

Original Research Article

Perinatal taurine supplementation enhances insulin sensitivity in the offspring of female diabetic rats

Pornrut Rabintossaporn¹, Nuntiya Somparn¹, Suphaket Saenthaweesuk¹,
Atcharaporn Somparn², Atcharaporn Thaeomor^{3*}

¹Preclinical Science, Faculty of Medicine, Thammasat University, 12120, ²Office of General Education, Udon Thani Rajabhat University, Udon Thani 41000, ³School of Preclinical Science, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

*For correspondence: **Email:** atcharaporn@sut.ac.th

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Abstract

Purpose: To determine whether perinatal taurine supplementation enhances glucose transporter-4 (GLUT4) expression in the skeletal muscle of adult male offspring of rats born to diabetic mothers.

Methods: Female Wistar rats ($n = 9$) were maintained under standard laboratory conditions with free access to food and water. Diabetes was induced in six rats via a single intraperitoneal injection of streptozotocin (50 mg/kg), followed by 20 % glucose supplementation for 24 hours. The remaining three rats served as non-diabetic controls. After diabetes confirmation, all females were mated with healthy males (3:1 ratio). From conception to weaning, pregnant rats received either 3 % taurine in drinking water ($n = 16$) or plain tap water ($n = 16$), resulting in four groups: Non-DM ($n = 8$), non-DM + T ($n = 8$), DM ($n = 8$), and DM + T ($n = 8$). Following weaning, only male offspring in each group ($n = 8$) were selected and maintained on a standard diet and water. At 16 weeks of age, fasting blood glucose, plasma insulin, lipid profiles, and skeletal muscle GLUT4 protein expression were assessed.

Results: Male offspring of diabetic mothers exhibited significantly elevated fasting glucose and triglyceride levels and reduced insulin levels compared to non-diabetic controls (non-DM; $p < 0.05$). Perinatal taurine supplementation in diabetic dams significantly reduced triglyceride levels, increased plasma insulin, and enhanced GLUT4 expression in offspring skeletal muscle compared to non-diabetic (non-DM; $p < 0.05$) and diabetic groups (DM; $p < 0.05$).

Conclusion: Perinatal taurine supplementation may mitigate metabolic disturbances in the offspring of female diabetic rats by improving insulin levels, reducing triglycerides, and upregulating GLUT4 expression in skeletal muscle.

Keywords: Perinatal, Taurine, Maternal diabetic, GLUT4

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INTRODUCTION

The health of a mother during pregnancy and lactation plays a critical role in shaping the long-term metabolic outcomes of her offspring. A growing body of evidence has linked maternal diabetes to a higher incidence of metabolic

disorders, such as type 2 diabetes and cardiovascular disease, across all age groups of offspring [1,2]. Moreover, the presence of maternal hyperglycemia has been associated with increased risk for both maternal and fetal complications, particularly those affecting the cardiovascular system [3]. Exposure to

gestational hyperglycemia may predispose offspring to conditions such as hypertension, dyslipidemia, and glucose intolerance later in life, partly due to vascular inflammation and endothelial dysfunction, which are early markers of cardiovascular disease [4]. Therefore, preventing diabetes during pregnancy and lactation is vital to reduce the risk of long-term health complications in the next generation.

Taurine, a sulfur-containing amino acid, is involved in numerous physiological processes spanning from fetal development through adulthood in both humans and rodents [5]. During pregnancy and lactation, maternal taurine levels may fall short of the increased metabolic demands, potentially compromising fetal development. Taurine deficiency during the prenatal period has been linked to low birth weight and a higher susceptibility to metabolic conditions, including diabetes and hypertension, in adulthood. Epidemiological data also indicate that insufficient dietary taurine intake correlates with a greater risk of metabolic syndrome and cardiovascular disease—conditions that mirror those observed in the offspring of diabetic mothers [2,4]. To counteract these outcomes, taurine-rich foods such as shellfish (particularly scallops), red meat, and fish (e.g., tuna) are recommended during these critical periods.

Taurine supplementation has demonstrated multiple health benefits, including antioxidant activity, support for growth and development, and the modulation of blood pressure, glucose, and lipid levels [6,7]. These properties suggest its potential to improve metabolic health in the offspring of diabetic mothers.

Glucose uptake in peripheral tissues is largely regulated by the glucose transporter protein family (GLUT), which includes 14 isoforms. Among them, GLUT4 is particularly important in insulin-sensitive tissues such as skeletal muscle and adipose tissue. Insulin facilitates glucose uptake by promoting the translocation of GLUT4 from intracellular storage to the cell membrane. Experimental models have shown that increased expression of GLUT4 enhances insulin sensitivity and glucose homeostasis [8,9]. In fact, boosting GLUT4 expression has been proposed as a promising strategy to combat insulin resistance and type 2 diabetes [10,11].

Given taurine's multifaceted metabolic roles and the critical function of GLUT4 in insulin action, this study aims to explore whether perinatal taurine supplementation prevents the onset of diabetes mellitus in adult offspring of diabetic

rats, potentially through the modulation of GLUT4 expression.

EXPERIMENTAL

Animal model

All animal procedures were conducted in accordance with institutional guidelines and approved by the Animal Ethics Committee of Suranaree University of Technology (approval no. SUT-IAUC-09/2013). Nine female Wistar rats were bred and housed in the university's animal facility under controlled conditions: A 12-h light/dark cycle, ambient temperature of 24 ± 1 °C, and relative humidity of 60 ± 5 %. Animals had free access to standard rat chow and tap water throughout the study.

Diabetes was induced in the female rats by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight. To prevent hypoglycemic shock, rats were given 20 % glucose in tap water for the subsequent 24 hours. Three days post-injection, diabetes was confirmed by measuring fasting blood glucose levels from tail vein samples, with values ranging between 280 and 350 mg/dL.

After confirmation, diabetic ($n = 6$) and non-diabetic ($n = 3$) female Wistar rats, weighing 250 - 330 g, were mated with healthy males ($n = 3$), weighing between 350 - 450 g, at a ratio of 3:1 (females to males). From the day of confirmed conception until the end of lactation, dams were provided with either 3 % taurine in their drinking water (DM + T and non-DM + T groups) or plain tap water (DM and Non-DM groups) [12].

Following weaning, only male offspring were included in further analyses to avoid potential sex-related metabolic differences. In the Non-DM and Non-DM + T groups, five to six male pups were selected per dam. In contrast, due to low birth rate, only two to three male pups per dam were selected in the DM and DM + T groups. All offspring continued to receive standard chow and tap water post-weaning. Each group consisted of eight male rats ($n = 8$).

At 16 weeks of age, male offspring were anesthetized via intraperitoneal injection of thiopental (50 mg/kg body weight). Blood samples were collected through cardiac puncture. The gastrocnemius muscle and retroperitoneal adipose tissues were carefully dissected, blotted using Whatman No. 1 filter paper, weighed, and immediately snap-frozen in liquid nitrogen. All tissue samples were stored at -80°C until further analysis.

Biochemical assay

Fasting blood glucose levels were measured immediately after blood collection from rat tail vein using a glucometer (Accu-CHECK, Roche Diagnostics). At the end of the experiment, additional blood samples were obtained via cardiac puncture. Plasma was then separated by centrifugation and stored at -20°C until further analysis. Plasma insulin concentrations were quantified using a commercially available Rat/Mouse Insulin ELISA kit (Cat. #EZRM1-13K, Merck Millipore, USA), following the manufacturer's protocol. Plasma lipid parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were analyzed at the clinical biochemistry laboratory of Thammasat University Hospital, Pathum Thani, Thailand.

Histological examination of the pancreas

Pancreatic tissues were promptly harvested and fixed in 4 % paraformaldehyde for 24 hours at room temperature. Fixed tissues were then processed, embedded in paraffin, and sectioned at 5 μm thickness. Tissue sections were stained with hematoxylin and eosin (H&E) using standard protocols. The stained sections were examined under a light microscope to assess the histological architecture of the pancreatic islets of Langerhans.

Western blot analysis

Tissue samples were rinsed in ice-cold phosphate-buffered saline (PBS) and homogenized in radioimmunoprecipitation assay (RIPA) buffer supplemented with a protease inhibitor cocktail. The total protein concentration in the lysates was quantified using the Pierce™ Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific, USA), following the manufacturer's instructions.

Equal amounts of protein (15 μg per sample) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Boston, MA, USA). Membranes were blocked with 5 % non-fat dry milk in Tris-buffered saline containing 0.1 % Tween-20 (TBS-T) for 2 hours at room temperature to prevent nonspecific binding.

After blocking, the membranes were incubated overnight at 4°C with primary antibodies diluted 1:1,000 in TBS-T: anti-GLUT4 (sc-53566 HRP)

and anti- β -actin (sc-47778 HRP), both obtained from Santa Cruz Biotechnology (USA). Following three washes with TBS-T, membranes were subjected to enhanced chemiluminescence (ECL) detection using the Amersham™ ECL™ Prime Western Blotting Detection Reagent (GE Healthcare, USA).

Protein band intensities were visualized and analyzed using Image Studio version 2.1 software (LI-COR® Biosciences, USA). β -actin served as an internal loading control to ensure equal protein loading across all samples [13].

Statistical analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). Comparisons among the four groups were conducted using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range post hoc test to determine statistically significant differences between groups. Statistical analysis was performed using StatMost32 software (version 3.6, Dataxiom, CA, USA). A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Effect of maternal diabetes on body weight and blood biochemistry in male adult offspring

At 16 weeks of age, the four groups had no significant differences in body, heart, or kidney weights (Table 1).

Table 1: Body, kidney, and heart weights in male offspring rats

Treatment	Body (g)	Heart (g)	Kidney (g)
Non-DM	385 $\pm$ 5.42	0.36 $\pm$ 0.02	1.39 $\pm$ 0.04
Non-DM+T	388 $\pm$ 7.02	0.35 $\pm$ 0.01	1.35 $\pm$ 0.05
DM	372 $\pm$ 5.4	0.38 $\pm$ 0.02	1.32 $\pm$ 0.03
DM+T	374 $\pm$ 7.03	0.37 $\pm$ 0.03	1.36 $\pm$ 0.02

Note: Data are mean $\pm$ SEM. There were no significant differences among groups: Control (Non-DM), Control plus taurine supplementation (non-DM + T), Diabetes (DM), Diabetes plus taurine supplementation (DM + T), *n* = 8 each group

In the DM group, fasting blood glucose was higher, whereas insulin level was lower compared to non-DM group (*P* < 0.05; Figure 1). However, taurine supplementation (DM + T group) showed lowered fasting blood glucose and higher insulin levels compared with the DM group (*P* < 0.05; Figure 1).

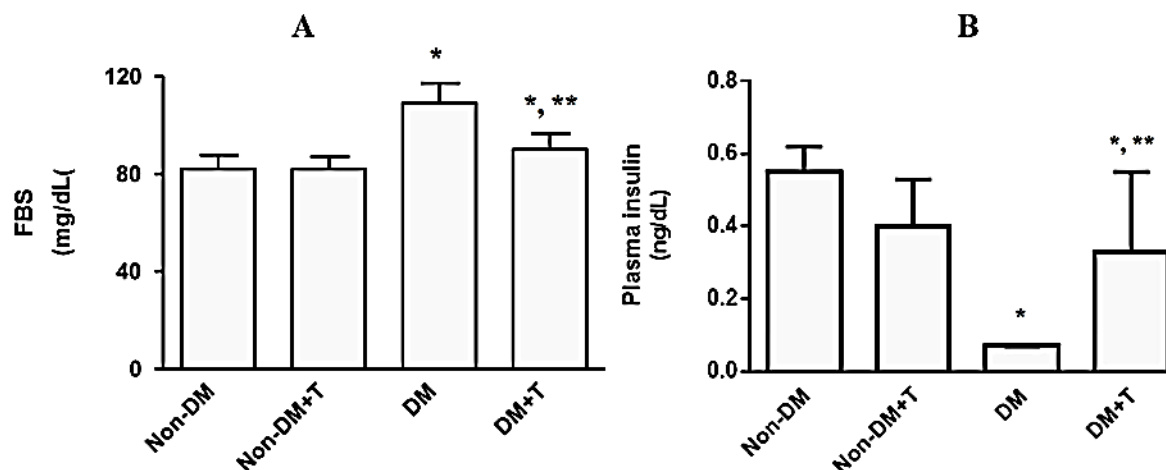


Figure 1: Effect of taurine supplementation on (A) Fasting blood sugar (FBS) and (B) Plasma insulin level of adult male offspring. **Key:** Control (Non-DM), Control plus taurine supplementation (Non-DM + T), Diabetes (DM), Diabetes plus taurine supplementation (DM + T). Each bar represents the mean $\pm$ SEM, $n = 8$. Statistically significant difference vs Control (Non-DM): * $P < 0.05$ and ** $P < 0.05$ vs DM

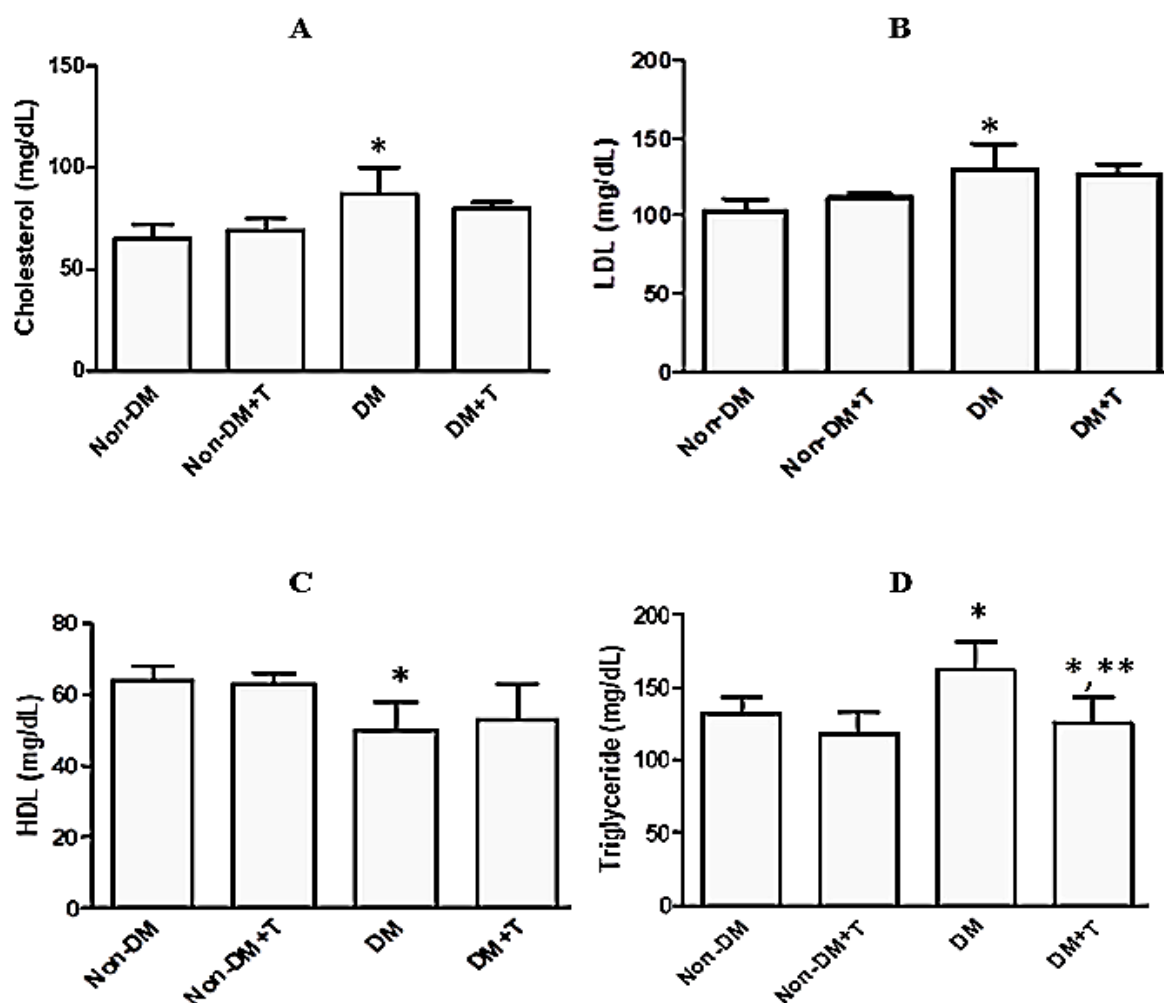


Figure 2: Effect of taurine supplementation on lipid profiles of adult male offspring. A. Cholesterol, B. LDL, C. HDL, D. Triglyceride. **key:** Control (Non-DM), Control plus taurine supplementation (Non-DM + T), Diabetes (DM), Diabetes plus taurine supplementation (DM + T). Each bar represents the mean $\pm$ SEM, $n = 8$. Statistically significant difference vs Control (Non-DM): * $P < 0.05$ and ** $P < 0.05$ vs DM

For lipid profile, triglyceride level increased in the DM group compared to the other three groups (Figure 2). Whereas triglyceride level in taurine supplementation group (DM + T) was significantly lowered compared to the DM group ($p < 0.05$). Cholesterol and LDL levels tend to be significantly lower in the taurine supplementation group (DM + T) compared to the DM group ($p > 0.05$). Additionally, perinatal taurine supplementation did not adversely affect blood chemistry parameters in the control groups (non-DM compared to non-DM + T).

Histology staining of pancreatic islets

Figure 3 illustrates the histopathology of rat pancreas. The results indicate that the non-DM and non-DM + T groups exhibit the typical appearance of islets of Langerhans (Figure 3). In contrast, diabetic rats display pathological changes characterized by an irregular shape and obvious shrinkage in the islets of Langerhans. However, diabetic rats supplemented with perinatal taurine exhibit more intact and typical architecture of the islet cells (Figure 3).

Effect of taurine supplementation on GLUT 4 protein expression in skeletal muscle

Perinatal taurine supplementation in diabetic conditions led to a notable increase in GLUT4 expression, as evidenced by Western blot

analysis of GLUT4 protein in skeletal muscle ($P < 0.05$; Figure 4). However, perinatal taurine supplementation did not affect GLUT4 expression in the control group.

Effect of taurine supplementation on GLUT4 protein expression in adipose tissue

In the adipose tissue, a trend to decreased levels of GLUT4 expression (Figure 5) was noted in DM compared with the non-DM group ($P = 0.08$). Additionally, no significant differences in GLUT4 expression were observed between the DM and DM + T groups.

DISCUSSION

Previous studies have identified maternal diabetes mellitus as a key risk factor for the development of metabolic disorders in offspring, including dyslipidemia, type 1 and type 2 diabetes mellitus, and hypertension in adulthood [2,4]. In the present study, type 1 diabetes was induced in pregnant rats using streptozotocin (STZ), a compound that selectively destroys pancreatic β -cells, leading to insulin deficiency and maternal hyperglycemia. As a result, adult offspring of diabetic dams exhibited hyperglycemia and dyslipidemia - hallmarks of metabolic syndrome.

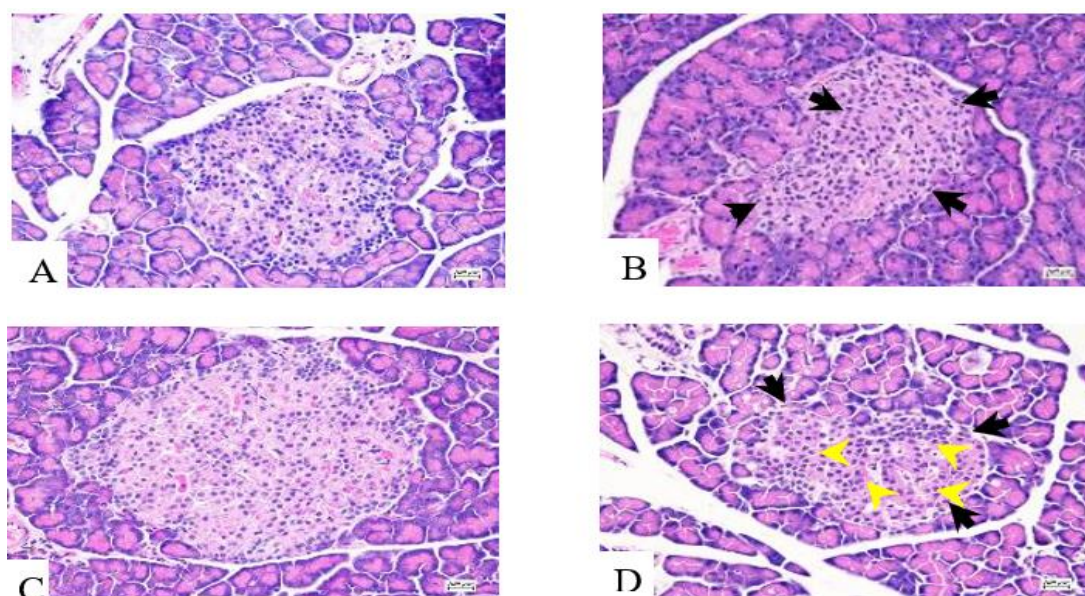


Figure 3: Histopathological observations of taurine-treated pancreas in streptozotocin-induced diabetic rats. (A) Non-DM, Presence of normal-sized, circular islets of Langerhans and pancreatic islet cells. (B) DM, induced by STZ, shows an irregular shape and obvious shrinkage in the islets of Langerhans (black arrow). (C) Non-DM + T, represents taurine-treated rats, show normally sized, circular islets of Langerhans. (D) DM + T, the morphological changes in the islets of Langerhans were improved in the DM group treated with taurine compared to the DM group. The cells in the islets of Langerhans showed an increase in size, appearing rounder (yellow arrowhead) than those in the DM group

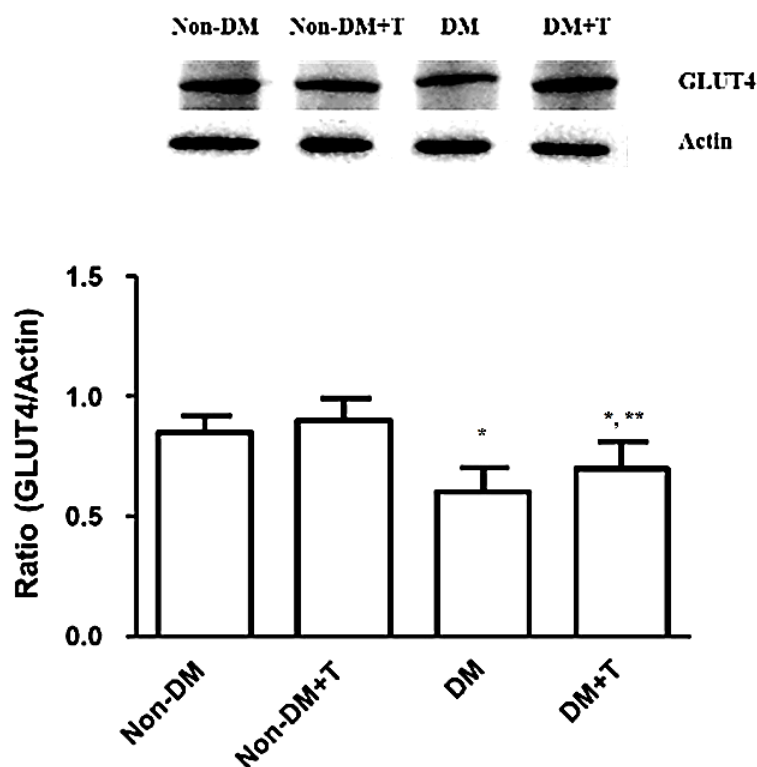


Figure 4: Effect of taurine supplementation on GLUT 4 protein expression in skeletal muscle of adult male offspring. **Key:** Each bar represents the mean $\pm$ SEM, $n = 8$. Statistically significant difference vs Control (Non-DM): * $P < 0.05$ and ** $P < 0.05$ vs DM. Non-Control (Non-DM), Control plus taurine supplementation (Non-DM + T), Diabetes (DM), Diabetes plus taurine supplementation (DM + T)

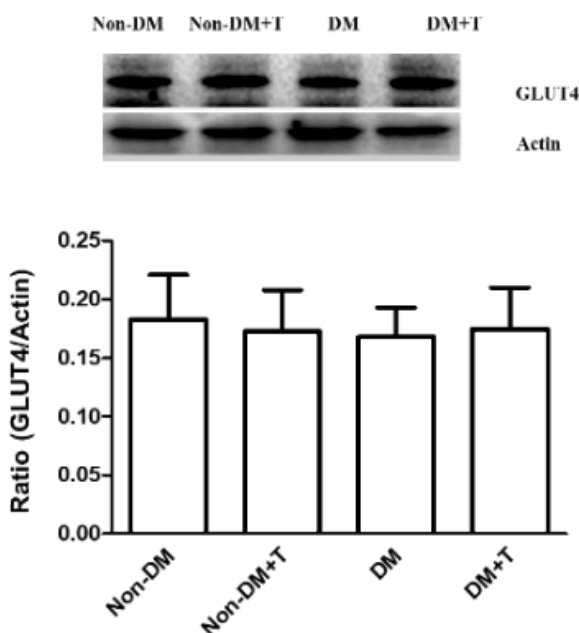


Figure 5: Effect of taurine supplementation on GLUT4 protein expression in adipose tissue of adult male offspring. **Key:** The expression of GLUT4 proteins was determined by Western blot analysis; representative images. Each bar represents the mean $\pm$ SEM, $n = 8$. Non-Control (Non-DM), Control plus taurine supplementation (Non-DM + T), Diabetes (DM), Diabetes plus taurine supplementation (DM + T)

These outcomes are likely attributable to both genetic predisposition and adverse intrauterine environmental conditions, which may promote insulin resistance, β -cell dysfunction, impaired glucose metabolism, and reduced insulin sensitivity [14,15].

Taurine supplementation during the perinatal period appeared to attenuate some of these adverse outcomes. In our study, maternal taurine intake improved insulin levels and preserved islet morphology in adult offspring. Prior studies have shown that taurine protects pancreatic β -cells from glucotoxicity by regulating mitochondrial calcium homeostasis and stabilizing protein conformation. Moreover, taurine administration has demonstrated a dose-dependent protective effect against STZ-induced β -cell damage in rodent models [16,17]. Data further suggest that taurine may ameliorate hyperglycemia and dyslipidemia in type 1 diabetes by reducing lipid peroxidation and lowering circulating lipid levels [18]. Supporting these findings, a study involving non-obese diabetic pregnant mice receiving a taurine-enriched diet through weaning reported enhanced islet mass and protection against β -cell cytotoxicity and hypoinsulinemia in their adult offspring [19]. Additionally, taurine has been shown to improve insulin sensitivity and regulate lipid metabolism by modulating GLUT4

expression in models of diet-induced metabolic dysfunction [20]. In line with these reports, our data demonstrated that taurine supplementation in diabetic dams led to increased plasma insulin levels and restoration of islet architecture in adult offspring. These improvements in insulin function suggest that perinatal taurine supplementation may offer protective effects against hyperglycemia and dyslipidemia - two key metabolic disturbances commonly associated with maternal diabetes.

CONCLUSION

Perinatal taurine supplementation prevents dyslipidemia and hyperglycemia, which are adverse effects of maternal diabetes in adult offspring. These effects may be attributable to increased GLUT4 expression in muscle tissue and associated increased plasma insulin level with restoration of beta cell function.

DECLARATIONS

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Ethical approval

All procedures were approved by the Suranaree University of Technology's Animal Care and Use Committee and conducted following the National Institutes of Health guidelines (license no. SUT-IAUC-09/2013).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors have accepted responsibility for the entire

content of this manuscript and approved its submission. Protocol/project development: Pornrut Rabintossaporn, Nuntiya Somporn and Atcharaporn Thaeomor. Data collection or management: Pornrut Rabintossaporn, Nuntiya Somporn, Suphaket Saenthaweesuk, Atcharaporn Somporn and Atcharaporn Thaeomor. Data analysis: Pornrut Rabintossaporn, Nuntiya Somporn, Suphaket Saenthaweesuk, Atcharaporn Somporn, and Atcharaporn Thaeomor. Manuscript writing/editing: Pornrut Rabintossaporn, Nuntiya Somporn and Atcharaporn Thaeomor.

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