

Pancreatic Weakness in Zucker Fatty Rats, a Genetic Obese Model

^{1,3}Takeshi Ohta, ²Katsuhiro Miyajima and ^{1,3}Takahisa Yamada

¹Laboratory of Animal Breeding and Genetics, Graduate School of Agriculture,
Kyoto University, Sakyo-Ku, 606-8502 Kyoto, Japan

²Department of Pathology, Tokai University School of Medicine,
259-1193 Isehara, Japan

³Laboratory of Animal Genetics, Graduate School of Science and Technology,
Niigata University, Nishi-Ku, 950-2181 Niigata, Japan

Abstract: Pancreatic dysfunction is a pivotal factor on incidence and development of diabetes mellitus and especially, the pancreatic weakness is a key defect contributing to diabetes mellitus. In this study, we investigated the pancreatic weakness in obese Zucker Fatty (ZF) rats. Different doses of Streptozotocin (STZ) were administered to ZF rats and the biochemical and the pancreatic related parameters were examined. Wistar rats were used as control animals. Before STZ injection, ZF rats showed a tendency to increase of insulinogenic index as compared with that in Wistar rats but the disposition index in ZF rats was significantly decreased as compared with that in Wistar rats. Moreover, the islets of STZ-untreated ZF rats were larger and had irregular boundaries. ZF rats treated with STZ at a dose level of 20 mg kg⁻¹ were diabetic within one week after the treatment whereas Wistar rats got the diabetic after 30 mg kg⁻¹ STZ was received.

Key words: Diabetes, pancreas, streptozotocin, ZF rat, dysfunction, weakness

INTRODUCTION

Type 2 diabetes and Impaired Glucose Tolerance (IGT) result from an imbalance between insulin sensitivity and the ability to secrete insulin. Increased obesity is a major determinant of insulin resistance but the insulin resistance alone is insufficient to cause type 2 diabetes (Szoke *et al.*, 2008; Goran *et al.*, 2008). It is reported that poor β -cell function is predictive of the development of type 2 diabetes independent of insulin resistance (Weyer *et al.*, 1999, 2000). Moreover, pancreatic islet β -cell dysfunction occurs well before frank hyperglycemia occurs (Xu *et al.*, 2008; Holman, 1998). Pancreatic function is a pivotal factor for considering the incidence and the progression of diabetes mellitus. Zucker Fatty (ZF) rat is a well-studied genetic model of obesity characterized by hyperlipidemia, hyperinsulinemia, glucose intolerance and insulin resistance (Inoescu *et al.*, 1985; Hiramatsu *et al.*, 1995). In the fasting state, the plasma insulin level of ZF rats is abnormally high. Relative to the basal hyperinsulinemia, the rise in plasma insulin after a bolus injection of glucose is blunted in mature fatty rats as compared with age matched lean rats (Inoescu *et al.*, 1985; De Souza *et al.*, 1995). Moreover, pancreatic islets from ZF rats show some changes by chronic exposure to low or

high glucose levels (Chan *et al.*, 1996). The glucokinase V_{max} of ZF rat islets was lower under all conditions, thereby limiting the potential increase in insulin secretion and the islets were easily desensitized. In this study, we investigated pancreatic weakness in ZF rats by Streptozotocin (STZ) treatment. STZ is known to be a diabetogenic agent which acts by causing selective destruction of pancreatic β -cells (Szkudelski, 2001).

MATERIALS AND METHODS

Animal: Male ZF rats and Wistar rats (Charles River Japan, Yokohama, Japan) were used for the study. ZF rats or Wistar rats were divided into two groups, a control group and a STZ-treated group. Rats were housed in suspended bracket cages and given a standard laboratory diet (CRF-1, Oriental yeast co., ltd., Tokyo, Japan) and water *ad libitum* in a controlled room for temperature, humidity and lightning.

STZ treatment: ZF rats (18 weeks of age) or Wistar rats (12 weeks of age) received a single intravenous injection of 10 mg kg⁻¹ STZ (Sigma, St. Louis, MO, USA) freshly dissolved in saline. Dosage of STZ has been gradually increased from 10-30 mg kg⁻¹ every week.

Biophysiological parameters: Body weights and blood chemical parameters such as glucose, insulin, Triglyceride (TG) and Total Cholesterol (TC) levels were examined every week. Free Fatty Acid (FFA) level was measured only before STZ injection. Blood samples were collected from the tail vein of non-fasted rats. Serum glucose, TG, TC and FFA levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin level was measured with a rat-insulin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). In ZF rats (18 weeks of age) or Wistar rats (12 weeks of age) before STZ injection, Oral Glucose Tolerance Test (OGTT) was performed. Glucose solution (2 g kg⁻¹) was administered to overnight-fasted rats. Blood samples were collected before and 30, 60 and 120 min after glucose loading. Serum glucose and insulin levels were measured as described above. Glucose AUCs and insulin AUCs were also calculated. To evaluate the insulin response to glucose during OGTT, the insulinogenic index (Δ insulin/ Δ glucose) was calculated using incremental serum insulin and glucose level for 0-30 min after glucose loading. Furthermore, disposition index (insulinogenic index/insulin level at 0 min) was calculated to characterize insulin secretion in the context of insulin sensitivity.

Pancreatic histology: In STZ-untreated group, necropsy was performed at 3 weeks after first STZ injection. The pancreas was fixed in 10% neutral buffered formalin. After resection, the tissue was paraffin-embedded by standard techniques and thin-sectioned (3-5 μ m). The sections were stained with Hematoxylin and Eosin (HE). The islet size was examined histopathologically. One slide per animal was analyzed, representing regions of pancreas. All islets within slide were circled and islet size was calculated using Win ROOF Ver. 5.01 software (Mitani corporation, Fukui, Japan).

Statistical analysis: Results of biophysiological parameters and pancreatic islet size were expressed as the mean \pm Standard Deviation (SD). Statistical analysis of differences between mean values was performed using the F-test followed by the Student's t-test or Aspin-Welch's t-test. Differences were defined as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Biological parameters (before STZ treatment): ZF rats at 18 weeks of age showed the increase of body weight, blood insulin, TC and FFA levels as compared with those

levels in Wistar rats at 12 weeks of age. The TG level tended to increase but not significant. Basal glucose level in ZF rats at 18 weeks of age was comparable to that level in Wistar rats at 12 weeks of age (Table 1). In OGTT, ZF rats showed the increase of blood glucose and insulin levels after glucose loading and the glucose AUC and the insulin AUC increased as compared with those levels in Wistar rats (Table 1). Insulinogenic index in ZF rats tended to increase but did not show a significant difference from that in Wistar rats. Moreover, the disposition index significantly decreased as compared with that in Wistar rats.

Effects on STZ treatment: Both ZF and Wistar rats did not develop diabetes at 10 mg kg⁻¹ STZ. All ZF rats showed hyperglycemia (516.0 mg dL⁻¹ in average) after 20 mg kg⁻¹ STZ injection (Fig. 1a). Whereas in Wistar rats, 20 mg kg⁻¹ of STZ failed to elevate blood glucose (except one of four rats). All Wistar rats showed hyperglycemia (552.0 mg dL⁻¹ in average) after 30 mg kg⁻¹ STZ injection (Fig. 1a). After 20 mg kg⁻¹ STZ injection, insulin levels in ZF rats decreased rapidly (Fig. 1b). Blood TG and TC levels did not show a significant change by STZ treatment (Fig. 1c, d). Body weights in ZF rats tended to decrease after 20 mg kg⁻¹ STZ injection but there was no significant change (Fig. 1e).

Pancreatic histology: Pancreatic islet size in ZF rats at 21 weeks of age was about three times as compared with that in Wistar rats at 15 weeks of age (mean value: ZF rat, 45183 μ m²; Wistar rat, 14249 μ m²) (Fig. 2 and 3a, b). Furthermore, irregular boundaries of islets in pancreas of ZF rats were observed (Fig. 3a, b). ZF rat is a genetic obese model which shows hyperinsulinemia and hyperlipidemia. Also, ZF rats at 18 weeks of age in this study showed the increase of body weight and blood insulin and lipid levels as compared with those levels in Wistar rats at 12 weeks of age. But the blood glucose

Table 1: Biophysiological and pancreatic related parameters in Zucker fatty rats (18 weeks of age) and Wistar rats (12 weeks of age)

Parameters	Zucker fatty rat	Wistar rat
Body weight (g)	685.1 \pm 99.5*	429.8 \pm 21.10
Glucose (mg dL ⁻¹)	130.0 \pm 22.5	125.0 \pm 7.400
Insulin (ng mL ⁻¹)	36.1 \pm 9.6**	2.5 \pm 0.70000
Triglyceride (mg dL ⁻¹)	948.8 \pm 551.8	179.9 \pm 115.6
Total cholesterol (mg dL ⁻¹)	236.1 \pm 85.5**	82.1 \pm 7.9000
Free fatty acid (mEq L ⁻¹)	0.549 \pm 0.016**	0.375 \pm 0.032
Glucose AUC (mg \cdot min dL ⁻¹)	30952 \pm 4112**	17886 \pm 661.0
Insulin AUC (ng \cdot min dL ⁻¹)	5986 \pm 1702**	221 \pm 144.000
Insulinogenic index	0.086 \pm 0.067	0.038 \pm 0.033
Disposition index	0.002 \pm 0.002*	0.057 \pm 0.034

Data represents means \pm SD (n = 4 or 5); * $p < 0.05$ ** $p < 0.01$; significantly different from Wistar rat; Glucose AUC, insulin AUC; insulinogenic index and disposition index were determined in oral glucose tolerance test

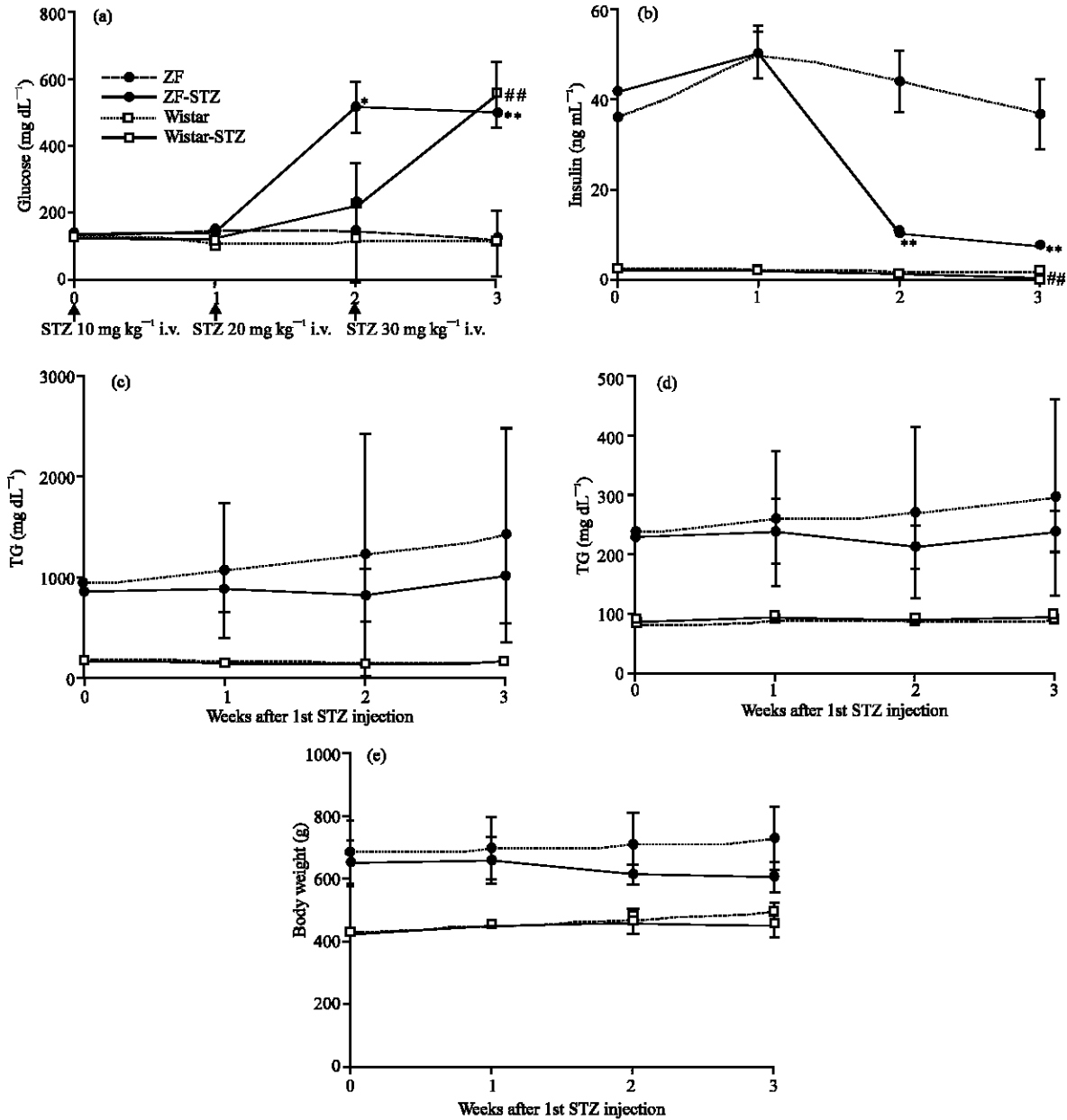


Fig. 1: Effects on: a) blood glucose; b) insulin; c) triglyceride; d) total cholesterol levels and e) body weight in ZF rats or Wistar rats with injection of streptozotocin. Data represent means \pm SD (n = 4). *p<0.05, **p<0.01; significantly different from the control ZF rats. #p<0.05, ###p<0.01; significantly different from the control Wistar rats

level in ZF rats was similar with that in Wistar rats (Table 1). Moreover, the ZF rats showed glucose intolerance and a tendency to increase of insulinogenic index. In obese animal models, the impaired glucose tolerance and the insulin over-secretion after glucose loading were observed (Upton *et al.*, 1998; Mori *et al.*, 1992; Ikeda *et al.*, 1981).

In this study, STZ was used as diabetogenic agent. STZ induced selective destruction of pancreatic β -cells and it is used to induce both type 1 and 2 diabetes (Szkudelski, 2001). The dosage of 10, 20 and 30 mg kg⁻¹ STZ was performed one by one. Both ZF and Wistar rats did not develop diabetes at 10 mg kg⁻¹ STZ. All ZF rats showed hyperglycemia after 20 mg kg⁻¹ STZ injection

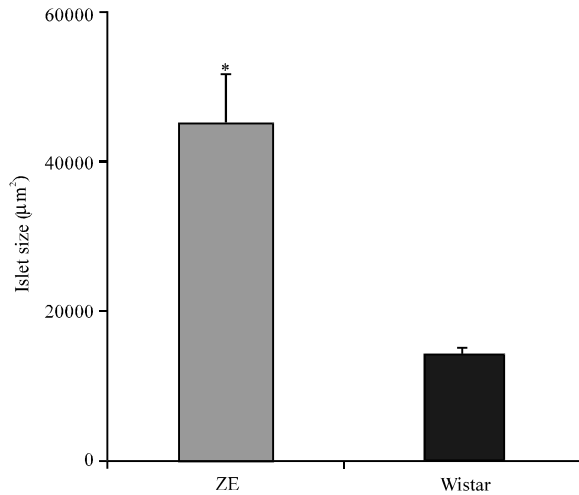


Fig. 2: Pancreatic islet size in the control ZF rats at 21 weeks of age and the control Wistar rats at 15 weeks of age. Data represent means±SD (n = 4). *p<0.05; significantly different from the control Wistar rats

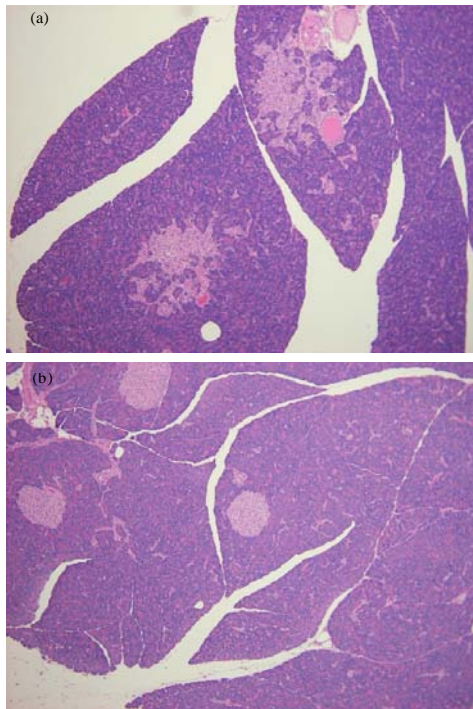


Fig. 3: Pancreas. HE stain. Original magnification x 100. a) control ZF rats at 21 weeks of age; b) control Wistar rats at 15 weeks of age. Pancreatic islets in ZF rats are large and have irregular boundaries

(Fig. 1a). Whereas in Wistar rats, 20 mg kg⁻¹ of STZ failed to elevate blood glucose. All Wistar rats showed hyperglycemia after 30 mg kg⁻¹ STZ injection (Fig. 1b). After 20 mg kg⁻¹ STZ injection, insulin levels in ZF rats decreased rapidly with the diabetes onset (Fig. 1b). Since, ZF rats with STZ at a dose level of 20 mg kg⁻¹ showed a significant hyperglycemia, the rats did not receive 30 mg kg⁻¹ STZ. Interestingly, a lower dosage of STZ (20 mg kg⁻¹) made ZF rats diabetic. It is considered that a pancreatic weakness exists in ZF rats.

We evaluated the appropriateness of β-cell function in relation to insulin sensitivity by calculating the disposition index. It is reported that the disposition index was lower in subjects with pre-diabetes as the presence of either Impaired Fasting Glucose (IFG) or IGT (Szoke *et al.*, 2008; Goran *et al.*, 2008; Weyer *et al.*, 1999). ZF rats also showed a decrease of the disposition index (Table 1) and the decrease suggested the pancreatic dysfunction in ZF rats. In further study, it is necessary to investigate mechanism of the pancreatic dysfunction.

Pancreatic islet size in ZF rats was about three times as compared with that in Wistar rats (Fig. 2 and 3). There are many reports concerning the size of pancreatic islet or β-cell. Pancreatic islet size in Otsuka Long-Evans Tokushima Fatty (OLETF) rats from 6-28 weeks of age was large as compared with that in control lean rats (Jia *et al.*, 2004). In Zucker Diabetic Fatty (ZDF) rats, pancreatic β-cell mass was large from 6-10 weeks of age as compared with that in control lean rats and the β-cell mass at 12 weeks of age was similar size with that in the lean rats (Finegood *et al.*, 2001). In obese rats, the size of pancreatic islet or β-cell grew with hyperphagia and hyperinsulinemia.

On the other hand, in non-obese rats such as STZ-diabetic rats and Goto-Kakizaki (GK) rats, the β-cell mass was small as compared with the control rats (Fernandez-Alvarez *et al.*, 2004; Movassat *et al.*, 1997). Pancreatic weakness in ZF rats exists, although the pancreas has large islet and an enough insulin-secretion property. Irregular boundaries of islets in pancreas of ZF rats were observed (Fig. 3) and the similar change was observed in OLETF rats (Jia *et al.*, 2004). One of the reasons for pancreatic dysfunction in ZF rats is considered to be lipotoxicity.

Blood FFA level in ZF rats was elevated (Table 1) and increase of blood TG levels was sustained during the experimental period (Fig. 1). It is reported that prolonged elevation of FFA has an impairing (lipotoxic) effect on β-cell function in rats and humans (Goh *et al.*, 2007; Hirose *et al.*, 1996; Lee *et al.*, 1994; Leung *et al.*, 2004).

The pancreatic weakness in ZF rats might be caused by the hyperlipidemia. Based on the defects in β -cell mass and function seen in diabetes mellitus, increased knowledge about these parameters and especially changes in them could be important for the early diagnosis and for treatment of diabetes in human.

CONCLUSION

In ZF rats, the diabetes was induced by a lower dosage of STZ and the existence of pancreatic weakness was suggested. ZF rat is a useful model for studies of not only insulin resistance but also pancreatic dysfunction.

REFERENCES

- Chan, C.B., J.M. Lowe and W.J. Debertin, 1996. Modulation by glucose of insulin secretion and glucose phosphorylating activity in cultured pancreatic islets from obese (*fa/fa*) Zucker rats. *Int. J. Obes. Relat. Metab. Disord.*, 20: 175-184.
- De Souza, C.J., J.H. Yu, D.D. Robinson, R.G. Ulrich and M.D. Meglasson, 1995. Insulin secretory defect in Zucker *fa/fa* rats is improved by ameliorating insulin resistance. *Diabetes*, 44: 984-991.
- Fernandez-Alvarez, J., A. Barbera, B. Nadal, S. Barcelo-Batllori and S. Piquer *et al.*, 2004. Stable and functional regeneration of pancreatic beta-cell population in nSTZ-rats treated with tungstate. *Diabetologia*, 47: 470-477.
- Finegood, D.T., M.D. McArthur, D. Kojwang, M.J. Thomas, B.G. Topp, T. Leonard and R.E. Buckingham, 2001. β -cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. *Diabetes*, 50: 1021-1029.
- Goh, T.T., T.M. Mason, N. Gupta, A. So and T.K.T. Lam *et al.*, 2007. Lipid-induced β -cell dysfunction *in vivo* in models of progressive β -cell failure. *Am. J. Physiol. Endocrinol. Metab.*, 292: E549-E560.
- Goran, M.I., C. Lane, C. Toledo-Corral and M.J. Weigensberg, 2008. Persistence of pre-diabetes in overweight and obese Hispanic children: Association with progressive insulin resistance, poor beta-cell function and increasing visceral fat. *Diabetes*, 57: 3007-3012.
- Hiramatsu, S., K. Inoue, Y. Sako, F. Umeda and H. Nawata, 1995. Secretion of insulin and glucagon by the perfused pancreas of genetically obese (*fa/fa*) Zucker rats and its alteration with aging. *Endocr. J.*, 42: 563-567.
- Hirose, H., Y.H. Lee, L.R. Inman, Y. Nagasawa, J.H. Johnson and R.H. Unger, 1996. Defective fatty acid-mediated beta-cell compensation in Zucker diabetic fatty rats. Pathogenic implications for obesity-dependent diabetes. *J. Biol. Chem.*, 271: 5633-5637.
- Holman, R.R., 1998. Assessing the potential for alpha-glucosidase inhibitors in prediabetic states. *Diab. Res. Clin. Pract.*, 40: S21-S25.
- Ikeda, H., A. Shino, T. Matsuo, H. Iwatsuka and Z. Suzuoki, 1981. A new genetically obese-hyperglycemic rat (Wistar fatty). *Diabetes*, 30: 1045-1050.
- Inoescu, E., J.F. Sauter and B. Jeanrenaud, 1985. Abnormal oral glucose tolerance in genetically obese (*fa/fa*) rats. *Am. J. Physiol. Endocrinol. Metab.*, 248: E500-E506.
- Jia, D., M. Yamamoto, M. Otani and M. Otsuki, 2004. Bezafibrate on lipids and glucose metabolism in obese diabetic otsuka long-evans Tokushima fatty rats. *Metabolism*, 53: 405-413.
- Lee, Y., H. Hirose, M. Ohneda, J.H. Johnson, J.D. McGarry and R.H. Unger, 1994. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: Impairment in adipocyte-beta-cell relationships. *Proc. Natl. Acad. Sci.*, 91: 10878-10882.
- Leung, N., T. Sakae, A. Carpentier, K. Uffelmann, A. Giacca and G.F. Lewis, 2004. Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. *Diabetologia*, 47: 204-213.
- Mori, Y., J. Yokoyama, M. Nemoto, S. Katoh, M. Nishimura and Y. Ikeda, 1992. Characterization of a new genetically obese-hyperglycemic OLETF rat. *Jikeikai Med. J.*, 39: 349-359.
- Movassat, J., C. Saulnier, P. Serradas and B. Portha, 1997. Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. *Diabetologia*, 40: 916-925.
- Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol. Res.*, 50: 537-546.
- Szoke, E., M.Z. Shrayyef, S. Messing, H.J. Woerle and T.W. van Haeften *et al.*, 2008. Effect of aging on glucose homeostasis: Accelerated deterioration of beta-cell function in individuals with impaired glucose tolerance. *Diabetes Care*, 31: 539-543.

- Upton, R., P.S. Widdowson, S. Ishii, H. Tanaka and G. Williams, 1998. Improved metabolic status and insulin sensitivity in obese fatty (*fa/fa*) Zucker rats and Zucker diabetic fatty (ZDF) rats treated with the thiazolidinedione, MCC-555. *Br. J. Pharmacol.*, 125: 1708-1714.
- Weyer, C., C. Bogardus, D.M. Mott and R.E. Pratley, 1999. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J. Clin. Invest.*, 104: 787-794.
- Weyer, C., R.L. Hanson, P.A. Tataranni, C. Bogardus and R.E. Pratley, 2000. A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: Evidence for a pathogenic role of relative hyperinsulinemia. *Diabetes*, 49: 2094-2101.
- Xu, L., C.D. Man, B. Charbonnel, G. Meninger and M.J. Davies *et al.*, 2008. Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on beta-cell function in patients with type 2 diabetes: A model-based approach. *Diabetes Obes. Metab.*, 10: 1212-1220.