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Review

Cardiomyopathy associated with noninsulin-dependent diabetes

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Summary

Cardiovascular disease represents the major cause of morbidity and mortality in noninsulin-dependent diabetic patients. While it was once thought that atherosclerotic vascular disease was responsible for all of these adverse effects, recent studies support the notion that one of the major adverse complications of diabetes is the development of a diabetic cardiomyopathy characterized by defects in both diastolic and systolic function. Contributing to the development of the cardiomyopathy is a shift in myosin isozyme content in favor of the least active V_3 form. Also defective in the noninsulin-dependent diabetic heart is regulation of calcium homeostasis. While transport of calcium by the sarcolemmal and sarcoplasmic reticular calcium pumps are minimally affected by noninsulin-dependent diabetes, significant impairment occurs in sarcolemmal $Na^+ - Ca^{2+}$ exchanger activity. This defect limits the ability of the diabetic heart to extrude calcium, contributing to an elevation in $[Ca^{2+}]_i$. Also promoting the accumulation of calcium by the diabetic cell is a decrease in Na^+, K^+ ATPase activity, which is known to increase $[Ca^{2+}]_i$ secondary to a rise in $[Na^+]_i$. In addition, calcium influx via the calcium channel is stimulated. Although the molecular mechanisms underlying these defects are presently unknown, the possibility that they may be related to aberrations in glucose or lipid metabolism are considered. The evidence suggests that classical theories of glucose toxicity, such as excessive polyol production or glycosylation, appear to be insignificant factors in heart. Also insignificant are defects in lipid metabolism leading to accumulation of toxic lipid amphiphiles or triacylglycerol. Rather, the major defects involve membrane changes, such as phosphatidylethanolamine N-methylation and protein phosphorylation, which can be attributed to the state of insulin resistance.

Characteristics of diabetes

Diabetes mellitus is a heterogenous population of diseases involving defects in either insulin supply or action. As early as 1936, Himsworth suggested the existence of at least two forms of diabetes [1]. However, it was not until the development of a bioassay for plasma insulin that his clinical findings were confirmed. Today, diabetics are classified into two major categories, insulin-dependent and noninsulin-dependent diabetes mellitus [2–4]. Both types of diabetes are characterized by fasting hyperglycemia or glucose intolerance to a glucose challenge. However, the pathology of the two diseases are different. While the pancreatic β cells of the insulin-dependent diabetic are usually completely destroyed by either autoimmune or environmental factors, the β cells remain functional, although defective, in the noninsulin-dependent diabetic. Thus, the insulin-dependent diabetic has an absolute requirement for insulin to prevent life-threatening bouts of hyperglycemia and ketoacidosis; the degree

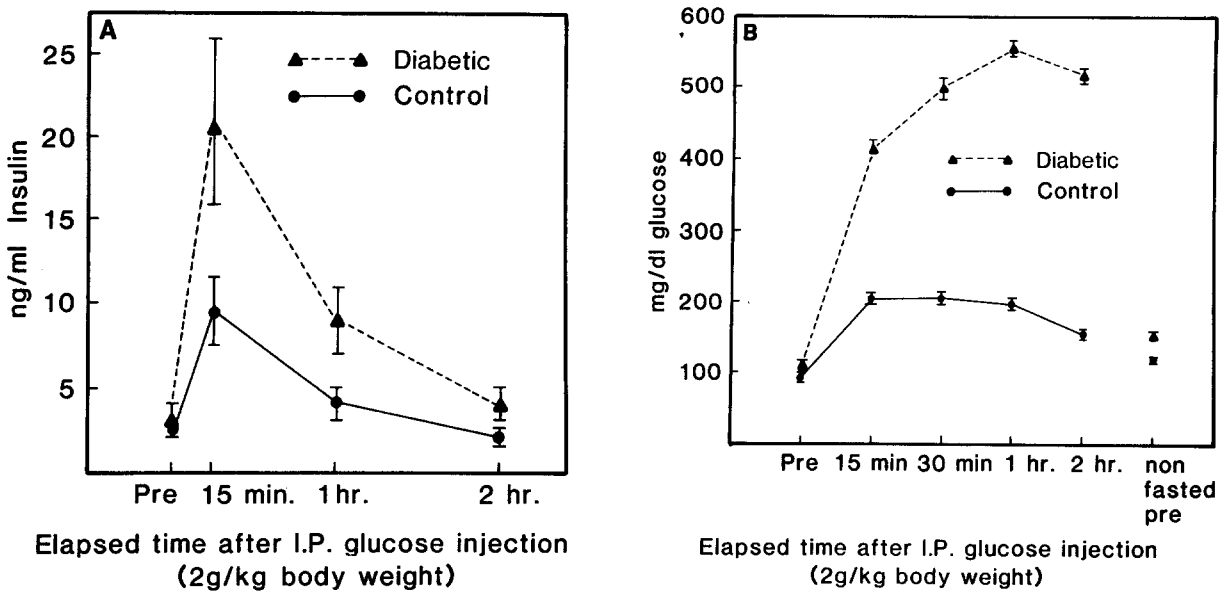

Fig. 1. Plasma insulin levels (A) and glucose content (B) in response to an intraperitoneal glucose tolerance test (2 g/kg body wt) in 6 month old noninsulin-dependent diabetic (▲--▲) and nondiabetic (●—●) rats following a 16 hour fast. All values represent means ± S.E.M. of either 10 (glucose) or 5 (insulin) animals. Reproduced from Schaffer *et al.* [62] with the permission of the American Physiological Society.

of hyperglycemia is usually less severe in the noninsulin-dependent diabetic and ketoacidosis is rare.

Although all patients with fasting hyperglycemia and insulin resistance are classified as noninsulin-dependent diabetics, it is recognized that noninsulin-dependent diabetes is a heterogeneous disease. Because some of these differences affect the heart, for the purpose of this review, two subsets of noninsulin-dependent diabetics will be considered. One group, which represents approximately 80% of the noninsulin-dependent diabetics in Western countries, are obese and exhibit enhanced rates of lipolysis and elevated levels of plasma free fatty acids [5]. This condition is frequently associated with very severe glucose intolerance. By comparison, the other group, lean noninsulin-dependent diabetics, exhibit much lower plasma free fatty acid levels and are usually less severely glucose intolerant than the obese group.

Animal models of noninsulin-dependent diabetes

Much of what is known about the pathogenesis of diabetic complications has been gleaned from studies using animal models. Most of these studies have utilized young adult rats administered either streptozotocin or alloxan to produce a diabetic model characterized by severe insulinopenia, glycosuria, polydipsia, hyperglycemia and muscle wasting (properties commonly associated with insulin-dependent diabetes).

Identification of an animal model which appropriately mimics the human condition of noninsulin-dependent diabetes has been problematic. While there are a number of animal models which carry a genetic predisposition to develop noninsulin-dependent diabetes, it is common for these species to develop abnormalities independent of diabetes; *e.g.*, the obese mouse (C57BL/6J ob/ob) exhibits several endocrine abnormalities, including exaggerated glycemic sensitivity to stress [6, 7] while the desert sand rat shows significant variability in its susceptibility to develop hyperinsulinemia and hyperglycemia [8]. The obese Zucker rat has been proposed by some investigators as a model of noninsulin-dependent diabetes [9], although it is a bet-

ter model of obesity and insulin resistance than noninsulin-dependent diabetes [10, 11].

A recently introduced chemical model of noninsulin-dependent diabetes overcomes many of the shortcomings of the genetic models [12–14]. The chemical model is produced by intraperitoneal injection of neonatal rats (0 – 2 days of age) with 90 mg/kg of streptozotocin. The pancreatic toxin causes partial β cell destruction accompanied by hyperglycemia and severe decreases in plasma insulin [14]. However, unlike the condition produced in young adult rats exposed to streptozotocin, in the neonatal rat the overt diabetic phase is merely transient. Within a week, plasma glucose and insulin levels return to normal, presumably because of the unique ability of the neonatal rat to regenerate β cells. Following a period of normoglycemia, nonfasting blood glucose levels of these animals slowly rise. By six months of age, these animals exhibit normal fasting glucose levels, but slightly elevated nonfasting glucose levels compared to age-matched controls. More importantly, these animals become markedly glucose intolerant when subjected to a glucose challenge. It is common for these animals to exhibit severe hyperglycemia and hyperinsulinemia following administration of glucose (Fig. 1). The abnormal response to a glucose challenge has been attributed to a specific defect in the sensitivity of the β cell to glucose. Analogous to patients with noninsulin-dependent diabetes [5, 12, 15, 16], glucose-induced, first phase insulin release is dramatically depressed or absent in pancreas isolated from chemically-induced noninsulin-dependent diabetic rats, while second phase insulin release often is exaggerated. Significantly, in the neonatal diabetic model, responsiveness of the β cells to other secretagogues of insulin release, such as arginine, glyceraldehyde, sulfonylureas, isoproterenol and glucagon remain seemingly normal [17]. Insulin resistance also develops in this model, but it is preceded by a defect in insulin secretion. Glucose clamp studies reveal that only after several months of abnormal insulin secretion will these animals exhibit impaired suppression of hepatic glucose production by insulin [14, 18]. Also abnormal in these older animals is insulin-mediated stimulation of glucose uptake and utilization by peripheral tissues, such as cardiac muscle and adipose tissue [19, 20]. These metabolic characteristics indicate that the chemically-induced noninsulin-dependent diabetic rat serves as an excellent model of the lean noninsulin-dependent diabetic subset.

Cardiomyopathy in noninsulin-dependent diabetics

Clinically apparent heart dysfunction is a common complication of noninsulin-dependent diabetes [21–24]. In a 1977 pioneering study, Regan *et al.* [25] performed catheterizations in noninsulin-dependent diabetics with and without symptoms of heart failure. These patients exhibited abnormally high end-diastolic pressure to volume ratios, indicative of decreased left ventricular compliance. Furthermore, they were characterized by a low stroke volume index and reduced response to an afterload stress, consistent with impaired contractile reserve.

Noninvasive techniques, such as echocardiography, radionuclide ventriculography and determination of systolic time intervals, have generally been supportive of the original pioneering studies by Regan *et al.* [25]. Left ventricular systolic time intervals are abnormal in many noninsulin-dependent diabetics showing no evidence of coronary heart disease or hypertension [26, 27]. In a recent study by Mustonen *et al.* [28], it was found that systolic defects can be partially reversed in noninsulin-dependent diabetics exhibiting impaired insulin secretion capacity by treatment with insulin.

In addition to systolic dysfunction, abnormalities in left ventricular diastolic function, such as reduced diastolic filling rate, prolonged isovolumic relaxation period and mitral diastolic closure rate, are also frequently observed in noninsulin-dependent diabetes [29, 30]. It has been suggested that diastolic dysfunction may contribute to the high cardiac morbidity and mortality among diabetic patients [31, 32].

Cardiac energy metabolism in noninsulin-dependent diabetes

Present-day dogma regarding the effects of dia-

betes on cardiac metabolism has largely been shaped by the classical studies of Randle [33]. According to the Randle hypothesis, the major bottleneck limiting myocardial glycolytic flux in the insulin-dependent diabetic is inhibition of the glycolytic enzyme, phosphofructokinase. The activity of this key enzyme is not directly affected by diabetes; rather, reduced flux through the enzyme occurs secondary to the accumulation of citrate, a potent inhibitor of phosphofructokinase. In the insulin-dependent diabetic, the series of reactions which culminate in the elevation of myocardial citrate levels and inhibition of glycolysis, originate with the massive mobilization of fatty acids from adipose tissue. The resulting elevation of plasma fatty acids leads to an increase in myocardial fatty acid uptake and oxidation. One of the consequences of enhanced β-oxidation is the accumulation of citrate. Besides the inhibition of glycolytic flux, the block at phosphofructokinase also results in a rise in glucose-6-phosphate levels and a diversion of substrate into glycogen synthesis.

Another important step of glucose metabolism affected by insulin-dependent diabetes is the enzyme complex, pyruvate dehydrogenase [34–37]. This enzyme plays a major role in glucose oxidation by serving as the initial reaction in the metabolism of pyruvate by the mitochondria. Because of its important link between glycolysis, the citric acid cycle and fatty acid metabolism, it is not surprising that its activity is tightly regulated by a number of factors. Inactivation of the enzyme is largely accomplished by the phosphorylation of a specific serine residue (site 1) on the α-chain of the decarboxylase [35]. However, two additional α-chain serine residues (sites 2 & 3) can be phosphorylated by pyruvate dehydrogenase kinase. While phosphorylation of sites 2 and 3 probably do not appreciably contribute to inactivation of the enzyme, they do affect the rate at which the phosphorylated enzyme can be reactivated by pyruvate dehydrogenase phosphatase; the rate is significantly slower in the fully phosphorylated enzyme [38, 39]. Interconversion between the active, unphosphorylated form and the inactive, phosphorylated form of the enzyme in the nondiabetic, intact heart is largely controlled by tissue levels of pyruvate, the mitochondrial ratios of $NADH/NAD^+$ and acetyl CoA/CoA, mitochondrial calcium content, the extent of cardiac work and the rate of oxygen consumption [40–44]. Because diabetes increases fatty acid metabolism, with a resulting increase in myocardial acetyl CoA/CoA and $NADH/NAD^+$ ratios, it has been hypothesized that reduced pyruvate dehydrogenase activity in diabetic heart may be related to changes in the mitochondrial levels of these regulators [45]. This concept is supported by the observation that inhibitors of fatty acid oxidation, such as sodium tetradecylglycidate, reverses the effects of diabetes on the percentage of active pyruvate dehydrogenase complex by lowering the acetyl CoA/CoA ratio [46]. Moreover, perfusion of the nondiabetic rat heart with fatty acids leads to an increase in tissue $NADH/NAD^+$ and acetyl CoA/CoA ratios, which is associated with decreases in pyruvate oxidation and the activity of the pyruvate dehydrogenase complex [42]. However, these factors are not the only regulators contributing to reduced pyruvate dehydrogenase activity in the diabetic heart. Kobayashi and Neely [37] have shown that the activity of pyruvate dehydrogenase is lower in the diabetic heart than anticipated on the basis of the ratios of $NADH/NAD^+$ and acetyl CoA/CoA. Moreover, the diabetic heart is resistant to activating effects of other regulators, such as increased work load, enhanced oxygen consumption and elevated tissue pyruvate levels, suggesting that some other factor must contribute to the regulation of pyruvate dehydrogenase in the diabetic heart. This notion was initially introduced by Randle and coworkers [36, 46], who observed that pyruvate dehydrogenase was significantly less active in diabetic heart than hearts oxidizing fatty acids and ketone bodies. Further studies by Randle and coworkers have revealed that diabetic heart contains a protein, known as kinase activator protein, which activates pyruvate dehydrogenase kinase [46, 47]. The activity of this factor in heart is increased by diabetes and starvation by a process involving cytoplasmic protein synthesis [47]. The combination of elevated kinase activator protein and the mitochondrial ratios of acetyl CoA/CoA and $NADH/NAD^+$, lead to a significant stimulation in pyruvate dehydrogenase kinase activity. As

a result, all three serine residues (sites 1–3) of the pyruvate dehydrogenase complex become phosphorylated and the activity of the enzyme complex becomes virtually inactive [48].

There seems little doubt that obese noninsulin-dependent diabetics belong to a subset of noninsulin-dependent diabetics in which the Randle mechanism also operates [5]. These individuals have elevated plasma fatty acid levels; therefore, cardiac rates of fatty acid oxidation are most likely elevated in comparison to that observed in the nondiabetic [49]. By the same argument, myocardial citrate levels presumably increase, thereby inhibiting flux of substrate through phosphofructokinase and diverting substrate into the glycogen synthetic pathway. One other consequence of increased fatty acid availability will be the elevation in mitochondrial acetyl CoA/CoA ratio, with the resulting inhibition of pyruvate dehydrogenase and reduction in glucose and pyruvate oxidation [50, 51].

Table 1. Effect of noninsulin-dependent diabetes on tissue metabolites

Metabolite	Tissue content (μmol/g dry weight)	
	Nondiabetic	Diabetic
Oxaloacetate	0.03 ± 0.003	0.03 ± 0.001
Malate	0.25 ± 0.04	0.30 ± 0.04
Citrate	0.39 ± 0.05	0.40 ± 0.03
α-Ketoglutarate	0.37 ± 0.03	0.40 ± 0.02
Acetyl CoA	0.02 ± 0.005	0.02 ± 0.005
CoA	0.61 ± 0.02	0.66 ± 0.06
Long chain fatty acyl CoA	0.10 ± 0.006	0.10 ± 0.003
Long chain fatty acylcarnitine	0.28 ± 0.04	0.22 ± 0.01
Carnitine	5.57 ± 0.23	4.99 ± 0.28
Aspartate	14.7 ± 2.2	17.7 ± 3.3
Glutamate	22.0 ± 2.4	26.0 ± 3.0
Alanine	2.1 ± 0.2	2.4 ± 0.2
ATP	21.1 ± 0.5	21.0 ± 0.6
Phosphocreatine	20.7 ± 1.3	19.3 ± 0.5
Glucose-6-phosphate	0.39 ± 0.02	0.40 ± 0.03
Fructose 1,6-bisphosphate	0.11 ± 0.01	0.07 ± 0.01*
Glycogen	115.0 ± 12.0	88.0 ± 11.0*

Hearts from noninsulin-dependent diabetic and nondiabetic rats were perfused with Krebs-Henseleit buffer containing 11 mM glucose. After 15 min the hearts were rapidly frozen with Wollenberger clamps precooled in liquid nitrogen. Samples were extracted and assayed. Values are means ± S.E.M. of 5 hearts. * denotes significant difference from nondiabetic ($p < 0.05$).

In contrast to the obese noninsulin-dependent diabetic population, lean diabetics with moderate fasting hyperglycemia belong to a diabetic subset which do not exhibit elevated plasma fatty acid levels and whose basal rates of lipid oxidation are not increased [5]. Most information on the function and metabolism of the lean noninsulin-dependent diabetic heart has been gleaned from studies utilizing the chemically-induced noninsulin-dependent diabetic rat model.

The metabolic pattern of the noninsulin-dependent diabetic rat heart is largely regulated by a few insulin-dependent, rate-limiting steps. However, unlike other types of diabetes, such as insulin-dependent and obese noninsulin-dependent diabetes, the lean noninsulin-dependent diabetic does not experience massive fatty acid mobilization and accompanying stimulation of fatty acid oxidation. Therefore, cardiac metabolism in these diabetics is not affected to any appreciable extent by the Randle mechanism. This prediction is borne out by analysis of key cardiac metabolic intermediates. The Randle hypothesis proposes that promotion of fatty acid oxidation will cause cardiac levels of long chain fatty acylcarnitine, long chain fatty acyl CoA, citrate, glucose-6-phosphate and glycogen to dramatically increase [33]. However, this pattern does not develop in the noninsulin-dependent diabetic rat (Table 1). While glycolytic flux is inhibited in these animals, this is not caused by an elevation in citrate levels and the development of a bottleneck at phosphofructokinase. Moreover, there is no diversion of substrate away from glycolysis and into the glycogen synthetic pathway [20]. In fact, myocardial glucose-6-phosphate levels remain unchanged while glycogen levels are reduced, presumably as a result of lower glycogen synthase activity (Table 1).

In the lean noninsulin-dependent diabetic, glucose utilization is depressed, but this abnormality appears to be largely linked to an impairment in glucose transport (Fig. 2). Although several factors are capable of modifying glucose transport [52, 53], recent studies suggest that the diabetes-mediated

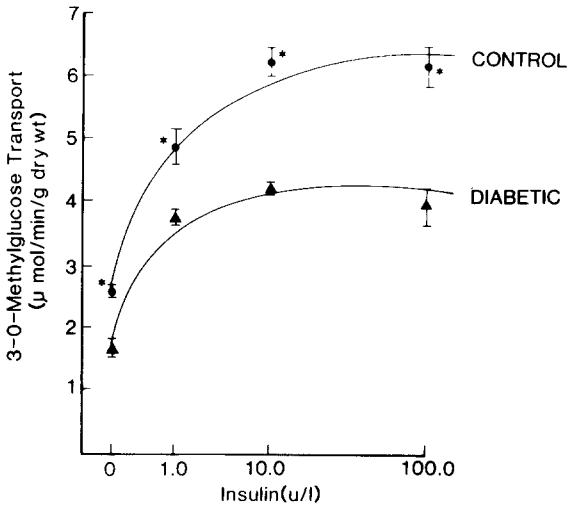

Fig. 2. Effect of noninsulin-dependent diabetes on glucose transport. Diabetic (▲—▲) and nondiabetic (●—●) hearts were perfused with buffer containing either 0, 1.0, 10.0 or 100 U/l insulin. Although the rate of 3-O-methyl-D-glucose transport was accelerated by insulin in both the diabetic and nondiabetic, the response was severely reduced in the diabetic. * denotes significant difference from diabetic ($p < 0.05$). Values represent means ± S.E.M. of four hearts. Reproduced from Schaffer *et al.* [20] with the permission of the American Diabetes Association.

defect may involve a reduction in the amount of glucose transporter associated with the plasma membrane [54–56]. Normal heart contains two glucose transport isoforms, the major type exhibiting a marked ability to translocate to the plasma membrane in response to insulin [54, 57]. Because the translocation process is important in insulin-mediated stimulation of glucose transport, it is not surprising that this transporter is the predominant form, not only in heart, but also in other insulin-responsive tissues. Expression of this transporter is regulated by insulin; therefore, in diabetic animals its levels become markedly reduced [58]. Although such a decrease in cell membrane content of the transporter has been confirmed in skeletal muscle and adipose tissue from insulin-dependent diabetic rats, it remains to be shown that glucose transporter content is depressed in the noninsulin-dependent diabetic heart. Nevertheless, this mechanism or a similar one is probably operative in the noninsulin-dependent diabetic heart because of the dramatic decrease in insulin responsiveness of the glucose transport system of these animals (Fig. 2).

While genetic diabetes found in C57BL/KsJ mice is associated with degeneration of mitochondria [59], no evidence of mitochondrial dysfunction is seen in the chemically-induced, noninsulin-de-

Table 2. Effect of noninsulin-dependent diabetes on metabolic flux

Rate process	Nondiabetic	Diabetic
Rate (μmol glucose equivalents/g dry wt/hr)		
Glycolysis	307	190
Glycogenolysis	0	0
Lactate output	128	102
Rate (μmol O_2/g dry wt/hr)		
Oxidation of pyruvate + glycolytic NADH	994	468
Tricarboxylic acid cycle	2500	2017
Palmitate β-oxidation	808	750
Rate (μmol ATP/g dry wt/hr)		
ATP synthesized from glucose	6585	3191
ATP from fatty acids	14874	13787
Total ATP synthesis	21459	16978

Hearts from noninsulin-dependent diabetic and nondiabetic rats were perfused with buffer containing 11 mM glucose. Perfusate was collected and used to measure the rates of lactate and pyruvate production from triose content and coronary flow rate. Glucose utilization was determined by measuring the extent of tritium release from [3-^{3}H]-glucose into water. The changes in glycogen content of the perfused heart over a 20 minute perfusion period was used as the measure of glycogenolysis. Glycolytic flux was calculated from the rates of glucose utilization and glycogenolysis. The rate of oxygen consumption was calculated from the flow rate and the A-V difference measured by use of a Clark oxygen electrode. Glucose oxidation was assessed from the difference between glycolytic flux and lactate plus pyruvate production. Its contribution to oxygen consumption, along with that of pyruvate, was based on theoretical oxygen equivalents. Palmitate oxidation refers to that portion of oxygen consumption not due to glucose oxidation and represents endogenous fatty acid oxidation. The ratios of ATP produced to oxygen consumed utilized in calculating ATP synthesis are: glucose, 3.17 and palmitate 2.8. The net yield of ATP from lactate output was assumed to be 2 μmol of ATP/μmol of glucose, whereas pyruvate output was assumed to be 8 μmol of ATP/μmol of glucose. Reproduced in part from Schaffer *et al.* [20] with permission of the American Diabetes Association.

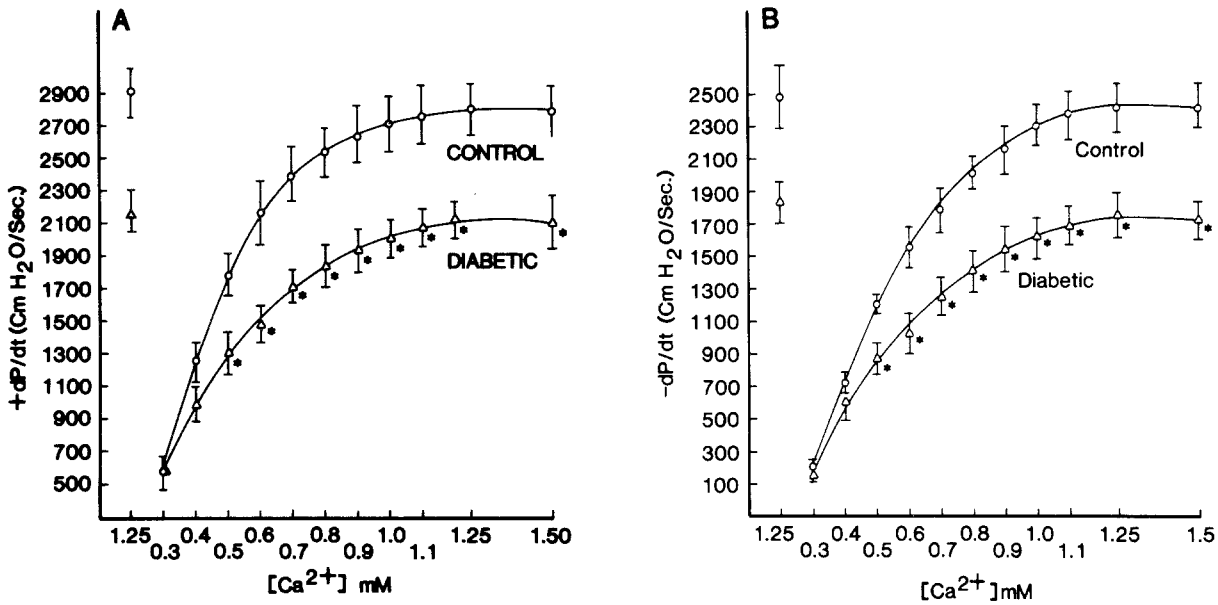

Fig. 3. Effect of noninsulin-dependent diabetes on myocardial contractility and relaxation. Hearts from noninsulin-dependent diabetic (△—△) and nondiabetic (○—○) rats were perfused with buffer containing various concentrations of calcium. At each calcium concentration, + dP/dt (A) and − dP/dt (B) were measured. Each data point represents the mean ± S.E.M. of 5–7 hearts. * denotes significant difference from nondiabetic ($p < 0.05$). Reproduced from Schaffer *et al.* [62] with permission of the American Physiological Society.

pendent diabetic rat. The slight reduction in oxygen consumption, oxidative metabolism and citric acid cycle flux appears to be secondary to the decline in mechanical function [20]. Estimates of high energy phosphate generation reveal that a greater percentage of ATP synthesis is derived from oxidative metabolism in the noninsulin-dependent diabetic rat heart than in age-matched nondiabetic controls (Table 2). Thus, the diabetic heart compensates for the decrease in nonoxidative metabolism by promoting oxidative metabolism, enabling the heart to maintain normal high energy phosphate reserves (Table 1). Also significant in these animals is the absence of changes in myocardial levels of key citric acid cycle and amino acid intermediates (Table 1), implying that noninsulin-dependent diabetes does not mediate an unspanning of citric acid cycle flux [60].

Mechanical dysfunction in noninsulin-dependent diabetic rat

Mechanical defects in the chemically-induced, noninsulin-dependent diabetic rat develop very slowly. Four months following treatment with streptozotocin, the diabetic rats show no detectable mechanical dysfunction or metabolic abnormalities [61]. However, by 8–12 months, cardiac function of the diabetic group is significantly depressed relative to nondiabetic age-matched controls. When perfused with standard Krebs-Henseleit buffer containing 1.25 mM calcium and paced at 300 beats/minute, hearts from diabetic rats exhibit significantly lower rates of pressure development and relaxation, maximal systolic pressure, cardiac output and cardiac work than comparable age-matched control hearts (Fig. 3). These effects are not reversed by adding high levels of insulin to the perfusion medium, providing an alternate substrate to the heart or increasing calcium content of the medium [61, 62]. Thus, the noninsulin-dependent diabetic heart appears to have undergone significant biochemical changes.

While there are many similarities between the mechanical aberrations of insulin-dependent and noninsulin-dependent diabetes, one major difference exists between the two diseases. Hearts from

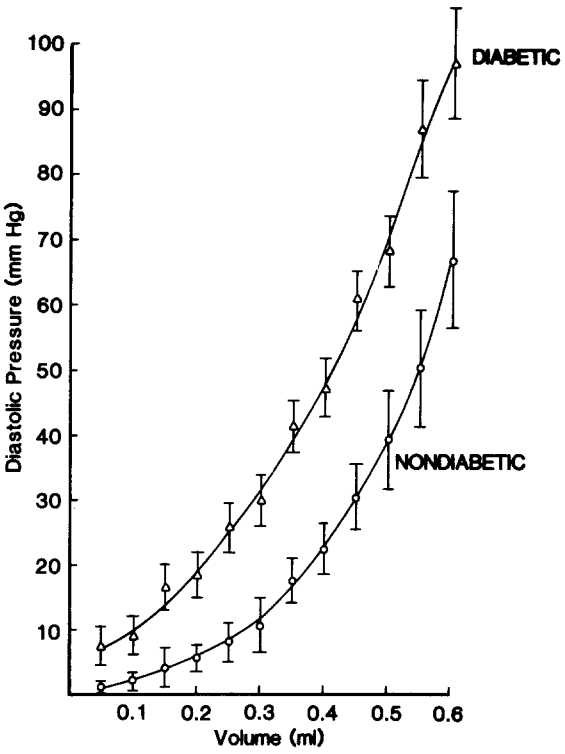

Fig. 4. Effect of noninsulin-dependent diabetes on diastolic compliance. A deflated balloon was inserted into the left ventricle of noninsulin-dependent diabetic (△—△) and nondiabetic (○—○) rats. After determining base-line pressure, 50 μl increments of saline were injected into the balloon and pressure within the balloon recorded. At a given volume, diastolic pressure was significantly greater in the diabetic. Each data point represents the mean ± S.E.M. of 8–12 hearts. Reproduced from Schaffer *et al.* [62] with the permission of the American Physiological Society.

noninsulin-dependent diabetic rats exhibit a dramatic decrease in diastolic compliance, as evidenced by a shift of the pressure-volume relation toward the pressure axis (Fig. 4). By contrast, the pressure-volume relation of the insulin-dependent diabetic rat heart is shifted away from the pressure axis [63, 64]. Yet, recent work by Litwin *et al.* [64] has shown that due to increases in end diastolic pressure, operating chamber stiffness is actually increased in the insulin-dependent diabetic rat heart, at least during the early phases of the diabetic condition. Thus, although passive elastic behavior differs in the two models, diastolic function is impaired in both. In this regard, it is significant that

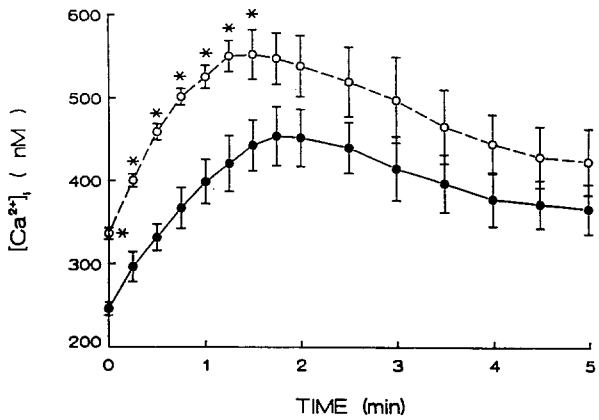

Fig. 5. Effect of noninsulin-dependent diabetes on $[Ca^{2+}]_i$ of quiescent, calcium-tolerant cardiomyocytes. Isolated myocytes prepared from nondiabetic (●—●) and noninsulin-dependent diabetic (○--○) rat heart were loaded with the calcium probe fura 2. At time zero, the cells were depolarized with 30 mM KCl. Both the initial cytosolic free calcium concentration, as well as the rate of rise in $[Ca^{2+}]_i$ following depolarization were elevated in the diabetic. Values shown represent means ± S.E.M. of four different preparations. * denotes significant difference from nondiabetic ($p < 0.05$). Reproduced with the permission of the American Physiological Society.

diastolic dysfunction is observed in humans with noninsulin-dependent diabetes and is thought to adversely affect ventricular filling and account for many of the hemodynamic changes seen in the diseased heart [65].

Table 3. Effect of noninsulin-dependent diabetes on myocardial Ca^{2+}

Condition	Na^+,K^+ ATPase Activity (μmol P_i/hr/mg)	Total Myocardial Ca^{2+} Content (μmol/g wet wt)	$[Ca^{2+}]_i$ (nM)
Nondiabetic	32.1 ± 2.5	0.85 ± 0.06	246 ± 8
Diabetic	18.1 ± 2.2*	1.15 ± 0.10*	336 ± 7*

Na^+, K^+ ATPase represents ouabain sensitive activity of isolated sarcolemma assayed in the presence of 1 mg alamethicin/mg sarcolemmal protein. Myocardial calcium content was determined after homogenization and extraction of calcium. $[Ca^{2+}]_i$ was measured in isolated myocytes prepared from nondiabetic and noninsulin-dependent diabetic rats using the fura 2 method. Diabetes was found to mediate a reduction in Na^+,K^+ ATPase activity and a rise in total myocardial calcium content and $[Ca^{2+}]_i$. Values shown represent means ± S.E.M. of 4–6 hearts. * denotes significant difference from nondiabetic ($p < 0.05$).

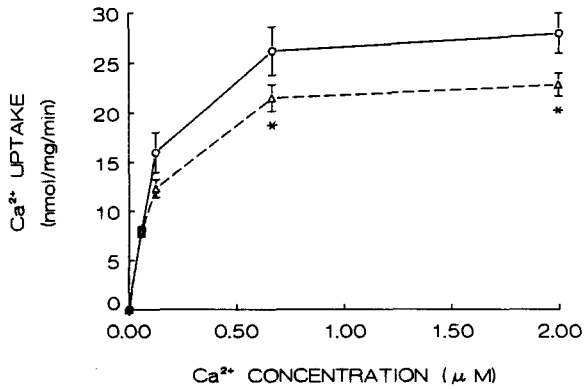

Fig. 6. Effect of noninsulin-dependent diabetes on sarcoplasmic reticular calcium transport. ATP-dependent calcium uptake into enriched sarcoplasmic reticulum from noninsulin-dependent diabetic (△– △) and nondiabetic (○—○) rats was determined over a free calcium concentration of 0.06–2.0 μM. Diabetes was associated with a slight decrease in calcium transport. Data points represent means ± S.E.M. of 5 preparations. * denotes significant difference from age-matched nondiabetic control preparations ($p < 0.05$).

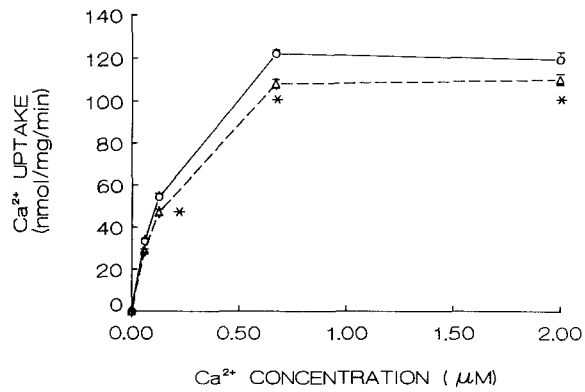

Fig. 7. Effect of noninsulin-dependent diabetes on calcium transport by the sarcolemmal calcium pump. ATP-dependent calcium uptake into sarcolemma from noninsulin-dependent diabetic (△--△) and nondiabetic (○—○) rats was determined over a free calcium concentration range of 0.06–2.0 μM. Diabetes was associated with a modest decrease in calcium transport at the higher calcium concentrations examined. Each data point represents mean ± S.E.M. of 5–7 preparations. * denotes significant difference from age-matched nondiabetic control ($P < 0.05$).

Abnormal calcium homeostasis in the noninsulin-dependent diabetic heart

It has been proposed that one of the factors contributing to the development of muscle stiffness in the noninsulin-dependent diabetic rat heart is partial activation of actomyosin caused by cytoplasmic calcium overload [62]. There seems little doubt that total myocardial calcium content is elevated in the noninsulin-dependent diabetic (Table 3). Less clear is the existence of cytoplasmic calcium overload. $[Ca^{2+}]_i$ measurements using the calcium probe, fura 2, provide the most compelling evidence that noninsulin-dependent diabetic rat heart contains higher cytoplasmic levels of calcium than the nondiabetic age-matched control heart. As seen in Fig. 5 dispersed, quiescent myocytes prepared from noninsulin-dependent diabetic rats contain 30% more $[Ca^{2+}]_i$ than myocytes of age-matched controls. Moreover, both the rate and extent of cellular calcium accumulation following K^+-induced depolarization is greater in diabetic myocytes. While the exactness of the fura 2 method as a quantitative measure of $[Ca^{2+}]_i$ has been questioned, most investigators agree that the technique is very effective in detecting highly significant qualitative differences in $[Ca^{2+}]_i$ [66]. Thus, it is reasonable to conclude that handling of Ca^{2+} by diabetic myocytes is abnormal.

The process of tissue calcium overload is a complex phenomenon, usually involving impairment in the movement of calcium by one or more calcium transporters [67–69]. In the normally functioning myocardium, the sarcoplasmic reticular calcium pump serves as the primary mechanism for removal of calcium from the cytoplasm. Abnormalities in this transporter have been shown to have a profound impact on both myocardial contractility and relaxation [67–69]. Nevertheless, the diabetes-mediated defect in calcium homeostasis does not appear to involve impaired sarcoplasmic reticular calcium transport. First, calcium transport by isolated sarcoplasmic reticulum obtained from noninsulin-dependent diabetic heart is only slightly depressed in comparison to nondiabetic, age-matched controls (Fig. 6). Second, although the sarcoplasmic reticulum is capable of decreasing cytoplasmic calcium levels, it is incapable of extruding calcium from the cell. Therefore, calcium accumulation by the diabetic heart cannot be caused by a defect in sarcoplasmic reticular calcium transport. Similar

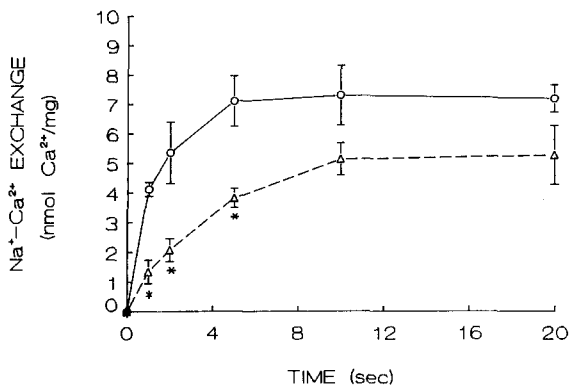

Fig. 8. Effect of noninsulin-dependent diabetes on sarcolemmal $Na^+ - Ca^{2+}$ exchange. Enriched sarcolemma from noninsulin-dependent diabetic (△--△) and nondiabetic (○—○) rat heart was loaded with buffer containing 140 mM NaCl. The exchange was initiated by placing the loaded vesicles into Na^+-free buffer containing 30 μM $^{45}Ca^{2+}$. Diabetic sarcolemma exhibited dramatically lower rates of $Na^+ - Ca^{2+}$ exchange than the nondiabetic. Each data point represents mean ± S.E.M. of 5 preparations. * denotes significant difference from nondiabetic preparations ($p < 0.05$). Reproduced with the permission of the American Physiological Society.

arguments can be raised regarding the involvement of the sarcolemmal calcium pump. Figure 7 reveals that sarcolemmal ATP-dependent calcium transport is only modestly affected by noninsulin-dependent diabetes. Thus, although this transporter is capable of extruding calcium from the cell, the degree of impairment is insufficient to appreciably affect calcium homeostasis. Moreover, the capacity of the pump to transport calcium from the cell is extremely limited and even in the uninhibited state its contribution to maintenance of tissue calcium homeostasis is negligible [69].

In contrast to the sarcolemmal and sarcoplasmic reticular calcium pumps, the transport of calcium by the $Na^+ - Ca^{2+}$ exchanger is severely impaired (Fig. 8). It is generally accepted that this high capacity calcium transporter normally functions to pump calcium into the cell during the action potential and extrude calcium from the cell during diastole [70]. Wier [69] estimates that during the course of the contraction-relaxation cycle, approximately 6 μmoles/l calcium exits the cell via the $Na^+ - Ca^{2+}$ exchanger. Because the same amount of calcium enters the cell via the Ca^{2+} channel, a decrease in flux through the exchanger could significantly compromise the ability of the diabetic heart to maintain calcium homeostasis and contribute to cellular accumulation of calcium. The $Na^+ - Ca^{2+}$ exchanger is also defective in the insulin-dependent diabetic heart [71]; however, due to the significant impairment in both sarcoplasmic reticular and sarcolemmal calcium pump activity [72–74], on a relative basis, the contribution of the $Na^+ - Ca^{2+}$ exchanger to the imbalance in calcium homeostasis is presumably less in insulin-dependent diabetes.

Another factor which may contribute to the elevation in tissue calcium levels in the diabetic heart is increased $[Na^+]_i$. Although myocyte sodium content has not been directly measured, it has been shown that the activity of the Na^+, K^+ ATPase is dramatically reduced in the diabetic heart (Table 3). Because this enzyme indirectly regulates intracellular calcium levels through its modulation of $[Na^+]_i$ [67, 69], the diabetes-linked decrease in Na^+, K^+ ATPase activity would be expected to mediate net increases in both $[Na^+]_i$ and $[Ca^{2+}]_i$.

Elevated intracellular calcium levels can also arise as a result of accelerated flux through the sarcolemmal calcium channel. Sperelakis and coworkers [75] have proposed that the response of the calcium channel to voltage activation depends upon its phosphorylation state. According to this hypothesis, the calcium channel remains inoperative in its dephosphorylated state. Phosphorylation of the calcium channel or a contiguous regulatory phosphoprotein changes the properties of the calcium channel by either facilitating the opening of the activation gate upon depolarization or effectively increasing the diameter of the pore, thereby enhancing influx of calcium [75, 76]. Because the sarcolemma contains phosphoprotein phosphatases capable of dephosphorylating the calcium channel, the life span of the phosphorylated channel in the normal heart is likely to be only a few seconds [75, 77, 78]. However, in the noninsulin-dependent diabetic myocardium, protein phosphatase 1 activity is dramatically reduced, which presumably prolongs the life span of the phosphorylated channel [78]. In support of this idea it has been shown that sarcolemma prepared from noninsulin-dependent diabetic rat heart is

phosphorylated more extensively by either endogenous protein kinases or skeletal muscle cAMP-dependent protein kinase than nondiabetic membrane [78]. SDS polyacrylamide gel electrophoresis analysis reveals that one of the sarcolemmal proteins which is phosphorylated more extensively in diabetic membrane is a 57 kD protein (Fig. 9). It is probably no coincidence that skeletal muscle calcium channel contains a 52 kD subunit, which is phosphorylated by cAMP-dependent protein kinase [79]. Although further study is required to identify the nature of the 57 kD polypeptide, it is significant that Nobe *et al.* [80] observed a prolongation of action potential duration in insulin-dependent diabetic rats as a result of stimulation in slow inward current.

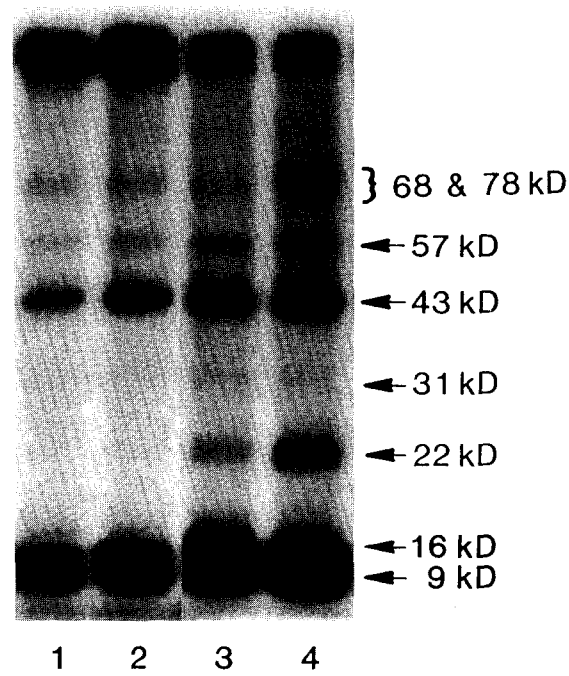

Fig. 9. Effect of noninsulin-dependent diabetes on sarcolemmal protein phosphorylation. Sarcolemma from noninsulin-dependent diabetic (Lanes 2 and 4) and nondiabetic (Lanes 1 and 3) were phosphorylated in medium containing [^{32}P]-ATP incubated in the presence (Lanes 3 and 4) or absence (Lanes 1 and 2) of cAMP-dependent protein kinase. Shown is the autoradiogram of membrane proteins subjected to polyacrylamide gel electrophoresis. Diabetes increases the amount of [^{32}P] incorporated into the sarcolemma. Although not shown this difference is eliminated by inclusion of a phosphatase inhibitor into the reaction medium. Reproduced from Allo and Schaffer [78] with permission of Biochimica Biophysica Acta.

Diabetes-linked alterations in myosin isozyme content

While alterations in calcium homeostasis are thought to contribute to the appearance of certain myocardial defects in diabetes, there is reason to believe that altered myosin isozyme content may also contribute to the development of the cardiomyopathy. Normal rat heart contains three myosin isozymes; myosin V_1 consists of two myosin heavy A chains, myosin V_2 of one myosin heavy A chain and one myosin heavy B chain and myosin V_3 of two myosin heavy B chains [81]. In younger animals the dominant form is myosin V_1, which exhibits the highest Ca^{2+} – stimulated ATPase activity [82]. Aging promotes a shift in myosin composition in favor of the least active V_3 isozyme form. This shift also occurs in hypothyroidism, hypertension, heat stress and pathological conditions, such as aortic stenosis, chronic volume overload and genetic myopathy [82–87]. Alpert and coworkers [81] have proposed that conditions leading to an elevation in the V_3 isozyme decrease the rate of isometric force development and the velocity of shortening, rendering the contractile machinery slow, but energy efficient. By contrast, hearts with elevated V_1 myosin isozyme content produce a fast, but less economic contractile unit.

Both insulin-dependent and noninsulin-dependent diabetes are associated with a shift in myosin isozyme content in favor of the least active V_3 form [62, 88–91]. This defect can be reversed by treatment of the insulin-dependent diabetic animal with insulin [92], but not with an oral agent commonly used in treating noninsulin-dependent diabetic patients [93]. In addition to insulin, Dillmann [94, 95] recently showed that either fructose feeding or treatment of diabetic rats with methyl palmoxirate, an inhibitor of fatty acid oxidation, is capable of preventing the loss in myosin V_1 isozyme content. Because insulin levels are not affected by these metabolic modulators, it appears that changes in the metabolic status of the heart can affect the levels of mRNA which code for specific chains of

myosin [96]. In a similar fashion, exercise improves the metabolic status of the insulin-dependent diabetic rat heart, while promoting a shift in myosin in favor of its active V_1 isozyme form [97, 98]. While most of these studies have focused on the shift in myosin isozyme content, some studies have examined both shifts in myosin isozyme content and changes in myocardial mechanical function. Generally, treatment protocols which improve contractile function of the insulin-dependent diabetic heart also increase myosin Ca^{2+} ATPase activity [99–101]. However, some exceptions have been observed, indicating that the shift in myosin isozyme content is not the only factor contributing to the impairment in mechanical function [91, 93].

Glucose hypothesis and diabetic complications

One of the most widely debated clinical issues is whether hyperglycemia leads to secondary biochemical abnormalities in target tissues. This question is very important because of evidence that tight control of glucose reduces the incidence of diabetic complications, including diabetes-mediated myocardial contractile defects [102]. Advocates of this theory, known as the glucose hypothesis, assume that high glucose levels are toxic to cells and can initiate a sequence of events culminating in altered tissue function. Several potentially relevant biochemical sequelae to hyperglycemia have been identified in tissue susceptible to diabetic complications.

1. Sorbitol or polyol hypothesis

A theory which has generated considerable attention is the so-called sorbitol or polyol hypothesis. In many tissues, high levels of glucose lead to the accumulation of sorbitol through a reaction catalyzed by the enzyme aldose reductase. Elevated sorbitol levels were initially thought to cause an osmotic imbalance, leading to cellular edema. However, with the possible exception of diabetic cataract formation [103], this theory has not held up. Nevertheless, evidence that inhibitors of aldose reductase produce modest improvements in neuronal, renal, retinal and cardiac function of diabetic patients and animals support the notion that increased polyol pathway activity is related to a number of diabetic complications [104–111]. Although the mechanism underlying the adverse effects of the polyol pathway have not been established, in the last few years considerable attention has focused on the reciprocal relationship between tissue myo-inositol and sorbitol content. Greene and coworkers [112, 113] have argued that sorbitol interferes with the ability of tissues to accumulate myo-inositol, thereby depleting myo-inositol levels. While questions have been raised about the relationship between myocardial levels of myo-inositol and sorbitol in diabetic rats, there seems to be little doubt that myo-inositol plays some role in the development of the insulin-dependent diabetic cardiomyopathy. Xiang *et al.* [101] have shown that treatment of insulin-dependent diabetic rats with myo-inositol improves myocardial function, an effect they attributed to a reduction in plasma and myocardial triacylglycerol levels. However, more recent studies suggest alternate mechanisms for the effects of myo-inositol supplementation. It is now widely accepted that inositol-containing lipids play a very important role in signal transduction. Through the promotion of phosphoinositide turnover a number of hormones and neurotransmitters yield two cellular messengers: (1) inositol 1, 4, 5 triphosphate, a second messenger involved in calcium mobilization from sarcoplasmic reticular stores, and (2) diacylglycerol, an activator of protein kinase C. Recognizing the link between phosphoinositide turnover and cellular calcium movement, Bergh *et al.* [114] explored the effects of diabetes on phosphoinositide metabolism by muscarinic agonists. They found that [^{3}H] myo-inositol incorporation into cardiac IP, IP_2 and IP_3 pools was reduced in the insulin-dependent diabetic rat and speculated that the regulation of these pools by insulin could modulate cardiac function. They also measured carbachol-mediated release of inositol-1-phosphate, but unfortunately the effect of diabetes on inositol 1, 4, 5 triphosphate formation was not determined, leaving unexplored the possibility that impaired calcium homeostasis may be associated

with changes in phosphoinositide metabolism. Another area which deserves some consideration is that myo-inositol deficiency may interfere with the actions of insulin. There is an increasing body of evidence that myo-inositol is a component of a unique glycolipid, which may function as a second messenger of insulin action [115].

2. Nonenzymatic and enzymatic glycosylation hypothesis

It has been known for some time that carbohydrates can react nonenzymatically with proteins to yield a Schiff base, which can subsequently be converted into a ketoamine via an Amadori rearrangement. The formation of these glycosylated protein adducts has been implicated in several diabetic complications, including retinopathy, nephropathy and cataract formation [116–118]. Another pathological consequence of hyperglycemia is the stimulation of basement membrane synthesis [119]. While these reactions may be important in the pathology of some tissues, neither reaction appears to contribute to the development of diabetes-mediated heart abnormalities. As reported by Ganguly *et al.* [120] nonenzymatic glycosylation products do not accumulate in insulin-dependent diabetic heart. Moreover, consistent with the extremely low glucosyl- and galactosyltransferase activity in the heart [121], no change in membrane glycoprotein content of the insulin-dependent diabetic heart is observed [120].

Involvement of lipids in diabetic complications

1. Accumulation of toxic amphiphiles

The presence of high myocardial levels of triacylglycerol, free fatty acids, long-chain acyl CoA and long-chain acyl carnitine in insulin-dependent diabetic animals has been recognized for some time [33, 122]. In the micromolar range, the long-chain acyl esters have been shown to inhibit several membrane transporters, such as the sarcoplasmic reticular calcium pump, the sarcolemmal Na^+, K^+ antiport and the mitochondrial adenine nucleotide translocase [123–124]. Lopaschuk *et al.* [125] have reported that levels of long-chain acyl carnitine associated with isolated sarcoplasmic reticulum are 30–50% greater in insulin-dependent diabetic animals, implying that the accumulation of these long chain esters in the insulin-dependent diabetic heart may contribute to alterations in sarcoplasmic reticular calcium transport. Support for this view comes from the observation that treatment of insulin-dependent diabetic rats with either carnitine or the carnitine palmityl transferase inhibitor, methyl palmoxirate, reduces total tissue long-chain acyl carnitine content, while increasing sarcoplasmic reticular calcium transport [23]. However, these treatment protocols do not affect the functional abnormality, indicating that other factors are responsible for mechanical dysfunction in insulin-dependent diabetes [23]. In the lean noninsulin-dependent diabetes model, no elevation in myocardial long-chain acyl CoA or long-chain acyl carnitine levels are observed (Table 1), ruling out this mechanism as a cause of the noninsulin-dependent diabetic cardiomyopathy. Nevertheless, these data do not exclude the possibility that diabetes may mediate changes in levels of other toxic lipid amphiphiles. For example, Makino *et al.* [71] recently reported that diabetic sarcolemma contains 77% more lysophosphatidylcholine than nondiabetic membrane.

2. Diet hypothesis as basis for diabetic complications

Until recently the standard diabetic diet has contained high levels of lipid and low levels of carbohydrate, with little attention given to the type of lipid consumed. However, it has become apparent that the high intake of saturated fats may contribute in an important way to the development of certain diabetic complications. While initial studies have focused on the potential link between macrovascular complications and high saturated fat intake, more recently it has been shown that diabetes is characterized by an impairment in essential fatty acid metabolism [126–128]. The potential impor-

tance of fatty acid deficiency in the development of the insulin-dependent diabetic cardiomyopathy has been recently recognized by Black *et al.* [129], who reported that treatment of insulin-dependent diabetic rats with omega 3 fatty acids (Promega) restores sarcoplasmic reticular calcium pump activity, while significantly reducing the degree of myocardial mechanical dysfunction. Because unsaturated fatty acid composition of the membrane phospholipids is reduced during diabetes, it seems reasonable that omega 3 supplementation normalizes the fatty acid profile, thereby influencing membrane structure and function [130, 131].

Another membrane change which is likely to affect membrane structure and function of the diabetic heart is an alteration in the membrane phosphatidylcholine/phosphatidylethanolamine ratio arising from an impairment in phosphatidylethanolamine N-methylation [132]. Gupta *et al.* [133] have provided convincing evidence that the N-methylation reaction regulates the activity of key calcium transporters, such as the sarcolemmal $Na^+ - Ca^{2+}$ exchanger and the sarcoplasmic reticular calcium pump. Because the promotion of this reaction in the sarcoplasmic reticulum is thought to enhance myocardial contraction, this defect could contribute to the diabetes-mediated decrease in myocardial contractility.

3. Lipid peroxidation as factor in diabetic cardiomyopathy

It has been proposed that changes in the activity of either the antioxidant defense system and/or free radical producing reactions may mediate specific complications in diabetic patients [134]. In the heart, there are only a few studies that have addressed this issue. Wohaieb and Godin [135, 136] recently reported that the activity of catalase and glutathione reductase increase significantly in either spontaneously diabetic BB rats or streptozotocin-treated insulin-dependent diabetic rats. Because the activity of these antioxidative enzymes can be restored to the normal range in animals treated with insulin, it has been proposed that the diabetic heart may be subjected to increased oxidative stress. Compatible with this view is an earlier study by Matkovics *et al.* [137], who found elevated levels of lipid peroxidation products in chemically-induced insulin-dependent diabetes. Also, there is reason to suspect that free radical production arising from oxidation of catecholamines [138] and/or the mitochondria may be greater. Recently, Pieper [139] has reported that diabetes-mediated changes in highly reactive oxygen species may affect the response of the heart to ischemia-reperfusion injury. However, insufficient evidence is presently available to evaluate their contribution to the development of the cardiomyopathy.

Defective membrane phosphorylation in diabetes

Pioneering work by Rosen, Kahn, Larner, Roth and others [140] indicate that the insulin receptor is a hormone-sensitive protein kinase capable of phosphorylating tyrosine residues. Some, but not all experimental evidence, support a direct role for the tyrosine-dependent kinase in mediating specific actions of insulin [141]. Also implicated in the actions of insulin is the phosphorylation of certain serine residues.

One of the serine kinases intimately involved in the actions of insulin is protein kinase C. In several insulin-sensitive tissues, insulin increases protein kinase C activity by promoting the accumulation of the protein kinase C activator, diacylglycerol [115]. In turn, protein kinase C serves as a positive modulator of a number of insulin-sensitive steps, including glucose transport, pyruvate dehydrogenase activity and acetyl CoA carboxylase activity [115]. Although the heart is an insulin-sensitive tissue, a role for protein kinase C in the actions of insulin has not been established. This confusion arises in part because protein kinase C exhibits both insulin-like and anti-insulin activity. Also unclear is the status of protein kinase C in the diabetic heart. While Van der Weeve *et al.* [141] have shown that translocation and activation of protein kinase C is defective in the myocardium of the insulin-resistant obese Zucker rat, in the insulin-dependent diabetic heart, protein kinase C activity is presumably elevated as a result of an increase in diacylglycerol

levels [142] and in the noninsulin-dependent diabetic heart no change in sarcolemmal protein kinase C activity is detected [78]. Regardless of the nature of the protein kinase C change, questions can be raised regarding its impact on the diabetic heart. Although a decrease in protein kinase C activity should reduce glucose transport, $Na^+ - K^+$ ATPase activity and phospholipid N-methylation (insulin-like effects), it may also stimulate myocardial contractility, which would represent an anti-insulin action [141, 143].

A link between insulin and the cAMP-dependent protein kinase cascade system is also well established. Although insulin has no effect on either cAMP levels or the cAMP-dependent protein kinase activity ratio in perfused heart obtained from normal rats, it potentiates the response of epinephrine [144]. Similarly, diabetes attenuates both the positive inotropic effect and the rise in cAMP levels in hearts perfused with epinephrine [145]. However, the diabetes-linked defect in basal contractility is apparently unrelated to the cAMP system because no effect on the activity ratio of cAMP-dependent protein kinase can be detected in the diabetic heart [145].

Also affecting the serine phosphorylation state of proteins in diabetic heart is the activity of various phosphoprotein phosphatases. According to the classification of Ingebretsen *et al.* [146], four different types of protein phosphatases exist; heart contains considerable levels of protein phosphatase 1 and 2A, but only small amounts of protein phosphatase 2B and 2C. In diabetes, protein phosphatase 1 appears to assume a very important role. Miller *et al.* [147] have shown that the activity of glycogen synthase phosphatase, a protein phosphatase 1 which regulates glycogen synthase activity, decreases in the insulin-dependent diabetic heart. Similarly, Allo and Schaffer [78] describe a specific protein phosphatase 1 associated with sarcolemma which exhibits less activity in the noninsulin-dependent diabetic heart. Because the phosphatase 1 group of enzymes is thought to contribute to the regulation of energy metabolism, as well as the regulation of troponin [148], myosin [149], phospholamban [150, 151] and the sarcolemmal calcium channel [77], it would be attractive to suggest that reductions in the activity of this group of enzymes in the diabetic heart may contribute in a very meaningful way to the development of the cardiomyopathy.

In conclusion, a cardiomyopathy develops in both noninsulin-dependent and insulin-dependent diabetes. While many of the characteristics of the two diseases are similar, they differ in some important and significant ways, including energy metabolism and diastolic function. It is also significant that the pathogenesis of both cardiomyopathies is presently unknown. Changes in calcium homeostasis and muscle protein function have been found. However, the classical theories of glucose toxicity appear to be insignificant factors in heart. Rather, the major defects appear to involve changes which can be attributed to either insulin resistance (noninsulin-dependent diabetes) or insulinopenia (insulin-dependent diabetes).

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