Original Research Article

Maternal Obesity and Malnutrition in Rats Differentially Affect Glucose Sensing in the Muscles and Adipose Tissues in the Offspring

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Abstract:

Background: The altered maternal/fetal metabolism appears to be associated with a diabetogenic effect in the adult offspring even in the absence of genetic predisposition.

Aim: The study aimed to investigate the effect of maternal obesity and malnutrition on the peripheral glucose sensing and mitochondria biogenesis in F1 offspring. Effect of postnatal diet was also evaluated.

Methods: Three groups of female Wistar rats were used (control, obese and malnourished). After the pregnancy and delivery the offspring were weaned to control diet or high-caloric (HCD) diet and followed up for 30 weeks.

Every 5 weeks OGTT was constructed and serum and tissues were obtained for assessment of glucose homeostasis parameters, mTFA, mtDNA, UCP2, insulin receptor (IR), phospho-insulin receptor (Phosho-IR) and GLUT4.

Results: The results indicated that maternal obesity impair glucose tolerance and sensing in the offspring from the 15th week of age even under control diet and the situation is worse under HCD these defects were precede by significant decline in mtDNA copy number in muscle and adipose tissues as early as 5th week of age . The offspring of malnourished mothers show normal and even better glucose tolerance and sensing and normal mtDNA copy number under control diet, while those offspring under HCD show impaired glucose sensing and tolerance only at older age than obese group.

Conclusion: maternal obesity and malnutrition differentially affect glucose sensing and tolerance, mtDNA copy number and the expression of genes involved in the mitochondrial biogenesis and function in the muscles and adipose tissues in the F1 offspring with the postnatal feeding appears to play a central role in these differential effects. The male F1 offspring appear to be more sensitive for fetal diabetogenic programming than female offspring.

Key Words:

Fetal origin, Diabetes, Epigenetic, Glucose sensing, Insulin resistance, UCP2, mitochondria

1. Introduction

The intra-uterine environment is the vital element that programs to a certain extent the health of an individual throughout life. This effect has been called "fetal origin of adult disease". The altered maternal/fetal metabolism appears to be associated with a diabetogenic effect in the adult offspring even in the absence of genetic predisposition.[1,2]

The fetal origin hypothesis has been supported by evidence that fetal nutrient availability, other intrauterine factors, and external environmental factors can cause serious consequences in later life by permanently reprogramming the functional capacity of organs.[3]

During early fetal development the metabolic response to nutrient supply is tightly regulated and any alterations in its availability, sensing and metabolism can affect fetal development.[4]. Accumulating body of evidences support the link between maternal nutritional status and the offspring susceptibility to metabolic syndrome and type 2 diabetes mellitus (T2DM) [2–7]. However, the exact molecular mechanism(s) of the fetal programming is still unclear. Another unclear subject is how two opposite maternal nutritional states (over- and mal-nutrition) program the offspring for the same diabetic phenotype. It was documented that, offspring of maternal obesity or malnutrition suffer from impaired glucose tolerance and insulin resistance. [5–8]

Glucose and insulin homeostasis is based on glucose sensing and metabolism in β-cell of pancreas and peripheral tissues. In muscle and adipose tissues the glucose sensing and uptake is controlled by activation of insulin receptor (IR) by insulin which cause autophosphorylation of β-subunit of the receptor to form phospho-IR then activates downstream substrates which finally end by translocation of GLUT4 from inside the cell through the plasma membranes [9].

Mitochondria fundamentally control cellular energetic metabolism and homeostasis especially in the context of glucose metabolism.[10], [11] Mitochondrial biogenesis, dynamics, homeostasis and regulation of oxidative-phosphorylation are regulated by the nuclear encoded genes. One of these genes is mitochondrial transcriptions factor A [12](mtTFA) which is essential for biogenesis, mitochondrial DNA (mtDNA) expression, replication and maintenance. Uncoupling protein 2 (UCP2) is another nuclear encoded protein that control mitochondrial functions [11, 13]. Many studies theorize a potential role of these proteins in the pathogenesis of T2D. Recently, we found that the fetal adverse environments can prenatally affecting the expression of genes that linked to the development of type 2 diabetes including genes controlling glucose sensing and metabolism. Prenatally, maternal obesity and malnutrition appear to affect these genes and proteins differentially [14]. In this study we follow-up the offspring of maternal obesity and malnutrition in an attempt clarify how different maternal nutritional status produce similar phenotype in the offspring (diabetogenic tendency).

2. Animals and Method:

2.1 Chemicals:

Rat insulin ELISA kit obtained from Mercodia (Sweden), rat insulin receptor and phospho-insulin receptor β-subunit (Tyr 1162/1163) star ELISA kit were purchased from Millipore (USA) and rat Glucose transporter 4 ELISA Kit was purchased from EIAab (China). Streptozotocin, and collagenase were obtained

from Sigma-Aldrich Chemie GmbH (Germany). GenJet RNA isolation kit obtained from Fermentas life science (EU). One-step RT-PCR kit using RT/PCR Master Mix Gold Beads obtained from Bioron (Germany). Primers of UCP2 and mtTFA obtained from Bioneer Co (Bioneer, USA).

2.2 Animals:

Three groups of female Wistar rats were used (control, obese and malnourished); obesity was induced by maintaining female neonates under obesogenic diet (Table 1) for two months after weaning, the female rats that were 20% heaver than control rats of the same age referred to as obese rat. Malnutrition was induced by maintaining female neonates under low-protein diet (8% protein, Table 1) for two months after weaning. The female rats that were 20% lighter than control rat of the same age referred to as malnourished rat.

Pregnancy was established by mating the females with normal healthy male rats. After the pregnancy and delivery the offspring were weaned to control diet (CD) or high-caloric diet (HCD, Table 1) and followed up for 30 weeks. So the resulted offspring groups were as following:

- F1offspring of control mother under control diet (CF1-CD)
- F1offspring of control mother under HCD (CF1-HCD)
- F1offspring of obese mother under control diet (OF1-CD)
- F1 offspring of obese mother under HCD (OF1-HCD)
- F1 offspring of malnourished mother under control diet (MF1-HCD)
- F1 offspring of malnourished mother under HCD (MF1-HCD)

Every 5 weeks post-natal, 10 pups (5 males and 5 females) of each subgroup were used for construction of oral glucose tolerance test (OGTT). After overnight fasting blood samples were obtained after that rat were injected with insulin i.p. (10 mU/kg) and then sacrificed after 30 minutes. The rats were dissected out to obtain quadriceps muscle and abdominal white adipose

2.3 Methods:

2.3.1 Total RNA isolation

Total RNA were isolated from the different tissues using GenJet RNA isolation kit (Fermentas, EU) according to the manufacturer instructions.

2.3.2 RT-PCR analysis:

For semi-quantitative determination of the gene expression of mitochondrial transcription factor A (mtTFA)[12] and UCP2 [15], the One-step RT-PCR Master Mix Gold Beads were used. For mtTFA the following primer set were used; forward 5'-GCT TCC AGG AGG CTA AGG AT-3' and reverse 5'-CCC AAT CCC AAT GAC AAC TC-3'. The primer set of UCP2; forward 5`-CAAACAGTTCTACACCAA-3` and reverse 5`-CGAAGGCAGAAGTGAAGTTGG-3`. To standardize the amount of mRNA in each sample, RT-PCR of Glyceraldehyde-3-

phosphate dehydrogenase (GAPDH) was performed in parallel using the following primer set; forward 5'-AATGTGTCCGTCGTGGATCTGA-3' and reverse 5'-GATGCCTGCTTCACCACCTTCT-3'. After the end of the program, the RT-PCR product was run on 1.5 % agarose and stained with ethidium bromide. The bands were visualized by using UV plate. The bands were scanned and the data were analyzed using UVP DOC-ITLS ™ Image & acquisition and analysis software (Ultra-Violet product, Ltd. Cambridge, UK) that analyze the relative band density to GAPDH band (as internal control).

2.3.3 Mitochondrial DNA copy number:

The mitochondrial DNA (mtDNA) content relative to the nuclear gene; peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) was measured using real-time PCR as described previously [16]. Primers for mtDNA were as follow: forward 5'-ACACCAAAAGGACGAACCTG-3' and reverse 5'-ATGGGGAAGAAGCCCTAGAA-3'; and for PGC1α, forward 5'-ATGAATGCAGCGGTCTTAGC-3' and reverse 5'-AACAATGGCAGGGTTTGTTC-3'. Reactions were carried out in using SYBR Green PCR Master Mix (Applied Biosystems), 0.5 μM of each forward and reverse primer, and 50 ng genomic DNA with the following conditions: 95°C for 10 min followed by 40 cycles of 95°C for 15 min, 60°C for 30 min and 72°C for 30 min. The relative mtDNA copy number was calculated using the ΔΔCt method as described previously [17].

2.3.4 ELISA measurements:

Plasma insulin was assayed using ELISA kit ((Mercodia, Sweden). The tissues homogenates were used for the assessment of phospho-insulin receptor β-subunit (Tyr 1162/1163) star ELISA kit (Millipore, USA) and GLUT4 by Rat Glucose transporter 4 ELISA Kit (EIAab, China) according to the manufacturer's instructions.

2.3.5 Statistical analysis:

All data are presented as mean ± SEM. Statistical analysis was performed using SPSS software (SPSS, Chicago,IL). Student's t test and ANOVA were used to assess differences. Probability values (p) <0.05 were considered to be statistically significant

3. Results:

3.1 Pregnancy outcome at delivery

Maternal obesity and malnutrition significantly decrease the number of viable pups per litter especially in malnourished mothers. Also the pup's weight was affected, as pups of malnourished mothers had lower, while those of obese mother had higher birth weight compared to control pups (Table 2).

The male and female offspring of obese and malnourished mothers have higher weights with age compared to control offspring especially those under HCD (Figure 1)

3.2 Glucose homeostasis parameters

The offspring of obese mother under CD or HCD and offspring of malnourished mothers only under HCD showed a significant elevation of fasting blood sugar (FBS) compared to control offspring. The offspring under HCD showed the elevation earlier (20th week) than those under CD (25th week). Also, male appears to show higher level than female (Figure 2). The offspring of malnourished mother under CD show no significant change in fasting blood sugar irrespective of sex and age (Figure 2)

At age of 30th week, OGTT of the offspring of maternal obesity and malnutrition (under control or high caloric diet) showed impaired glucose tolerance (IGT) after 30 minutes of glucose administration, the highest level of blood glucose was detected on the OF1 under HCD followed by those under CD then MF1 under HCD and then MF1 under CD. We can also note that, males are more affected than females (Figure 3)

Fasting insulin level showed age-dependent increase in the all offspring groups (Figure 4). The offspring of obese mother under HCD showed higher fasting insulin level from 15th week of age while those offspring under CD show the higher level at later age (25th week) in males and females. The offspring of malnourished mother under HCD show the similar pattern as OF1-HCD. In contrast, those MF1 offspring under CD show near control or even lower levels of fasting insulin (Figure 4).

The index of insulin resistance calculated as HOMA showed an age-dependent increase in all offspring groups (Figure 5). The increase in insulin resistance became significant as early as 10th week of age in male and female offspring of maternal obesity and malnutrition under HCD and with aging the increase became more apparent (Figure 5). The offspring of obese mother under CD showed significant higher HOMA from 15th week in male and 20th week of age in female. On the other hand, the offspring of malnourished mothers maintained under control diet showed no significant change compared to control offspring, irrespective of sex and age (Figure 5 A and B).

3.3 Peripheral glucose sensing parameters:

The muscle content of insulin receptor (IR) showed lower levels in the male offspring of obese mother under CD and HCD and offspring of malnourished mother under HCD form 10th week of age and thereafter with the lowest level observed in MF1-HCD, while MF1-CD showed near normal values (Figure 6A). In females, muscle IR content showed lower level mainly in the offspring under HCD (from obese or malnourished mothers) from 10th week of age, with aging also OF1 under CD showed significantly lower level and also control offspring under HCD showed a significant lower level of IR compared to CF1-CD. As in male the MF1-CD offspring show normal values or even higher values at certain ages (25th and 30th week) (Figure 6B).

The adipose tissue content of IR in male offspring showed similar pattern of changes as that observed in muscle but it start as early as 5th week of age in all offspring irrespective of diet even the control offspring under HCD showed

lower level. With aging the lower IR level became limited to the OF1-CD, OF1-HCD and MF1 under HCD; the later offspring showed the lowest level (Figure 8A). The female offspring of obese and malnourished mothers only under HCD showed significant lower level of IR at age 20th week and thereafter (Figure 8B).

The muscle phospho-IR showed an age-dependent down regulation in the offspring of obese mothers; males and females under HCD, the significant lower level was detected at the 20th week of age in males and at 25th week in females (Figure 7A and B). The adipose tissue phospho-IR showed significantly low levels in the offspring of obese mothers under CD and HCD and in the offspring of malnourished mothers under HCD from the age of 25 week in males and 20 week in females (Figure 9A and B).

The muscle and adipose tissue contents of Glut4 were down regulated in the male and female offspring; OF1-CD, OF1-HCD and MF1-HCD. These offspring showed a significant lower levels mainly at 25th week of age, the offspring under HCD showed lower level earlier than other offspring (Figure 10 and 11).

3.4 Mitochondrial parameters

The mitochondrial DNA (mtDNA) copy number in muscle showed significantly lower level in the offspring of obese and malnourished mothers under HCD from the 10th week of age in males and 15th week of age in females and thereafter, also OF1-CD showed lower level but at later ages, the decrease was apparent in those offspring under HCD (Figure 12). The adipose tissue showed similar pattern of mtDNA copy number change but it starts later than muscle (at 25th week of age in males and 20th week of age in females) (Figure 13).

The expression of *mTFA* in muscle was around the control level (CF1-CD) in the all studied offspring except in the offspring of obese mothers under HCD which showed up regulation from 15th week of age in males and females (Figure 14). In adipose tissue, no significant changes were detected in the different offspring except at age 30 week in the male OF1-HCD which showed up regulation of *mTFA* gene expression (Figure 15).

The expression of *UCP2* gene in the muscle tissue showed no significant change in the female offspring from the control value irrespective of the type of diet (Figure 16B). Only male OF1-HCD showed up regulation at 25th week of age (Figure 16A). The expression of *UCP2* gene in the adipose tissue was significantly up regulated in the control male offspring under HCD from 25th week of age compared to the control value (Figure 17A), while female offspring showed this up regulation earlier than males (15th week of age) (Figure 17B).

4. Discussion

The molecular mechanism(s) of intra-uterine programming for T2DM in the offspring of obese or malnourished mothers is (are) unclear. Also, how two different maternal nutritional extremities (over- and mal-nutrition) produce similar diabetogenic phenotype need detailed study of the different pathways involved in glucose sensing and metabolism. The results of this study indicated that the altered peripheral glucose

sensing and mitochondrial biogenesis may play a role in this programming. Also, the post-natal high caloric diet (HCD) may be involved in exacerbating this programming.

Maternal obesity and malnutrition appear to affect pregnancy outcome as it caused significant decrease in the number of pups per litter especially in the malnourished pregnancies. The decreased number of pups may results from increased fetal deaths and resorption [14] or decreased quality of the maternal ova. It was documented that, maternal metabolic disturbances, like a negative energy balance or obesity and type II diabetes are associated with ovarian dysfunction. Changes in the growth pattern of the ovarian follicle, due to suboptimal metabolic conditions, can affect oocyte quality. Furthermore, maternal metabolic disorders (nutritionally induced or caused by obesity) may alter the endocrine and biochemical composition of the microenvironment of the growing and maturing female gamete (follicular fluid). Any perturbation in oocyte's direct environment has the potential to reduce the oocyte's developmental competence. Also embryo quality is significantly reduced due to maturation in adverse conditions [18]. The results indicated that, pups of maternal obesity have significantly higher while; pups of maternal malnutrition have lower weight than control pups. In agreement with these results, Snoeck et al. demonstrated a link between the maternal low protein diet and lower birth weights in the offspring as well as decreased beta-cell proliferation and altered adipocyte properties [19]. Also, using intrauterine artery ligation (as a model of intrauterine impaired fetal nutrition) similar results were obtained [4,8].

Previous reports examined the effect of maternal health challenges during gestation and the pregnancy outcomes and offspring health. It was reported that the maternal health even during pre-gestation alone can result in diabetogenic and obesogenic tendency in the offspring [20,21]. This could create different developmental windows of diabetogenic programming namely; pre-gestational, gestational and post-natal. In this study the maternal obesity or malnutrition were induced pre-gestational and continued through gestation and lactation which resembles to large extend the human situations, however we manipulated the pos-natal feeding using normal balanced control diet (CD) and high-caloric diet (HCD) to examine the role of the type of nutrition on the intra-uterine programming.

Follow up of F1 offspring indicated that, the male offspring of maternal obesity and malnutrition became over weight from the 15th week of age and thereafter and post-natal feeding with HCD further increase the weight gain. These indicated obesogenic behavior of the offspring which consider a risk factor for the development of insulin resistance and T2DM. The offspring of malnourished dams maintained under control diet showed normal glucose tolerance and insulin sensitivity while those maintained under high caloric diet showed age-dependant decline in glucose tolerance and insulin sensitivity which became significant from the 15th week of age especially in male. On the other hand the offspring of obese mothers showed age-dependent impaired glucose tolerance, increased insulin and insulin resistance even when they maintained under control diet but at later age than those offspring under HCD. OGTT showed impaired glucose tolerance from 15th week of age in the male and 20th week of age in female offspring of obese mother under HCD while those offspring under CD showed this impairment 5 weeks later (Data not shown). With age 30 week, mild hyperglycemia was detected in the male offspring of maternal obesity or malnutrition (under HCD only) while in female no hyperglycemia were detected and IGT was

aggravated. These results imply that maternal nutritional status alone is not sufficient to induce T2DM at this young age while post-natal feeding play important role in accelerating the development of insulin resistance and T2DM phenotype in the F1 offspring especially in the offspring of maternal malnutrition.

Many studies previously demonstrated IGT and insulin resistance in the offspring of maternal obesity [22–26]. Human study by Catalano et al 2009, demonstrated that offspring of obese mothers were more insulin resistance than offspring of lean mothers (using cord blood measurements) suggesting increased insulin secretion during in-utero gestational period [27]. Also, in sheep it was documented that maternal obesity resulted in increased fetal pancreatic weight and number of insulin positive cells which reflect early β-cell maturation [28]. These alterations in insulin sensitivity during early age could contribute to enhanced adiposity in later life.

On the other hand, the maternal malnutrition is a known inducer of offspring metabolic syndrome and diabetes risk. It was documented that gestational caloric and protein restriction resulted in metabolic alterations, reduced pancreatic cell and insulin and diabetogenic tendency in the offspring[19, 29,30]. Isganaitis et al develop a mouse model of low birth weight (LBW) by mother food restriction by 50% from gestational days 12.5–18.5 [31]. In this model, LBW mice with accelerated post- natal growth have increased adiposity in early life and increased risk of diabetes and of obesity in adulthood. Also, they confirm the role of early postnatal feeding in this programming.

The observed shifts in glucose homeostasis were associated with changes in the peripheral sensing of glucose in muscle and adipose tissues which start at insulin receptor and ended by translocation of Glut4 to the plasma membrane of the cell. The muscle and adipose tissue contents of insulin receptor (IR) were significantly lowered in the offspring of obese mothers from the 10th and 5th week of age; respectively, irrespective of postnatal diet (Figure 6 and 8). The offspring of malnourished mothers maintained under control diet showed similar or even higher level of IR in muscle tissues especially in females while in adipose tissue the IR was lower during the first 15 week of age after which its level become insignificant from control offspring. Those offspring under HCD showed the lowest level of IR in muscle and adipose tissues especially in males which show the least level and at earlier age than females.

The regulation of IR gene expression occurs at different levels of control; transcription, post-transcription and/or post-translational. Insulin is known to down regulate IR protein by inducing its degradation [32] and by inhibiting its gene transcription[33]. So, the observed down regulation of IR in the offspring of obese and malnourished mothers may explained by the increased insulin level in those offspring.

Insulin activation of IR leads to tyrosine (Tyr) autophosphorylation of both β-subunits with the major 3 sites at Tyr-1158, Tyr-1162 and Tyr-1163 [34]. In the present study we used a kit for assessment of Tyr-1162/1163 phosphorylated β-subunit (Phospho-IR) after 30 minutes of insulin injection and the result indicated down regulation from 20th week age in male and 25th week in female in the muscle of the offspring of obese mothers maintained under HCD compared to control. Prenatally at gestational day 17 we reported an up regulation of phospho-IR in the fetuses of malnourished mothers [14]. This prenatal effect may activates negative mechanisms to down regulate this up regulation by inducing expression of protein tyrosine phosphatases 1B and this

epigenetic change may persist post-natal while even though no hyperglycemia present, but this assumption need experimental prove.

Another important observation is the absence of significant changes in the phospho-IR level in the muscle and adipose tissues earlier than 20th week of age while insulin resistance (by HOMA) and IGT (by OGTT) were detected at earlier age which may indicate that resistance to insulin action at this age may occur downstream the insulin receptor activation.

The whole homogenate content of Glut4 was assayed, the results indicated a lower level of Glut4 in muscle tissues of male and female offspring of obese mothers maintained under HCD at age 20 week and at age 25 week for those under CD and only offspring of malnourished mother under HCD show the same pattern of change while those under CD show normal comparable level with control offspring. In adipose tissues, only those offspring under HCD showed significantly lower level of Glut4. This decrease of Glut4 protein may results from increased degradation and/or down regulation of the expression. There are many evidences for the decreased total Glut4 content in many animal model of insulin resistance and obesity like GK rats and obese Zucker rats [35,36].

The important mediators in the glucose sensing and metabolism are mitochondria [37]. Mitochondrial transcription factor A (mTFA) is nuclearly encoded transcription factor which act as a key regulator of mitochondrial transcription and mtDNA replication [38]. The relative gene expression of mTFA in muscle tissues of the offspring of maternal obesity under HCD showed up regulation compared to control offspring from the 15th week of age in males and females. This up regulation may be a compensatory mechanism to counteract the decrease in mtDNA copy number that detected in muscle tissues from the 10th week of age in male and 15th week of age in females. The offspring of malnourished mother maintained under HCD showed the least level of mtDNA copy number in muscle and adipose tissues but without change in the relative gene expression mTFA. The decrease in mtDNA copy number is more apparent, in the males than females, and in muscle earlier than adipose tissue (Figure 12 and 13), that may explain the metabolic defects observed in those offspring. In contrast to the offspring of maternal obesity or malnutrition under HCD, the offspring of maternal malnutrition under control diet showed normal or even higher mtDNA copy number that may indicate better metabolic fitness than other offspring. The decreased mtDNA copy number could impair the function and metabolism in the muscle and adipose tissues and may considere the prime event in the development of insulin resistance. It was documented that maternal obesity altered expression of the genes involved in muscle metabolism and reduced muscle force [39]. Also, In line with these results Park el al demonstrated that low protein diet during gestation significantly decrease mtDNA number in liver and muscles tissues of the offspring [40].

Many studies relate the reduced mitochondria in the muscles and adipose tissue to the pathogenesis of T2DM. Mitochondria in adipocytes play pivotal role in the regulation of lipolysis as fatty acids resulting from lipolysis can be oxidized by the fatty acid β-oxidation. This process of removal of fatty acids within white adipocytes would protect against fatty acid leakage out of adipocytes [37]. So, the reduced mtDNA and function would result in enhanced efflux of fatty acid into circulation that contributes to the insulin resistance, since fatty acids impair muscle and liver insulin sensitivity [41].

The expression of *UCP2* in muscle showed no significant change during the study period in any of the offspring with the exception of male offspring of maternal obesity

of HCD which show up regulation at 25th week of age. While in adipose tissues the control offspring under HCD showed up regulation of *UCP2* expression at mRNA level especially in females. This up regulation may considered an adaptive mechanism to eliminate the excess fuel intake and inhibit production of reactive oxygen species however this mechanism not operated in the other offspring of obese or malnourished mothers that made them highly vulnerable for adiposity, oxidative stress and insulin resistance.

From the above results and discussion we can suggest that maternal obesity and malnutrition pre-gestational and gestational differential affect the studied parameters. While maternal obesity caused IGT and even hyperglycemia in the offspring with disturbed regulation of glucose sensing parameters and mtDNA copy number in muscle and adipose tissues, maternal malnutrition have no effect or even ameliorate the glucose sensing and insulin sensitivity in the offspring. Post-natal feeding is pivotal factor in the programming of the offspring; in the offspring of obese mother post feeding with HCD escalate the effects of intra-uterine diabetogenic programming while in those offspring of malnourished mother HCD feeding reverse the situation completely; as the offspring showed the same pattern as that observed in those offspring of maternal diabetes under HCD or worsen. There are many hypotheses that could explain this contradictory figure observed in offspring of maternal malnutrition. The first one is "thrifty phenotype" which suggests that poor intra-uterine environment causes the development of fetal organs (brain) at the expense of other organs like pancreas and cardiomyocytes which result in survival of fetuses under this poor condition [42]. But this hypothesis can't explain the development of insulin resistance observed in the offspring. It was also postulated that fetus develop insulin resistance to ensure proper supply of the brain with glucose and amino acids [4]. Catch-up growth is another hypotheses which assumes that the mismatch between nutritionally poor intra-uterine environment and post-natal nutrient enrich environment results in early post-natal catch-up growth associated with long-term adverse metabolic effects including insulin resistance and IGT [4,43,44].

From the results of this study we can concluded that maternal obesity and malnutrition differentially affect glucose sensing and tolerance, mtDNA copy number and the expression of genes involved in the mitochondrial biogenesis and function in the muscles and adipose tissues in the F1 offspring with the postnatal feeding appears to play a central role in these differential effects. The male F1 offspring appear to be more sensitive for fetal diabetogenic programming than female offspring. Also, the study point out to the role of adipose tissues in triggering the programming for development of T2D.

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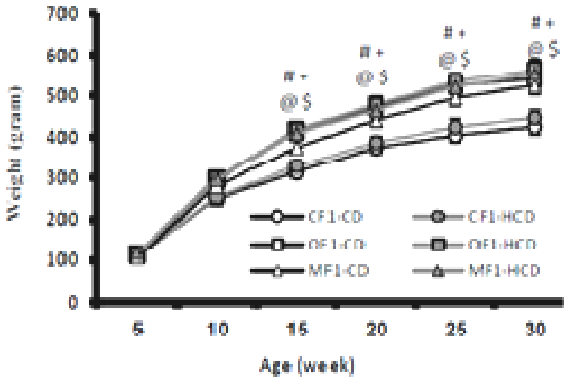
Table (1) Different type of diet used in the study

Macronutrients (g/kg diet)	Control diet (CD)	Low-protein diet (LPD)	Obesogenic diet (OD)	High-caloric diet (HCD)
Protein	220	90	300	244
Carbohydrates • **Dextrose** • **Corn starch** • **Sucrose**	631	761	105 106 140	593
Fat • **Lard** • **corn oil**	43	43	195 70	48 20
Cellulose	54	54	-	50
Vitamin mix	10	10	30	10
Mineral mix	40	40	40	35
Total energy (kcal/g diet)	3.8	3.8	5.4	4.7

Table (2): Pregnancy outcomes of different pregnancies at delivery

	Control	Obese	Malnourished
No. of litters	29	28	36
No. of viable pups	362	283	216
No. of pups/litter	12±3	10±3[a]	6±3[a]
Pups weight (gm)	5.9±0.6	6.1±0.5[a]	5.3±0.38[a]

Data of No of pups/litter and pups weight are presented as Mean±SEM
[a] *Significant different from control b y ANOVA followed by Fisher's LSD Post hoc test (P<0.05)*



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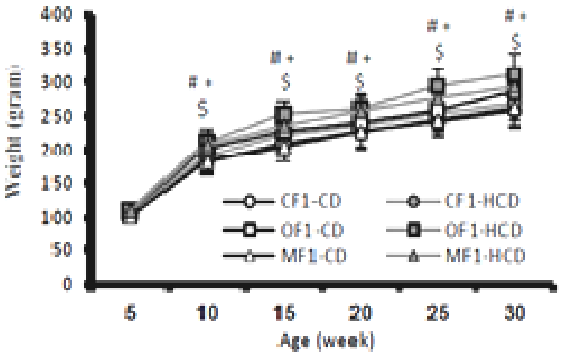

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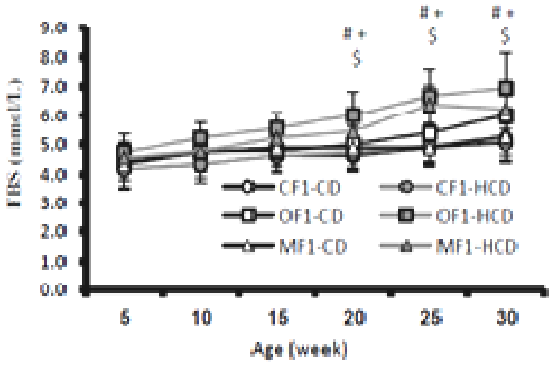

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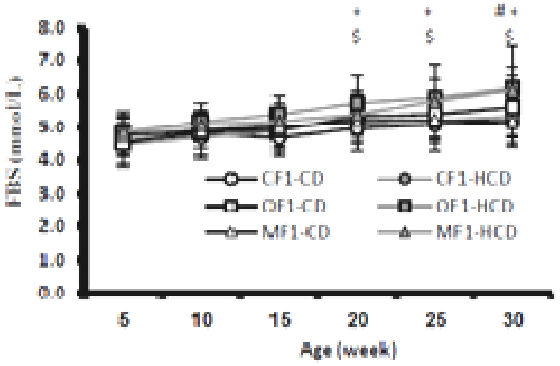

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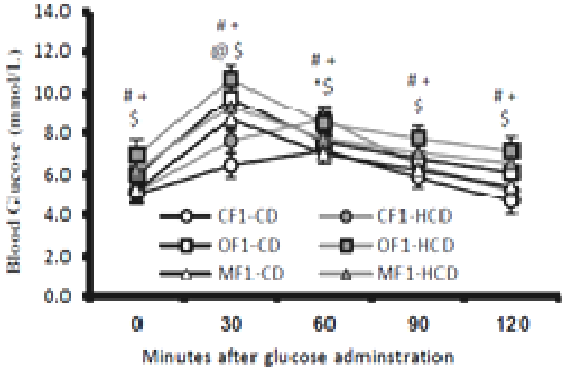

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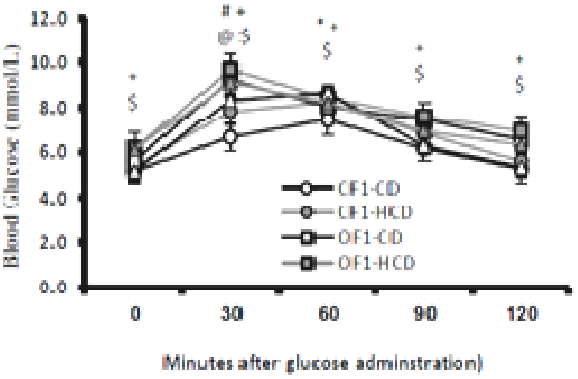

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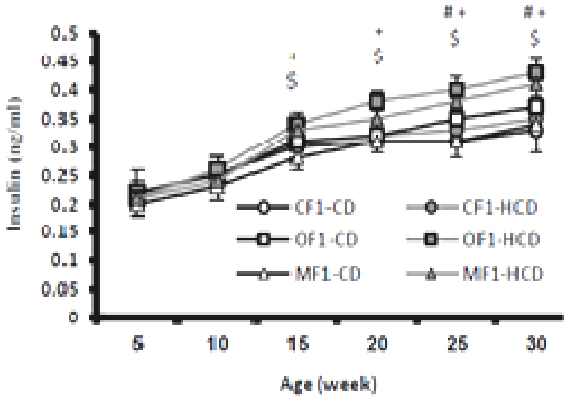

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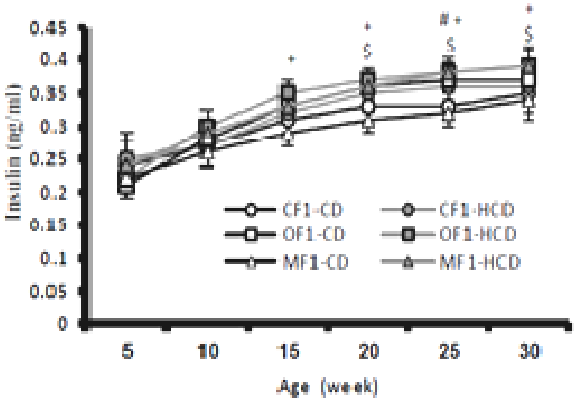

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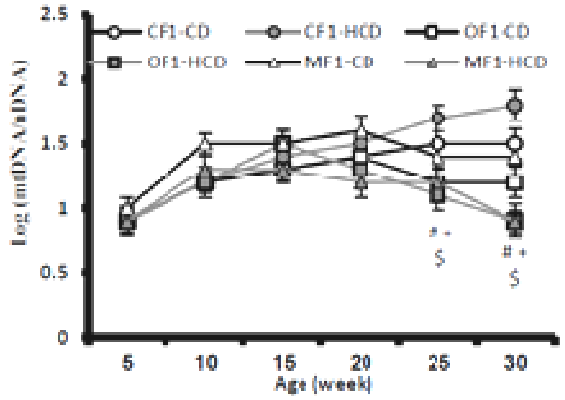

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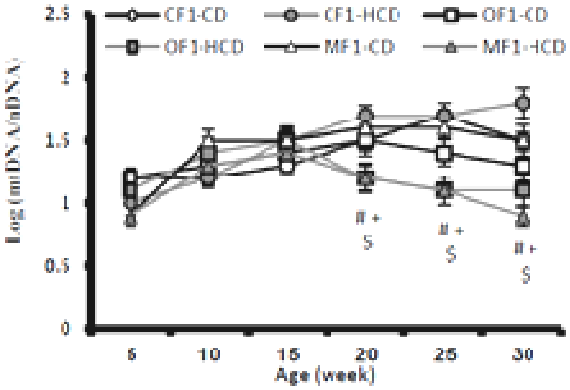

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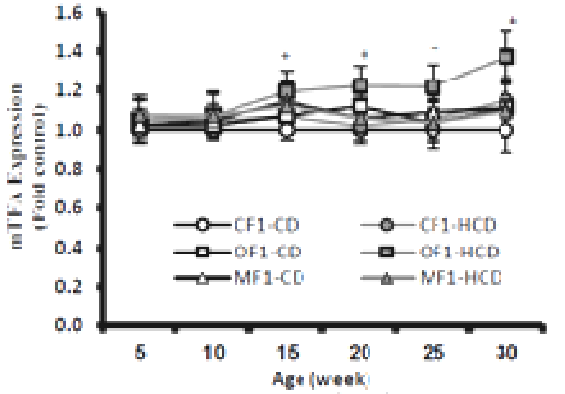

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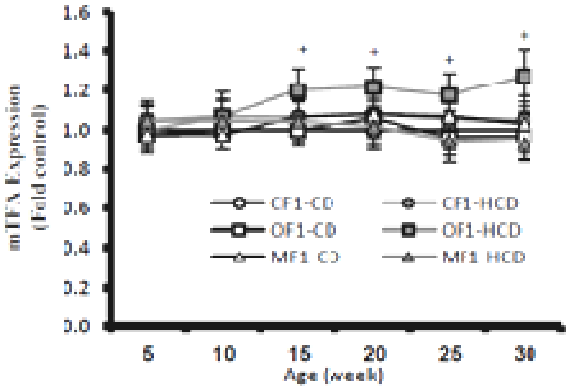

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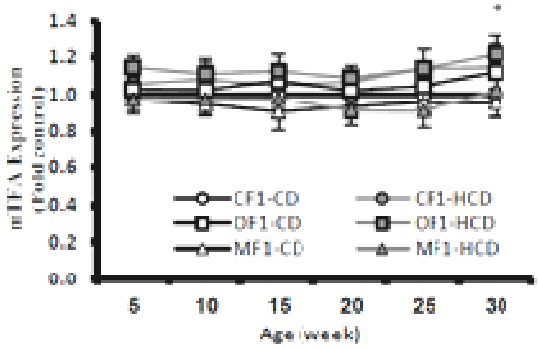

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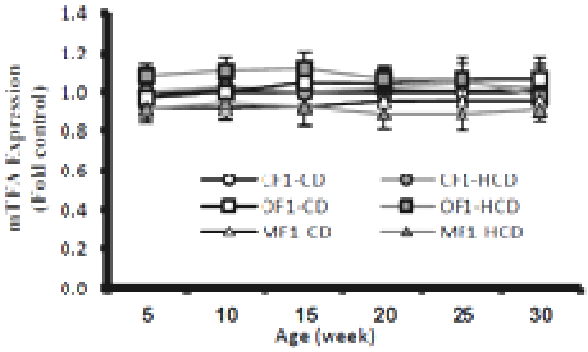

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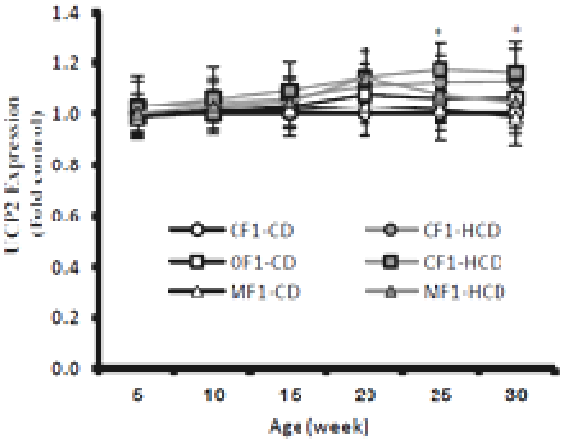

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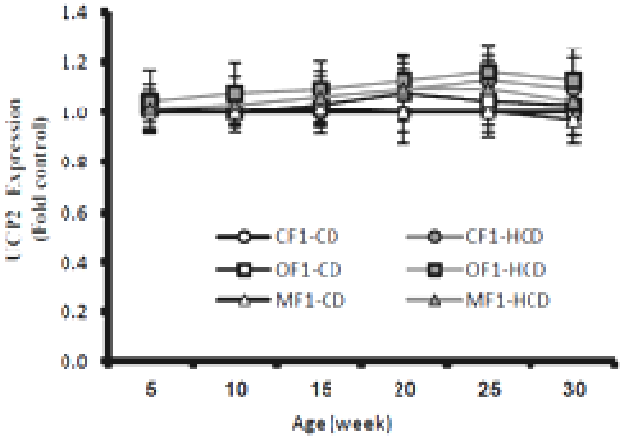

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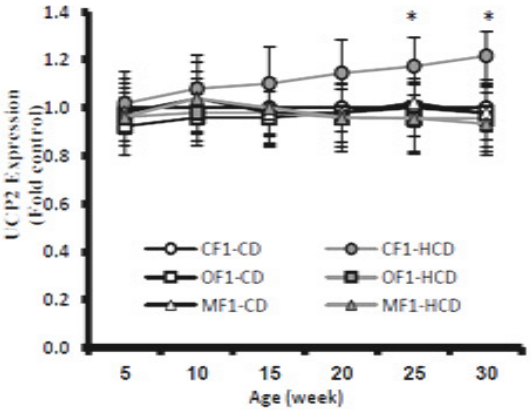

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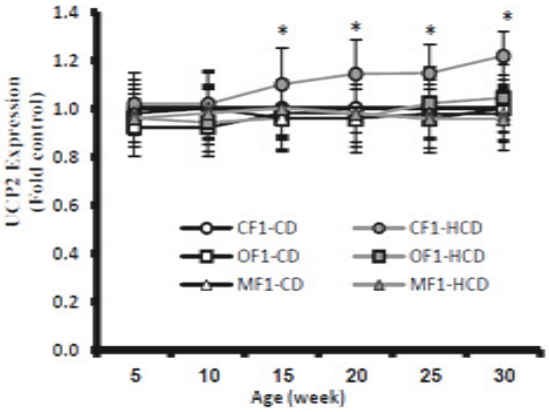

Figure (17B)

<u>Figure legends:</u>

Figure (1):

Age-dependent change of body weight in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (2):

Age-dependent change of FBS in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (3):

OGTT of male (A) and female (B) F1 offspring under control and HCD diet at 30 week old

Figure (4):

Age-dependent change of Insulin in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (5):

Age-dependent change of HOMA in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (6):

Age-dependent change of muscle IR in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (7):

Age-dependent change of Muscle Phospho-IR in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (8):

Age-dependent change of adipose tissue IR in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (9):

Age-dependent change of adipose tissue Phospho-IR in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (10):

Age-dependent change of muscle Glut4 in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (11):

Age-dependent change of adipose tissue Glut4 (ug/mg protein) in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (12):

Age-dependent change of muscle mTFA in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (13):

Age-dependent change of adipose tissue mTFA in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (14):

Age-dependent change of muscle UCP2 expression in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (15):

Age-dependent change of adipose tissue UCP2 in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (16):

Age-dependent change of muscle mitochondrial DNA copy number in male (A) and female (B) F1 offspring under control or HCD-diet

Figure (17):

Age-dependent change of adipose tissue mitochondrial DNA copy number in male (A) and female (B) F1 offspring under control or HCD-diet

Figure Notes:

* Significant difference between CF1-HCD and CF1-CD at each age by ANOVA ($p<0.05$)

Significant difference between OF1-CD and CF1-CD at each age by ANOVA ($p<0.05$)

+ Significant difference between OF1-HCD and CF1-CD at each age by ANOVA ($p<0.05$)

@ Significant difference between MF1-CD and CF1-CD at each age by ANOVA ($p<0.05$)

$ Significant difference between MF1-HCD and CF1-CD at each age by ANOVA ($p<0.05$)