TRANSLATIONAL PHYSIOLOGY

The antioxidant tempol attenuates pressure overload-induced cardiac hypertrophy and contractile dysfunction in mice fed a high-fructose diet

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Chess DJ, Xu W, Khairallah R, O’Shea KM, Kop WJ, Azimzadeh AM, Stanley WC. The antioxidant tempol attenuates pressure overload-induced cardiac hypertrophy and contractile dysfunction in mice fed a high-fructose diet. Am J Physiol Heart Circ Physiol 295: H2223–H2230, 2008; doi:10.1152/ajpheart.00563.2008.—We have previously shown that high-sugar diets increase mortality and left ventricular (LV) dysfunction during pressure overload. The mechanisms behind these diet-induced alterations are unclear but may involve increased oxidative stress in the myocardium. The present study examined whether high-fructose feeding increased myocardial oxidative damage and exacerbated systolic dysfunction after transverse aortic constriction (TAC) and if this effect could be attenuated by treatment with the antioxidant tempol. Immediately after surgery, TAC and sham mice were assigned to a high-starch diet (58% of total energy intake as cornstarch and 10% fat) or high-fructose diet (61% fructose and 10% fat) with or without the addition of tempol [0.1% (wt/wt) in the chow] and maintained on the treatment for 8 wk. In response to TAC, fructose-fed mice had greater cardiac hypertrophy (55.1% increase in the heart weight-to-tibia length ratio) than starch-fed mice (22.3% increase in the heart weight-to-tibia length ratio). Treatment with tempol significantly attenuated cardiac hypertrophy in fructose-fed TAC mice (18.3% increase in the heart weight-to-tibia length ratio). Similarly, fructose-fed TAC mice had a decreased LV area of fractional shortening (from 38 ± 2% in sham to 22 ± 4% in TAC), which was prevented by tempol treatment (33 ± 3%). Markers of lipid peroxidation in fructose-fed TAC hearts were also blunted by tempol. In conclusion, tempol significantly blunted markers of cardiac hypertrophy, LV remodeling, contractile dysfunction, and oxidative stress in fructose-fed TAC mice.

antioxidants; myocardial oxidative damage; systolic dysfunction

RECENT EPIDEMIOLOGICAL STUDIES have demonstrated that diets with a high glycemic load increase the risk for coronary heart disease (5, 16, 21). Overconsumption of highly refined carbohydrates and sugar-sweetened beverages raise postprandial glycemia and may contribute to the development of insulin resistance, dyslipidemia, obesity, and diabetes, all of which are independent risk factors for cardiovascular disease. The effects of sugar intake on the development and progression of heart failure are unclear, as are the direct effects on the myocardium. There is growing evidence from animal models of hypertension-induced cardiac hypertrophy to suggest that high-carbohydrate diets exacerbate the development of heart failure.

Hypertensive Dahl salt-sensitive rats displayed increased mortality and worsened left ventricular (LV) hemodynamics when fed diets high in fructose or sucrose (~60% of energy intake from sugar) compared with a low-carbohydrate diet (35, 36). A high-fructose diet increased mortality, decreased mitochondrial enzyme activities, and dramatically upregulated myosin heavy chain (MHC-β) expression in mice subjected to transverse aortic constriction (TAC) compared with those fed a high-starch diet (8). Furthermore, LV hypertrophy and remodeling caused by abdominal aortic banding in rats was worsened with a high-sucrose diet compared with a low-carbohydrate diet (12). Thus, dietary carbohydrate composition may have a profound impact on heart failure pathogenesis.

A potential link between high carbohydrate intake and heart disease progression is oxidative stress. Studies have shown that the consumption of diets high in simple sugars decrease the capacity of SOD in the heart (6) and facilitate oxidative damage (24). While cellular mechanisms leading to sugar-induced oxidative stress are unclear, one potential contributor is the oxidative pentose phosphate pathway. A rate-controlling enzyme in this pathway, glucose 6-phosphate dehydrogenase (G6PDH), oxidizes glucose to provide cytosolic reducing equivalents to glutathione reductase to enhance the intrinsic antioxidant system. However, under pathological conditions, such as in the severely hypertrophied or failing heart, G6PDH flux may increase secondary to greater cardiac glucose uptake, paradoxically fueling superoxide production by NADPH oxidases (9). Upregulation of G6PDH activity was observed in heart failure patients and in a canine model of heart failure and was associated with increased cardiac superoxide levels (14, 15). High levels of ROS can increase lipid peroxidation and cardiomyocyte apoptosis and decrease nitric oxide availability, leading to ventricular remodeling and contractile dysfunction (40). It is unclear if a high-sugar diet increases oxidative damage in the myocardium under conditions of chronic cardiac stress (e.g., hypertension or postmyocardial infarction) or if long-term treatment with an antioxidant prevents the acceleration in cardiac pathology observed with high sugar consumption.

The present study examined the role of dietary carbohydrate composition on oxidative stress, cardiac hypertrophy, and heart failure under conditions of chronic pressure overload. We...
hypothesized that high-fructose feeding would increase cardiac oxidative damage, hypertrophy, LV remodeling, and systolic dysfunction, which would be blunted by treatment with an antioxidant. Experiments were performed in an established mouse model of chronic pressure overload and LV dysfunction (7, 8), and animals were fed a high-starch diet or a high-fructose diet with or without the SOD mimetic tempol. Cardiac function was assessed by echocardiography, and LV tissue was analyzed for markers of lipid peroxidation, the activities of the pentose phosphate pathway and mitochondrial enzymes, and the expression of fetal genes known to be upregulated in heart failure.

METHODS

Study design. The goal of this study was to determine if high-fructose feeding increased myocardial oxidative damage and exacerbated systolic dysfunction after TAC and if this effect could be attenuated by treatment with the antioxidant tempol. One day after surgery, TAC and sham mice were assigned to a high-starch diet or a high-fructose diet with or without the addition of tempol to the chow. Each of the eight treatment groups had 12–16 mice/group at the onset. Dietary and drug treatments were maintained for 8 wk, after which the animals were subjected to echocardiography and terminal surgery. All analyses were performed with the investigator blinded to the treatment.

Induction of pressure overload. TAC was performed on male C57BL/6J mice (20–25 g) as previously described (18) using a 27-gauge needle. The sham procedure was identical but without aortic ligation. This study was conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23) and was approved by the Institutional Animal Care and Use Committee of the University of Maryland (Baltimore, MD). Animals were maintained on a reverse 12:12-h light-dark cycle.

Diets. All diets were custom formulated by Research Diets (New Brunswick, NJ) and matched for caloric and micronutrient content. As a percentage of total energy, both diets contained 10% fat, 70% carbohydrate, and 20% protein, with the carbohydrate source as 58% cornstarch, 12% maltodextrin, and 31% fructose and 9% cornstarch in the starch and fructose diets, respectively. Diets containing antioxidant tempol were identical to those without drug except for the addition of 0.1% (wt/wt) tempol (an approximate dose of 150 mg•kg⁻¹•day⁻¹ assuming an average daily food consumption of 0.15 g/g body wt). Animals were fed institutionally available rodent chow (Harlan Teklad 2014, Global 14% Protein Rodent Maintenance Diet) for 1 wk to allow for stabilization. Mice were then subjected to surgery and assigned randomly to one of four diets: starch, starch + tempol, fructose, or fructose + tempol. Food and water were provided ad libitum for the remaining 8 wk of the study.

Echocardiography. Echocardiographic measurements of LV size and function were performed at 8 wk postsurgery using a VisualSonics Vevo 770 In Vivo Imaging System (Toronto, ON, Canada). LV end-diastolic areas (EDA) and systolic areas (ESA) were planimetered from long-axis cine loops and used to calculate the area of fractional shortening (aFS) using the following equation: aFS = [(EDA – ESA)/EDA] × 100%. Analyses were performed with the investigator blinded to the surgical and dietary treatment.

Terminal surgery. After 8 wk of treatment, animals were subjected to terminal surgery in the fed state between 3 and 6 h after the initiation of the dark phase. Mice were weighed and anesthetized with 1.5–2.0% isoflurane. The thoracic cavity was opened, and the heart was removed, dissected, weighed, freeze clamped, and stored at −80°C. Pooled blood was collected from the thoracic cavity and separated into serum and plasma. Epididymal, retroperitoneal, and subcutaneous fat was removed and weighed to assess differences in fat mass.

Metabolic measurements. Plasma glucose, insulin, free fatty acids, and triglycerides were measured using commercially available kits as previously described (7, 8). Activities of medium chain acyl-CoA dehydrogenase (MCAD), citrate synthase (CS), isocitrate dehydrogenase (ICDH), aconitase, G6PDH, and 6-phosphogluconate dehydrogenase (6PGDH) were measured using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA). Briefly, 25 μg of powdered LV tissue were homogenized in 250 μl of cold buffer (0.1 M Tris–HCl, 15 mM tricarboxylic acid, and 0.1% Triton X-100; pH 7.8) for 3 min at 30 s⁻¹ using a MixerMill 300 (Qiagen). MCAD and CS activities were measured as previously described (31) to assess fatty acid and overall mitochondrial oxidative capacity. To determine the effect of surgery and diet on oxidative pentose phosphate pathway flux, the activities of G6PDH and 6PGDH were assayed using a modified two-substrate method (13, 41). For G6PDH, 20 μl of a 10% homogenate were added to a buffer containing 50 mM HEPES-Tris, 3.3 mM MgCl₂, 0.5 mM NADP⁺, and 0.5 mM 6-phosphogluconate. Absorbance was read continuously at 340 nm at 37°C for 5 min. For total dehydrogenase activity (6PGDH + G6PDH), 0.5 mM glucose-6-phosphate was added rapidly to the 6PGDH reaction mixture, and the absorbance was read a second time at 340 nm for 5 min. G6PDH activity was then calculated by subtracting the activity of 6PGDH from total dehydrogenase activity. Aconitase and ICDH activities were measured due to their established link with cardiac hypertrophy and oxidative stress (3). To assay aconitase, 10 μl of 10% LV homogenate were added to 390 μl of an incubation mixture containing 5 mM citrate, 0.5 mM MgCl₂, 0.5 mM NADP⁺, and 1 U/ml ICDH at pH 7.4 (28). Absorbance was read at 340 nm for 5 min. ICDH activity was measured using a modified previously described method (23). Two microliters of 10% homogenate were added to 1 ml of an incubation mixture containing 5.6 mM triethanolamine, 4 mM isocitrate, 3.5 mM MgCl₂, and 0.5 mM NADP⁺, and absorbance was read continuously at 340 nm for 5 min.

Assessment of LV oxidative damage. The lipid peroxidation products malondialdehyde and 4-hydroxynonenals were determined using a colorimetric microplate assay (Oxford Biomedical Research, Oxford, MI). Powdered LV tissue was analyzed according to the manufacturer’s protocol, with the results normalized to total protein content.

mRNA expression analysis. RNA isolation and cDNA synthesis were performed as previously described (8). The expression of atrial natriuretic factor (ANF; Nppa), MHC-α (Myh6), MHC-β (Myh7), and peptidylpropyl isomerase A (Ppia, internal control) were assessed using the following Applied Biosystems TaqMan Gene Expression Assays: Mm01255747_g1 (Nppa), Mm00440354_m1 (Myh6), Mm00600555_m1 (Myh7), and Mm02342430_g1 (Ppia). Semiquantitative RT-PCR was performed using an Applied Biosystems Prism 7900HT Sequence Detection System with the TaqMan Universal PCR master mix and the following protocol: 2 min at 50°C, 10 min at 95°C, and 40 cycles at 95°C for 15 s and 1 min at 60°C. Transcript levels were normalized to cyclophilin A (Ppia), which did not change with surgery, diet, or tempol treatment. Results are expressed as fold increases relative to the starch-fed sham group.

Statistical analysis. Two-way ANOVA with the Bonferroni post hoc adjustment was used to compare the effects of diet and drug treatment groups within the sham and TAC groups separately and to assess the interaction between dietary and drug treatment groups. Statistical comparisons were not made between sham and TAC groups. All data are presented as means ± SE. P < 0.05 was accepted as statistically significant.

RESULTS

Survival. All-cause mortality at 8 wk was similar among sham groups: 0%, 6%, 0%, and 8% for starch, starch + tempol, fructose, and fructose + tempol groups, respectively. The mortality in the diet + tempol groups was due to a loss of 1 animal/group and was not significantly different from the
groups without drug treatment. TAC induced the greatest mortality in the fructose group (29%), which was blunted by tempol (14%). A similar reduction in mortality was observed with tempol in starch-fed animals (9% vs. 19% without drug). No significant differences were observed among surgical, dietary, or drug treatment groups.

**Body and fat masses.** Despite no significant differences in food consumption (data not shown), the fructose diet significantly decreased body mass independent of surgical or drug treatment (Fig. 1A). The fructose diet also led to decreased weight gain compared with the starch diet in both the sham and TAC groups independent of drug treatment (Fig. 1B).

Epididymal, retroperitoneal, and subcutaneous fat as well as the sum of these three fat masses as a percentage of body mass were significantly lower in the fructose-fed compared with starch-fed sham groups (Fig. 2, A–D). Within TAC groups, animals on tempol treatment had significantly greater retroperitoneal fat mass compared with TAC animals without drug (Fig. 2B).

**Heart mass.** TAC increased the total heart weight (normalized to tibia length) in both starch- and fructose-fed animals (22.3% and 55.1% increase with respect to sham, respectively; Fig. 3A). Treatment with tempol had a main effect in lowering the heart weight-to-tibia length ratio within TAC groups regardless of diet and also significantly blunted cardiac hypertrophy in fructose-fed TAC mice (Fig. 3A).

There was a strong trend for tempol to reduce LV hypertrophy in both starch-fed and fructose-fed groups within TAC; however, this decrease did not reach statistical significance. Right ventricular mass was decreased by tempol within TAC groups independent of diet and was significantly blunted in fructose-fed TAC mice (Fig. 3C). Atrial mass was also significantly decreased by tempol within the fructose-fed TAC group (Fig. 3D).

**mRNA expression.** No differences were observed in the expression of cyclophilin A (Fig. 4A), which was used to normalize the mRNA levels of other genes. There were no differences in ANF (Fig. 4B), MHC-α (Fig. 4C), or MHC-β (Fig. 4D) among groups. Treatment with tempol showed a strong trend to blunt the fructose-induced increase in MHC-β.

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**Fig. 1.** Terminal body masses (A) and percent changes in body mass from baseline (B) in sham (n = 12–16 animals/group) and transverse aortic constriction (TAC; n = 9–11 animals/group) mice. Pluses and minuses indicate the presence or absence of dietary and/or tempol treatment. Data are means ± SE. Separate two-way ANOVAs were used to determine differences within sham and TAC groups and to assess the interaction between diet and drug treatment. Statistical significance is shown by brackets. *P < 0.05 compared with TAC groups without tempol.

**Fig. 2.** Masses of epididymal fat (A), retroperitoneal fat (B), and subcutaneous fat (C) as well as their sum as a percentage of body mass (D) taken during terminal surgery in sham (n = 12–16 animals/group) and TAC (n = 9–11 animals/group) mice. Pluses and minuses indicate the presence or absence of dietary and/or tempol treatment. Data are means ± SE. Separate two-way ANOVAs were used to determine differences within sham and TAC groups and to assess the interaction between diet and drug treatment. Statistical significance is shown by brackets. *P < 0.05 compared with TAC groups without tempol.
expression within TAC groups, although this reduction did not reach statistical significance ($P = 0.072$).

LV dimensions and performance. Heart rates were similar across all surgical, dietary, and/or drug treatment groups (Fig. 5A). No differences were observed in echocardiographic measurements among sham animals (Fig. 5). EDA was unchanged by diet or drug within TAC groups (Fig. 5B). Tempol decreased ESA (Fig. 5C) and increased aFS (Fig. 5D) within fructose-fed TAC mice.

Metabolic parameters. Plasma free fatty acids were lower in fructose-fed compared with starch-fed sham animals. Within TAC groups, tempol increased plasma glucose and insulin concentrations independent of diet (Table 1).

Fig. 3. Terminal heart weight (HW; A), left ventricular (LV; B), right ventricular (RV; C), and atrial weight (D) normalized to tibia length in sham (n = 12–16 animals/group) and TAC (n = 9–11 animals/group) mice. Pluses and minuses indicate the presence or absence of dietary and/or tempol treatment. Data are means ± SE. Separate two-way ANOVAs were used to determine differences within sham and TAC groups and to assess the interaction between dietary and drug treatment. *$P < 0.05$ compared with the respective control groups (main effect); †$P < 0.05$ compared with the fructose-fed TAC group without tempol. No differences were observed within sham groups.

Fig. 4. Semiquantitative RT-PCR expression data from genes in LV tissue in sham (n = 12–16 animals/group) and TAC (n = 9–11 animals/group) mice. No differences were observed in the mean cycle threshold of cyclophilin A (A), which was used to normalize the mRNA expression of atrial natriuretic factor (ANF; B), myosin heavy chain (MHC)-α (C), and MHC-β (D). Pluses and minuses indicate the presence or absence of dietary and/or tempol treatment. Data are means ± SE. Separate two-way ANOVAs were used to determine differences within sham and TAC groups and to assess the interaction between dietary and drug treatment. No differences were observed within sham groups.
**Enzyme activities.** Within sham and TAC groups, there were no statistically significant differences in the activities of MCAD, CS, aconitase, ICDH, 6PGDH, or G6PDH (Table 2).

**Markers of oxidative damage.** The elevated lipid peroxidation observed in fructose-fed TAC hearts was significantly decreased by tempol (Fig. 6). No differences were observed among sham groups.

**DISCUSSION**

We (8) have previously shown that high-sugar diets increase mortality and LV dysfunction during pressure overload. The present study examined whether treatment with the antioxidant tempol decreases myocardial oxidative damage and prevents the deterioration of systolic function after TAC in fructose-fed mice. Our results demonstrate that tempol effectively prevented cardiac hypertrophy, LV remodeling, contractile dysfunction, and myocardial lipid peroxidation in fructose-fed TAC mice.

Fructose feeding during chronic pressure overload induced hypertrophy, LV dilation, contractile dysfunction, and the upregulation of ANF and MHC-β. This effect was absent in the sham groups, suggesting that fructose adversely affects the chronically stressed myocardium but not the unstressed myocardium. Chronic pressure overload switches myocardial oxidative fuel from fatty acids to glucose (42) and impairs mitochondrial ATP production and the transfer of ATP to the contractile element (27). When superimposed on that phenotype, a high-fructose diet may further impair cardiac energy transfer and/or accelerate pathological processes. The results of the present study and others suggest that antioxidant therapy with tempol or similar drugs might be effective therapy for the prevention and treatment of heart failure. Randomized clinical trials have shown no benefit of antioxidant supplementation on reducing cardiovascular disease outcomes (22, 43, 46), but such trials have not targeted patients with heart failure, hypertension, or cardiac hypertrophy. When patients at high risk for cardiovascular events were given 400 IU vitamin E daily for 4–6 yr, there was no reduction in death, infarction, or stroke compared with patients on placebo (22, 46). Vitamin E supplementation in men with established coronary disease showed similar results (43). In these studies, no attempts were made to control for dietary vitamin E intake or macronutrient consumption, perhaps contributing to the ineffectiveness of antioxidant therapy. The clear ability of tempol to prevent LV hypertrophy

**Table 1. Plasma metabolite and hormone concentrations in the fed state after 8 wk of treatment**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Glucose, mM</th>
<th>Insulin, nM</th>
<th>Free Fatty Acids, nM</th>
<th>Triglycerides, mg/dl</th>
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<tr>
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<td>13</td>
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<tr>
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<td>14.2±0.7</td>
<td>0.28±0.09</td>
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<td>71.4±8.0</td>
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<tr>
<td>Control</td>
<td>15</td>
<td>14.0±0.5</td>
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<td>57.5±6.3</td>
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<td>14.2±0.6</td>
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<td>57.6±4.8</td>
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<td>Starch diet</td>
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<td>13.0±0.8</td>
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<td>0.32±0.09*</td>
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Values are means ± SE; n, no. of animals/group. TAC, transverse aortic constriction. *P < 0.05 compared with the respective control group.
Separate two-way ANOVAs were used to determine differences within sham and TAC groups and to assess the interaction between dietary and drug treatment. Data are means ± SE. †P < 0.05 compared with the fructose-fed TAC group without tempol. No differences were observed within sham groups.

Fig. 6. The lipid peroxidation products malondialdehyde (MDA) and 4-hydroxyalkenals (4-HA) normalized to total protein content were measured spectrophotometrically in LV homogenates in sham (n = 9–16 animals/group) and TAC (n = 9–11 animals/group) mice. Pluses and minuses indicate the presence or absence of dietary and/or tempol treatment. Data are means ± SE. Separate two-way ANOVAs were used to determine differences within sham and TAC groups and to assess the interaction between dietary and drug treatment. †P < 0.05 compared with the fructose-fed TAC group without tempol. No differences were observed within sham groups.

Table 2. Spectrophotometric measurements of enzyme activities from left ventricular homogenates

<table>
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<tr>
<th></th>
<th>n</th>
<th>MCAD, μmol·g wet wt⁻¹·min⁻¹</th>
<th>CS, μmol·g wet wt⁻¹·min⁻¹</th>
<th>MCAD-to-CS Ratio</th>
<th>Aconitase, μmol·g wet wt⁻¹·min⁻¹</th>
<th>ICDH, μmol·g wet wt⁻¹·min⁻¹</th>
<th>6PGDH, μmol·g wet wt⁻¹·min⁻¹</th>
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<td>Starch diet</td>
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<tr>
<td>Control</td>
<td>13</td>
<td>15.7 ± 0.7</td>
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<td>0.278 ± 0.012</td>
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<tr>
<td>Control</td>
<td>15</td>
<td>16.7 ± 0.6</td>
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<td>0.279 ± 0.012</td>
<td>18.5 ± 0.4</td>
<td>55.5 ± 1.7</td>
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<td>59.0 ± 2.3</td>
<td>0.49 ± 0.02</td>
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<td><strong>TAC group</strong></td>
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Values are means ± SE; n, no. of animals/group. MCAD, medium-chain acyl-CoA dehydrogenase; CS, citrate synthase; ICDH, isocitrate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase.

and dysfunction in the present study, coupled with the recent findings of Moens et al. (26) showing a reversal of LV hypertrophy and heart failure in TAC mice treated with the same dose of tempol, suggest that a SOD mimetic may be an effective treatment for hypertrophy and heart failure.

The mechanisms responsible for fructose-induced cardiac dysfunction under conditions of pressure overload are unclear, as are the precise sources of ROS production. Specifically, the role of G6PDH in the generation of ROS and maintenance of the cardiac redox state remains controversial. The purpose of this study was not to measure differences between sham and TAC animals; however, to investigate a previously unidentified TAC effect on G6PDH activity compared with sham, we employed a three-way ANOVA. Compared with sham, TAC increased G6PDH activity from 10% to 20% with no additional dietary or drug effects (Table 2). This modest increase is far less than the two- to fourfold increase observed in dogs or humans with advanced heart failure (14, 15). Oxidative stress is neutralized by reduced glutathione, which donates an electron to hydrogen peroxide and lipid peroxides and prevents the formation of toxic hydroxyl and peroxyl radicals. G6PDH can exert an antioxidant effect by generating NADPH, a cofactor for glutathione reductase, which itself protects against oxidative stress-induced cardiac dysfunction during pathological conditions (19, 20). NADPH is also a cofactor for nitric oxide synthase, which could have beneficial effects in heart failure. Mice deficient in G6PDH are more susceptible to myocardial injury and contractile dysfunction during postischemic reperfusion, again associated with decreased total and reduced glutathione concentrations. These findings suggest that G6PDH is essential for maintaining intracellular reduced glutathione and cardiomyocyte contractility. On the other hand, G6PDH may enhance superoxide production in the failing heart, as suggested by Gupta et al. (14, 15), who found elevated myocardial oxidized glutathione, superoxide, and NADPH concentrations and increased G6PDH activity in dogs and patients with heart failure. Superoxide production was blunted by the NADPH oxidase inhibitor gp91dsat and the G6PDH inhibitor 6-aminonicotinamide, suggesting that G6PDH fuels paradoxical superoxide production via NAPDH oxidase and worsens contractile function. Thus, G6PDH could be either protective or detrimental depending on the severity of hypertrophy and contractile impairment. Further studies are needed with high-sugar diets and pharmacological or genetic manipulation of G6PDH activity within experimental heart failure to clarify the role of this enzyme.

The effects of a high-sugar diet in heart failure may be determined by the relative activities of pro- and antioxidant enzymes. Antioxidant enzymes are known to be downregulated in the failing heart, suggesting that reduced scavenging of ROS contributes to disease pathogenesis (10, 17, 38, 39). As tempol is a SOD mimic, it may have increased hydrogen peroxide production, especially in the presence of diminished catalase activity. Future studies should assess the effects of high-sugar diets on myocardial antioxidant enzymes and hydrogen peroxide levels to determine if there is a direct connection between diet-induced oxidative stress and heart failure.

Despite the increase in G6PDH activity with TAC, there was no specific dietary effect, suggesting another mechanism for the deleterious effects of fructose feeding. Although not addressed in the present study, a potential link between high-
fructose diets and cardiac hypertrophy and failure is insulin signaling. It has been demonstrated that the activation of insulin signaling mediates growth in the postnatal heart (2). Furthermore, effectors of insulin signaling, notably, phosphoinositide 3-kinase and Akt, are regulated by nutritional status and induce cardiomyocyte protein synthesis (37). These data suggest that chronic insulin signaling, perhaps through the consumption of high-carbohydrate diets, may exacerbate hypertrophy and expedite the transition to contractile failure. Feeding studies in the well-established cardiac insulin receptor knockout mouse would clarify the impact of diet on pathological growth and remodeling. It has also been reported that fructose feeding increases angiotensin type I receptor expression, oxidative stress, and inflammation in the rat aorta, leading to decreased vascular reactivity and hypertension (1, 29). Thus, the hypertrophy and ventricular remodeling observed in fructose-fed TAC animals in the present study may be attributed to increased macrovascular oxidative damage and dysfunction.

Although there were no differences in food consumption, animals fed the high-fructose diet had significantly lower body mass and weight gain compared with starch-fed mice (Fig. 1, A and B). Consistent with these data, epididymal, retroperitoneal, and subcutaneous fat masses were significantly lower in fructose-fed compared with starch-fed sham animals (Fig. 2, A-C). These data suggest a greater thermogenic potential for high fructose compared with high starch. Within TAC, however, despite causing significantly decreased weight gain from baseline, fructose feeding did not decrease fat depot masses as a percentage of body mass (Fig. 2D). These results imply a surgical effect on body composition, perhaps through behavioral changes associated with the development of hypertrophic cardiomyopathy and contractile dysfunction in TAC animals compared with sham animals. Thus, the complex interplay between diet, lifestyle modifications coupled with disease progression, and the resultant metabolic abnormalities requires further examination.

It is important to consider the possibility that the beneficial effects of tempol may have originated from a reduction in blood pressure, although it is unknown if tempol has antihypertensive effects independent of its antioxidant properties. Studies in deoxycorticosterone acetate salt, spontaneously hypertensive, ANG II-induced hypertensive, and normotensive Sprague-Dawley rats have demonstrated that tempol can lower blood pressure (4, 25, 30, 32–34, 44, 45). In mice, tempol blunts the elevation in systolic blood pressure induced by chronic ANG II infusion but has no blood pressure-lowering effects in normotensive animals (11). In the present study, TAC precluded tail cuff blood pressure measurements, as aortic ligation decreases downstream pressure. Other methods of hemodynamic assessment, such as aortic or LV catheterization, require anesthesia and invasive surgical maneuvers that could trigger acute systemic and myocardial oxidative stress. Thus, these measurements were not performed. Since the aortic hypertension induced by TAC is unlikely to be due to any of the mechanisms involved in the tempol-induced reduction in blood pressure, there is little reason to speculate that tempol lowered aortic blood pressure in TAC mice in the present study. Furthermore, any potential antihypertensive effect of tempol would be consistent across groups, thereby denoting diet as the main factor contributing to the TAC-induced cardiac phenotype.

In conclusion, the antioxidant tempol significantly blunted markers of cardiac hypertrophy, LV remodeling, contractile dysfunction, and oxidative stress in fructose-fed TAC mice. Additional studies are needed to identify the effects of diet on ROS-generating pathways in cardiac hypertrophy and heart failure.

GRANTS
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REFERENCES


