

# The study on the effect of hyperglycemia on offspring fatty tissue metabolism during pregnancy

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**Abstract. – OBJECTIVE:** In this study, we adopted maternal diabetes model on rats, which induced by streptozotocin to explore the metabolism changes of rat adipose tissue during the neonatal period.

**MATERIALS AND METHODS:** The female rats were induced as diabetes models by streptozotocin (STZ), and mated with the normal male rats when they entered into adulthood. The chosen male offspring rats were executed at week 12 and the epididymis and subcutaneous fat pad were obtained. Then the adipose cells were extracted and the foundation level and absorbing of insulin induced 2-deoxyglucose (2DG) were assessed.

**RESULTS:** Compared with normal control group, the body weight, fat pad weight of the epididymis and diameter of lipid cells for maternal diabetes offspring rats all increased. Lipid cells of epididymis and the intake of glucose induced by insulin increased. At the same time, glucose was oxidized to CO<sub>2</sub> and increased lipid. However, there was no change in the capacity of *in vitro* lipid decomposition. Also, GLUT4, insulin receptor (IR $\beta$ ), acetyl coenzyme A (ACC), etc. increased in fat pad of maternal offspring of diabetes.

**CONCLUSIONS:** Maternal diabetes had effect on fat metabolism of offspring; lipid storage capacity increased but the ability of lipid decomposition had no change.

*Key Words:*

Pregnancy, Diabetes mellitus, Lipid metabolism, Offspring.

## Introduction

Obesity is one of the main risk factors for diabetes mellitus, high blood pressure, hyperlipidemia, cerebral apoplexy, coronary disease and many other chronic diseases. The correlation

between diabetes mellitus and obesity is the most closed. Some studies showed that obesity in childhood was closely related to the obesity and chronic diseases in adulthood. Therefore, the perinatal period was the key stage of intervention on obesity. The hyperglycemia and hyperinsulinemia in uterus could influence the internal environment of fetal growth and development, which could change its related metabolism and had an effect on the function of many organs and tissues<sup>1</sup>. Exposed to such a high blood glucose levels in the fetal environment, even if the baby had a normal weight when he was born, the risk of overweight or obesity was significantly higher in adults than in normal adults<sup>2,3</sup>. At present, some studies have established the animal model of gestational diabetes mellitus. When a part of  $\beta$  cell of islet was broken, the remaining cells were compensated for differentiation, and secreted insulin to keep normal levels of blood glucose. When  $\beta$  cell of islet completely lost its function, then hypoinsulinemia and hyperglycemia appeared<sup>4-8</sup>. At present, the study has confirmed that the diabetes model of rats could lead metabolic and functional disorders of white adipose tissue in adulthood. While the metabolic phenotype of offspring was not the same, the offspring derived from type 1 maternal diabetes mellitus had the common features of type 2 diabetes mellitus, such as obesity in adulthood, overweight, etc.<sup>9,10</sup>. Some studies described other features of offspring. However, there was still a lack of study on the effect of maternal diabetes on adipose tissue metabolism of offspring. Therefore, in this study, we explored metabolism changes of adipose tissue of neonatal rats by using maternal diabetes models induced by streptozotocin (STZ). It is believed to provide a certain reference and guiding significance to the clinical diagnosis and treatment.

## Materials and Methods

### *The Establishment of Animal Models and Grouping*

There were 15 male SB rats, 12-15 weeks old, the weigh was 200-220 g, which were bought from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd. (Beijing, China). All the rats were raised under standard animal feeding condition. Diluted STZ in citrate buffer solution (10 mM, ph 4.5) according to 140 mg/kg weight. Diabetes models were built for 10 rats by intraperitoneal injection. Only the equivalent buffer was injected to another 5 rats, which was regarded as a control group. The blood was obtained to detect the blood sugar by tail puncture for the rats which were treated by STZ after 3 weeks. Then the rats with blood glucose levels in excess of 150 mg/dl were chosen to mate with normal male rats. After that, the male offspring rats were chosen based on whether their maternal rats had diabetes mellitus. The rats were divided into experimental group (20 rats) and control group (10 rats). After suckling period, the maternal rats were weighted and executed. At the same time, the blood was collected for detecting and determining plasma glucose level and insulin (used specific rat radioimmunoassay kit in accordance with the specification of the product, Millipore, Billerica, MA, USA). The male offspring rats from two groups were raised under standard conditions; the ratio of carbohydrate, protein and lipid in their feedstuff was 4:2:1. All rats were in abrosia for 12 h in the week 14. Then sodium sulfide (25 mg/kg) was used to anesthesia. All rats were put to death. We collected and detected the trunk blood and detected the level of glucose and insulin. Groin and fat pad of epididymis region were obtained by celiotomize. The adipose cell was extracted from the obtained adipose tissues according to the reported methods<sup>17</sup>. All the operations of this study were informed and agreed by the Ethics Committee of our hospital.

### *D-[U-<sup>14</sup>C]-glucose Transport and the Release of <sup>14</sup>CO<sub>2</sub>*

The lipid cells that were isolated from fat pads were suspended in Krebs/Ringer/phosphate buffer (pH 7.4); they contained 1% bovine serum albumin (BSA), and 2 mM glucose. The final concentration was  $1 \times 10^6$  cells/ml, which was immersed in the mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. 450 ml cell suspension was incu-

bated in D-[U-<sup>14</sup>C]-glucose, with or without 10 nM insulin for 1 h at 37°C. 200 ml 8N H<sub>2</sub>SO<sub>4</sub> was added, and 200 ml alcohol was used to wet the released <sup>14</sup>CO<sub>2</sub>, which remained on filter paper for 30 min.  $\beta$  counter for reading radioactive value was applied on filter paper. Then, 2.5 ml dole's reagent (isopropanol: heptane: H<sub>2</sub>SO<sub>4</sub> 4:1:0.25), 1.5 ml normal heptane, and 1.5 ml distilled water were added to the residual reactant and decanted for 5 min. 500 ml upper layer liquid were collected and the radioactivity in lipid was tested. The result was expressed as the nano-molar number released by <sup>14</sup>CO<sub>2</sub> and glucose content of every  $10^6$  cells  $\times$  h entered into lipid.

### *Detection of Lipid Decomposition*

According to the description in the literature, the lipid decomposition for the adipose cells separated from fat pad was detected. Then the glycerol content in incubation buffer was analyzed. The data was expressed as the nano-molar release amount of glycerol for  $10^6$  cells per h.

### *The Expression of IR $\beta$ , ACC, GLUT4 in Fat Pad*

Firstly, low temperature cracking buffer was used to homogenization treatment for the obtained fat pad (the content of lysate included 20 mM Tris-HCl, pH 7.4; 150 mM NaCl, 10% glycerol; Nonidet P-40; 1 mM EDTA; 20 mM rluorol; 30 mM sodium pyrophosphate; 0.2% SDS; 0.5% sodium deoxycholate; 1 mM phenyl methyl sulfide; 1 mM sodium vanadate; 50  $\mu$ M leupeptin and 50  $\mu$ M trasylol). The samples were incubated on the ice for 15 min, then centrifuged for 15 min at 4°C. The protein concentration in liquid supernatant was detected by protein determination method of Bio-Rad Company (Hercules, CA, USA). 50 ug protein was dissolved in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose filter, then sealed off in sealing fluid for 1 h at 22°C. After that, the samples were incubated overnight with primary antibody (IR $\beta$  insulin receptor  $\beta$  subunit), ACC (Acetyl-CoA carboxylase), GLUT4 (glucose transporter 4) and  $\beta$  actin (cell signal was 4970) at 4°C. After incubation, the membrane was washed and re-incubated with secondary antibody combined with peroxidase for 1 h. The density value of band was read by using image density meter of Bio-Rad Company (Hercules, CA, USA).

### Statistical Analysis

SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used to process the data statistics and analysis. The result was expressed as the form of average value  $\pm$  standard deviation. The comparison among groups was made by factor analysis of variance; multiple comparison between groups was performed using *t*-test. If  $p < 0.05$ , the difference had statistical significance.

## Results

### Weight, Food-Intake, Glucose and Insulin Levels of Offspring Rats

The result showed no difference between experimental group and control group in weights when the rats were born. However, the weight of experimental group in the week 12 was higher than that of control group, the difference had statistical significance ( $p < 0.05$ ). 2 weeks before the end of this study, the food-intake of experimental group was also much higher than that of control group ( $p < 0.05$ ). There was no difference in glucose tolerance and insulin levels for these two groups of rats ( $p > 0.05$ ) (Figure 1). Also, the average fat pad weight in the epididymis fat pad of experimental group was significantly higher than that of control group ( $p < 0.05$ ) (Table I).

### The Comparisons of Adipose Tissue and Metabolism Between Two Groups

The average fat diameter in the epididymis fat pad of experimental group was higher than that of control group (83  $\mu$ m vs. 75  $\mu$ m,  $p < 0.05$ ). However, there was no significant difference in fat cell diameter of subcutaneous fat pad and fat pad weight ( $p > 0.05$ ). In absorption detection of 2-deoxyglucose (2DG), we found that the levels of glucose turning into the lipid and oxidation in fat pad of epididymis were higher than that

of control group, no matter whether it was stimulated by insulin ( $p < 0.05$ ) (Figure 1-2). There was no difference in subcutaneous fat pad for two groups ( $p > 0.05$ ) (Figure 3). There was no difference in the lipid decomposition activity of lipid cells in the subcutaneous and epididymis fat pads between these two groups ( $p > 0.05$ ) (Figure 4). To assess the increased of 2DG absorption and the molecular mechanism of the increased amount of glucose entering the lipid under the stimulation of insulin in experimental group, we tested the protein content of IR $\beta$ , GLUT4 and ACC in fat pad of epididymis. The result showed that compared with control group, the protein levels of these three indexes were higher than that of the control group ( $p < 0.05$ ) (Figure 5).

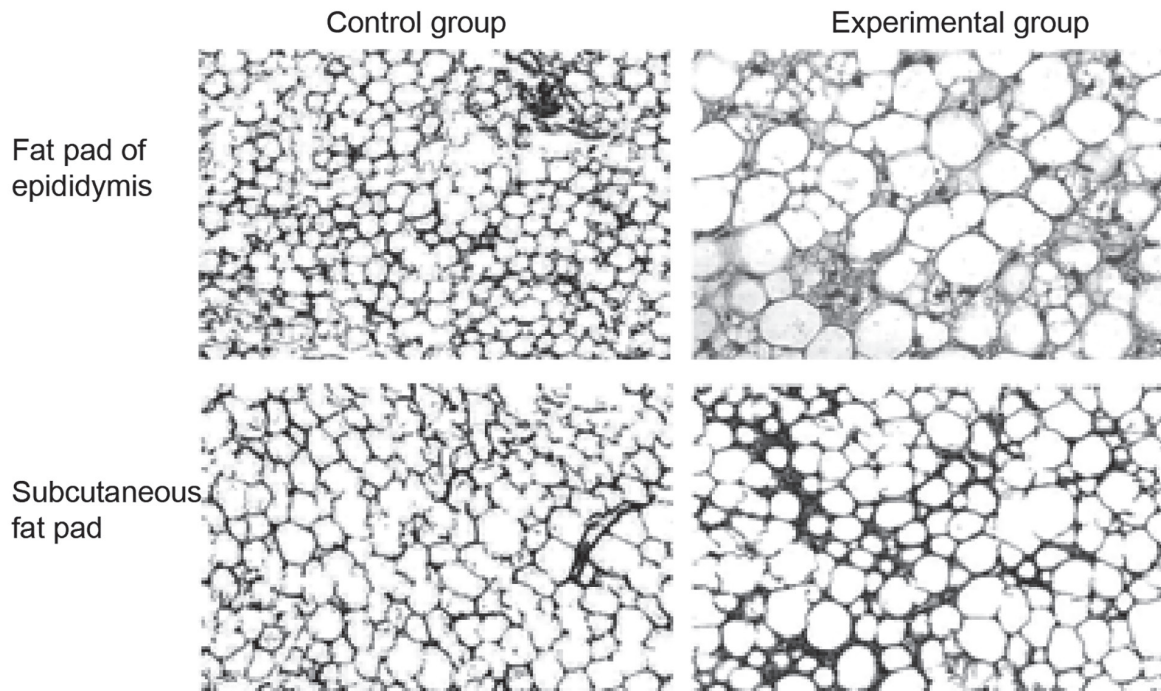
## Discussion

Epidemiology and experimental research evidence showed that the early life events of the fetus and neonatal period could influence the susceptibility to chronic diseases after adulthood. However, the related presupposition assumed that high blood glucose environment in the womb of the fetus could make the physiological and metabolic changes of the fetus. In this work, we used STZ to induce type 1 diabetes mellitus. We found that the blood sugar level, insulin level and weight were all normal when the offspring male rats were born. Nevertheless, overweight and obesity appeared from 14-week old, which had significant difference compared with control group. Some studies confirmed that high blood glucose internal environment of maternal generation could lead the metabolism changes of fetal glucose homeostasis<sup>11,12</sup>. In some researches, diabetes mellitus of maternal generation was induced on the 5<sup>th</sup> day after pregnancy. Moreover, the study found that obesity of offspring appeared in the 14<sup>th</sup> week,

Table I. Basic data statistics.

Item	Control group	Experimental group
Birth weight (g)	8.33 $\pm$ 0.14	8.38 $\pm$ 0.12
Body weight at 12 weeks (g)	261.8 $\pm$ 3.4	332.6 $\pm$ 12.6*
Fat pad weight of epididymis (g)	3.32 $\pm$ 0.3	4.44 $\pm$ 0.25*
Subcutaneous fat pad weight (g)	3.4 $\pm$ 0.26	3.94 $\pm$ 0.26
Food intake (g/day)	17.82 $\pm$ 0.65	28.66 $\pm$ 0.32*
Blood sugar level (mg/dl)	81.77 $\pm$ 4.34	107.8 $\pm$ 10.54
Insulin (ng/ml)	1.19 $\pm$ 0.8	0.98 $\pm$ 0.17

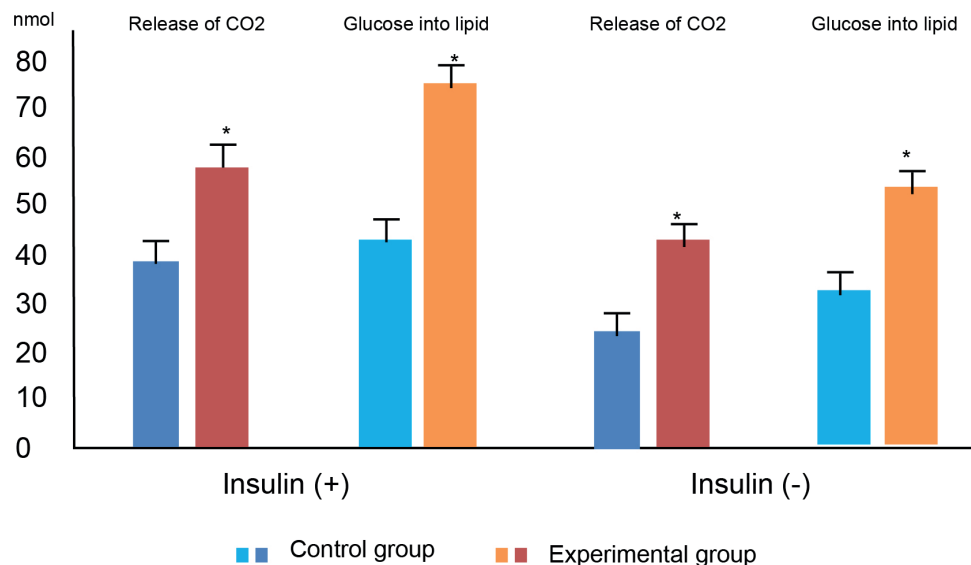
\*Meant the difference between two groups had statistical significance.



**Figure 1.** Macroscopic comparison of cell diameter for two adipose tissues between experimental group and control group under electron microscope. Magnification  $\times 10000$ .

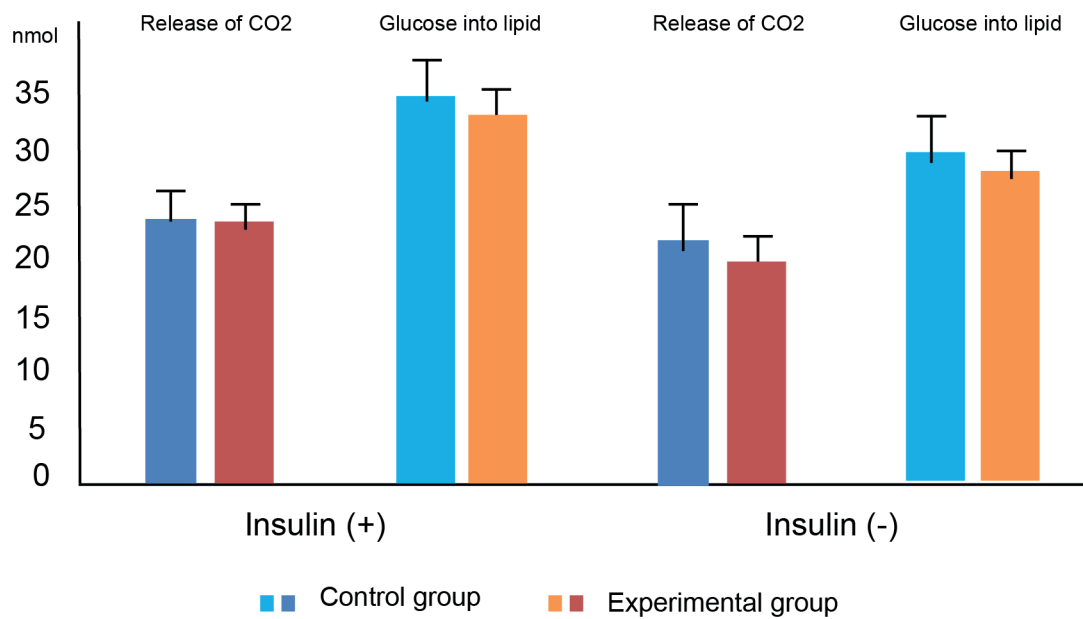
while some of them had normal weight<sup>13</sup>. The increasing of subcutaneous and epididymis fat pad and hypertrophy of lipid cells were the main manifestations of the increasing body weight for offspring rats. In this study, we indirectly detected the ability of lipid cells to combine glucose into lipid to assess the ability of lipid cells to synthesize

and accumulate lipid. The results showed that fat pad of epididymis for offspring rats contained 2-deoxyglucose, and its related transfer was not influenced by insulin. There was no difference of subcutaneous adipocytes between experimental group and control group. The similar performance was the glucose oxidized into  $\text{CO}_2$ . Subcutaneous



**Figure 2.** The comparison of glucose turned into lipid and the release of  $^{14}\text{CO}_2$  with/without insulin stimulation on fat pad of epididymis for two groups.

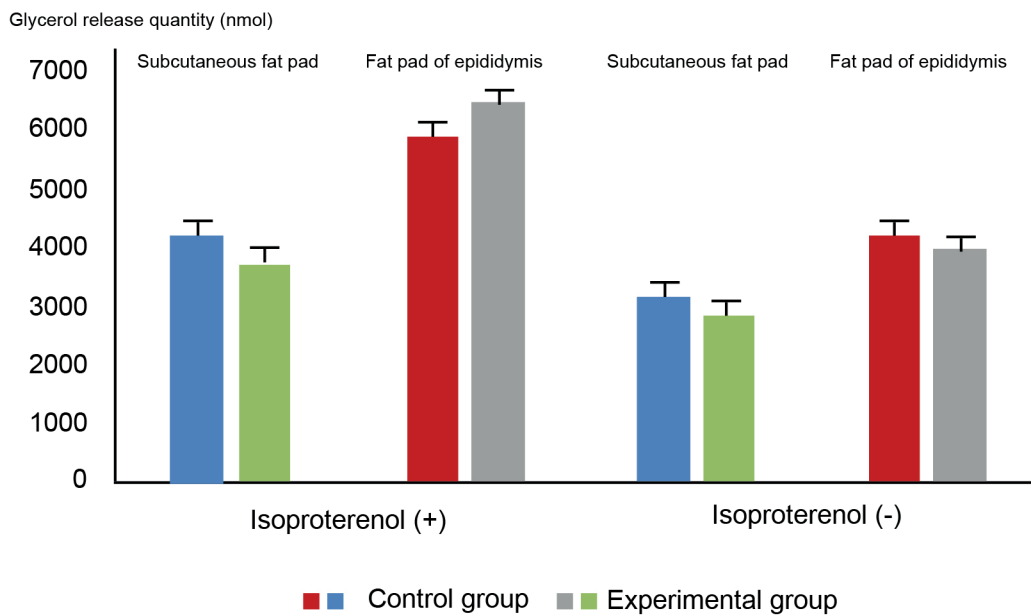




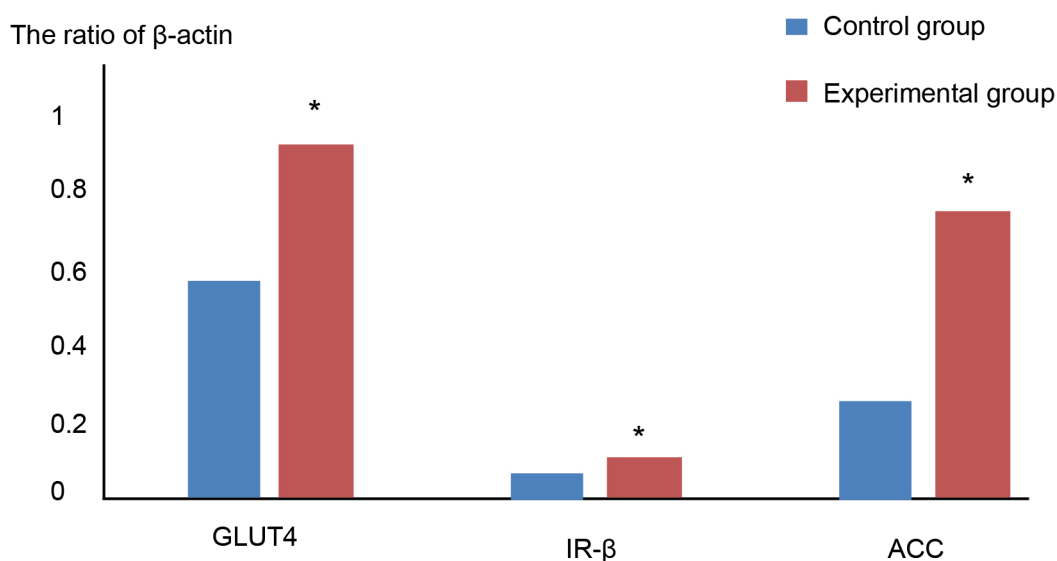
**Figure 3.** The comparison of glucose turned into lipid and the release of <sup>14</sup>CO<sub>2</sub> with/without insulin stimulation on subcutaneous fat pad for two groups.

ous and visceral fat accumulation also showed difference; fat accumulation in epididymis was regarded as a part of visceral fat. Rat visceral lipid cells showed increased levels of gene expression related lipogenesis, lipoclasia and fatty acid oxidation. The study confirmed that visceral adipose tissue was the main driving force of metabolic disorders<sup>14-18</sup>. In this study, the glucose content

of the extracellular matrix was kept constant, so that it controlled the glucose transport volume in cases with or without insulin stimulation. Glucose transporter (GLUT4) was the main glucose transporter for insulin regulation, which could always be found in adipose tissues and striated muscle, when the expression content of local cell membrane had positive correlation to the quantity of



**Figure 4.** The comparison on glycerol content of subcutaneous tissue and fat pad of epididymis between two groups in the condition of with or without adrenaline stimulation.



**Figure 5.** The protein content comparison of IR $\beta$ , GLUT4 and ACC between two groups of rats.

glucose transport into cells. In this paper we found glucose metabolism in lipid cells more tended to the generation of fat. Its related mechanism might be activated by the effect of ACC and FAS in the internal environment of gestational diabetes mellitus. However, it was not influenced by glucose transporter. The results of the study indicated that ACC protein content in the epididymis fat pad of the diabetic offspring rats was indeed higher than that of the control group, which coincide with the above hypothesis. Some investigations used the subcutaneous and visceral adipose tissue of obese sheep fetus. It was found that the gene expression of ACC enzyme increased, which caused the compound of aliphatic acid in adipose tissue increased. After adulthood, the weight, food intake and obesity of the lambs produced by the fat mother were all higher than that of the lambs produced by non obese sheep<sup>19,20</sup>. The increased insulin sensitivity of lipid cells derived from the offspring of diabetic mothers, also reflected in the glucose uptake the absorption of 2DG depended on GLUT4 density in cell membrane<sup>21</sup>. We applied the unit of nmol/cm<sup>2</sup> for describing hexose flow through the membrane surface flow. The results showed the absorbed dose of 2DG in fat pad of experimental group increased under the stimulation of insulin. We further detected the expression level of GLUT4, IR- $\beta$  and ACC protein, which showed their expressions all increased. Thereafter, it was consistent with previous results. Gave high-fat diet to fat offspring rats for 20 weeks after ablactation showed decreased GLUT4 gene

expression in adipose tissue. At the same time, the study found GLUT4 gene expression and protein content in fat sheep fetus all increased<sup>22</sup>.

## Conclusions

We observed that compared with control group, the food-intake for offspring of diabetic rat increased, which was related to the changes in the metabolic mechanisms. The adult male rats showed increased weight and fat accumulation. In conclusion, maternal diabetes can have an effect on fat metabolism of offspring. The lipid storage capacity increased but there was no change in the ability of lipid decomposition.

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## Conflict of Interest

The Authors declare that they have no conflict of interests.

## References

- 1) VRACHNIS N, ANTONAKOPOULOS N, ILIODROMITI Z, DAFOPOULOS K, SIRISTATIDIS C, PAPPA KI, DELIGEOROGLOU E, VITORATOS N. Impact of maternal diabetes on epigenetic modifications leading to diseases in the offspring. *Exp Diabetes Res* 2012; 2012: 538474.

- 2) TAKADA J, MACHADO MA, PERES SB, BRITO LC, BORGES-SILVA CN, COSTA CE, FONSECA-ALANIZ MH, ANDREOTTI S, LIMA FB. Neonatal streptozotocin-induced diabetes mellitus: a model of insulin resistance associated with loss of adipose mass. *Metabolism* 2007; 56: 977-984.
- 3) DE OLIVEIRA AC, ANDREOTTI S, FARIAS TDA S, TORRES-LEAL FL, DE PROENÇA AR, CAMPAÑA AB, DE SOUZA AH, SERTIÉ RA, CARPINELLI AR, CIPOLLA-NETO J, LIMA FB. Metabolic disorders and adipose tissue insulin responsiveness in neonatally STZ-induced diabetic rats are improved by long-term melatonin treatment. *Endocrinology* 2012; 153: 2178-2188.
- 4) RISKIN-MASHIAH S, DAMTI A, YOUNES G, AUSLENDER R. First trimester fasting hyperglycemia as a predictor for the development of gestational diabetes mellitus. *Eur J Obstet Gynecol Reprod Biol* 2010; 152: 163-167.
- 5) DHARURI H, 'T HOEN PA, VAN KLINKEN JB, HENNEMAN P, LAROS JF, LIPS MA, EL BOUZZAOUI F, VAN OMMEN GJ, JANSSEN I, VAN RAMSHORST B, VAN WAGENSVELD BA, PIJL H, WILLEMS VAN DIJK K, VAN HARMELEN V. Downregulation of the acetyl-CoA metabolic network in adipose tissue of obese diabetic individuals and recovery after weight loss. *Diabetologia* 2014; 57: 2384-2392.
- 6) TAKADA J, FONSECA-ALANIZ MH, DE CAMPOS TB, ANDREOTTI S, CAMPANA AB, OKAMOTO M, BORGES-SILVA CD, MACHADO UF, LIMA FB. Metabolic recovery of adipose tissue is associated with improvement in insulin resistance in a model of experimental diabetes. *J Endocrinol* 2008; 198: 51-60.
- 7) HUNTER WA, CUNDY T, RABONE D, HOFMAN PL, HARRIS M, REGAN F, ROBINSON E, CUTFIELD WS. Insulin sensitivity in the offspring of women with type 1 and type 2 diabetes. *Diabetes Care* 2004; 27: 1148-1152.
- 8) SANKAR P, SUBHASHREE S, SUDHARANI S. Effect of *Trigonella foenum-graecum* seed powder on the antioxidant levels of high fat diet and low dose streptozotocin induced type II diabetic rats. *Eur Rev Med Pharmacol Sci* 2012; 16: 10-17.
- 9) LINDSAY RS, NELSON SM, WALKER JD, GREENE SA, MILNE G, SATTAR N, PEARSON DW. Programming of adiposity in offspring of mothers with type 1 diabetes at age 7 years. *Diabetes Care* 2010; 33: 1080-1085.
- 10) CHIMIN P, FARIAS TS, TORRES-LEAL FL, BOLSONI-LOPES A, CAMPAÑA AB, ANDREOTTI S, LIMA FB. Chronic glucocorticoid treatment enhances lipogenic activity in visceral adipocytes of male Wistar rats. *Acta Physiol (Oxf)* 2014; 211: 409-420.
- 11) ALONSO-VALE MI, BORGES-SILVA CN, ANHE GF, ANDREOTTI S, MACHADO MA, CIPOLLA-NETO J, LIMA FB. Light/dark cycle-dependent metabolic changes in adipose tissue of pinealectomized rats. *Horm Metab Res* 2004; 36: 474-479.
- 12) BORGES-SILVA CN, FONSECA-ALANIZ MH, ALONSO-VALE MI, TAKADA J, ANDREOTTI S, PERES SB, CIPOLLA-NETO J, PITHON-CURI TC, LIMA FB. Reduced lipolysis and increased lipogenesis in adipose tissue from pinealectomized rats adapted to training. *J Pineal Res* 2005; 39: 178-184.
- 13) SCHWARTZ R, TERAMO KA. Effects of diabetic pregnancy on the fetus and newborn. *Semin Perinatol* 2000; 24: 120-135.
- 14) TAIN YL, LEE WC, HSU CN, LEE WC, HUANG LT, LEE CT, LIN CY. Asymmetric dimethylarginine is associated with developmental programming of adult kidney disease and hypertension in offspring of streptozotocin-treated mothers. *PLoS One* 2013; 8: e55420.
- 15) MERZOUK H, MADANI S, HICHAMI A, PROST J, MOUTAIROU K, BELLEVILLE J, KHAN NA. Impaired lipoprotein metabolism in obese offspring of streptozotocin-induced diabetic rats. *Lipids* 2002; 37: 773-781.
- 16) BJORN DAL B, BURRI L, STAAL ESEN V, SKORVE J, BERGE RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *J Obes* 2011; 2011: 490650.
- 17) SHI KL, QI L, MAO DB, CHEN Y, QIAN JY, SUN YB, GUO XG. Impact of age on epicardial and pericoronary adipose tissue volume. *Eur Rev Med Pharmacol Sci* 2015; 19: 3257-3265.
- 18) DEVEAUD C, BEAUVOIT B, SALIN B, SCHAEFFER J, RIGOUTLET M. Regional differences in oxidative capacity of rat white adipose tissue are linked to the mitochondrial content of mature adipocytes. *Mol Cell Biochem* 2004; 267: 157-166.
- 19) PALOU M, PRIEGO T, SANCHEZ J, RODRÍGUEZ AM, PALOU A, PICÓ C. Gene expression patterns in visceral and subcutaneous adipose depots in rats are linked to their morphologic features. *Cell Physiol Biochem* 2009; 24: 547-556.
- 20) LONG NM, RULE DC, ZHU MJ, NATHANIELSZ PW, FORD SP. Maternal obesity upregulates fatty acid and glucose transporters and increases expression of enzymes mediating fatty acid biosynthesis in fetal adipose tissue depots. *J Anim Sci* 2012; 90: 2201-2210.
- 21) BADON SE, DYER AR, JOSEFSON JL; HAPO STUDY COOPERATIVE RESEARCH GROUP. Gestational weight gain and neonatal adiposity in the hyperglycemia and adverse pregnancy outcome study-North American region. *Obesity (Silver Spring)* 2014; 22: 1731-1738.
- 22) HAPO STUDY COOPERATIVE RESEARCH GROUP, METZGER BE, LOWE LP, DYER AR, TRIMBLE ER, CHAOVARINDR U, COUSTAN DR, HADDEN DR, McCANCE DR, HOD M, McINTYRE HD, OATS JJ, PERSSON B, ROGERS MS, SACKS DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; 358: 1991-2002.