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Original Article

Beneficial Effects of Megadoses of Biotin in Streptozotocin-induced Gestational Diabetes Mellitus in Rats – A Preliminary Study

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Abstract

Gestational diabetes (GDM) is one of the most commonly observed obstetrical complications, affecting between 5–18% of all pregnancies worldwide. In pregnancy, hyperglycemia poses both short-term and long-term risks to the health of women and their offspring. Country-specific estimates have further brought out that India had the highest number of women affected by hyperglycemia in pregnancy. Despite much general progress in clinical management, GDM still represents a major medical challenge. Proper management of GDM can reduce the fetal mortality and morbidity and also better postpartum lifestyle for women. The present study investigates the beneficial effect of megadoses of biotin in streptozotocin-induced gestational diabetes mellitus in rats. Female virgin rats were injected with 50 mg/kg body wt. streptozotocin and were allowed to mate. Some pregnant rats were treated with 1 mg/kg body wt. biotin daily for 19 days. On the 19th day the rats were sacrificed, after withdrawal of blood. Hyperglycemia and hypercholesterolemia were observed in the diabetic rats. Treatment with biotin normalised these values. Biotin treatment improved maternal reproductive performances as well fetal outcomes. Biotin may be beneficial in the prevention/ management of gestational diabetes.

Introduction

Gestational diabetes (GDM) is one of the most commonly observed obstetrical complications, affecting between 5–18% of all pregnancies worldwide (1). The risk of developing GDM is highest among Asian (particularly South Asian), black, American Indian, and Hispanic women (2).

In pregnancy, hyperglycemia poses both short-term and long-term risks to the health of women and their offspring. Experimental and clinical studies have demonstrated that hyperglycemia of maternal diabetes is associated with early intrauterine growth retardation and deformities in embryos which results in increased prematurity, infant mortality and morbidity rates. Individuals born to mothers with GDM have higher risk of obesity and type 2 diabetes as adults (3).

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Country-specific estimates have further brought out that India had the highest number of women affected by hyperglycemia in pregnancy with an estimated 5.7 million cases in 2013, followed by China with 1.2 million. In India, GDM prevalence rates ranges from around 5% to 18% (4). A recent observational study from Tamil Nadu revealed that GDM was prevalent in 16.3% of the study population (5).

Thus, GDM is not only of clinical relevance, but is also an important public health issue. Despite much general progress in clinical management, GDM still represents a major medical challenge. Current management of GDM includes changes in diet and exercise, and administration of human insulin to those patients for whom dietary advice fail to achieve desired glycemic goals. Prescription of rapid acting insulin analogues (insulin lispro, insulin aspart) is also increasing (6, 7). The National Institute for Health & Care Excellence (NICE) clinical practice guidelines recommend use of metformin and glyburide instead of insulin if life style interventions fail to control glycemic levels (6). However, there has been much debate about efficacy and safety of oral antidiabetic drugs for use in GDM patients.

In pregnancies complicated by diabetes, hyperglycemia and lipid metabolism alterations are associated with both maternal and fetal complications (8). Proper management of GDM can reduce the fetal mortality and morbidity and also better postpartum lifestyle for women.

Biotin is a water-soluble B complex vitamin. Biotin has stimulatory effects on genes whose action favors hypoglycemia such as insulin, insulin receptor, pancreatic and hepatic glucokinase; on the other hand, biotin decreases the expression of hepatic phosphoenolpyruvate carboxykinase, a key gluconeogenic enzyme that stimulates glucose production by the liver (9). Pharmacologically, biotin can reduce type I and type II diabetes blood sugar levels, improve the experimental rat's glucose tolerance and insulin resistance (10). Pharmacological concentrations of biotin reduce serum glucose, triglycerides and cholesterol (11). Biotin is important for normal embryonic growth (12).

The present study is aimed at investigating the antidiabetic effect of megadoses of biotin and to analyse the reproductive outcome and fetal outcome in diabetic rats. The objectives are to determine the effects of megadoses of biotin on

1. Plasma glucose, insulin, and cholesterol level in maternal rats

2. Reproductive outcomes- quantal pregnancy, gestational index, post implantation loss, and birth index

3. Fetal weight, crown rump (CR) length and external malformations

Materials and Methods

Chemicals and reagents

Streptozotocin was purchase from Sigma Chemical Co., St. Louis, MO, USA. Kits for glucose and total cholesterol were purchased from Wako Pure Chemical Industries. Insulin RIA kit was purchased from Shionogi & Co. (Osaka, Japan). All other chemicals and reagents used were of analytical grade and were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai, India.

Animals and animal treatment

This study was conducted in accordance with the Guide for Care and Use of Laboratory Animals, and was approved by the IRB and IAEC. Female virgin adult Wistar rats weighing 150-180 gm were used for the study. The animals were kept under standard laboratory conditions (22±3°C, 12-h light/dark cycle), fed with standard rat chow, and tap water ad libitum.

Induction of Diabetes

Diabetes was induced in rats as described earlier (13). Adult virgin Wistar rats (150-180 gm body wt.) were injected i.p. streptozotocin (50 mg/kg body wt.in freshly prepared citrate buffer 0.1 M, pH 4.4). Control rats were administered citrate buffer alone. Three days later blood sample was drawn from the lateral

tail vein of rats and glucose was measured by portable glucometer (One Touch Ultra Johnson & Johnson, USA). Rats with fasting blood glucose > 300 mg % were chosen for the study. Food intake, non-fasting blood glucose concentrations and urine sugar were recorded once weekly. The body weight was measured twice weekly. Two weeks later the diabetic rats/control rats were placed for mating.

Establishment of pregnancy

For mating, the female diabetic /healthy and healthy male rats were placed together in the cage in the ratio of 3: 1 and checked daily for the presence of vaginal plaque (VP). The presence of VP was considered to be at zero day of pregnancy. The mating protocol was followed for 15 consecutive days, i.e. approximately three estrous cycles. The females that failed to become pregnant during this period were considered infertile and excluded from the study (14).

Biotin treatment protocol

VP positive healthy rats were randomized into three groups and treated as follows for 19 days

Group I (7 rats) - saline alone

Group II (6 rats) - 1 mg/kg body wt. biotin i.p.

Group III (6 rats) - 2 mg/kg body wt. biotin i.p.

VP positive diabetic rats were randomized into three groups and treated as follows for 19 days

Group IV (22 rats) - saline alone

Group V (8 rats) - 1 mg/kg body wt. biotin i. p.

Group VI (8 rats) - 2 mg/kg body wt. biotin i. p.

The dose of biotin was chosen as described earlier (15). Body weight was recorded twice weekly.

Experimental protocol

On day 19 of pregnancy, rats were weighed, anesthetized with sodium thiopental and blood was obtained by cardiac puncture. Laparotomy was then performed to remove the uterine horns. Maternal blood was collected for assays of insulin, glucose, and cholesterol. The uteri were removed and examined in situ for the presence and location of resorption sites and for live and dead foetuses. Foetuses were removed, weighed, and examined for external malformations.

Measurement of the plasma metabolic parameters

The concentrations of plasma glucose, and total cholesterol were determined by using kits purchased from Wako Pure Chemical Industries. The plasma insulin concentration was measured by a radioimmunoassay, using a kit purchased from Shionogi & Co. (Osaka, Japan).

Calculation of reproductive outcomes

The reproductive performances such as quantal pregnancy, implantation index, post implantation loss, birth index, and gestational index were calculated as described previously (16). Quantal pregnancy = (number of pregnant dams/number mated) × 100; implantation index = (total number of implants/ number mated) × 100; post-implantation loss = [(number of implantations – number of viable implantations)/number of implantations] × 100; birth index = (number of pups born/number of implantations) × 100; and gestation index = (number of live pups/number of pregnant dams) × 100.

Collection of fetal data

The fetuses were weighed, and CR length was measured. Fetuses were evaluated under a dissecting microscope for congenital anomalies.

Statistical analysis

The Kruskal–Wallis test, followed by Dunn's test, was used for comparison between experimental groups as regards the number of implantations, birth index, post implantation loss, fetal weight and CR length. For biochemical parameters and maternal weight gain Analysis of Variance (ANOVA) was

applied, followed by Bonferroni test. The percentage values were calculated by the Fisher Exact Test (17). Differences were considered statistically significant when $p<0.05$.

Results

Effect of biotin treatment on plasma glucose, cholesterol, and insulin

Plasma glucose and cholesterol were significantly higher in the diabetic rats as compared with control. Treatment with biotin normalised the values to almost control values (Fig. 1). Plasma insulin was higher in the diabetic rats as compared with control. However, the result was not statistically significant. Treatment of diabetic rats with biotin normalised the plasma insulin level to control values.

Effect of biotin treatment on maternal reproductive performance

Since there was no significant difference in the serum biochemical parameters between the 1 mg and 2 mg/kg body wt. biotin treated diabetic groups, we used the data from 1 mg /kg body wt. treated groups for further analysis. The effect of biotin treatment on maternal reproductive performance is shown in Table I. Maternal weight gain was significantly lower in the

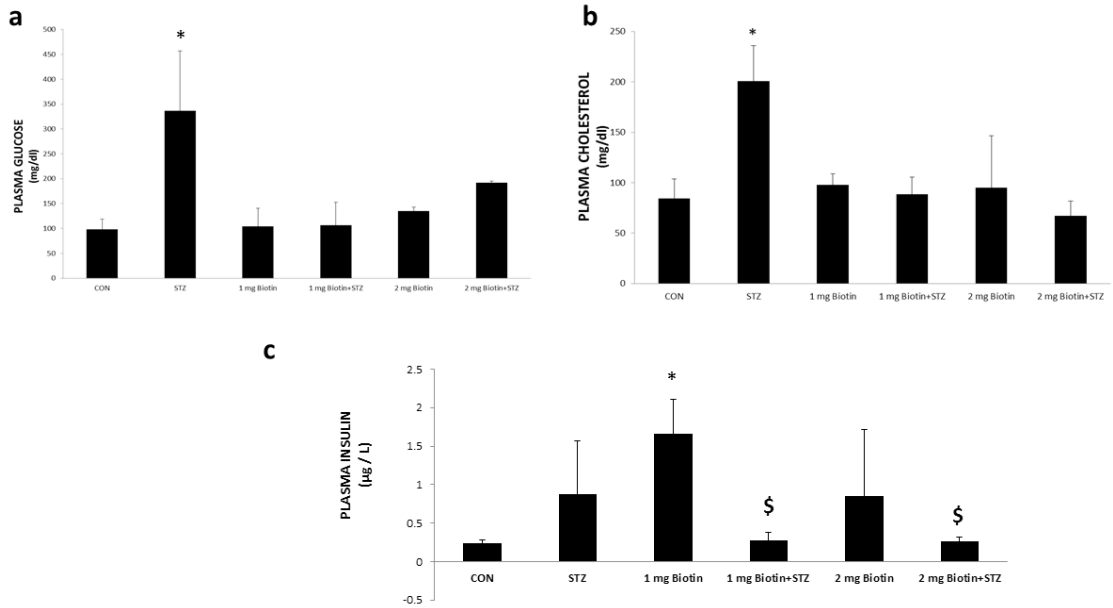

Fig. 1: a. Plasma glucose levels in the control rats and experimental rats. Data represent Mean±SD, n=6 in each group. *p<0.01 when compared with control values. b. Plasma cholesterol levels in the control rats and experimental rats. Data represent Mean±SD, n=6 in each group. *p<0.01 when compared with control values. c. Plasma insulin levels in the control rats and experimental rats. Data represent Mean±SD, n=6 in each group. *p<0.01 when compared with control values. $p<0.05 when compared with 1 mg / body wt. biotin treated group.

TABLE I: Effect of biotin treatment in reproductive outcomes in rats.

Parameter	*Control (n=8)*	*Biotin (n=6)*	*STZ (n=22)*	*Biotin + STZ (n=8)*
Maternal weight gain (g)	47.33±10.21	46.67±11.52	26.41±4.62$^{*,\$}$	38.89±11.32$^{\#}$
Quantal Pregnancy (%)	90.3	100	20.7$^{*,\ \$}$	100$^{\#}$
Gestational Index (%)	440.55	416.75	433.57	460.86
Post Implantation Loss (%)	10.8	13.5	30.3$^{*,\$}$	2.6$^{*,\ \#,\$}$
Number of foetus/Pregnancy	4.4±2.2	4.7±2.7	4.4±2.0	5.1±2.8
Implantation Index (%)	374.24	366.96	177.55$^{*,\$}$	420.96 $^{\#,\$}$
Birth Index (%)	85.4	86.3	66.6$^{*,\$}$	92.3$^{\#}$

N = number of rats. Data represent Mean±SD and proportions (%). * P<0.05 vs control, # P<0.05 vs. STZ, and $ P<0.05 vs. biotin.

diabetic rats as compared with control. Treatment of diabetic rats with biotin significantly increased the body weight .Quantal pregnancy, implantation index, and birth index were significantly lower in the diabetic rats as compared with control. Post implantation loss was higher in the diabetic rats. Treatment with biotin improved these reproductive outcomes significantly.

Effect of biotin treatment on fetal weight

The weights of the fetuses of diabetic mothers was significantly lower than that of control rats. Treatment of diabetic rats with biotin significantly increased the weights of fetuses (Fig. 2)

Effect of biotin treatment on gross fetal malformations

There were no gross malformations in the fetuses of control rats, biotin alone treated rats and STZ+ biotin treated rats. Limbs and nostrils were not fully formed in 10 % of the fetuses of STZ treated rats. Ears, eyes, and mouth were not fully developed in 15 % of the fetuses of STZ treated rats. The appearance of the foetuses is shown in Fig. 3.

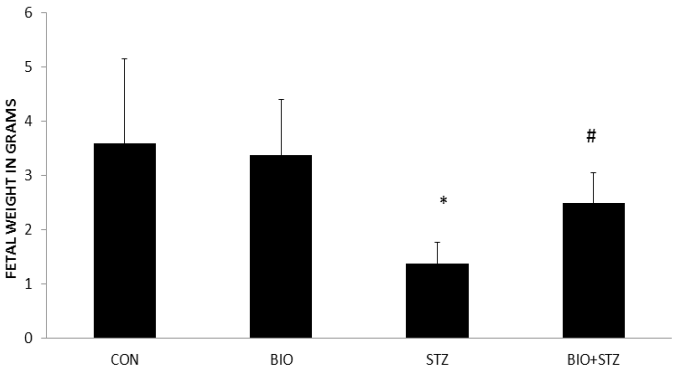

Fig. 2 : Effect of biotin treatment on fetal weight .Data represent Mean±SD, n=12 in each group. *p<0.05 when compared with control, #p<0.05 when compared with STZ.

Effect of biotin treatment on CR length

CR length was reduced in the fetus of diabetic rats. However, it was not statistically different from the fetuses of control rats. Treatment of diabetic rats with biotin increased the CR length in the fetuses to control values (Fig. 4)

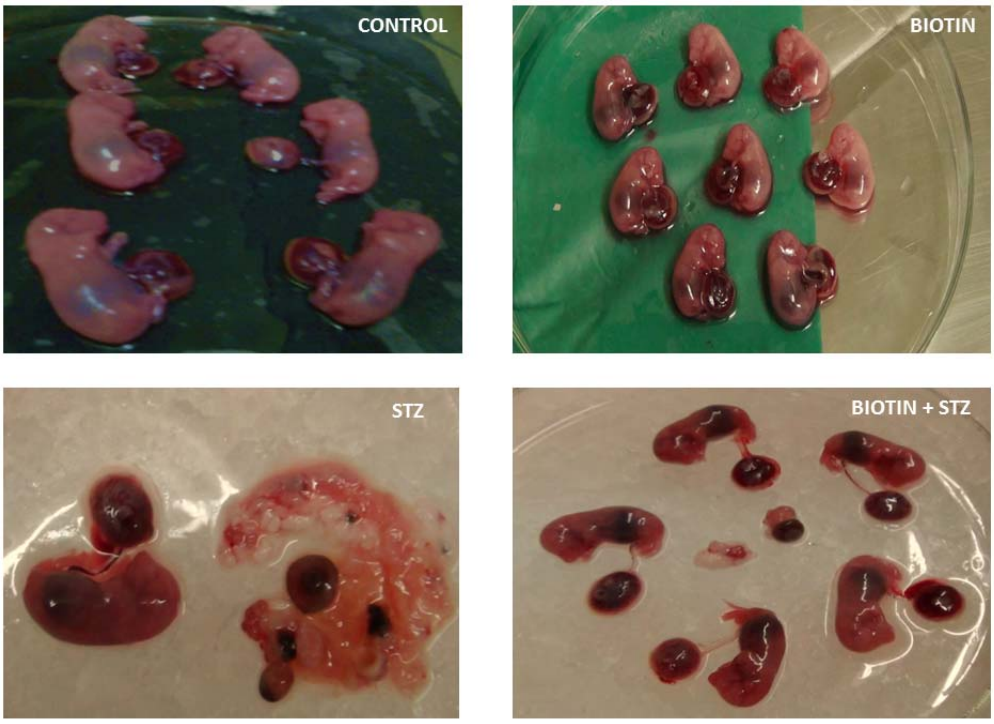

Fig. 3 : Representative images of foetuses of control rats, biotin treated rats, STZ treated rats and biotin + STZ treated rats. Control rats had 6 normal foetuses, biotin treated rats had 8 normal foetuses, STZ treated rats had one abnormal foetus and one aborted foetus, and biotin + SYZ treated rats had 5 normal fetuses.

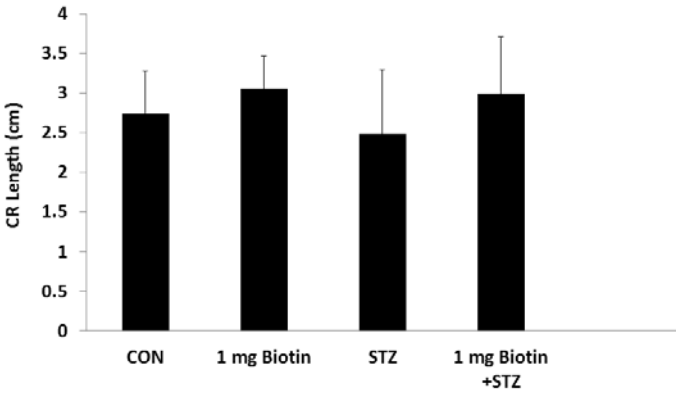

Fig. 4 : Effect of biotin treatment on CR length of foetuses. Data represent Mean±SD, n=12 in each group.

Discussion

GDM is not only of clinical relevance, but is also an important public health issue. Despite much general progress in clinical management, GDM still represents a major medical challenge. Proper management of GDM can reduce the fetal mortality and morbidity and also better postpartum lifestyle for women. Pharmacological doses of biotin have been shown to possess hypoglycemic effect (10). Likewise, pharmacological doses of biotin appear to decrease plasma lipid level and modify lipid metabolism. Several studies have shown that pharmacological concentrations of biotin reduce hyperglycemia and hypertriglyceridemia in type 1 and 2 diabetic patients (18). The blood sugar lowering capacity of biotin is now established in human clinical trials and animal models of Type 2 diabetes studies (19-21).

Although the antidiabetic effects of biotin have been proven in type 1 and type 2 diabetes, its beneficial effects in gestational diabetes has not been reported yet to the best of our knowledge. The results of the present study show that biotin has antidiabetic effect as evidenced by its ability to decrease plasma glucose and cholesterol levels. Biotin may act in multiple ways to exert its hypoglycemic effect. Biotin has stimulatory effects on hepatic glucokinase, whose action favors hypoglycemia (15); on the contrary, biotin decreases the expression of hepatic phosphoenolpyruvate carboxykinase, a key gluconeogenic enzyme that stimulates glucose production by the liver (9). This may decrease the need for insulin in the biotin treated rats. Recent studies have shown that pharmacological concentrations of biotin enhance insulin secretion and the expression of genes and signaling pathways that favor islet function (22). In addition, biotin has been shown to enhance ATP synthesis in pancreatic islets of the rat, resulting in facilitation of glucose-induced insulin secretion (23).

Biotin treatment improved reproductive outcomes in the diabetic rats, improved fetal weight and reduced the incidence of fetal malformations.

The effects of biotin on carbohydrate metabolism and the lack of toxic effects of the vitamin at pharmacological doses suggest that biotin could be used in the development of new therapeutics in the management of GDM (24). We are currently investigating the molecular mechanism of the antidiabetic effect of biotin in GDM.

Conclusion

Megadose of biotin can reduce serum glucose levels in rat model of gestational diabetes. In addition megadose of biotin can improve reproductive outcomes in the diabetic rats, fetal weight and reduce the incidence of fetal malformations.

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Conflicts of interest

There are no conflicts of interest.

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