed insulin resistance in LP and LPF groups

Male and female rats were subjected to euglycemic hyperinsulinemic clamp at 4 months of age. During the clamp glucose concentrations were maintained at a steady state of ~ 6 mmol/L. The glucose infusion rate was nearly 2-fold lower in LP males and 3-fold lower in LPF males when compared to the controls (9.1 mmol/[kg*min] in LP ($p<0.01$), 4.9 mmol/[kg*min] in LPF ($p<0.0001$) vs Control (19.4 mmol/[kg*min]) ($n = 5-6$) (Fig. 7A, B, C, D). In females, the glucose infusion rate was nearly 6-fold lower in LP and 8-fold lower in LPF when compared to the controls (3.8 mmol/[kg*min] in LP ($p<0.01$), 3.1 mmol/[kg*min] in LPF ($p<0.0001$) vs Control (24.0 mmol/[kg*min]) ($n = 5-6$) (Fig. 7E, F, G, H).

Discussion

In utero exposure to adverse conditions have been implicated in programming offspring to cause changes in physiology and metabolism thereby increasing the risk for developing various metabolic diseases [20–31]. This increased susceptibility is the result of fetal adaptations in utero in response to altered nutrient availability [9–11]. We have shown that LP diet during pregnancy leads to T2D with a lean phenotype with progressively worsening insulin resistance and glucose tolerance [16]. Folate is essential for embryo development and normal infant birth weight [12]. Our present results show that both male and female LPF groups displayed similar growth characteristics, BMI and body fat to that of LP group. Thus, folate treatment did not alter the lean phenotype and low birth weight of the LP offspring. Earlier studies had suggested that folic acid supplementation could reduce the risk of intrauterine growth restriction in human [32, 33]. However, in rats, prenatal folate treatment did not show any improvement in the litter weights [34] similar to our observation, strongly suggesting folate alone cannot improve fetal weight gain in the absence of sufficient dietary protein.

Various reports show that folate treatment during gestation alleviate or neutralize the effect of diet induced gestational programming [10–15]. Further, folate is a source of methyl group and is essential for DNA methylation, and low folate levels are associated with lower DNA methylation [35]. DNA methylation is highly active during early embryogenesis immediately after implantation [36] and hence the developing fetus may be highly susceptible to uterine environmental modifications induced by maternal diet [37]. Several animal models suggest that the programming effect during early development is due to

epigenetic changes via altered DNA methylation patterns and subsequently affecting the expression of related genes [38]. Many rodent models of maternal insult show that specific genes of interest carry promoter methylation that correlates with their expression [39–41]. It is therefore conceivable that folate, being a methyl donor could reverse the programming effects caused by methylation defects if supplemented during fetal development.

Our GTT results show that folate treatment restored overall glucose intolerance in both males and females but to varying degrees especially in early stages. However, insulin measurements during the GTT show higher insulin secretion in response to glucose bolus in LPF offspring. Interestingly, the pancreatic weights of LPF rats were higher and could be attributed for the increased insulin production. This strongly suggests that the apparent restoration of glucose intolerance in folate treated group is due to compensatory hyperinsulinemia and larger pancreas may assist in greater secretion of insulin in this group. Pancreatic β cells may initially compensate for the insulin resistance by upregulating insulin production/secretion especially at a younger age. Compensatory insulin production may ultimately fail due to inadequate β cell expansion and/or due to the failure of the β cells to sense and respond to glucose [42]. However, the precise mechanism of compensatory insulin production or secretion in LPF group is not known and warrants further investigation.

In our earlier publications in this lean type 2 diabetic rats, we had shown sex differences in the progression, severity, and mechanisms of the disease [16–18]. Interestingly, our present euglycemic hyperinsulinemic clamp data shows that dietary folate supplementation caused a greater insulin resistance in males whereas it made no differences in females. It is not clear how folate affects insulin resistance in a sex specific manner. Recently, folate has been shown to alter the gene expression of 11β -hydroxysteroid dehydrogenase type 2 in placenta by affecting the promoter methylation in a sex selective fashion [43]. It is possible that folate might act via altering genes involved in insulin signaling in a sex specific manner. Our data from basal insulin levels, HOMA-IR, HOMA-IS and euglycemic hyperinsulinemic clamp clearly show that both LP and LPF rats are insulin resistant.

Thus, the underlying peripheral insulin resistance in the programmed offspring could not be reversed indicating that folate treatment is not sufficient to reverse LP induced metabolic programming. Further, prenatal folate supplementation could be detrimental to males. Folate is routinely given to women during pregnancy. It is not known if folate causes sex specific effects in humans. If such a scenario were to be true especially in an underdeveloped or developing country where poor nutrition is common, it would be important to revisit the folate intake recommendations. Compounds capable of making epigenetic changes such as folate could cause epigenetic reprogramming and may lead to the development or worsening of metabolic diseases.

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Abbreviations:

LP	Low protein
LPF	Low protein with folate supplementation
GTT	Glucose tolerance test
IR	Insulin Resistance

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Figure 1: Maternal feed intake and body weights of control, LP and LPF groups. (A) Maternal feed intake during pregnancy. (B) Maternal weight gain in control, LP and LPF diet fed rats from day 4 to day 22 of pregnancy. Results are presented as mean $\pm$ SEM, $n = 7-8$ per group. Results are presented as mean $\pm$ SEM.

Figure 2:

Body weight and BMI of control, LP and LPF offspring. Figures showing the body weights of control and LP programmed male (A) and female (C) offspring and their respective body mass index (BMI) (B and D) from birth to 4 months of age. *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ and ****= $p < 0.0001$. (n= 23–31 for males and 23–30 for females for weights and BMI)

Figure 3:
Pancreatic weights of male and female offspring. Figure showing the pancreatic weights of male (A) and female (C) offspring along with their respective % body weights (B and D).
(n= 10–13 for males and 11–13 for females)

Figure 4: Glucose tolerance test (GTT) in males. Figure showing glucose and insulin levels along with their respective area under the curve (AUC) during GTT in control, LP and LPF offspring at 1 (A-D), 2 (E-H) and 3 months (I-L) of age. (n=5–6 per group).

Figure 5: Glucose tolerance test (GTT) in females. Figure showing glucose and insulin levels along with their respective area under the curve (AUC) during GTT in control, LP and LPF offspring at 1 (A-D), 2 (E-H) and 3 months (I-L) of age. (n=5–6 per group).

Figure 6:

Fasting Insulin and HOMA in males. Fasting insulin levels and HOMA-IR and HOMA-IS in 1, 2 and 3 month of age belonging to control, LP and LPF groups. (n=4–5 per group).

Figure 7:

Euglycemic hyperinsulinemic clamp for control, LP and LPF group performed in 4 months old males and females. Figures showing the blood glucose and their corresponding glucose infusion rates for males (A and B) and females (E and F). Blood glucose and infusion rates were compared between the groups at the steady state in males (C and D) and females (G and H). (n=5–6 per group) *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ and ****= $p < 0.0001$.