Effect of Streptozotocin-Induced Diabetes on Bone and Heart Development in Juvenile Rats

Joo-Heon Kim[1,#], Youngjeon Lee[2,#], Sangun Lee[3], Takao Suzuki[3], Sang-Kil Lee[4], Taeyoung Kang[5] and Yonggeun Hong[2,4,†]

[1]*Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea*
[2]*Deaprtment of Rehabilitation Science in Interdisciplinary PhD Program, Graduate School of Inje University, Gimhae 621-749, Korea*
[3]*Department of Physical Therapy, Aomori University of Health and Welfare, Aomori 030-8505, Japan*
[4]*Cardiovascular & Metabolic Disease Center, College of Biomedical Science & Engineering, Inje University, Gimhae 621-749, Korea*
[5]*College of Veterinary Medicine, Jeju National University, Jeju 690-756, Korea*

ABSTRACT

Our objective of current study was to investigate the development of bone and heart in association with diabetes mellitus (DM). DM was induced by administering an intraperitoneal injection of streptozotocin (STZ; 60 mg/kg) to 4-week-old Sprague-Dawley rats. Body weight and blood glucose were monitored, and rats were sacrificed after 2 or 5 weeks. The left ventricle (LV), including the interventricular septum, was weighed, and body weight and tibial bone length were assessed. Young diabetic rats showed reduced growth in terms of tibial length and body weight compared to controls. Moreover, diabetic males showed more significant growth suppression and reduced LV size than diabetic females. Morphometric analysis of tibiae from diabetic rats revealed suppressed bone growth at 2 and 5 weeks, with no difference between genders. STZ-induced diabetes decreased bone growth and retarded pre-pubertal heart development. As a result, diabetes may increase cardiovascular risk factors and lead to eventual heart failure. Therefore, new therapeutic approaches are required for diabetic children exhibiting growth retardation. Heart growth factor, exercise, and cardiopulmonary physical therapy may be required to promote heart development and physiological function.

(Key words : Diabetes, Growth retardation, Hypertrophy, Streptozotocin(STZ), Tibial bone length)

INTRODUCTION

Juvenile insulin-dependent diabetes mellitus (IDDM) adversely affects linear growth and pubertal development (Chiarelli *et al.*, 2004). Although Mauriac syndrome, the most significant expression of growth alteration due to severe insulin deficiency in diabetic patients, is now rare, delayed puberty and reduced adult height in diabetic children are still occasionally reported (Vanelli *et al.*, 1992), and subtle abnormalities in growth and weight gain are frequently observed (Dunger *et al.*, 2002). These effects reflect persistent endocrine and metabolic abnormalities, even in children on appropriate insulin therapy (Dunger *et al.*, 2002).

Diabetes is characterized by increased blood glucose level as well as gradually reduction of blood insulin. Consequently, the ability to transport glucose across the cell membrane is impaired, and cells are compromised with respect to the growth-promoting properties of insulin. Diabetes is a chronic progressive disease that results in microvascular and macrovascular complications (Fonarow, 2005). Diabetes mellitus (DM) is one of the most important risk factors for cardiovascular disease (Bauters *et al.*, 2003; Fonarow, 2005), and numerous risk factors exist for patients with both diabetes and heart failure (Ho *et al.*, 1993). Diabetes is a major predictor of heart failure. Diabetic myopathy, including of the heart and skeletal muscle, is related to hyperglycemia, but the mechanisms by which hyperglycemia causes the pathological remodeling of myocytes, leading to tissue atrophy and cell dysfunction, are not understood. Programmed cell death, or apoptosis, plays a critical role in cardiac pathogenesis.

Approximately 15~25% of patients with heart failure are diabetic (Ryden *et al.*, 2000), and it has been sugge-

* This work was supported by a grant (Code No. 20100301-061-100-001-03-00 to Y. Hong) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

# These authors contributed equally.

† Corresponding author : Phone: +82-55-320-3681, E-mail: yonghong@inje.ac.kr

sted that DM may play an important role in the pathogenesis, prognosis, and treatment response in heart failure (Solang *et al.*, 1999). However, little is known regarding the mechanisms of pathological heart remodeling, cardiac dysfunction, and heart failure.

Recent studies have suggested that DM may cause left ventricular (LV) dysfunction and a subsequent increase in heart failure. Factor *et al.* (1996) noted that the structural and functional changes associated with diabetic cardiomyopathy are only observed when hypertension coexists with diabetes. Recently, comorbid type 2 DM and hypertension were shown to be associated with LV hypertrophy (Palmieri *et al.*, 2001). Comorbid hypertension and DM may further increase the risk of cardiovascular target organ damage and decrease clinical responsiveness (Sowers, 2004).

Increased blood glucose increases blood viscosity and hemodynamic stress on the heart, leading to pathological heart remodeling and development disability, in association with growth retardation caused by insulin deficiency (Salerno *et al.*, 2003). We examined whether prepubertal diabetes induces cardiac hypertrophy using a streptozotocin (STZ)-induced rat model of type 1 DM. Furthermore, we examined whether gender-specific differences exist on body growth and heart development between juvenile male and female diabetic rats.

MATERIALS AND METHODS

Experimental Design

Experiments were designed to determine whether diabetes reduces bone growth or causes heart hypertrophy. Eight male and twelve female rats were randomly assigned to the control (3 males, 5 females) or diabetic (5 males, 7 females) group.

Animal Care and Housing

All experiments were approved by the Ethics Committee for Animal Research at Inje University. Four-week-old Sprague-Dawley rats (Samtaco, Korea) of both sexes were used. All rats were housed two per cage, grouped according to gender, under controlled environmental conditions (23℃), with an established photoperiod of 12/12 h light/dark cycle (lights on: 06:00 h). Animals were provided standard rodent chow (Hyochang Science, Korea) and water *ad libitum* throughout the study. Blood glucose levels and body weight were monitored in both of the designated 2- and 5-week experimental groups.

STZ Injections and Blood Glucose Assessment

Diabetes was chemically induced via an intraperitoneal injection of 60 mg/kg STZ (Sigma, St. Louis, MO, USA), a specific pancreatic β-cell toxin, dissolved in sodium citrate buffer (pH 4.5). Three days later, blood was collected via tail vein puncture, and blood glucose was determined using a glucometer (Super Glucocard, ARKRAY, Japan). We adopted a minimum blood glucose level of 200 mg/dl as our criterion for having established moderate diabetes. Animals with blood glucose levels below 200 mg/dl received a second STZ injection and were reassessed for blood glucose. Non-responders were omitted from the study. All blood sampling was performed between 09:00 and 11:00 hr on fed, unanesthetized animals. All animals were observed daily for signs of poor health. Blood glucose was measured prior to sacrifice at 2 or 5 weeks (Fig. 1).

Tissue Collection and Analysis

At 2 and 5 weeks, one or two animal from the control and three or four animals from the diabetic group were sacrificed. All animals were weighed and then anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). Hearts and tibiae were collected and stored at −70℃ (Revco, USA) until analysis. After that, hearts were weighed to the nearest 0.01 g on an electronic balance. The LV, including the interventricular septum, was isolated by removing the atria and the free wall of the right ventricle and then weighed. Both the right and left tibiae were removed and cleaned of muscle and connective tissue. Bone length, from the top of the tibial plateau to the bottom of the lateral malleolus process, was measured using a Vernier caliper (Mitutoyo, Japan).

Statistical Analysis

Data were collected from each experiment and are presented as mean±SD. One-way ANOVA used for statistical analysis. Differences were considered to be significant at $p<0.05$. All analyses were performed using the Statistical Package for the SPSS software (ver. 17.0).

RESULTS

Body Weight

Diabetic rats showed a significant decrease in body weight gain compared to the age-matched controls. Interestingly, diabetic male rats showed weight loss compared to baseline (Fig. 2). Furthermore, diabetes resulted in decreases of 66 and 229% in final body weight in females and males, respectively, compared to healthy rats at the end of the 5-week experiment. These results suggest that diabetes reduces the rate of somatic growth in both sexes, and that males are affected to a greater degree than females.

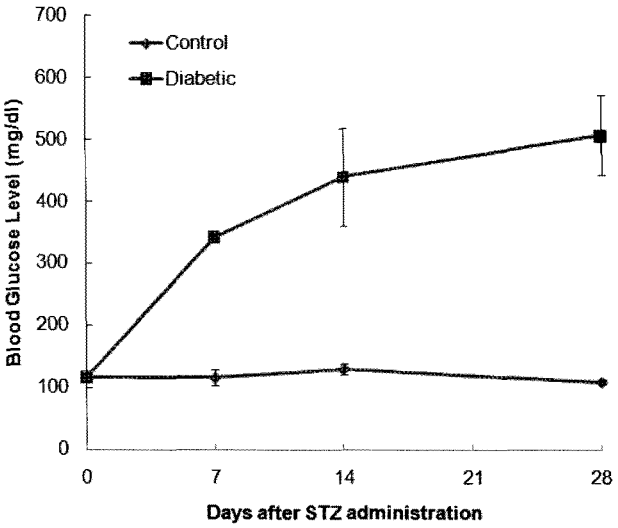


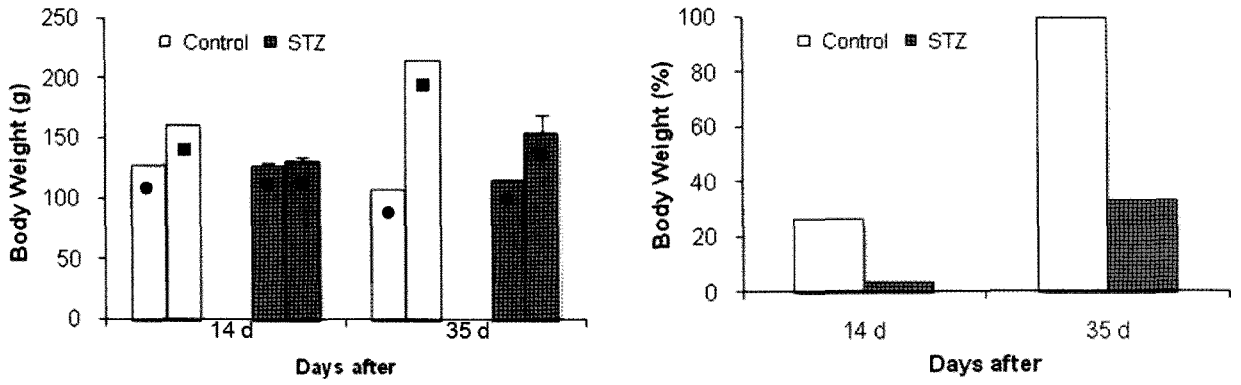
Fig. 1. Blood glucose levels of diabetic rats. Blood glucose level of STZ-induced diabetic vs. control rats.

Morphometric Analysis of Tibiae

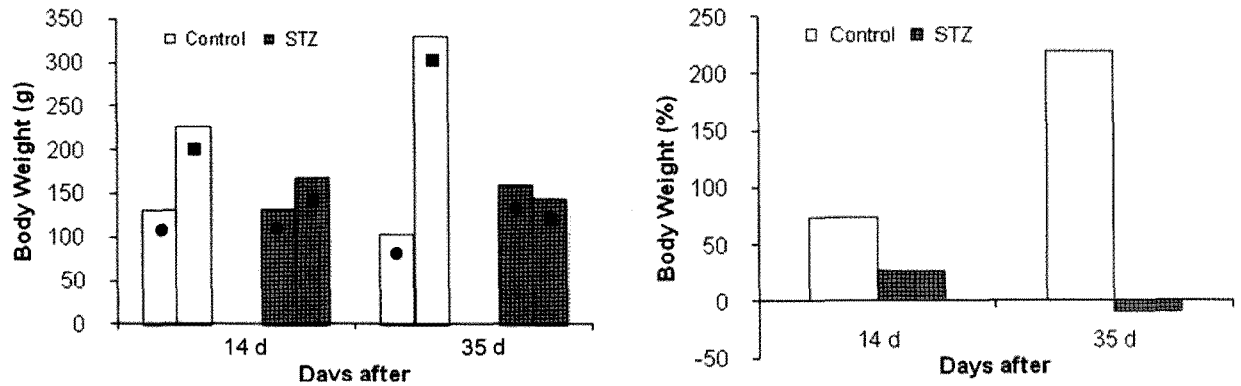
Compared to the control, diabetes suppressed tibial bone growth in both sexes at 2 and 5 weeks after STZ induction into animals (Fig. 3). Male diabetic rats appeared to show a lesser degree of suppression than female rats, but this difference of tibial bone length was not significant. These results indicate that diabetes affects bone growth to a similar degree in both sexes (Fig. 4).

LV Weight/Body Weight (BW) Index

Among female rats, heart weight increased approximately 7.9% in control rats, whereas diabetic induction decreased heart weight approximately 8% (Fig. 5A). In males, however, heart weight decreased approximately 5.9% in control rats, whereas heart weight increased 1.2% in diabetic rats (Fig. 5B). The gender difference in LV/BW in control animals reflects the marked difference in body weight gain shown in Fig. 2; that is, control males showed a greater body weight gain than control females over both 2 and 5 weeks. In contrast, among diabetic rats, males showed much lower body weight gain or weight loss over 2 and 5 weeks (Fig. 2B), respectively, resulting in an increase in LV/BW index (Fig. 5B). These results imply that diabetes results in abnormal heart development with res-

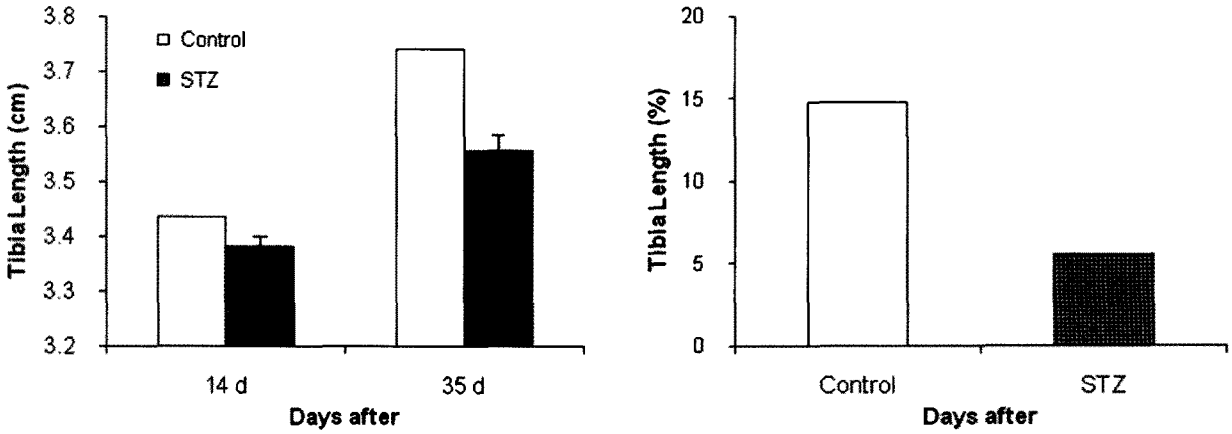


(A) Female

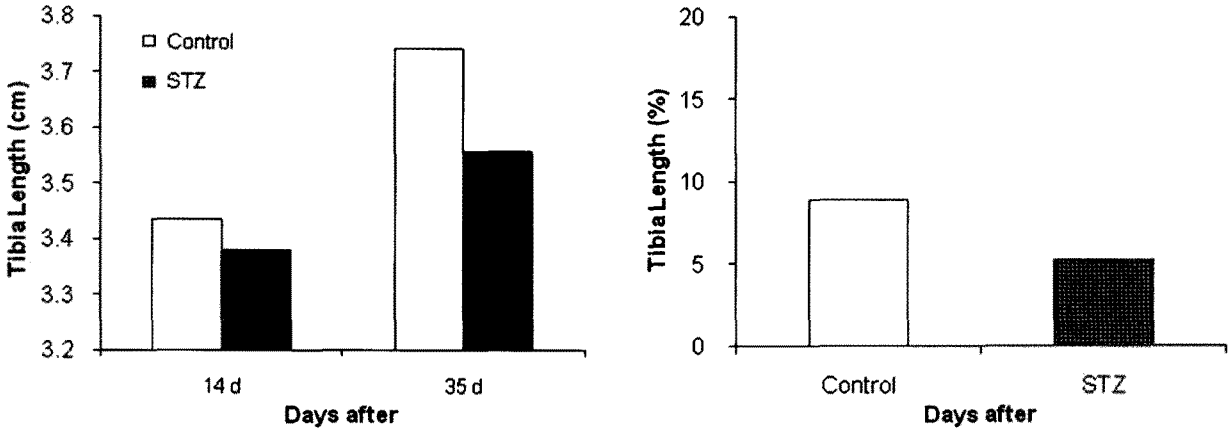


(B) Male

Fig. 2. The effect of diabetes on body weight of male and female control and STZ induced diabetic rats. Diabetes present reduced increase in body weight on growing, and male diabetic rats showed even loss of body weight at final experimental day. All data represent means± S.E.M. (● before; ■ after experiment).



(A) Female



(B) Male

Fig. 3. The effect of diabetes on tibial bone length of male and female diabetic rats induced by STZ. Diabetic rats pronounced a suppression in tibial length maturation. All data represent means±S.E.M.

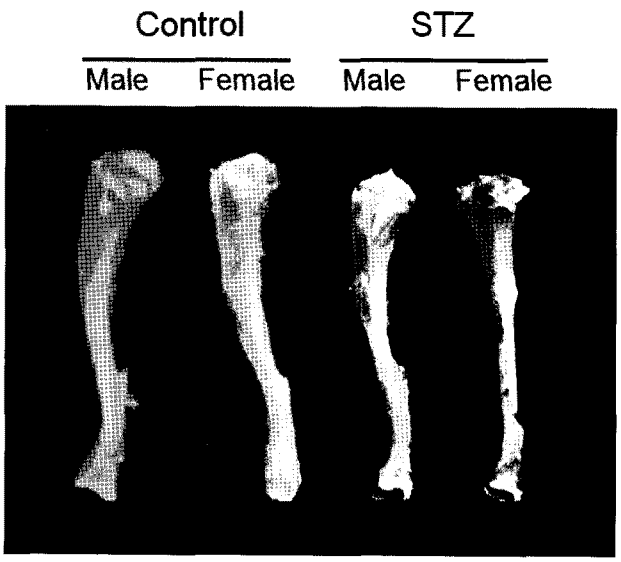


Fig. 4. Morphology of tibia in control and STZ-induced diabetic male and female rats, showing suppressed bone growth appearance.

pect to body weight in both sexes, although the change in LV/BW in male diabetic rats was opposite to that in female diabetic rats.

LV/Tibial Length (TL) Index

Among female control rats, the LV/TL index increased 45% from 2 to 5 weeks, whereas diabetic females showed a decrease of approximately 13% (Fig. 6, Fig. 7A). In contrast, the LV/TL index decreased approximately 43 and 16% in control and diabetic males, respectively (Fig. 7B). These data suggest that diabetes induced cardiac hypertrophy, most likely due to high blood glucose leading to increased blood viscosity and subsequent hypertension. However, we observed no increase in LV mass, and the LV/TL index appeared to decrease (Fig. 6).

DISCUSSION

We investigated whether diabetes affects bone and

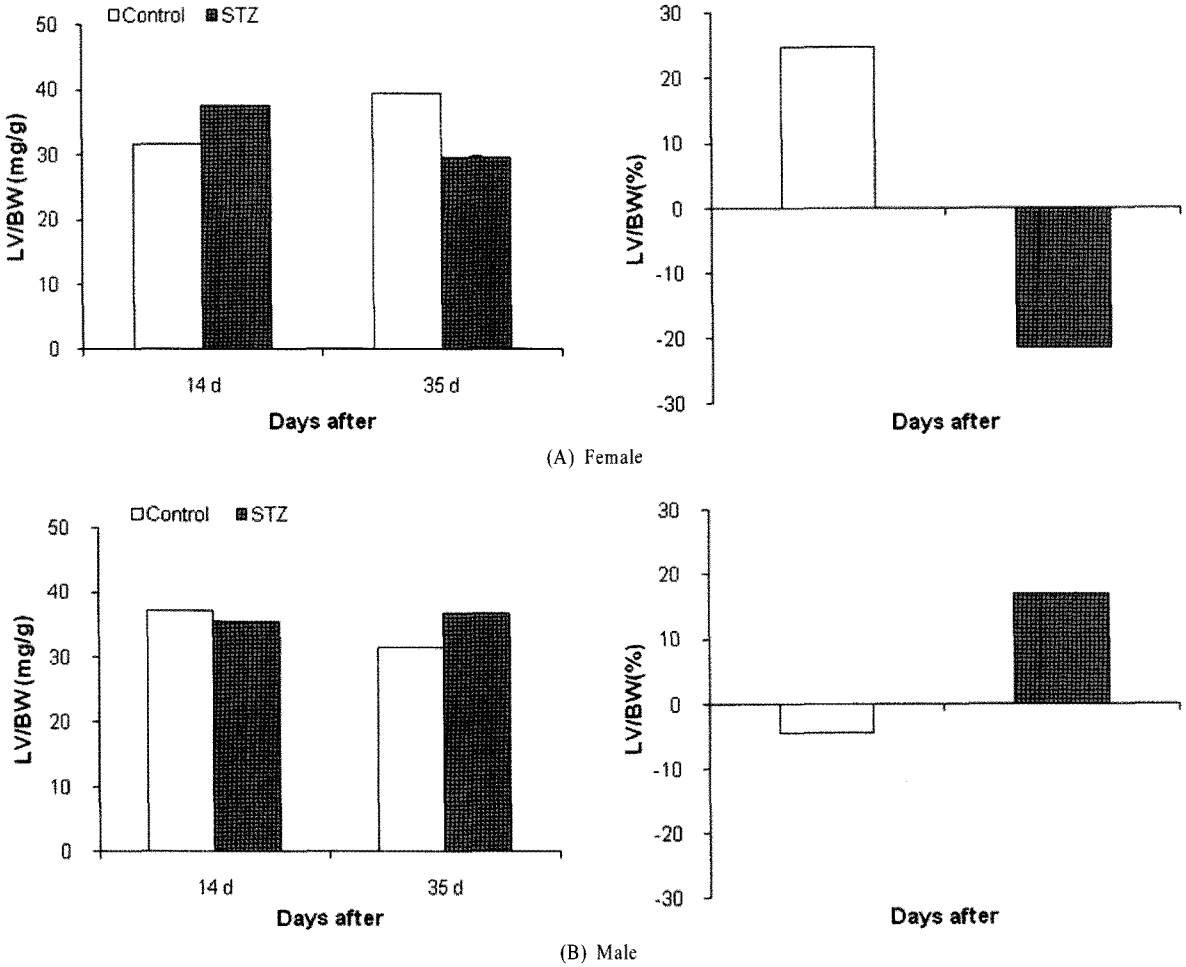


Fig. 5. The effect of diabetes on left ventricle mass to tibial bone length (LV/TBL) index in female and male control and STZ-induced diabetic rats. All data represent means±S.E.M.

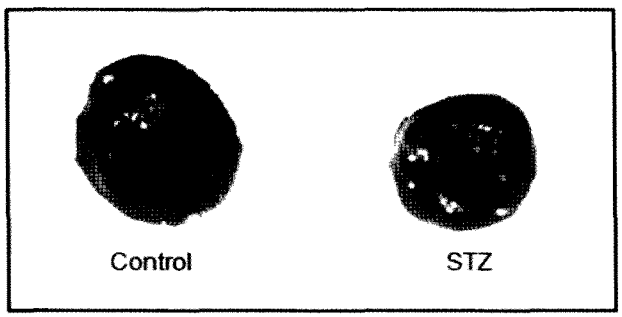


Fig. 6. Left ventricle of control and STZ-induced diabetic rats, showing diabetes at early age causes suppresses heart development.

heart growth during puberty in rats. We found that diabetic rats showed higher glucose levels and lower body weight gain, LV weight, and tibial length than control rats. Regarding body weight, control rats showed greater body weight gain and overall weight compared to diabetic rats, suggesting that diabetes retards somatic growth. Moreover, males showed a greater degree of suppression and even decreased in weight after 5 weeks. In a previous study, diabetes slowed pubertal development due to abnormalities in the growth hormone (GH)/insulin-like growth factor -1 (IGF-1) axis; digestion was also slowed as a result of hepatic growth hormone receptor impairment (Blaas *et al.*, 2010). In another study, male diabetic rats were affected to a greater degree than female rats in terms of linear growth and body composition (Cortright *et al.*, 1996).

Previous studies have shown a tendency for greater increases in weight rather than height in diabetic children. Body composition may also change with the development of leptin resistance, leading to a gradual increase in fat deposition, particularly in girls (Dunger *et al.*, 2002). In contrast, men show a notable decrease in body volume. The leptin hormone is produced by adipocytes and regulates appetite and food intake. Previous research has shown that leptin resistance and fat

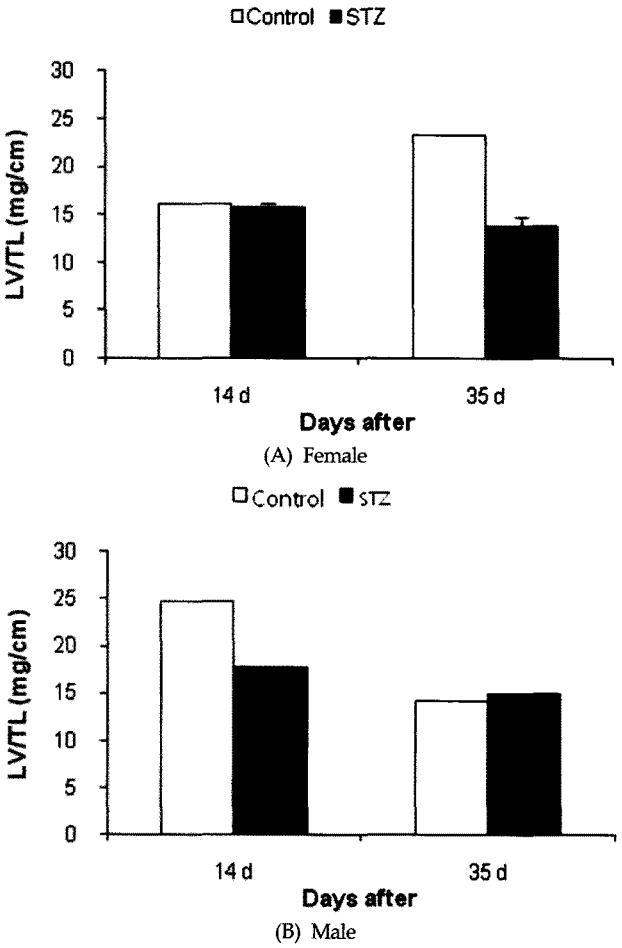


Fig. 7. The effect of diabetes on left ventricle mass to tibial bone length (LV/TBL) index in female and male control and STZ-induced diabetic rats. All data represent means±S.E.M.

mass are relatively high in individuals with an elevated body mass index (BMI) (Reinehr *et al.*, 2009). For women, increased fat mass and high leptin levels are related to insulin levels. In men, high testosterone levels increase leptin activity, leading to a decrease in fat mass. Therefore, body weight differs between men and women as a result of sex steroids (Rolland *et al.*, 2006). However, additional research has indicated that treating diabetes patients with growth hormone (GH) increases the speed of growth (Taverna *et al.*, 2000).

In this study, diabetic rats showed reduced tibial bone growth. Previous studies have shown that kidney malfunction and subsequent decreases in calcium reabsorption result in a 2~5 fold increase in calcium excretion in diabetic rats compared to healthy controls (Thébault *et al.*, 2006). The resultant decrease in available calcium may explain decreased long bone growth in diabetes. In addition, Maor and Karnieli (1999) suggested that low IGF-1 levels in diabetics may further decrease glucose levels in developing bone through the lack of IGF-1 receptor-mediated regulation of GLUT4, leading to retarded bone growth. Maor and Karnieli (1999) showed that the insulin-sensitive glucose transporter (GLUT4) is involved in early bone growth in control and diabetic animals. Data from previous studies suggest that defects in the glucose transport system, particularly GLUT4, are associated with morphological changes and slower bone growth, which is in accordance with Verhaeghe *et al.* (1999). Previous studies have shown that diabetic mice are particularly susceptible to skeletal growth retardation. Within skeletal growth centers, immature chondrocytes were conspicuously lacking and the growth center was dominated by hypertrophied cells, suggesting that diabetic insult likely results in changes in chondrocyte differentiation and the uncoupling of proliferative and differentiative processes necessary to bone development (Maor *et al.*, 1993). Furthermore, increasing evidence suggests that early DM results in decreased bone mass or osteopenia in humans (Al-Qadreh *et al.*, 1996).

Our results indicate that pre-pubertal diabetes results in growth delay rather than hypertrophy of the heart. GH and IGF-I are important for normal heart growth and for maintaining cardiac mass and function (Isgaard, 2004). Diabetes is associated with LV structural and functional abnormalities, as demonstrated in previous population studies (Devereux *et al.*, 2000; Palmieri *et al.*, 2001).

New insulin treatment regimens involving multiple daily injections and adjusted insulin doses according to blood glucose levels have resulted in substantial improvements in terms of the weight of diabetic children (Donaghue *et al.*, 2003). In terms of height, improved treatment has eliminated the discrepancy between children with type 1 DM and their unaffected peers at all ages. Palmieri *et al.* (2003) reported that diabetic patients with LV hypertrophy showed increased levels of fibrinogen and C-reactive protein, markers of systemic inflammation, and were more likely to exhibit microalbuminuria. These results suggest that diabetics are at a relatively greater risk for endothelial damage and atherothrombosis.

In this study, juvenile rats showed suppressed bone and heart development as a result of diabetic induction. Although hypertension causes cardiac hypertrophy, pre-pubertal diabetes appears to retard cardiac development rather than cause hypertrophic remodeling. These results imply that growth factors such as insulin play an important role in heart development. It is possible that similar retardation in heart development occurs in humans. If so, diabetic children may benefit from various therapies to promote normal development and physiological function, such as growth factor supplementation, exercise, or cardiopulmonary physical therapy.

Further studies are required to determine the mecha-

nisms underlying these abnormalities in heart development at the molecular level. In addition, further research is required to determine whether this pre-pubertal condition increases the risk of pathological remodeling and eventual heart failure.

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(Received: 7 May 2010 / Accepted: 23 June 2010)