Insulin resistance and mechanical dysfunction in hearts of Wistar rats with streptozotocin-induced non-insulin-dependent diabetes mellitus

S. W. Schaffer¹ and G. L. Wilson²

¹Department of Pharmacology, and

²Department of Structural and Cellular Biology, University of South Alabama, Mobile, Alabama, USA

Summary. The present study correlates the insulin resistance seen in the myocardium of rats with streptozotocin-induced non-insulin-dependent diabetes mellitus with insulin secretory defects, hyperglycaemia and disease duration. Two-day-old male Wistar rats were given a bolus injection of streptozotocin (0.09 mg/g body weight) which caused glucose intolerance when these animals reached adulthood. Although these rats developed a progressive resistance to the actions of insulin in the heart this did not correlate with the development of glucose intolerance. However, a correlation

was seen with a shift in insulin secretory response from hyper- to hypo-secretion which developed between 6 and 14 months of age. Moreover, this shift in secretory pattern can be associated with the onset of a cardiac mechanical malfunction.

Key words: Glucose intolerance, insulin resistance, insulin secretion, cardiomyopathy, non-insulin-dependent diabetes mellitus.

Non-insulin-dependent diabetes mellitus is characterized by defects in both pancreatic insulin secretion and peripheral insulin action. Although it is generally accepted that these metabolic abnormalities trigger the onset of non-insulin-dependent diabetes, the relationship between these metabolic abnormalities remains unclear. One problem in clarifying the role of each defect in the pathogenesis of non-insulin-dependent diabetes is that both metabolic parameters improve in association with weight loss, sulphonylurea therapy or insulin treatment [1–3]. Moreover, procedures such as the insulin clamp technique, which are useful to quantify insulin action are unable to adequately quantify the contribution of each parameter [4–6].

To gain more information on the aetiology of non-insulin-dependent diabetes, animal models have been developed. One useful non-insulin-dependent diabetes model can be induced by treatment of neonatal rats with streptozotocin [7, 8]. These animals quickly develop acute diabetes characterized by overt hyperglycaemia and reduced pancreatic insulin stores. However, the pancreas is able to partially regenerate and within 3–4 weeks basal glucose levels are normalized. As these animals age they slowly become glucose intolerant and insulin resistant [9].

Portha et al. [9] have proposed that the development of peripheral insulin resistance in these animals requires frank hyperglycaemia (200 % above control values) and hypoinsulinaemia (50 % reduction). Yet, insulin resis-

tance relative to cardiac glucose transport has been noted in a milder form of the disease, but only after prolonged disease exposure [10]. In order to gain insight into the pathogenic progression towards insulin resistance, the effect of disease duration on glucose intolerance, insulin status and peripheral insulin resistance was monitored.

Materials and methods

Non-insulin-dependent diabetes mellitus model. Two-day-old Wistar rats were injected i. p. with 0.09 mg of streptozotocin (the generous gift of Dr. A. Chang, The Upjohn Company, Kalamazoo, Mich., USA) per g of body weight.

Glucose tolerance tests. Rats were fasted for 16 h after which time they were given an intraperitoneal injection of 2 g glucose per kg body weight. Blood samples were drawn from the tail immediately before the glucose challenge and 15, 60 and 120 min thereafter. Plasma glucose concentrations were determined using a Beckman 2 Glucose Analyzer (Fullerton, Calif., USA). Serum insulin concentrations were determined using a Linco Rat Insulin RIA Kit (Linco Research, Inc., St. Louis, Mo., USA).

Heart perfusions. Hearts from non-insulin-dependent diabetic and non-diabetic rats were perfused on a typical isolated working heart apparatus with Krebs-Henseleit buffer containing 10 mmol/l glucose and varying concentrations of insulin. After-load of the working heart apparatus was fixed at 110 cm water, while pre-load was either maintained at 15 cm H₂O or was varied over a range of 10 to 30 cm H₂O. All hearts were paced at 300 beats per min.

Fig. 1 A, B. Effect of duration of diabetes on glucose utilization and cardiac work of isolated, working rat heart. Hearts from 6- and 14-month-old non-insulin-dependent diabetic and non-diabetic rats were perfused with Krebs-Henseleit buffer containing 10 mmol/l [$3\text{-}^3\text{H}$]-glucose and insulin varying from 0 to 9 U/l. Effluent was collected and glucose utilization determined from the rate of tritium appearance into water (A). Cardiac work of these same hearts was calculated from aortic pressure, aortic output and coronary flow determined by standard methods (B). Six-month-old non-diabetic (●-●); 6-month-old diabetic (○-○); 14-month-old non-diabetic (▲-▲); 14-month-old diabetic (△-△) rats were studied. Data represent mean $\pm$ SEM of five hearts and are expressed as a percent increase over initial value after exposure to insulin. Asterisks denote significant difference from age-matched non-diabetic control ($p < 0.05$)

Glucose utilization. Glucose utilization was determined at varying perfusate insulin concentrations by collecting perfusate which had passed through the heart and measuring the [^3H]- H_2O content. The rate at which the heart promotes the formation of [^3H]- H_2O from [$3\text{-}^3\text{H}$]-glucose is a measure of flux through the glyceraldehyde-3-phosphate dehydrogenase step [11].

Cardiac function. Typical pressure-cardiac work curves were generated by varying pre-load over a range of 10–30 cm H_2O and measuring aortic pressure, aortic output and coronary flow. Aortic pressure and output were obtained by placing a Statham P23 Gb pressure transducer and flowmeter, respectively, above the aorta. Left ventricular dP/dt was measured with a Statham P23 Gb pressure trans-

ducer by inserting a 22 gauge needle through the ventricular wall. Coronary flow was quantitated by collecting the effluent exiting the pulmonary artery. Cardiac work was calculated from cardiac output (sum of aortic output and coronary flow) and aortic pressure as described previously [12, 13].

Statistical analysis

Differences between diabetic and their age-matched control rats were evaluated by Student's *t*-test. Analysis of variance was used to detect changes in cardiac metabolism and mechanical function. The Student-Newman-Keuls multiple range test was performed to establish significant differences ($p < 0.05$).

Results

To establish that insulin resistance was present in the myocardium of these rats glucose utilization was examined as a function of perfusate insulin concentration. In the non-diabetic animals, at both 6 and 14 months of age, glucose utilization increased progressively to a value of approximately 80% over a range of 0 to 9 units per litre of insulin (Fig. 1A). In the 6-month-old diabetic animals similar increases in glucose utilization were achieved with this range of insulin concentrations. By contrast, in the 14-month-old diabetic animal, elevations of insulin led to significantly decreased response that reached a peak of only 20% increase at the maximal concentration of insulin. Insulin-mediated changes in contractile function paralleled the alterations in glucose metabolism. Maximal stimulation of cardiac work by insulin was about 30% in the hearts of the 6-month-old diabetic, 6-month-old non-diabetic and 14-month-old non-diabetic rats, but only about 10% in the 14-month-old diabetic heart (Fig. 1B). Therefore, a resistance to insulin had developed in the myocardium of these animals by 14 months.

The relationship between insulin resistance and secretion was examined in 6- and 14-month-old diabetic and control animals administered glucose tolerance tests. Both sets of animals were injected i.p. with a bolus of 2 g/kg body weight glucose (Figs. 2 and 3). This produced a prolonged hyperglycaemia in the diabetic animals, however, glucose values of the control animals returned to near normal by 2 h. By comparison, when insulin secretion in these animals was determined, the 6-month-old diabetic rats were actually hyperinsulinaemic compared to control rats 15 min after glucose injection. Insulin levels in both the control and diabetic animals returned to near normal values by 2 h even though the diabetic animals were markedly hyperglycaemic. In contrast to these findings 14-month-old diabetic animals were hypoinsulinaemic compared to age-matched control animals at all time points after glucose injection. Therefore, the development of insulin resistance in the myocardium can be correlated temporally with the shift that we see from hyper- to hypo-insulin secretion in response to a glucose challenge.

To determine whether these changes in insulin secretion and action can be associated with changes in heart function, we generated typical pressure cardiac work rela-

Fig. 2. **A** Plasma glucose concentrations in response to an intraperitoneal glucose challenge (2 g glucose/kg body weight) in 6-month-old streptozotocin-treated (SZ; $n = 5$, O—O) and control ($n = 5$, ●—●) Wistar rats following a 16-h fast. Values represent the mean $\pm$ SEM ($*p < 0.05$). **B** Plasma insulin concentrations in response to an intraperitoneal glucose challenge in 6-month-old streptozotocin-treated (SZ; $n = 5$, O—O) and control ($n = 5$, ●—●) Wistar rats following a 16-h fast. Values represent the mean $\pm$ SEM ($*p < 0.05$).

Fig. 3. **A** Plasma glucose concentrations in response to an intraperitoneal glucose challenge (2 g glucose/kg body weight) in 14-month-old streptozotocin-treated (SZ; $n = 5$, Δ—Δ) and control ($n = 5$, ▲—▲) Wistar rats following a 16-h fast. Values represent the mean $\pm$ SEM ($*p < 0.05$). **B** Plasma insulin concentrations in response to an intraperitoneal glucose challenge in 14-month-old streptozotocin-treated (SZ; $n = 5$, Δ—Δ) and control ($n = 5$, ▲—▲) Wistar rats following a 16-h fast. Values represent the mean $\pm$ SEM ($*p < 0.05$).

tionships by varying pre-load over the range of 10 to 30 cm of water (Fig. 4). Stepwise increases in pre-load led to progressive increases in cardiac work in all the animals examined. In the 6-month-old diabetic and 6- and 14-month-old control animals, there were no differences in the curves generated. However, in the 14-month-old diabetic animals cardiac work was significantly depressed at all pre-load values examined in comparison to all the other three groups. Both $+dP/dt$ (a measure of contractility) and $-dP/dt$ (a measure of cardiac relaxation) were altered in the 14-month-old diabetic rat. While $+dP/dt$ of the 14-month-old non-diabetic rat was 2820 ± 140 cm H₂O/s, the age-matched diabetic animals showed a value of 2380 ± 110 cm H₂O/s. Corresponding values for $-dP/dt$ were 2410 ± 80 and 2010 ± 100 cm H₂O/s for 14-month-old non-diabetic and diabetic rats, respectively. No differences in either $+dP/dt$ or $-dP/dt$ were noted in the 6-month-old non-diabetic and diabetic rats; $+dP/dt$ was 2480 ± 50 and 2450 ± 40 cm H₂O/s in non-diabetic and diabetic rats, respectively, while $-dP/dt$ was 2450 ± 40 and 2360 ± 70 cm H₂O/s in the same two groups. Thus, cardiac function is normal in the 6-month-old diabetic rat but is markedly de-

pressed by 14 months indicating that the changes in cardiac function follow the alterations in insulin action and secretion.

Discussion

Since the initial reports by Portha et al. [7] and Weir et al. [8] of the streptozotocin-induced neonatal model of non-insulin-dependent diabetes, a variety of investigations have shown that insulin resistance develops in peripheral tissues [10, 14–17] including our own work with the heart [10]. The present study has attempted to delineate the factors involved in insulin resistance by examining the temporal association between the development of hypoinsulinaemia and impaired insulin-stimulated glucose utilization in the heart. We found that insulin-stimulated glucose utilization was normal in the 6-month-old diabetic animals but was markedly defective in the 14-month-old diabetic animals. Although both the 6- and 14-month-old animals were markedly glucose intolerant, the 6-month-old animals actually had an enhanced insulin

Fig. 4. Effect of non-insulin-dependent diabetes on myocardial contractile function. Hearts from rats which were 6-month-old non-diabetic (●—●), 6-month-old diabetic (○—○), 14-month-old non-diabetic (▲—▲) and 14-month-old diabetic (△—△) were perfused on a working heart apparatus. Myocardial function was evaluated at varying pre-load by determining cardiac work. Values represent means $\pm$ SEM of five hearts. (* $p < 0.05$)

secretory response to the glucose challenge. In contrast, the 14-month-old animals had a blunted insulin response to glucose. Portha et al. [9] observed progressive degrees of insulin resistance and glucose intolerance in female Wistar rats administered streptozotocin 0, 2 and 5 days after birth. Their studies revealed a narrow window between development of overt hyperglycaemia and hypoinsulinaemia and milder degrees of dysfunction. Three important differences existed between their protocol and ours. First, their rats were treated with 80 mg/kg streptozotocin and ours with 90 mg/kg streptozotocin. Second, we used male Wistar rats and they used female Wistar rats. Finally, glucose tolerance and insulin response were measured at a much earlier time in their study than ours. Since strain and the time of streptozotocin administration have a profound influence on the final status of the animal, it is not surprising that sex and dosage also affect insulin status. The present model adds an interesting variation to the neonatal model because the transition in insulin secretory profile over time is reminiscent of that seen in the progression of human non-insulin-dependent diabetes [4, 5].

Previously, Portha et al. [16] found in clamp studies that insulin resistance developed in 10-week-old animals which had received a streptozotocin treatment protocol that produced frank hyperglycaemia and hypoinsulinaemia (80 mg/kg in 5-day-old neonates). Subsequently, they reported that protocols that caused milder basal hypoinsulinaemia and very mild hyperglycaemia actually increased responsiveness to insulin in 3- to 5-month-old rats [18]. They concluded that hypoinsulinaemia causes insulin resistance. The present work shows that even in mild conditions where basal hyperglycaemia does not develop and only glucose intolerance is found, insulin resistance can be detected after prolonged periods [9]. Thus, this work agrees with the basic tenant of the Portha group in that hypoinsulinaemia causes insulin resistance but it also

shows that insulin resistance can develop under milder conditions, in which only a defect in insulin secretion rather than basal hypoinsulinaemia is seen. A similar correlation between attenuated insulin secretion and insulin resistance has been reported for adipocytes [14]. In that study, as in ours, basal insulin levels were not severely depressed. In contrast, Portha and colleagues [18, 19] have reported that they found diminished insulin secretion does not cause insulin resistance in hepatocytes of 3- to 5-month-old animals. However, based on the results of our study it is conceivable that sufficient time was not allowed for the hepatocytes to progress into insulin resistance. It seems likely that there may be heterogeneity in the development of insulin resistance in peripheral tissues, with adipocytes and skeletal muscle becoming refractory to insulin much faster than the liver or heart under similar conditions.

A final important observation that resulted from this study is that cardiac dysfunction correlated more closely with the insulin secretory defect rather than hyperglycaemia. This would suggest that the cardiomyopathy that occurs in these animals is not the result of glucose toxicity but rather a problem relating to the direct action of insulin on the heart. In support of this notion is our finding that myoinositol levels in the diabetic heart do not correlate with sorbitol concentrations (unpublished observations).

Acknowledgements. This work was supported by research grants from the National Institutes of Health DK 36440 and ES 03456 and a grant from the Pfizer Pharmaceutical Corporation. The authors wish to thank Ms. N.J. Patton for skilled technical assistance.

References

1. Savage PJ, Bennion LJ, Flock EJ et al. (1979) Diet-induced improvement of abnormalities in insulin and glucagon secretion and in insulin receptor binding in diabetes mellitus. *J Clin Endocrinol Metabol* 48: 999–1007
2. Andrews WJ, Vasquez B, Nagulesparan M et al. (1984) Insulin therapy in obese, noninsulin-dependent diabetes induced improvements in insulin action and secretion that are maintained for two weeks after insulin withdrawal. *Diabetes* 33: 634–642
3. Kolterman OG, Gray RS, Shapiro G, Scarlett JA, Griffin J, Olefsky JM (1984) The acute and chronic effects of sulfonylurea therapy in type II diabetic subjects. *Diabetes* 33: 346–354
4. Reaven GM (1984) Insulin secretion and insulin action in non-insulin-dependent diabetes mellitus: which defect is primary? *Diabetes Care* 7 [Suppl 1]: 17–24
5. DeFronzo RA (1988) The triumvirate: β -cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 37: 667–687
6. Garvey WT (1989) Insulin resistance and noninsulin-dependent diabetes mellitus: which horse is pulling the cart? *Diabetes Metab Rev* 5: 727–742
7. Portha B, Picon L, Rosselin G (1979) Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. *Diabetologia* 17: 371–377
8. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S (1981) Islet secretion in a new experimental model for noninsulin-dependent diabetes. *Diabetes* 30: 590–595
9. Portha B, Blondel O, Serradas P et al. (1989) The rat models of noninsulin dependent diabetes induced by neonatal streptozotocin. *Diabetes Metab (Paris)* 15: 61–75
10. Schaffer SW, Seyed-Mozaffari M, Cutcliff CR, Wilson GL (1986) Postreceptor myocardial metabolic defect in a rat model of non-insulin-dependent diabetes mellitus. *Diabetes* 35: 593–597

11. Neely JR, Denton RM, England PJ, Randle PJ (1972) The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in perfused rat heart. *Biochem J* 128: 147–159
12. Neely JR, Liebermeister H, Battersby EJ, Morgan HE (1967) Effects of pressure development on oxygen consumption by isolated rat heart. *Am J Physiol* 212: 804–814
13. Schaffer SW, Tan BH, Wilson GL (1985) Development of a cardiomyopathy in a model of non-insulin-dependent diabetes. *Am J Physiol* 246: H179–H185
14. Trent DF, Fletcher DJ, May JM, Bonner-Weir S, Weir GC (1984) Abnormal islet and adipocyte function in young B-cell-deficient rats with near-normoglycemia. *Diabetes* 33: 170–175
15. Fantus IG, Chayoth R, O'Dea L, Marliss EB, Yale JF, Grose M (1987) Insulin binding and glucose transport in adipocytes in neonatal streptozotocin-injected rat model of diabetes mellitus. *Diabetes* 36: 654–660
16. Blondel O, Bailbe D, Portha B (1990) Insulin resistance in rats with non-insulin-dependent diabetes induced by neonatal (5 days) streptozotocin: evidence for reversal following phlorizin treatment. *Metabolism* 39: 787–793
17. Karl IE, Gavin JR, Levy J (1990) Effect of insulin on glucose utilization in epitrochlearis muscle of rats with streptozotocin-induced NIDDM. *Diabetes* 39: 1106–1115
18. Melin B, Caron M, Cherqui G et al. (1991) Increased insulin action in cultured hepatocytes from rats with diabetes induced by neonatal streptozotocin. *Endocrinology* 128: 1693–1701
19. Giroix M-H, Portha B, Kergoat M, Bailbe D, Picon L (1983) Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes: a study with the perfused pancreas. *Diabetes* 32: 445–451

Received: 10 August 1992 and in revised form: 20 October 1992

Dr. G. L. Wilson
Department of Structural and Cellular Biology
University of South Alabama
Mobile, AL 36688
USA