Sucrose-induced cardiomyocyte dysfunction is both preventable and reversible with clinically relevant treatments

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Sucrose-induced cardiomyocyte dysfunction is both preventable and reversible with clinically relevant treatments. Am J Physiol Endocrinol Metab 286: E718–E724, 2004; 10.1152/ajpendo.00358.2003.—We recently identified cardiomyocyte dysfunction in the early stage of type 2 diabetes (i.e., diet-induced insulin resistance). The present investigation was designed to determine whether a variety of clinically relevant interventions are sufficient to prevent and reverse cardiomyocyte dysfunction in sucrose (SU)-fed insulin-resistant rats. Subsets of animals were allowed to exercise (free access to wheel attached to cage) or were treated with bezafibrate in drinking water to determine whether these interventions would prevent the adverse effects of SU feeding on cardiomyocyte function. After 6–8 wk on diet and treatment, animals were surgically prepared to assess whole body insulin sensitivity (intravenous glucose tolerance test), and isolated ventricular myocyte mechanics were evaluated (video edge recording). SU feeding produced hyperinsulinemia and hypertriglyceridemia, with euglycemia, and induced characteristic whole body insulin resistance. Both exercise and bezafibrate treatment prevented these metabolic abnormalities. Ventricular myocyte shortening and relengthening were slower in SU-fed rats (42–63%) compared with starch (ST)-fed controls, and exercise or bezafibrate completely prevented cardiomyocyte dysfunction in SU-fed rats. In separate cohorts of animals, after 5 wk of SU feeding, animals were either switched back to an ST diet or given menhaden oil for an additional 7–9 wk to determine whether the cardiomyocyte dysfunction was reversible. Both interventions have previously been shown to have favorable metabolic effects, and both improved myocyte mechanics, but only the ST diet reversed all indications of cardiomyocyte dysfunction induced by SU feeding. Thus phenotypic changes in cardiomyocyte mechanics associated with early stages of type 2 diabetes were found to be both preventable and reversible with clinically relevant treatments, suggesting that the cellular processes contributing to this dysfunction are modifiable.

Type 2 diabetes is a progressive, multifactorial disease that typically involves comorbidities such as dyslipidemia, obesity, hypertension, and insulin resistance (15). The type 2 form accounts for >90% of all diabetic cases and has recently been recognized as a serious health threat that is growing significantly worldwide (40). The increased incidence of insulin resistance and diabetes in young adults is particularly disturbing, given the potential for high rates of morbidity and mortality, predominantly due to heart disease (40).

It is well established that diabetes-related heart disease in humans (and animal models) involves ventricular dysfunction, with diastolic abnormalities developing earlier than changes in systole (2, 32, 35, 38). Diabetic cardiomyocyte dysfunction is characterized by phenotypic changes in ventricular myocytes that occur with or without coronary artery disease and is well described in animal models with long-term type 1 diabetes (27, 30). The existence of cardiomyocyte dysfunction has also been shown in some models of type 2 diabetes, (34, 37), but not in all type 2 models (23).

We have recently described abnormal cardiomyocyte excitation-contraction coupling in sucrose (SU)-fed, insulin-resistant rats (10). SU-fed animals exhibit metabolic abnormalities [such as hyperinsulinemia and hypertriglyceridemia [but with euglycemia (25, 28)]] consistent with insulin resistance in humans but without additional complicating factors that may alter cardiac physiology (e.g., obesity). In this model, ventricular myocyte shortening and relengthening are prolonged, and cytosolic Ca2+ removal is slowed, but these dysfunctions are seen only under nonphysiological conditions (i.e., slow rates of stimulus frequency and low extracellular Ca2+). This suggests that subtle cellular changes occur early in the disease process, before overt heart failure (4).

The benefits of exercise training and/or dietary modifications with respect to complications of type 2 diabetes and the related syndrome X have been recognized clinically (7, 43). Exercise has been shown to increase insulin sensitivity in insulin-resistant and type 2 diabetic patients (18, 45). Most evidence supports the view that increasing whole body insulin sensitivity reduces not only the hyperinsulinemia but also improves dyslipidemia (e.g., lowers triglycerides). Interventions designed to lower triglycerides (e.g., fibrates) have also been shown to improve insulin sensitivity (13, 21).

Lipid-lowering interventions (e.g., fish oil supplementation, bezafibrate, and exercise) also attenuate many of the metabolic abnormalities seen in insulin-resistant animals (1, 28, 39). Cortez et al. (3) reported improved metabolic status in exercised Zucker obese rats, and we (29) have previously shown that exercise prevents whole body insulin resistance in another diet-induced model (i.e., high-fat feeding). Although exercise had little effect on insulin sensitivity in control (starch-fed)
animals, it significantly increased insulin sensitivity in fat-fed animals (29). What is not known is whether exercise can also prevent insulin resistance and cardiomyocyte dysfunction in SU-fed animals. We have chosen to use the SU-fed, rather than the fat-fed model, because we (10) have already characterized the impact of the SU diet on cardiomyocyte function.

We undertook the present investigation to determine whether clinically relevant treatments, with different molecular targets, can be cardioprotective by either preventing or reversing whole body insulin resistance. We show that allowing animals to exercise or providing dietary supplementation with bezafibrate not only prevents SU-induced hyperinsulinemia, hypertriglyceridemia, and reduced insulin sensitivity but can also prevent the cardiomyocyte dysfunction as well. Furthermore, SU-induced insulin resistance and cardiomyocyte dysfunction can be reversed by returning animals to a normal (starch) diet or by supplementation with fish oil.

METHODS

All protocols were approved by the Institutional Animal Care and Use Committee at the University of New England. Male Wistar rats (120–140 g; Charles River Breeding Lab, Wilmington, MA) were housed individually in an animal facility under controlled conditions (12:12-h light-dark cycle). Animals were allowed water ad libitum and placed on a purified high-starch diet (ST; 68% of kcal from corn starch, 20% from protein, 12% from fat) for a 1-wk baseline period. After the baseline period, animals were continued either on the ST diet or fed a high-sucrose diet (SU; 68% of kcal from sucrose, 20% from protein, 12% from fat) for 6–8 wk (in the prevention protocol) or 12–14 wk (in the reversal protocol). Both diets were formulated by Research Diets (New Brunswick, NJ). It was previously shown that body weights are similar in ST- and SU-fed animals, so no groups were food restricted (28). All diets were based on recommendations of the American Institute of Nutrition.

Prevention and reversal protocols. Cohorts of animals were either treated concurrently with SU diet (prevention protocol) or allowed to develop insulin resistance and then treated (reversal protocol). The prevention protocol involved subsets of ST- and SU-fed animals (ST-EXE and SU-EXE), which were given free access to an exercise wheel attached to each cage [Lafayette Instruments, Lafayette, IN (27)] and whose activity (based on the number of revolutions/day) was monitored for 5 wk. Both groups exercised to the same extent, averaging ~2,400 m/day, with a total of 81,034 ± 5,444 m for the ST-fed animals and 86,293 ± 7,311 m for the SU-fed animals. Animals were allowed continued access to the exercise wheels until they were killed. A subset of sedentary SU-fed animals was treated with bezafibrate (SU-FIB) in the drinking water for 6–8 wk. Water consumption was monitored every other day, and the volume was adjusted to deliver ~30 mg/kg·day⁻¹ (39). There were no apparent differences in the amount of water consumed in any of the animals. The reversal protocol involved separate cohorts of animals maintained on diet for 12–14 wk. After 5 wk on the SU diet, animals were either maintained on the SU diet (SU), switched back to the ST diet (SU-ST), or maintained on SU supplemented with fish oil [SU-FO; same amount of fat as SU but one-half of it from menhaden oil (6% of total kcal)]. We (28) have previously shown that SU-ST reverses all of the metabolic abnormalities characteristic of whole body insulin resistance, whereas SU-FO reverses only the hypertriglyceridemia and not insulin resistance. Thus these interventions have subtle but important differences in their effects on metabolic abnormalities.

Measuring insulin sensitivity. An intravenous glucose tolerance test (IVGTT) was used to assess insulin responsiveness to a bolus glucose injection (42) in a subset of SU-fed and ST-fed animals to measure the efficacy of exercise and bezafibrate treatment. After the 6- to 7-wk dietary period, IVGTTs were performed. In preparation for the IVGTT, the left carotid artery and right jugular vein were cannulated. Briefly, animals were anesthetized by injecting intraperitoneally 80 mg/kg ketamine and 12 mg/kg xylazine, and cannulas (PE-50) were inserted into both the carotid artery (advanced up to the aortic arch) and the jugular vein (advanced up to the vena cava). The cannulas were then sutured to the respective vessel and exteriorized through the back of the neck. Animals were allowed ≥3 days to recover from surgery and were required to be at >90% of presurgery body weight to be studied.

IVGTTs were performed in unanesthetized, resting animals. On the day of the experiment, extensions were added to the cannulas for ease of sampling, and then animals were allowed to rest for 20 min. A baseline blood sample was drawn (500 μl), and then a glucose bolus of 0.4 g/kg body wt was slowly injected into the venous catheter (~1 min). Blood samples (300 μl) were taken from the carotid artery cannula and replaced with an equal volume of isotonic saline during the following times after the bolus glucose injection: 5, 12.5, 20, 27.5, 35, 50, 65, and 80 min. Blood samples were immediately centrifuged; some of the plasma was analyzed for glucose, and the remaining samples were frozen at ~70°C for subsequent insulin (at all times) and triglyceride (at time 0 only) analyses. Plasma glucose levels were determined by the glucose oxidase method (10, 28) using a Beckman glucose analyzer (Fullerton, CA). Plasma insulin was measured by radioimmunoassay (Lance Research, St. Louis, MO). Triglycerides were measured by assay kit 320-A (Sigma Chemical, St. Louis, MO). After the experiment, the animals were returned to their feeding protocol and used for isolated myocyte experiments ~1 day post-IVGTT.

Ventricular myocyte isolation and myocyte mechanics. All animals were anesthetized with the same ketamine-xylazine as that stated above (ip). Single ventricular myocytes were isolated by methods previously described (10). Myocytes were allowed to attach to glass coverslips coated with laminin (10 μg/ml; Collaborative Biochemical Products, Bedford, MA) and were maintained at 37°C in a 100% humidity and 5% CO₂ incubator until used (~6 h after isolation). Mechanical properties of ventricular myocytes were assessed using a high-speed video-based edge detection system (IonOptix, Milton, MA) as described previously (10). In brief, myocytes were electrically stimulated at 0.5 Hz and mechanical properties recorded at a sampling rate of 240 Hz. Cells were superfused with a buffer composed of (in mM) 131 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES at pH 7.4.

The indexes used to describe isotonic shortening have been previously reported (9, 31) and include peak fractional shortening (ps post hoc test, when appropriate).

 Statistical analyses. Data are presented as means ± SE. Statistical significance (P < 0.05) was determined for myocyte mechanics by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test, which was used for multiple comparisons (SYSTAT, Richmond, CA). Metabolic data in Fig. 1 and Table 1 were analyzed using a Kruskal-Wallis nonparametric test followed by Bonferroni’s post hoc test, when appropriate.

RESULTS

Efficacy of exercise and bezafibrate treatment on whole body insulin resistance. It has been well established that animals fed a diet high in sucrose develop whole body insulin resistance (25, 28), which can be prevented by treatment with the antiabetic agent metformin (10). The untreated groups (both ST-
and SU-fed animals) were repeated herewithin. We have extended our findings with metformin treatment to include evaluating the effectiveness of preventing insulin resistance with either exercise or bezafibrate treatment in SU-fed rats (SU-EXE and SU-FIB, respectively). Table 1 illustrates that SU feeding produces hyperinsulinemia and hypertriglyceridemia compared with ST-fed controls. Either exercise or bezafibrate treatment in SU-fed animals reduced basal triglyceride and insulin levels. All groups were euglycemic, which is characteristic of the SU-feeding model. Figure 1 shows both the time course and area under the curve (AUC) for plasma insulin and glucose following a bolus injection of glucose (IVGTT). As expected, the SU-fed animals exhibited elevated peak and prolonged plasma insulin levels in response to the glucose challenge (Fig. 1A), which was reflected in a larger AUC (Fig. 1B). Each of these measurements indicates impaired insulin sensitivity (insulin resistance). Exercise and bezafibrate treatment were both effective in preventing insulin resistance. As we have previously reported (29), exercise does not affect insulin sensitivity in the normal (i.e., ST-fed) group. We did not treat ST-fed animals with the fibrate because of the potential complications that might have arisen by lowering triglycerides below normal values. Plasma glucose levels were also measured for each group (Fig. 1, C and D) and tended to follow those of the insulin levels (i.e., in SU-fed animals peak glucose was higher and sustained for a longer time). There was a trend for the AUC for glucose to be significantly higher in the

Table 1. Body weights and resting plasma factors before IVGTTs in prevention protocol

<table>
<thead>
<tr>
<th></th>
<th>ST</th>
<th>SU</th>
<th>SU-EXE</th>
<th>SU-FIB</th>
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<tbody>
<tr>
<td>BW, g</td>
<td>455 ± 32</td>
<td>465 ± 34</td>
<td>422 ± 12</td>
<td>466 ± 16</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>33 ± 5</td>
<td>71 ± 9*</td>
<td>46 ± 3</td>
<td>44 ± 6</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>6.7 ± 0.5</td>
<td>7.4 ± 0.2</td>
<td>7.5 ± 0.5</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>192 ± 22</td>
<td>687 ± 31*</td>
<td>206 ± 58</td>
<td>224 ± 13</td>
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</table>

Data represent means ± SE from 3–4 animals/group. ST, starch-fed; SU, sucrose-fed; SU-EXE, exercised SU; SU-FIB, bezafibrate-treated SU for 6–8 wk; BW, presurgery body weight; TG, triglyceride; IVGTT, intravenous glucose tolerance test. *Significantly different from ST controls, P < 0.05.

Fig. 1. Plasma insulin and glucose levels during IVGTTs in prevention protocol (Fig. 1D), but these values did not reach statistical significance (P = 0.08). Again, both exercise and bezafibrate treatment prevented these effects, although there was more variability among the treated groups, which likely contributed to the lack of statistical significance. Collectively, these data show that both interventions prevented the insulin resistance associated with SU feeding; therefore, we evaluated the efficacy of these treatments on the development of cardiomyocyte dysfunction.

SU-induced cardiomyocyte dysfunction is prevented by exercise or bezafibrate treatment. Mechanical properties of isolated ventricular myocytes were evaluated to investigate whether SU-induced cardiomyocyte dysfunction could be prevented by either exercise or bezafibrate treatment. Figure 2A illustrates indexes used to assess myocyte mechanics. As previously shown, the rates of isotonic shortening (AC/PK and TPT) and relengthening (ARP/K and TR) were slower in myocytes isolated from SU-fed rats compared with ST-fed animals (Fig. 2, B–E). Both area and time indexes are shown because there were subtle differences among groups in subsequent experiments (see reversal protocol). All indexes in the ST-EXE group were comparable to those of sedentary controls (ST), consistent with the metabolic effects (29). Voluntary exercise completely prevented abnormalities of cardiomyocyte shortening and relengthening in SU-fed rats (SU-EXE; Fig. 2). Bezafibrate treatment also completely prevented cardiomyocyte dysfunction in SU-fed animals (SU-FIB; Fig. 2). In addition, peak fractional shortening (PS; expressed as a percentage of resting cell length) was significantly depressed in SU-fed animals (6.7 ± 0.6%) compared with the ST-fed group (12.5 ± 0.6%). Both exercise and bezafibrate treatment prevented this dysfunction (12.5 ± 0.5% and 12.1 ± 0.6%, respectively). It should be noted that resting cell length tended to be shorter in SU-fed animals than in ST-fed animals, thereby highlighting the importance of expressing peak shortening relative to cell length rather than absolute amplitude.

SU-induced cardiomyocyte dysfunction is reversed by dietary interventions. Reversibility of SU-induced cardiomyocyte dysfunction was investigated in animals fed either ST or SU diets for 12–14 wk. After 5 wk (a period sufficient to

**Fig. 1.** Plasma insulin and glucose levels during IVGTTs in prevention protocol (Fig. 1D).
induce myocyte dysfunction; data not shown), subsets of SU-fed animals were maintained either on the SU diet (SU), returned to normal diet (SU-ST), or placed on the SU diet supplemented with fish oil (SU-FO). Each of these interventions has been previously shown to either completely (i.e., SU-ST) or partially (i.e., SU-FO) reverse metabolic abnormalities associated with insulin resistance (28). Myocyte shortening (Fig. 3, A and B) and relengthening (Fig. 3, C and D) were slower in SU-fed animals than in age-matched ST animals. The clinically most important finding is that slowed myocyte shortening (A_CPK and TPT) was completely reversible with either dietary intervention (Fig. 3, A and B), not just preventable, as shown in Fig. 2. Slowed relengthening (A_RPK and TR) was also reversed by returning animals to a normal ST diet; however, fish oil supplementation restored relaxation to normal, as measured by the area index (Fig. 3C) but not the TR index (Fig. 3D). This may reflect the inability of fish oil treatment to completely reverse all metabolic complications associated with SU feeding (28). It should also be noted that the degree of myocyte dysfunction after 12–14 wk on the SU diet (Fig. 3) appears less severe than that after 6–8 wk on the SU diet (Fig. 2). Consistent with this observation is the finding that PS was not significantly depressed by SU feeding (7.8 ± 0.4%) compared with ST feeding (8.8 ± 0.3%). It was previously shown (24) that SU-fed animals do not progress to overt type 2 diabetes (i.e., hyperglycemia and insulinopenia), even after 30 wk on the diet. It is conceivable (but not yet determined) that the heart undergoes a compensatory phase, thus adjusting to prolonged insulin resistance. Conversely, there may be an effect of age on myocyte function, as illustrated by a slightly smaller peak fractional shortening in control animals.

**DISCUSSION**

There are two major findings from this investigation. First, clinically relevant interventions (i.e., exercise or bezafibrate treatment) known to lower triglycerides and increase insulin sensitivity can prevent cardiomyocyte dysfunction in SU-fed rats (Table 1 and Figs. 1 and 2). We (28) have previously shown that exercise (and fish oil supplementation) can prevent the metabolic changes associated with SU feeding (measured by hyperinsulinemic euglycemic clamps). We now show additionally that bezafibrate treatment is equally effective in pre-

![Fig. 2. Prevention protocol. A: representative traces of twitches of cardiomyocytes isolated from ST- and SU-fed rats and indexes used for evaluating mechanical properties. Myocyte mechanics from sedentary animals maintained on ST and SU diets (CTRL), ST and SU diets with voluntary exercise (EXE), and SU-FIB for ~7 wk. Myocyte isotonic shortening was evaluated by area under the shortening (contraction) phase, normalized to peak shortening amplitude (A_CPK; B) and time to peak twitch (TPT; C). Myocyte relengthening was evaluated by area under the relengthening (relaxation) phase, normalized to peak shortening amplitude (A_RPK) and time to relengthening (TR; D). Data represent means ± SE from 28–58 myocytes/group. *Statistical significance from all other groups (P < 0.05).](http://ajpendo.physiology.org/)

![Fig. 3. Reversal protocol. Cardiomyocyte mechanics from animals maintained on ST or SU diets for 12–14 wk, SU diet for 5 wk and then switched to either ST diet (SU-ST) or SU diet plus fish oil supplementation (SU-FO) for an additional 7–9 wk. Myocyte isotonic shortening was evaluated by A_CPK (A) and TPT (B). Myocyte relengthening was evaluated by A_RPK (C) and TR (D). Data represent means ± SE from 82–160 myocytes/group. *Statistical significance from all other groups; #statistical significance from ST cells.](http://ajpendo.physiology.org/)
venting the metabolic changes (Table 1 and Fig. 1). Second, and perhaps more importantly, dietary interventions can reverse some (or all) of the cardiomyocyte dysfunctions in SU-fed rats. Returning SU-fed animals to a normal ST diet completely reverses the metabolic effects associated with insulin resistance (28) and completely reverses the cardiomyocyte dysfunction (Fig. 2). On the other hand, fish oil supplementation normalizes only triglycerides and not the hyperinsulinemia associated with SU feeding (28) and was only partially effective in reversing myocyte dysfunction (Fig. 3).

The aim of this study was to make use of several clinically relevant interventions known to alter metabolic status associated with insulin resistance to explore the early pathogenesis of cardiomyocyte dysfunction. Chronic peripheral insulin resistance generally leads to hyperglycemia (glucose intolerance), followed eventually by a reduction in insulin secretion. Most animal models mimic end-stage type 2 diabetes (e.g., Zucker diabetic fatty and LA/N corpulent rats). However, chronic high-sugar diets produce whole body insulin resistance and hypertriglyceridemia without obesity or hyperglycemia (24, 44), thereby mimicking the metabolic changes that precede the development of type 2 diabetes. We (10) have previously shown that treatment with metformin prevents both whole body insulin resistance and cardiomyocyte dysfunction, thus establishing an association between the two. We have extended the metformin study by investigating other means of manipulating whole body insulin resistance (i.e., exercise or dietary interventions) to determine whether cardioprotection is specific to metformin’s effects or more generalizable to treating the metabolic effects of insulin resistance [independent of the drugs’ molecular target(s)]. The cellular mechanism(s) that underlie abnormal cardiomyocyte mechanics are presently unknown. However, we have preliminary evidence to suggest that impaired relengthening in cardiomyocytes from SU-fed rats stems, in part, from slowed sarcoplasmic reticulum Ca$^{2+}$ uptake (20). Future studies are being undertaken to investigate Ca$^{2+}$ regulation in these myocytes.

In our present study, we show that exercise prevents SU-induced whole body insulin resistance and cardiomyocyte dysfunction (Table 1 and Figs. 1 and 2). This is consistent with the clinical observation of a significant reduction in ventricular dysfunction and cardiac mortality among insulin-resistant and type 2 diabetic patients with regular physical exercise of moderate intensity (reviewed in Ref. 22). The mechanism of action underlying the beneficial effect of exercise on diabetic cardiac dysfunction is still poorly understood. However, it has been determined that exercise lowers plasma triglyceride levels and improves cardiac pump function in diabetic hearts (6, 26). Increased cardiac work during exercise is known to augment glucose oxidation and perhaps insulin sensitivity in the heart (8), which could be a component of exercise’s cardioprotective effects in our SU-fed rats. To our knowledge, there are no studies that have evaluated the direct effects of exercise on cardiomyocyte contractile properties in diabetes.

It is possible that exercise improves cardiac function through indirect actions on plasma lipid profiles and insulin sensitivity. Routine exercise has been demonstrated to reduce blood glucose, blood pressure, body weight, and body fat; improve lipid profiles; and reduce insulin resistance (18, 19). Exercise training enhances basal and insulin-stimulated glucose uptake in skeletal muscle by increasing translocation of the glucose transporter GLUT4 to the plasma membrane (18, 33). Exercise-induced increases in insulin sensitivity reduce the hyperinsulinemia associated with insulin resistance in animals (29) and humans (18), which is likely a reflection of improved glucose control. Exercise may also prevent the development of diet-induced insulin resistance by reducing triglycerides, since endurance training is known to enhance skeletal muscle lipid oxidation (29, 36). Whether the cardioprotection of exercise is due to direct effects on the heart or indirect effects through improving metabolic status in SU-fed rats remains to be determined.

Dietary interventions that reduce triglyceride concentrations can prevent insulin resistance or improve insulin sensitivity (28, 44). Bezafibrate supplementation is known to lower triglyceride levels in humans (13) and animals (Ref. 39 and Table 1). We now show that bezafibrate treatment can prevent the development of cardiomyocyte dysfunction in our SU-fed rats (Fig. 2), suggesting that elevated triglycerides play a key role in our model. Fibrates reduce total plasma cholesterol, low-density and very-low-density lipoprotein cholesterol, and triglyceride concentrations and increase high-density lipoprotein cholesterol and apolipoprotein AI and AII concentrations, thus reducing the risk of cardiovascular disease (16). These effects of fibrates are believed to be mediated through activation of peroxisome proliferator-activated receptor-α (PPARα), thereby enhancing fatty acid oxidation and improving insulin sensitivity in tissues such as skeletal muscle and adipocytes (5, 16, 17). It is likely that bezafibrate treatment is cardioprotective in SU-fed rats through the lowering of systemic lipids, rather than having direct effects on cardiomyocytes, since increased fatty acid metabolism has been shown to be detrimental to heart function (12). In support of this view, cardiac-specific activation of PPARα in transgenic mice exacerbates diabetic cardiomyopathy, whereas both cardiac-specific PPARα knockout and systemic lipid lowering strategies are cardioprotective (11).

Omega-3 fatty acids (found in fish oil) have a similar lipid-lowering profile to fibrates, but whether they act as ligands for PPARα has been questioned (5). It has been shown that n-3 fatty acids can inhibit the synthesis of several proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1 and -2, which could potentially destabilize atherosclerotic lesions. They can also reduce aggregational properties of blood platelets, leading to interference with thrombosis, and can modify the electrical activity of the heart, potentially reducing the risk of arrhythmias. At high doses, n-3 fatty acids may reduce serum triglyceride levels, further reducing the risk of cardiovascular disease (14, 21). Previous studies have shown a strong correlation between elevated triglyceride concentrations and insulin resistance (17, 41). Fish (menhaden) oil has been shown to lower triglyceride concentrations (1) and to prevent, but not reverse, SU-induced insulin resistance in rats (28). The inability of fish oil supplementation to completely reverse abnormal myocyte mechanics in our SU-fed animals (Fig. 3) lends support to the notion that hyperinsulinemia, not just hypertriglyceridemia, plays a role in the development of cardiomyocyte dysfunction. By employing various types of interventions (i.e., metformin, exercise, bezafibrate, fish oil, and carbohydrate composition of diet), we have attempted to tease out the effects of hypertriglyceridemia from...
those of hyperinsulinemia. To date, all of our treatments except fish oil have completely prevented/restored both of these metabolic abnormalities. Thus the fish oil treatment is the first intervention to be useful at delineating the differential effects of elevated lipids and insulin on cardiomyocyte function in early stages of type 2 diabetes.

In summary, this study supports the view that the pathogenesis of diabetic cardiomyocyte dysfunction is associated with elevated insulin and triglycerides, as well as whole body insulin resistance. Perhaps the most intriguing finding is that lowering triglycerides alone (e.g., by dietary supplementation with fish oil) is insufficient to completely reverse myocyte abnormalities. This study suggests that cardiomyocyte dysfunction, and thus cardiac impairment, is reversible if interventions are targeted toward improving insulin resistance.

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REFERENCES