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RESEARCH ARTICLE

L-ARGININE CONTROLS CARDIAC PARAMETER DEFECTS INDUCED BY PREGESTATIONAL DIABETES IN OFFSPRING

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ABSTRACT

Background: Offspring of diabetic mothers can have a transient cardiomyopathy, which is major complication of diabetes. L-arginine may aid in reducing the risk of cardiovascular-related complications such as high blood pressure, high cholesterol and other heart diseases. This observation indicates to examine the effect of arginine in off springs of diabetic mother with different physical andcardiac parameters.

Methods: Female mice were treated with streptozotocin (STZ) to induce pregestational diabetes prior to breeding with normal male to produce offspring. Off spring of diabetic mice were divided into two groups. The one group (Diabetic Arginine Group) of experimental offspring were given 50 mg L-arginine PO (free base, per kg body wt per day) for 2 months. Second group of animals were only STZ diabetic (Diabetic Group). Control group (non-diabetic) were given tap water. Body weight, blood glucose, blood pressure, heart rate, heart weight, LV weight and o- tyrosine level were measured.

Result: Bodyweight, heart weight, heart rate, LV weight, and O-Tyrosine, Serum Glucose concentration showed a significant increase ($p<0.05$) in diabetic group (DG) compared with control groups while diabetic treatment group that has received L-Arginine showed a significant ($p<0.05$) decrease compared with diabetic group.

Conclusion: Our data suggest that L-arginine controls the major biochemical abnormalities of diabetes in offspring caused by maternal hyperglycemia. It clearly indicates that accumulation of arginine can overcome the physical and cardiac parameters in diabetic offspring.

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INTRODUCTION

Numerous epidemiological studies have demonstrated that the presence of diabetes is a predictor of not only coronary artery disease, but also heart failure. The Framingham Heart Study showed the frequency of heart failure is twice in diabetic men and five-fold in diabetic women compared with age-matched non-diabetic cohorts (Kannel *et al*., 1974). The increased risk of heart failure in diabetic patients persisted after taking into account age, hypertension, hyper cholesterolemia, and coronary artery disease. Although coronary artery disease and hypertension are major causes of heart failure in patients with diabetes, a considerable number of diabetic patients without these complications develop heart failure. The cardiomyopathy seen in very poorly controlled gestational diabetes is likely different from the hypertrophic cardiomyopathy (HCM) found in better controlled IDM (Wolfe *et al*., 1977; Naeye *et al*., 1965; Breitweser *et al*., 1980; Cooper *et al*., 1992; Gutgesell *et al*., 1980). The HCM seen in infants of well controlled diabetic mothers is characterized by asymmetric septal hypertrophy with individual myocyte hypertrophy and limited myofiber disarray (Gutgesell *et al*., 1980) in vascular endothelilal cells (ECs), nitric oxide (NO) synthase (NOS) uses L-arginine to produce NO, which maintains blood flow and reduces inflammation (Farquhar *et al*., 1959 and Rizzo *et al*., 1991). Reduced availability of L arginine to NOS and the resultant reduction of NO production have been implicated in the vascular dysfunction associated with diabetes and other cardiovascular disease states (Rizzo and Arduini, 1991; Vela-Huerta, 2000). Acute L-arginine supplementation can prevent or reverse EC dysfunction and restore EC dependent vasodilation in diabetes (Brosnan *et al*., 1983). Reduced availability of L-arginine for NOS can occur via increased activity or expression of arginase, an enzyme that competes

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with NOS for L arginine, producing ornithine and urea. L-arginine plays a reverse critical role in collagen synthesis and fibrosis by providing the substrate (Pieper *et al*., 1997).

MATERIALS AND METHODS

Animals

C57BL/6 wild type mice were used. Animals in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH publ. no. 85–23, revised 1996).All procedures were performed within the regulations of the Animal Welfare Act and the National Institutes of Health Guide for the Care and use of Laboratory Animals.

Induction of Diabetes mellitus

Eight weeks old female mice were treated with streptozotocin (STZ, 80 mg/kg body weight) for 3 consecutive days. Mice treated with tap water served as controls. Non-fasting blood glucose levels were determined one week after STZ injection using a gluco-meter (One Touch Ultra2, Life Scan). Mice with blood glucose levels higher than 11 mmol/L were bred to normal adult males. Mating was verified by observation of a vaginal plug (Wender-Ozegowska *et al*., 2004). Body weight and glucose levels were noted. Their off springs were divided into two groups and used for treatment with L arginine. Further study was started at age of 12 week. The one group (Diabetic Arginine Group) of experimental offspring were given 50 mg L-arginine PO (free base, per kg body wt per day) for 2 months. Second group of animals were only diabetic (Diabetic Group). Control group were given tap water. All the animals were observed in good health. At the end of the experiment, animals were sacrificed by neck dislocation, and necropsies were performed. The body tissues were taken for biochemical investigations.

Measurement of blood pressure and Heart Rate

Blood pressure and heart rate were determined in conscious animals from all groups by an indirect tail-cuff method (Indirect blood pressure meter BP-98A, Soft on TM, Tokyo, Japan). Before the measurements, mice were placed in the restrainers for several times and the measures were always performed by the same person. The pressure records were made after a 15 to 20 min of quietude (Fisher *et al*., 2000). Three stable consecutive measurements of blood pressure and heart rate were averaged.

Determination of O-Tyrosine

We developed Tyrosine determination using stable isotope dilution reversed-phase HPLC. The advantages of this method are the very short sample preparation without derivatization, a short chromatographic run, the use of selective detection to eliminate interference, and the use of ideal internal standardization (stable isotope). Tyrosine was achieved by HPLC (Hewlett-Packard 1100 series) using a Hypersil C-184mm 3100mm column containing 3mm particles (Hewlett-Packard). The column temperature was held at 24 °C. The mobile phase was isocratic, at 93% solvent A(1 mL/L TFA in H2O) and 7% solvent B (1 mL/L TFA in 100 mL/L H2O–900 mL/L acetonitrile), the flow rate was 0.6 mL/min (Wender-Ozegowska *et al*., 2004).

Statistical Analysis

The statistical analysis was done by using the SAS system v. 6.11. Results are expressed as means ± SE. Differences between groups were analyzed by one-way ANOVA. *P*-values are two-sided and considered significant when $P < 0.05$.

RESULTS

Body Weights and Laboratory Data

Table 1 shows the body weights and laboratory data. The statistical analysis for Serum Glucose concentration (mg/dl) revealed that the Diabetic Group showed a significant increase ($P<0.05$) in serum glucose concentration (534 ±16.8 mg/dl) compared with Control Group in the same period (519 ±13.5 mg/dl).Meanwhile Diabetic Arginine Group was showed significant ($P<0.05$) decreased in serum glucose concentration at (532±18.3 mg/dl) compared with Diabetic Group in the same period.

Table 1. Physical parameters of study animals

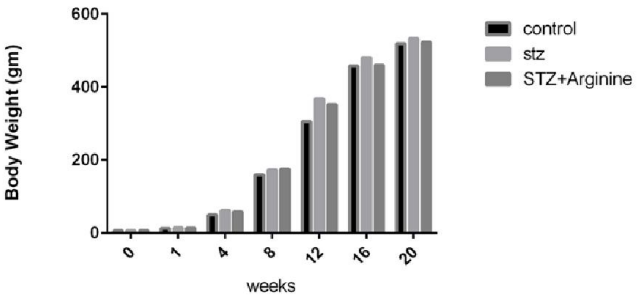
	Control	STZ	STZ + L-arg
Body Weight(gm)	519±13.5	534±13.8	532±18.3
Serum Glucose (mg/dl)	249±16	627±19	601±11
MABP (mmHg)	102±4	123±8	110±7
Heart Rate(BPM)	216±12	196±15	200±16

MAPB- Mean arterial blood pressure. BPM- Beats per minute. Values are mean ± SEM, n=10.

Table 2. Cardiac Parameters

	Control	STZ	STZ + L-arg
Heart Weight (gm)	2.27±0.31	2.36±0.10	2.31±0.21
LV Weight (mg)	86.3±2.2	91.7±2.1	88.3±4.2
O-tyrosine (nmol/L per 100gm heart tissue)	4.2	2.9	3.6

Figure 1. Body weight of the offspring at different weeks



In the diabetic group body weight, mean arterial blood pressure and heart rate were increased by STZ treatment but arginine treated animals were found decreased body weight, blood pressure and normal heart rate as shown in Table 1. Relatively heart weight(2.36±0.10), left ventricular weight(61.7±2) were found increased by STZ treatment but arginine treated animals

were found decreased heart weight (2.31±0.21), left ventricular weight (88.3±4.2) as shown in table 2. O-Tyrosine level was decreased in STZ treated group(2.8 nmol/L 100gm heart tissue) than the control group (4.2 nmol/L 100gm heart tissue)mean while at the same time arginine treated animals were found increased (3.6 nmol/L 100gm heart tissue) than STZ treated group.

DISCUSSION

L-arginine can help diabetics through a number of mechanisms. For example, it may aid in reducing the risk of cardiovascular-related complications such as high blood pressure, high cholesterol and heart diseases (Schrier *et al*., 1999). This is because L-arginine creates a chemical in body that works to relax blood vessels. Our study show that The Diabetic Group showed significant increase ($P<0.05$) in concentration of serum glucose concentration, body weight, heart weight, blood pressure compared with Control group in the same period that occur due to destroy of the ß-cells in pancreas and absent of insulin secretion (Elsner, 2006). Also results demonstrate that administration of L-Arginine to the diabetic arginine group showed significant ($P<0.05$) decrease in the serum glucose concentration, body weight, heart weight, blood pressure compared with diabetic group in the same period. Hyperglycemia causes increased oxidative stress because the production of several reducing sugars is enhanced via glycolysis and the polyol pathway (King *et al*., 1993). Oxidative stress plays a role in the development of diabetic complications (Engerman *et al*., 1977). In the body, the amino acid arginine changes into nitric oxide (NO). Nitric oxide is a powerful neurotransmitter that helps blood vessels relax and also improves circulation. Some evidence shows that arginine may help improve blood flow in the arteries of the heart (Brownlee *et al*., 2001). That may improve symptoms of arteries, chest or angina, and coronary artery disease. However, currently no data on how the long-term use of arginine affects cholesterol or heart health. L-Arginine normalize hyperglycemia and can act as anti oxidant by scavenge superoxide anion [O^{-2}] radical causing inhibition of oxidation process (Samuelsson *et al*., 2008). Analysis of the data showed a 4% increase in insulin sensitivity in the control group, compared to a 34% increase in the arginine group. And although the arginine didn't completely resolve insulin sensitivity issues, the change was a significant improvement. The arginine group as a whole also experienced a decrease in systolic blood pressure (Fukuzawa *et al*., 1999; Popov *et al*., 2002; Mendez and Balderas, 2001; Flynn, 2002). Analysis of the data showed a 4% increase in insulin sensitivity in the control group, compared to a 34% increase in the arginine group. And although the arginine didn't completely resolve insulin sensitivity issues, the change was a significant improvement. The arginine group as a whole also experienced a decrease in systolic blood pressure. Major effect of arginine can be seen on reducing o-tyrosine level with reduction in left ventricular weight (Brilla *et al*., 1990).

Ethical approval

No ethical approval was required for this review article.

Disclosure

The authors declare no conflict of interest

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