Taurine Supplementation Reduces Oxidative Stress and Improves Cardiovascular Function in an Iron-Overload Murine Model

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Background—Iron overload has an increasing worldwide prevalence and is associated with significant cardiovascular morbidity and mortality. Elevated iron levels in the myocardium lead to impaired systolic and diastolic function and elevated oxidative stress. Taurine accounts for 25% to 50% of the amino acid pool in myocardium, possesses antioxidant properties, and can inhibit L-type Ca^{2+} channels. Thus, we hypothesized that this agent would reduce the cardiovascular effects of iron overload.

Methods and Results—Iron-overloaded mice were generated by intraperitoneal injection of iron either chronically (5 days per week for 13 weeks) or subacutely (5 days per week for 4 weeks). Iron overload causes increased mortality, elevated oxidative stress, systolic and diastolic dysfunction, hypotension, and bradycardia. Taurine supplementation increased myocardial taurine levels by 45% and led to reductions in mortality and improved cardiac function, heart rate, and blood pressure in iron-overloaded mice. Histological examination of the myocardium revealed reduced apoptosis and interstitial fibrosis in iron-overloaded mice supplemented with taurine. Taurine mediated reduced oxidative stress in iron-overloaded mice along with attenuation of myocardial lipid peroxidation and protection of reduced glutathione level.

Conclusions—These results demonstrate that treatment with taurine reduces iron-mediated myocardial oxidative stress, preserves cardiovascular function, and improves survival in iron-overloaded mice. The role of taurine in protecting reduced glutathione levels provides an important mechanism by which oxidative stress–induced myocardial damage can be curtailed. Taurine, as a dietary supplement, represents a potential new therapeutic agent to reduce the cardiovascular burden from iron-overload conditions. **(*Circulation*. 2004;109:1877-1885.)**

Key Words: hemochromatosis ■ cardiomyopathy ■ taurine ■ stress, oxidative ■ glutathione

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Secondary iron overload and primary (hereditary) hemochromatosis are now being increasingly recognized as worldwide epidemics.[1–5] Iron overload is associated with progressive iron deposition in a variety of tissues, including the heart and endocrine organs, leading to cardiomyopathy and various endocrinopathies.[6–9] Iron-overload cardiomyopathy is characterized by marked diastolic dysfunction, increased propensity for arrhythmias, and an end-stage dilated cardiomyopathy.[6,8,10–12] Cardiac disease is the primary determinant of survival in patients with secondary iron overload[6,11,12] and occurs frequently in patients with primary hemochromatosis.[6,8,13] Acute iron toxicosis, a common cause of pediatric drug-related mortality, is also associated with myocardial injury and dysfunction.[14,15]

Iron overload is associated with increased free radical production and elevated oxidative stress and altered intracellular Ca^{2+} handling in cardiomyocytes.[16–21] As such, therapeutic strategies that attempt to minimize the oxidative damage and reduce perturbations in intracellular Ca^{2+} may protect the cardiovascular system from iron-mediated injury. The sulfur-containing β-amino acid taurine (2-aminoethanesulfonic acid) is found in relatively high concentrations in myocardium, where it accounts for $\approx$25% of the free amino acid pool in humans and 50% in rodents.[22,23] In mammals, taurine is neither metabolized nor incorporated into cellular proteins, suggesting an important requirement for free cytosolic taurine.[22,23] The essential role of taurine in cardiovascular function is illustrated by the dilated cardiomyopathies that have been linked to taurine depletion.[24–28] Emerging evidence supports 2 major mechanisms for the actions of taurine. First, taurine has a combination of effects on ion channels, transporters, and enzymes, leading to modulation of

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intracellular Ca^{2+} levels.[23,27,29–31] Second, taurine has been shown to have potent antioxidant properties under various pathophysiological conditions.[22,23,27,32]

Despite the vast amount of literature supporting the role of free radical–mediated oxidative stress in the pathogenesis of iron-mediated injury, the use of antioxidants as possible therapeutic agents has not been well studied. In this study, we developed a murine model of secondary iron overload (hemosiderosis), which is characterized by pronounced diastolic and systolic dysfunction associated with increased oxidative stress, intracellular iron deposition, interstitial fibrosis, and a significant reduction in survival. We found that dietary taurine supplementation reduced myocardial oxidative stress and damage, leading to a protection of cardiovascular function and survival, in our murine model of iron overload. Our results suggest that increases in dietary taurine may exert significant salutary effects in patients with iron overload.

Methods

Experimental Animals and Iron-Loading Protocols

Male B6D2F1 mice (Jackson Laboratory, Ann Harbor, Maine) 8 weeks of age were housed in temperature- and humidity-controlled rooms with 12-hour light-dark cycles. All experimental protocols confirmed to the standards of the Canadian Council On Animal Care. Mice were randomized to receive either taurine (0.1 mol/L) or vehicle (2.5% dextrose) in their drinking water starting 2 weeks before injections and throughout the course of the experiments. Mice were injected with either iron or placebo (0.1 mL 10% dextrose) subacutely (total duration of 4 weeks; total dose, ≈200 mg/25 g body wt) or chronically (total duration of 13 weeks; total dose, ≈200 mg/25 g body wt) as described previously.[21] In addition, some mice were injected with iron over a period of 6 weeks (subacute protocol)

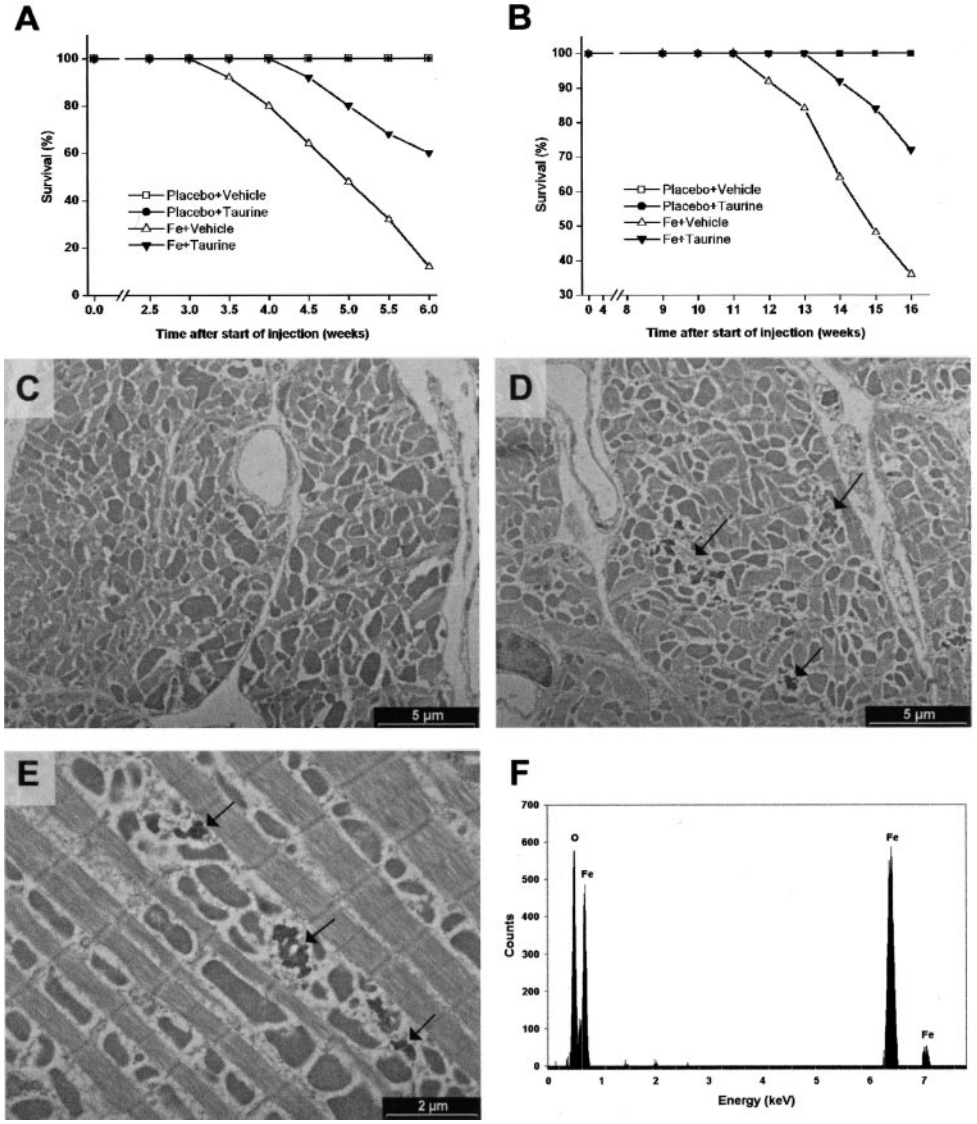

Figure 1. Survival and iron deposition in iron-overloaded mice. For survival analysis, mice were injected for 6 weeks (subacute protocol; A) and 16 weeks (chronic protocol; B); open squares, placebo+vehicle (n=10); closed circles, placebo+taurine (n=10); open triangle, iron+vehicle (n=25); closed triangle, iron+taurine (n=25); $P<0.001$ by Kaplan-Meier survival analysis for both subacute (A) and chronic (B) groups. Electron microscopic analysis of iron-overloaded myocardium showing transmission electron micrograph from cardiomyocytes in placebo+vehicle (C) and iron+vehicle (D, E) groups, showing multiple intracellular electron-dense deposits (arrows). Multiple electron-dense deposits from iron+vehicle group were shown to be iron deposition using x-ray microscopic analysis (F).

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TABLE 1. Body Weight, Organ Weight, and Myocardial Taurine Levels in Iron-Injected Mice

	Subacute (4 wk)				Chronic (13 wk)			
	Placebo+Vehicle	Placebo+Taurine	Iron+Vehicle	Iron+Taurine	Placebo+Vehicle	Placebo+Taurine	Iron+Vehicle	Iron+Taurine
Body weight, g	29.6±0.35	28.4±0.6	24.3±0.6*	27.2±0.4	33.5±0.75	33.2±0.38	31.1±1.1	30.3±0.2
HW/BW, mg/g	4.94±0.15	4.55±0.11	4.45±0.13	4.59±0.16	5.06±0.09	4.97±0.13	5.08±0.16	5.32±0.14
LIV/BW, mg/g	45.7±0.9	44.8±1.3	113.7±2.8*	96.6±2.3†	45.5±1.13	50.5±1.2	96.1±1.6†	90.3±2.7†
Taurine level, μmol/g	28.4±1.56	...	...	42.9±1.09‡	29.2±1.37	45.8±2.1‡	30.5±0.89	43.3±1.92‡

n=9 for all subgroups in the subacute and chronic categories. HW/BW indicates heart to body weight ratio; LIV/BW, liver-to–body weight ratio.
*$P<0.05$ vs all other groups.
†$P<0.05$ vs Placebo+Vehicle and Placebo+Taurine groups.
‡$P<0.05$ vs Placebo+Vehicle and Fe+Vehicle groups.

and 16 weeks (chronic protocol) to assess the impact of taurine on the survival of iron-overloaded mice.

Histology, Electron Microscopy, and TUNEL Assay

Hearts were removed from anesthetized mice, rinsed in PBS, and fixed with 10% buffered formalin. Thin sections (5 μm) were stained with Masson's trichrome, Prussian blue, and picrosirius red for morphometric analysis and intracellular iron deposition using computerized planimetry performed in a blinded manner as described previously.[21] Analytical electron microscopy and TdT-mediated dUTP nick end-labeling (TUNEL) assay were performed as described previously.[21]

Tissue Iron Levels

Tissues from the left ventricular (LV) myocardium (transmural section from mid LV free wall) and liver (left lobe) were excised, washed in PBS, and stored at −70°C. Iron levels in the tissue samples were measured by atomic absorption spectrophotometry (Perkin-Elmer 5000 flame atomic absorption spectrophotometer) at the provincial specialized trace metal laboratory (Trace Metals Laboratory, London, Ontario, Canada).

Echocardiography

Transthoracic 2D, M-mode, and Doppler echocardiographic examination was performed with an Acuson Sequoia C256 system equipped with a 15-MHz linear transducer (15L8) (Version 4.0, Acuson Corp) in mice anesthetized with isoflurane/oxygen (0.75%/100%).

Hemodynamic Indexes

Mice were anesthetized with intraperitoneal injections of ketamine (60 mg/kg) and xylazine (3 mg/kg). The right common carotid artery was exposed and cannulated with a 1.4F Millar catheter, which was advanced into the proximal aorta and then through the aortic valve and into the LV.

Measurement of Taurine and Aldehyde Levels

Ventricular myocardial tissue was homogenized and the supernatant ultrafiltered and diluted with an internal standard, methionine sulfone. Taurine concentrations were determined as described previously with a high-performance liquid chromatography system and a specific Pico-Tag column (Waters Operating Corp).[33] Plasma and heart aldehyde levels were analyzed as described previously.[21]

Measurement of Glutathione Levels (Reduced and Oxidized)

Total (GSH+GSSG), reduced (GSH), and oxidized (GSSG) myocardial glutathione and the redox ratio (GSH/GSSG) were measured as described previously.[34] Each sample was analyzed in duplicate, and the average value was used.

Statistical Analysis

All statistical analyses were performed with SPSS software (version 10.1). Survival data were compared by the Kaplan-Meier survival analysis. The effects of iron and the role of taurine were evaluated by ANOVA followed by the Student-Newman-Keuls test for multiple-comparison testing.

Results

Clinical and Cardiovascular Effects of Iron Overload

Mice injected with iron showed clinical signs of heart failure, including lethargy, ascites, and peripheral edema and increased mortality (Figure 1, A and B). Iron-overloaded mice also had reduced body weights and a 2-fold increase in ratio of liver to body weight, with no evidence of cardiac hypertrophy (Table 1). Electron microscopy analysis showed electron-dense intracellular bodies throughout the sarcoplasm of cardiomyocytes from mice injected with iron, whereas no such deposits were observed in cardiomyocytes from the placebo-injected mice (Figure 1, C–E). Energy-dispersive x-ray spectrometry confirmed that the electron-dense deposits contained iron on the basis of the signature energy spectrum of iron (Figure 1F).

Iron-overloaded mice have reduced blood pressures with modest reductions in heart rate (HR) compared with placebo-injected controls (Table 2). Iron overload led to impaired myocardial contractility, as shown by reduction ($P<0.01$) in $+dP/dt_{max}$ and $-dP/dt_{max}$ for the iron+vehicle compared with placebo+vehicle groups. HR-corrected echocardiographic recordings confirmed significant reductions of systolic function in iron-overloaded mice. Fractional shortening, HR-corrected velocity of circumferential shortening, and peak aortic velocity were all significantly reduced in the iron+vehicle group compared with placebo+vehicle mice (Table 2), without changes in ventricular wall thickness or diastolic dimension (data not shown). In association with impaired systolic function, iron-overloaded mice had diastolic dysfunction, as assessed by various hemodynamic indexes such as elevated LV relaxation time and LV end-diastolic pressure (Table 2). Echocardiographic assessment of the HR-corrected E/A ratio is also a useful measure of diastolic dysfunction (where E/A ratio is the ratio of early peak velocity of mitral diastolic flow [E wave] and the late peak velocity of mitral diastolic flow from atrial contraction [A wave]).[35,36] Indeed, HR-corrected E/A ratios were increased in the iron-overload mice when measured with either ketamine/xylazine or isoflurane/oxygen

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TABLE 2. Hemodynamic and Echocardiographic Parameters in Mice Chronically Injected With Iron Over a 13-Week Period

	Placebo+Vehicle	Placebo+Taurine	Iron+Vehicle	Iron+Taurine
SBP, mm Hg	112±4.6	119.5±6.7	70.2±3.8†	92.8±7.5‡
MABP, mm Hg	81.6±5	83.6±5.1	49.3±3.7†	68.9±4‡
LVEDP, mm Hg	8.11±0.59	8.63±1.4	16.15±1.85†	10.51±1.13
$+dP/dt_{max}$, mm Hg/s	6124±322	6020±272	2544±288†	5802±307
$-dP/dt_{max}$, mm Hg/s	5482±171	5241±178	1853±261†	4159±153‡
Tau_c, ms	8.12±0.36	7.72±0.29	15.3±1.7†	8.87±1.5
HR, bpm	501±12.3	509±11.2	365±13.1†	461±9.8‡
LVESD, mm	2.01±0.12	2.07±0.08	2.56±0.12†	1.98±0.09
FS, %	47.9±1.7	47.3±1.21	34.1±1.4†	48.6±1.43
VCF_c, circ/s	9.72±0.27	9.79±0.31	6.27±0.23†	9.88±0.31
PAV_c, cm/s	85.6±3.8	91.7±3.5	58.8±2.9†	89.1±2.8
E/A_c ratio (n=5)	2.28±0.13	2.36±0.11	1.67±0.14†	2.41±0.09
E/A_c ratio*	3.25±0.22	3.72±0.31	1.81±0.19†	3.81±0.27

n=9 for all groups. SBP indicates systolic blood pressure; MABP, mean arterial blood pressure; LVEDP, LV end-diastolic pressure; $\pm dP/dt_{max}$, positive and negative maximal rate of change of ventricular pressure; Tau_C, time needed for relaxation of 50% maximal LV pressure to baseline value corrected for HR; LVESD, left ventricular end-systolic and end-diastolic dimensions; FS, fractional shortening; VCF_C, velocity of circumferential shortening corrected for HR; PAV_C, peak aortic velocity corrected for HR; and E/A_C ratio, ratio of early diastolic filling (E-wave) to filling from atrial contraction (A-wave) corrected for HR.

*Using ketamine/xylazine (basal HR, 312±13 bpm).

†$P<0.01$ vs all other groups.

‡$P<0.01$ vs Placebo+Vehicle and Placebo+Taurine groups.

anesthesia, despite producing marked differences in HRs (Table 2).

Histological Characterization and Iron Deposition

Increased myocardial interstitial fibrosis is a prominent feature of the cardiovascular pathology in patients with iron overload. Hearts from mice injected chronically with iron displayed extensive interstitial fibrosis and myocyte vacuolar degeneration with mild inflammatory infiltrate (Figure 2, A–C). Quantitative morphometry confirmed a significant amount of myocardial damage, including inflammation and interstitial fibrosis, in the iron+vehicle group compared with the placebo+vehicle group ($P<0.01$) (Figure 2M). A more specific analysis of the interstitial fibrosis using picrosirius red staining confirmed increased collagen deposition in mice injected with iron chronically (Figure 2N). Immunoperoxidase TUNEL staining revealed significant increases in cardiomyocyte apoptosis in mice injected with iron chronically for 13 weeks (Figure 2, E–G and O). These histological changes suggest that impaired cardiac function in iron-overloaded mice could result from myocyte loss and replacement with interstitial fibrosis.

To investigate the mechanism whereby iron overload alters cardiac function, we examined the phenotype in mice injected with iron subacutely (4 weeks) (see Methods). Consistent with the effects seen with chronic iron overload, subacute iron overload for 4 weeks impaired systolic and diastolic cardiac function along with reductions in HR and blood pressure (Table 3) before the development of extensive myocardial fibrosis (data not shown). Prussian blue staining provided clear evidence of iron deposition both within and outside cardiomyocytes in subacutely iron-injected mice (Figure 2, I–K), which was confirmed by quantitative estimation of the intracellular iron deposition within cardiomyocytes (Figure 2P).

Increased Oxidative Stress in Iron-Overloaded Mice

The accumulation of iron inside cardiomyocytes is expected to lead to free radical–mediated oxidative stress.[37,38] To confirm the presence of increased oxidative stress, we quantified myocardial and plasma levels of the lipid peroxidation products malondialdehyde (MDA), hexanal (HEX), and 4-hydroxynonenal (HNE). Baseline MDA level in the heart was increased by 15-fold from a baseline value of 21.1±2 to 329±29.2 nmol/g ($P<0.01$) in mice injected with iron subacutely (Figure 3A), and myocardial HEX and HNE levels were also significantly elevated in iron-injected mice (Figure 3, B and C). Although these elevations in aldehydes occur before the development of significant interstitial fibrosis (subacute group), mice injected chronically with iron also had elevated aldehyde levels (Figure 3, A–C), albeit less pronounced than in the subacute hearts. Plasma MDA levels, which reflect a composite index of the whole-body oxidative stress, showed similar changes to aldehydes in the myocardium (Figure 3D). To confirm iron-induced oxidative damage in our murine model, we assessed myocardial glutathione status. As expected from the aldehyde measurements, iron overload was associated with a marked depletion of GSH (Figure 4A) with a corresponding increase in GSSG (Figure 4B) and elevation in GSH/GSSG ratio (Figure 4C), leading to a net reduction in GSH+GSSG (Figure 4D).

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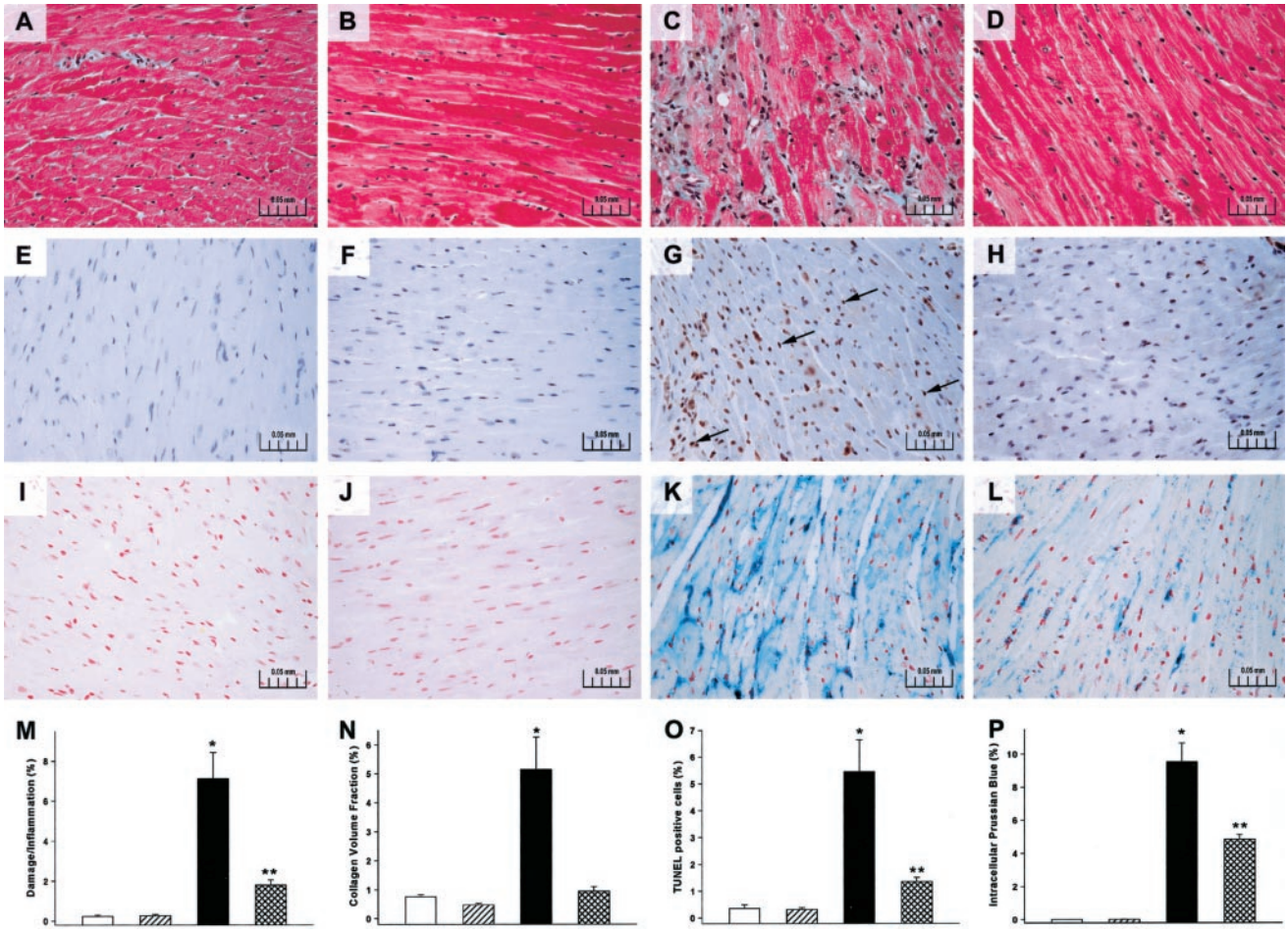

Figure 2. Histological characterization of myocardium from iron-overloaded mice. Representative trichrome-stained sections (A–D) and TUNEL immunoperoxidase staining for apoptosis (E–H) from mice injected with iron chronically and Prussian blue–stained sections (I–L) from mice injected subacutely in placebo+vehicle (A, E, I), placebo+taurine (B, F, J), iron+vehicle (C, G, K), and iron+taurine (D, H, L) groups. Bar graphs showing quantification of myocardial damage using trichrome staining (M), myocardial interstitial fibrosis using picrosirius red staining (N), and TUNEL-positive apoptotic cardiac myocytes (O) in mice injected chronically, and intracellular iron deposition by Prussian blue staining in mice injected subacutely (P); open bar, placebo+vehicle; hatched bar; placebo+taurine; closed bar, iron+vehicle; cross-hatched bar, iron+taurine. *$P<0.01$ vs all other groups; **$P<0.01$ vs placebo+vehicle and iron+vehicle; n=5 in all groups.

Taurine Protects Cardiac Function in Iron-Overloaded Mice

Taurine levels were elevated to similar extents (≈50%) in iron- and placebo-injected mice supplemented with taurine (Table 1), demonstrating that iron overload does not interfere with taurine loading of the myocardial tissue. Dietary supplementation with taurine caused significant improvement in body weight and a lowered liver wt/body wt ratio in the subacute iron-loaded group but had little effect in chronically iron-loaded mice (Table 1). Taurine supplementation in mice

TABLE 3. Hemodynamic Parameters in Mice Injected Subacutely With Iron Over a 4-Week Period

	Placebo+Vehicle	Placebo+Taurine	Iron+Vehicle	Iron+Taurine
HR, bpm	313.5±7.5	307±7.2	221±8.3*	258.5±8.2†
SBP, mm Hg	110.8±4.6	103.5±4.9	72.3±6.4*	101.3±5.1
MABP, mm Hg	82.1±5	81.6±4.6	55.9±5.7*	73.3±4†
LVEDP, mm Hg	5.53±1.22	6.11±1.8	12.93±1.31*	9.21±1.51†
$+dP/dt_{max}$, mm Hg/s	6985±323	7110±209	3127±311*	5610±257†
$-dP/dt_{max}$, mm Hg/s	6194±170	5856±207	2217±257*	4376±216†
Tau_c, ms	7.42±0.36	7.57±0.29	11.32±0.9*	7.89±0.45

n=9 for all groups; abbreviations as in Table 2.
*$P<0.01$ vs all other groups.
†$P<0.01$ vs Placebo+Vehicle group.

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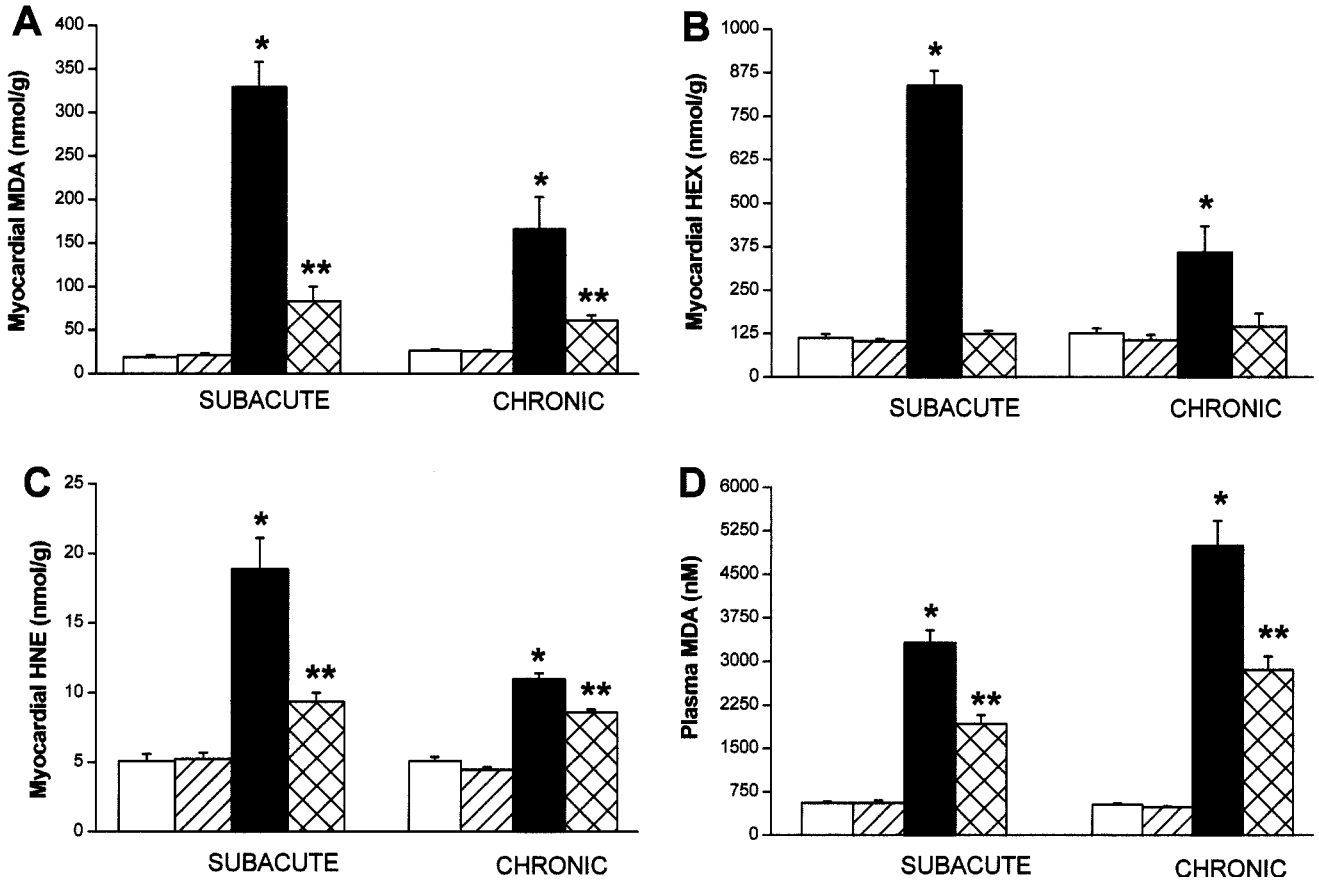

Figure 3. Myocardial MDA (A), HEX (B), and HNE (C) levels and plasma MDA (D) level as markers of oxidative stress as determined by gas chromatography–mass spectrophotometry in mice injected subacutely (subacute) and chronically (chronic). Open bar, placebo+vehicle; hatched bar, placebo+taurine; closed bar, iron+vehicle; crosshatched bar, iron+taurine. *$P<0.01$ vs all other groups; **$P<0.01$ vs placebo+vehicle and placebo+taurine groups; n=9 in all groups.

not given iron was not associated with any adverse effects on survival (Figure 1, A and B), cardiac structure and function (Figure 2, Tables 2 and 3), or oxidative stress (Figures 3 and 4).

Taurine supplementation resulted in a significant protection in survival in iron-overloaded mice (Figure 1, A and B) ($P<0.001$, Kaplan Meier survival analysis). Cardiac diastolic and systolic function in mice injected subacutely and chronically with iron was nearly completely protected by taurine, and blood pressure and HR were also partially protected (Tables 2 and 3). As expected from the improvement in survival and cardiac function, taurine treatment led to protection of the myocardium (Figure 2, D and M), and interstitial fibrosis was markedly lowered (Figure 2N). This improvement in the myocardial ultrastructure was associated with large reductions in the degree of apoptosis (Figure 2, H and O) ($P<0.01$; n=4).

Taurine Reduces Oxidative Stress and Protects GSH Levels

Taurine may protect myocardial structure and function in iron-overloaded hearts as a result of its potent antioxidant properties.[22,23,27,32] Consistent with these anticipated effects, taurine supplementation reduced myocardial MDA levels to 83.3±16.5 from 329±29.2 nmol/g and myocardial HEX levels to 124±9 from 871±49 nmol/g in mice injected subacutely with iron (Figure 3, A and B). Moreover, increased myocardial HNE levels were also reduced in this group (Figure 3C). In mice injected with iron chronically, a similar overall trend occurred in the myocardial and plasma aldehyde levels after taurine treatment (Figure 3, A–C). Plasma levels of MDA in iron-overload mice were also reduced by taurine supplementation, suggesting a global decrease in iron-induced oxidative damage (Figure 3D). These results establish that the protective effects of taurine on heart function are correlated with the amelioration of iron-induced oxidative stress.

Because the potent antioxidant property of taurine (and its metabolites) may be linked to its sulfur moiety,[22,32,39,40] we predicted that taurine may preserve levels of GSH, which is a fundamental defense mechanism in conditions of increased oxidative stress. Interestingly, taurine supplementation in iron-overloaded mice completely prevented the decreases in GSH ($P<0.01$) (Figure 4A) and increases in GSSG ($P<0.01$) (Figure 4B), thereby providing partial protection of the redox ratio (Figure 4C) and a normalization of GSH+GSSG levels (Figure 4D). Given the antioxidant effects of taurine and the importance of redox cycling in controlling iron entry into cells, we examined the impact of taurine on myocardial iron burden. Subacute iron-overloaded mice, as with chronically injected mice, showed marked elevations in cardiac and

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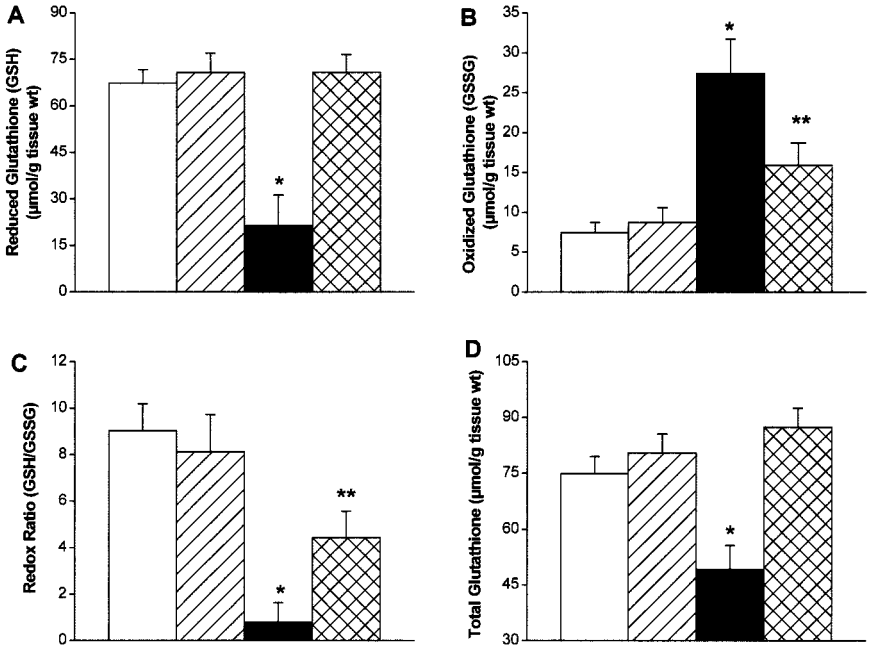

Figure 4. Myocardial GSH (A) and GSSG (B) levels, redox ratio (GSH/GSSG) (C), and GSH+GSSG levels (D) in mice injected subacutely. Open bar, placebo+vehicle; hatched bar, placebo+taurine; closed bar, iron+vehicle; crosshatched bar, iron+taurine. *$P<0.01$ vs all other groups; **$P<0.01$ vs placebo+vehicle and placebo+taurine groups; n=9 in all groups.

hepatic iron levels (Figure 5, A–D). Taurine supplementation was associated with reductions in myocardial iron levels in the subacute (Figure 5A) and chronic (Figure 5C) groups. Histological analysis confirmed that taurine treatment selectively reduced iron deposition in myocytes without affecting extramyocyte iron accumulation (Figure 2, L and P). By contrast, hepatic iron levels were unaffected by taurine treatment (Figure 5, B and D).

Discussion

Myocardial iron deposition and injury are the major determinants of survival in patients with secondary iron overload[6,11,12] and cause significant morbidity and mortality in patients with primary hemochromatosis.[6,8,13] In addition to chronic iron-induced cardiomyopathy, acute iron toxicosis is also associated with myocardial injury and dysfunction.[14,15] Therefore, the phenotypic diversity of iron-overload cardiomyopathy range from acute toxic effects of iron to chronic iron-mediated effects (increased interstitial fibrosis with inflammatory changes). In our murine model, we made a distinction between direct toxic effects of iron on the heart (subacute protocol) versus chronic iron-mediated myocardial damage (chronic protocol). Mice that were injected with iron subacutely had clear evidence for intracellular iron deposition in cardiomyocytes with minimal change in interstitial fibrosis. In contrast, chronically iron-overloaded mice showed progressive myocardial damage with increased interstitial (and perivascular) fibrosis coincident with increased apoptosis of iron-overloaded cardiomyocytes. The myocardial iron levels obtained in our murine model (iron+vehicle groups, 7.6 to 11.5 mg/g LV dry wt) are similar to myocardial iron levels (3.5 to 9.2 mg/g dry wt) reported in patients with iron-overload cardiomyopathy and heart failure.[6]

Free radical production and oxidative stress play a key role in the triggering and progression of iron-overload cardiomyopathy and in acute iron toxicosis, as shown in isolated cardiomyocytes,[16,41–43] papillary muscle preparations,[44] animal models,[18,21,45] and patients.[46,47] In both subacute and chronically iron-overloaded hearts, there was clear evidence of increased oxidative damage, as shown by marked increases in various lipid peroxidation products (aldehydes) and depletion of GSH (and GSH+GSSG) levels. Iron-overloaded mice had marked elevations in unsaturated (MDA and HNE) and saturated (HEX) aldehydes in the heart and plasma. These aldehyde products are generated by free radical–induced lipid peroxidation and participate in cytotoxic reactions, leading to cellular dysfunction[48,49] including altered excitation-contraction coupling and electrophysiology and defective contractile function.[20,42,43,50] Indeed, a distinct feature of iron-overload cardiomyopathy in humans is the increased susceptibility to various arrhythmias, including bradyarrhythmias and cardiac death.[8,11,12] In our study, iron-overloaded mice had increased mortality and displayed bradycardia. The effects of iron overload on mortality are consistent with observations showing that free radical–mediated lipid peroxidation alters cardiomyocyte electrophysiology[42,50] and Ca^{2+} handling[20,43] and blockade of electrical conduction and sudden death in iron-overloaded guinea pigs.[51]

Our strategy was to increase myocardial taurine levels to protect the cardiovascular system and prevent iron overload–induced cardiovascular dysfunction, even though myocardial taurine levels were not affected by iron overload. This rationale is based on the observation that in the setting of increased oxidative stress, there appears to be an increased (conditioned) demand for taurine.[22,23,27,30,32,52] Taurine supplementation resulted in protection of cardiac function and arterial blood pressure in iron-overloaded mice. The impressive benefit of taurine in mediating a survival advantage in iron-overloaded mice occurs in conjunction with a marked improvement in bradycardia. These protective actions were correlated with reductions in apoptosis and interstitial fibrosis, suggesting that taurine was able to decrease the toxic

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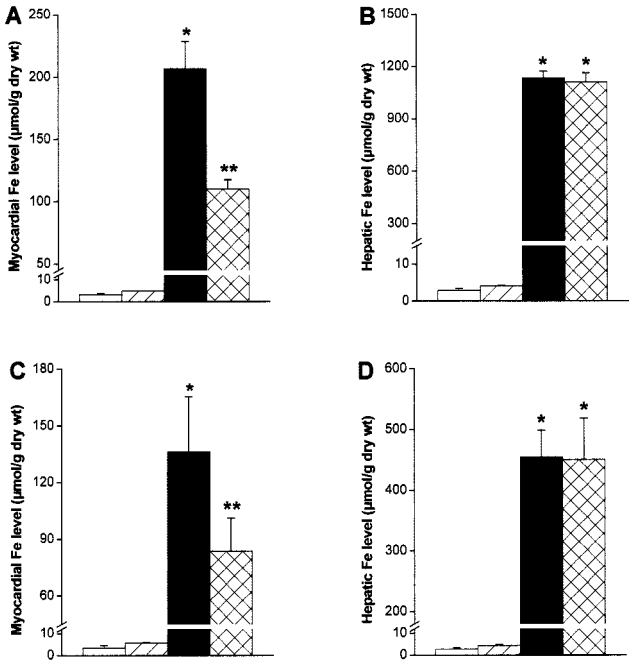

Figure 5. Myocardial and hepatic iron levels in mice injected subacutely (A and B) and chronically (C and D). Open bar, placebo+vehicle; hatched bar, placebo+taurine; closed bar, iron+vehicle; crosshatched bar, iron+taurine. *$P<0.01$ vs all other groups; **$P<0.01$ vs placebo+vehicle and placebo+taurine groups; n=9 in all groups.

effects of iron. These observations are similar to those of previous studies showing that taurine has cytoprotective effects against iron-induced damage in cell culture systems[53,54] and protects against apoptosis.[55–57] The protective effects of taurine have been linked previously to the antioxidant properties[22,23,32] of taurine and its ability to modulate excitation-contraction coupling and intracellular Ca^{2+} homeostasis in cardiomyocytes.[23,27,30] Moreover, antioxidants have been shown to reduce iron-mediated lipid peroxidation and the associated cellular damage in isolated ventricular myocytes[16,41] and in vivo.[45]

In support of the antioxidant properties of taurine in our studies, drastic reductions in the formation of aldehydes were observed in iron-overloaded mice. The potent antioxidant property of taurine may be linked to its sulfur moiety and its modulation of glutathione levels.[22,32,39,40] GSH plays an important role in the cellular defense against oxidative stress.[34,58,59] GSH depletion and accumulation of GSSG occur in the heart during oxidative stress caused by increased cellular demand and lead to impaired cell function because of a shift in redox state.[34,58,59] Interestingly, taurine supplementation completely prevented the iron-induced decrement in GSH levels while protecting glutathione redox ratio. These observations provide a novel mechanism of action of taurine and may explain its pleiotropic beneficial effects in conditions of increased oxidative stress. Taurine can also react directly with a variety of cytotoxic aldehydes, including MDA, suggesting that the protective effects of taurine on heart function may also be related to lowering of aldehyde levels per se.[23] Moreover, we cannot rule out a role of taurine in mediating anti-inflammatory effects,[60] maintaining cellular metabolism (via its effect on mitochondria),[61] and/or protection of coronary endothelium and blood flow.[62] In our murine model, taurine treatment also led to reductions in myocardial iron levels. Reduced cardiac iron levels may be related to the tonic inhibition of L-type Ca^{2+} channels by taurine,[29,31] which is an important transferrin-independent pathway for iron transport in iron-overload conditions.[21,63] Alternatively, antioxidant properties of taurine may play an important role in preventing iron overload in myocardium, possibly by the redox cycling of iron,[4,64] as shown previously using other antioxidants in isolated cardiomyocytes[16] and in an animal model of iron overload.[45]

In summary, our murine model of iron-overload cardiomyopathy shows that taurine supplementation has unequivocal beneficial effects on survival and cardiac structure and function, with marked reductions in iron-induced oxidative stress. Given the impressive benefit and absence of toxicity with taurine supplementation, we propose that increased dietary taurine intake represents an important nutritional modification that may prove to be a useful intervention to reduce the worldwide burden from iron-overload cardiovascular disease.

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References

1. Weatherall DJ, Clegg JB. Thalassemia: a global public health problem. *Nat Med*. 1996;2:847–849.
2. Barton JC, Bertoli LF. Hemochromatosis: the genetic disorder of the twenty-first century. *Nat Med*. 1996;2:394–395.
3. Olivieri NF. The beta-thalassemias. *N Engl J Med*. 1999;341:99–109.
4. Andrews NC. Disorders of iron metabolism. *N Engl J Med*. 1999;341:1986–1995.
5. Ballas SK. Iron overload is a determinant of morbidity and mortality in adult patients with sickle cell disease. *Semin Hematol*. 2001;38:30–36.
6. Buja LM, Roberts WC. Iron in the heart: etiology and clinical significance. *Am J Med*. 1971;51:209–221.
7. Jensen CE, Tuck SM, Old J, et al. Incidence of endocrine complications and clinical disease severity related to genotype analysis and iron overload in patients with beta-thalassaemia. *Eur J Haematol*. 1997;59:76–81.
8. Muhlestein JB. Cardiac abnormalities in hemochromatosis. In: Barton JC, Edwards CQ, eds. *Hemochromatosis: Genetics, Pathophysiology, Diagnosis, and Treatment*. Cambridge, UK: Cambridge University Press; 2000:297–310.
9. Strohmeyer G, Niederau C. Diabetes mellitus and hemochromatosis. In: Barton JC, Edwards CQ, eds. *Hemochromatosis: Genetics, Pathophysiology, Diagnosis, and Treatment*. Cambridge, UK: Cambridge University Press; 2000:268–277.
10. Liu P, Olivieri N. Iron overload cardiomyopathies: new insights into an old disease. *Cardiovasc Drugs Ther*. 1994;8:101–110.
11. Olivieri NF, Nathan DG, MacMillan JH, et al. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med*. 1994;331:574–578.
12. Brittenham GM, Griffith PM, Nienhuis AW, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med*. 1994;331:567–573.

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13. Niederau C, Fischer R, Purschel A, et al. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology*. 1996;110: 1107–1119.
14. Fine JS. Iron poisoning. *Curr Probl Pediatr*. 2000;30:71–90.
15. Tenenbein M, Kopelow ML, deSa DJ. Myocardial failure and shock in iron poisoning. *Hum Toxicol*. 1988;7:281–284.
16. Hershko C, Link G, Pinson A. Modification of iron uptake and lipid peroxidation by hypoxia, ascorbic acid, and alpha-tocopherol in iron-loaded rat myocardial cell cultures. *J Lab Clin Med*. 1987;110:355–361.
17. Lesnefsky EJ, Allen KG, Carrea FP, et al. Iron-catalyzed reactions cause lipid peroxidation in the intact heart. *J Mol Cell Cardiol*. 1992;24: 1031–1038.
18. Kadiiska MB, Burkitt MJ, Xiang QH, et al. Iron supplementation generates hydroxyl radical in vivo: an ESR spin-trapping investigation. *J Clin Invest*. 1995;96:1653–1657.
19. Hershko C, Link G, Cabantchik I. Pathophysiology of iron overload. *Ann N Y Acad Sci*. 1998;850:191–201.
20. Horackova M, Ponka P, Byczko Z. The antioxidant effects of a novel iron chelator salicylaldehyde isonicotinoyl hydrazone in the prevention of H_2O_2 injury in adult cardiomyocytes. *Cardiovasc Res*. 2000;47:529–536.
21. Oudit GY, Sun H, Trivieri MG, et al. L-type Ca^{2+} channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. *Nat Med*. 2003;9:1187–1194.
22. Huxtable RJ. Physiological actions of taurine. *Physiol Rev*. 1992;72: 101–163.
23. Sole MJ, Jeejeebhoy KN. Conditioned nutritional requirements and the pathogenesis and treatment of myocardial failure. *Curr Opin Clin Nutr Metab Care*. 2000;3:417–424.
24. Takihara K, Azuma J, Awata N, et al. Beneficial effect of taurine in rabbits with chronic congestive heart failure. *Am Heart J*. 1986;112: 1278–1284.
25. Pion PD, Kittleson MD, Rogers QR, et al. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. *Science*. 1987;237:764–768.
26. Moise NS, Pacioretty LM, Kallfelz FA, et al. Dietary taurine deficiency and dilated cardiomyopathy in the fox. *Am Heart J*. 1991;121:541–547.
27. Schaffer S, Solododushko V, Azuma J. Taurine-deficient cardiomyopathy: role of phospholipids, calcium and osmotic stress. In: Della Corte L, Huxtable RJ, Sgaragli, G, et al, eds. *Taurine 4. Taurine and Excitable Tissues (Advances in Experimental Medicine and Biology, vol 483).* New York, NY: Kluwer Academic/Plenum Publishers; 2000:57–69.
28. Alroy J, Rush JE, Freeman L, et al. Inherited infantile dilated cardiomyopathy in dogs: genetic, clinical, biochemical, and morphologic findings. *Am J Med Genet*. 2000;95:57–66.
29. Satoh H. Electrophysiological and electropharmacological actions of taurine on cardiac cells. In: Della Corte L, Huxtable RJ, Sgaragli, G, et al, eds. *Taurine 2. Basic and Clinical Aspects (Advances in Experimental Medicine and Biology, vol 403).* New York, NY: Plenum Press; 1996: 285–296.
30. Holloway C, Kotsanas G, Wendt I. Acute effects of taurine on intracellular calcium in normal and diabetic cardiac myocytes. *Pflugers Arch*. 1999;438:384–391.
31. Satoh H. $[Ca^{2+}]_i$-dependent actions of taurine in spontaneously beating rabbit sino-atrial nodal cells. *Eur J Pharmacol*. 2001;424:19–25.
32. Biasetti M, Dawson R. Effects of sulfur containing amino acids on iron and nitric oxide stimulated catecholamine oxidation. *Amino Acids*. 2002; 22:351–368.
33. Keith ME, Ball A, Jeejeebhoy KN, et al. Conditioned nutritional deficiencies in the cardiomyopathic hamster heart. *Can J Cardiol*. 2001;17: 449–458.
34. Hill MF, Singal PK. Right and left myocardial antioxidant responses during heart failure subsequent to myocardial infarction. *Circulation*. 1997;96:2414–2420.
35. Schmidt AG, Gerst M, Zhai J, et al. Evaluation of left ventricular diastolic function from spectral and color M-mode Doppler in genetically altered mice. *J Am Soc Echocardiogr*. 2002;15:1065–1073.
36. Kremastinos DT, Tsiapras DP, Tsetsos GA, et al. Left ventricular diastolic Doppler characteristics in β-thalassemia major. *Circulation*. 1993; 88:1127–1135.
37. Ball AM, Sole MJ. Oxidative stress and the pathogenesis of heart failure. *Cardiol Clin*. 1998;16:665–675.
38. Crichton RR, Wilmet S, Legssyer R, et al. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. *J Inorg Biochem*. 2002;91:9–18.
39. Sevier CS, Kaiser CA. Formation and transfer of disulphide bonds in living cells. *Nat Rev Mol Cell Biol*. 2002;3:836–847.
40. Woo HA, Chae HZ, Hwang SC, et al. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. *Science*. 2003; 300:653–656.
41. Link G, Konijn AM, Hershko C. Cardioprotective effect of alpha-tocopherol, ascorbate, deferoxamine, and deferiprone: mitochondrial function in cultured, iron-loaded heart cells. *J Lab Clin Med*. 1999;133:179–188.
42. Bhatnagar A. Electrophysiological effects of 4-hydroxynonenal, an aldehydic product of lipid peroxidation, on isolated rat ventricular myocytes. *Circ Res*. 1995;76:293–304.
43. Folden DV, Gupta A, Sharma AC, et al. Malondialdehyde inhibits cardiac contractile function in ventricular myocytes via a p38 mitogen-activated protein kinase-dependent mechanism. *Br J Pharmacol*. 2003;139: 1310–1316.
44. Artman M, Olson RD, Boucek RJ Jr, et al. Depression of contractility in isolated rabbit myocardium following exposure to iron: role of free radicals. *Toxicol Appl Pharmacol*. 1984;72:324–332.
45. Bartfay WJ, Hou D, Brittenham GM, et al. The synergistic effects of vitamin E and selenium in iron-overloaded mouse hearts. *Can J Cardiol*. 1998;14:937–941.
46. Young IS, Trouton TG, Torney JJ, et al. Antioxidant status and lipid peroxidation in hereditary haemochromatosis. *Free Radic Biol Med*. 1994;16:393–397.
47. Livrea MA, Tesoriere L, Pintaudi AM, et al. Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood*. 1996;88:3608–3614.
48. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med*. 1991;11:81–128.
49. Lee SH, Oe T, Blair IA. Vitamin C–induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science*. 2001;292: 2083–2086.
50. Nakaya H, Tohse N, Kanno M. Electrophysiological derangements induced by lipid peroxidation in cardiac tissue. *Am J Physiol*. 1987;253: H1089–H1097.
51. Schwartz KA, Li Z, Schwartz DE, et al. Earliest cardiac toxicity induced by iron overload selectively inhibits electrical conduction. *J Appl Physiol*. 2002;93:746–751.
52. Eley DW, Lake N, ter Keurs HE. Taurine depletion and excitation-contraction coupling in rat myocardium. *Circ Res*. 1994;74:1210–1219.
53. Dawson R Jr, Tang E, Shih D, et al. Taurine inhibition of iron-stimulated catecholamine oxidation. *Adv Exp Med Biol*. 1998;442:155–162.
54. Eppler B, Dawson R. Cytoprotective role of taurine in a renal epithelial cell culture model. *Biochem Pharmacol*. 2002;63:1051–1060.
55. Wu QD, Wang JH, Fennessy F, et al. Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis. *Am J Physiol*. 1999; 277:C1229–C1238.
56. Heller-Stilb B, van Roeyen C, Rascher K, et al. Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. *FASEB J*. 2002;231:16.
57. Golubnitschaja O, Moenkemann H, Kim K, et al. DNA damage and expression of checkpoint genes p21(WAF1/CIP1) and 14-3-3 sigma in taurine-deficient cardiomyocytes. *Biochem Pharmacol*. 2003;66: 511–517.
58. Forgione MA, Cap A, Liao R, et al. Heterozygous cellular glutathione peroxidase deficiency in the mouse: abnormalities in vascular and cardiac function and structure. *Circulation*. 2002;106:1154–1158.
59. Li S, Li X, Rozanski GJ. Regulation of glutathione in cardiac myocytes. *J Mol Cell Cardiol*. 2003;35:1145–1152.
60. Schuller-Levis GB, Park E. Taurine: new implications for an old amino acid. *FEMS Microbiol Lett*. 2003;226:195–202.
61. Suzuki T, Wada T, Saigo K, et al. Taurine as a constituent of mitochondrial tRNAs: new insights into the functions of taurine and human mitochondrial diseases. *EMBO J*. 2002;21:6581–6589.
62. Fennessy FM, Moneley DS, Wang JH, et al. Taurine and vitamin C modify monocyte and endothelial dysfunction in young smokers. *Circulation*. 2003;107:410–415.
63. Tsushima RG, Wickenden AD, Bouchard RA, et al. Modulation of iron uptake in heart by L-type Ca^{2+} channel modifiers: possible implications in iron overload. *Circ Res*. 1999;84:1302–1309.
64. Templeton DM, Liu Y. Genetic regulation of cell function in response to iron overload or chelation. *Biochim Biophys Acta*. 2003;1619:113–124.

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