Cardiac Function in Rats Exposed to Chronic Alcohol and Nutritional Deficiency Involving Selenium and Vitamin E

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KEY WORDS: alcohol, alcoholic cardiomyopathy, lipid peroxidation, selenium, vitamin E, cardiac function

ABSTRACT
Objective: The purpose of this study was to determine if a concurrent nutritional deficiency involving selenium and/or vitamin E is a prerequisite in the development of cardiac dysfunction by chronic alcohol.

Methods: Young adult rats were fed alcohol (5 gm/kg/day) and either a normal, selenium deficient, vitamin E deficient or a selenium- and vitamin E-deficient diet for 36 ± 2 weeks, followed by in vivo cardiac function assessment using the thermodilution cardiac output technique. Cardiac selenium content, vitamin E content and lipid peroxidation were measured. Histological studies of the left ventricle were also performed.

Results: In analyzing the interactive treatment effects of alcohol and the 4 diets using two-way ANOVA, only the alcohol, selenium-/vitamin E-deficient group shows significant decrease in cardiac output ($P=0.0007$), stroke volume ($P=0.03$) and rate of rise in left ventricular pressure over time ($P=0.0001$). The selenium and vitamin E contents of the left ventricle were reduced by 19 to 38% in the corresponding deficient diet groups ($P<0.05$). The index of lipid peroxidation as determined from the conjugated dienes were higher in both the alcohol, selenium-deficient rats and the alcohol, selenium-/vitamin-E deficient rats. Electron microscopy of the left ventricle revealed findings consistent with alcoholic cardiomyopathy (severe mitochondrial derangement, contraction band necrosis with replacement fibrosis, dilated sarcoplasmic reticulum and infiltration of mononuclear white blood cells).

Conclusions: Chronic heavy alcohol consumption plus a diet deficiency in both selenium and vitamin E leads to depression in cardiac contractile function. Together with the non-quantita-
tive histological findings of cardiomyopathy, our data suggest that simultaneous selenium- and vitamin E-deficiency are cofactors necessary in the development of alcoholic cardiomyopathy, probably by enhancing the alcohol-induced oxidative stress in the myocardium.

**INTRODUCTION**

Today, alcoholism continues to be among the top 5 public health problems in the United States. Alcoholism frequently causes cardiac muscle dysfunction and longstanding heavy alcohol abuse can lead to the development of severe congestive heart failure, clinically known as alcoholic cardiomyopathy (ACM).\(^1\) As a matter of fact, alcohol is now considered the number one identifiable cause of acquired form of cardiomyopathies.\(^2\) Chronic alcohol consumption induces oxidative stress by generation of free radicals\(^3\) and in the heart, chronic alcohol ingestion has been shown to cause an increase in lipid peroxidation (LP) in both humans and experimental animals.\(^3\) Although the exact mechanism involved in the development of ACM is not known, the role of nutritional deficiency has largely been discounted.\(^7\) Nonetheless, if there is a concurrent nutritional deficiency in the key antioxidants, a reduced antioxidant defense/reserve will ensue and the alcohol-mediated oxidative stress should be further increased, and this may potentially be the underlying mechanism for the development of ACM. Although such a notion has not been looked into clinically or experimentally, several converging lines of evidence lend support to such a hypothesis. First, several clinical studies have reported a 13 to 40% decrease in serum selenium, one of the key antioxidants, and a 27 to 47% decrease in serum vitamin E levels in chronic alcoholics with skeletal myopathy,\(^8\) possibly as a result of poor dietary intake and/or gut malabsorption.\(^9,11\) In patients with alcoholic cardiomyopathy without a history of significant malnutrition, the cardiomyopathy has been discounted as viral etiology instead.\(^14\) Second, cardiac biopsies from patients with a history of heavy alcohol intake show significant amounts of cardiac lipopigment (lipofuscin), a marker for oxidative stress.\(^5\) Third, established animal models of alcoholic cardiomyopathy indicate that an increase in oxidative stress is closely linked to the pathogenesis of alcoholic cardiomyopathy. Fourth, nutritional deficiency in selenium and vitamin E in swine results in a nutritional cardiomyopathy, known as mulberry heart disease (because of the mulberry appearance of the heart), and share a number of ultrastructural derangements similar to those of alcoholic cardiomyopathy, though the foci of myocardial necrosis are most severe in the atria.\(^5\) Conceivably, a compromised antioxidant system due to selenium and/or vitamin E deficiency could play a pivotal role in development of alcoholic cardiomyopathy by enhancing the alcohol-induced oxidative stress in the heart. Therefore, the primary objective of the present study is to determine if the alcohol-induced oxidative stress in the setting of nutritional deficiency involving selenium and/or vitamin E results in significant myocardial contractile dysfunction. The secondary goal is to see if such a hemodynamic derangement is associated with pathological changes in the affected myocardium.

**METHODS**

**Alcohol and Diet Feeding**

This feeding study was reviewed and approved by the University of Arizona institutional animal care committee (IACUC). Alcohol was given by gastric gavage twice daily to young male adult (150-200 gm) Sprague-Dawley rats (10 per group) and fed one of the following
4 diets (prepared by DYETS, Bethlehem, Pa): 1, normal; 2, selenium deficient (zero selenium content); 3, vitamin E deficient (corn oil-stripped, vitamin E content zero); or 4, selenium-and vitamin E-deficient diet (zero selenium and zero vitamin E). All 4 diets otherwise contained the same complete nutrients according to established AIN guidelines. For each diet group, a paired, non-alcohol control (10 rats each) was included. All rats were housed comfortably in cages with 12-hour light/dark cycles. Ethanol was initially administered by gastric gavage at 5% v/v concentration (1.25 gm/kg/day) for the first week, 10% v/v (2.5 gm/kg/day) during the second week, 15% v/v (3.75 gm/kg/day) during the third week, and 20% v/v (5 gm/kg/day) from the fourth week on. For the non-alcohol rats, alcohol is substituted isocalorically with Dextran-maltose. All 8 groups of animals were fed the assigned diet ± alcohol for 36 ± 2 weeks.

**Hemodynamic Assessment**

At the end of 36 ± 2 weeks of feeding, in vivo cardiac performance was assessed using the thermodilution technique. To avoid measuring any acute effect of alcohol on cardiac function, alcohol feeding was discontinued 1 to 2 days prior to cardiac catheterization; rat was anesthetized with 80-100 mg/kg intraperitoneal thiobutarbital (Inactin, Byk Gulden Pharmaceutical, Konstanz, Germany) and placed on a makeshift constant-temperature (37°C) surgical bed, a 3 F micromanometer-tip catheter (Millar Instruments Inc., Houston, Texas) was inserted into left ventricle (LV) via the right carotid artery. The left ventricular rate of rise of pressure (dp/dt) was obtained with physiological recorder (Model 2400, Gould Instruments Co, Cleveland, OH). To measure cardiac output, a fluid-filled polyethylene catheter (PE 50) was inserted into the right atrium via the right jugular vein by cut-down technique. A 3.5 F microprobe (Columbus Instruments, Columbus, OH) was inserted into the left femoral artery by cut-down technique and advanced to the descending aorta. After baseline left ventricular, central aortic pressure and heart rate were obtained, cardiac output was then measured in triplicate by injecting 0°C saline into the right atrium via the PE 50 catheter. The mean arterial pressure was calculated as diastolic pressure + one-third pulse pressure.

**Tissue Harvest**

At the end of hemodynamic study and with the rat still fully anesthetized, the entire heart was quickly excised, aortic remnant trimmed off, blotched dry, and weighed. The left ventricle was isolated and divided equally into several pieces. One piece was fixed in glutaraldehyde and the rest placed in liquid nitrogen and stored at -86°C.

**Lipid Peroxidation Assay**

Left ventricular lipid peroxidation was measured using the conjugated diene

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Table 1. Peak Blood Alcohol Level (BAL) Determined from Each of the 4 Groups of Alcohol Treated Rats

<table>
<thead>
<tr>
<th>Diet Treatment</th>
<th>Normal</th>
<th>Selenium deficient</th>
<th>Vitamin E deficient</th>
<th>Selenium deficient, Vitamin E deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak BAL (mg/dL)</td>
<td>205, 405</td>
<td>252, 262</td>
<td>285</td>
<td>175, 260</td>
</tr>
</tbody>
</table>

The results indicated the number of rats studied.
fluorescence method; left ventricular tissue was weighed and homogenized in Folch’s reagent at 30 mL/gm of tissue. The homogenate was placed in a screw top test tube and capped using a silica septum, mixed again and allowed to stand for 16 hours. The proteins usually settled to the bottom of the tube within 30 minutes. If this separation did not occur, 1 mL of methyl alcohol was added and the homogenate was again shaken and allowed to stand for 16 hours and centrifuged for 15 minutes. The homogenate was stored in freezer at -86°C and analyzed in batches for conjugated dienes.

**Determination of Serum Alcohol Concentration**

Toward the end of alcohol and diet feeding, blood alcohol levels were measured in randomly selected alcohol-treated rats (1-2 per group).

**Determination of Selenium**

Left ventricular selenium content was determined in Dr. AJ Blotcky’s laboratory using the neutron activation technique. Briefly, selenium extraction from left ventricular tissue was performed as follows: the heart tissue was homogenized in 4 volume of 10 mmol/L HEPES/0.25 mol/L sucrose buffer (pH 7.5) and centrifuged at 5,000 x g for 7 minutes at 4°C to remove non-disrupted cells. Supernatant was used for measurement of selenium concentration by neutron activation analysis. The samples and standards were irradiated in heavy water nuclear reactor for 24 hours. After two weeks of decay, 77Selenium was counted by a high-purified germanium detector.

**Determination of Vitamin E (α-tocopherol)**

Left ventricular vitamin E (α-tocopherol) content was determined using the high pressure liquid chromatography (HPLC) technique. To extract the α-tocopherol, BHT (1±2 mg), 400 microliter of PBS, and 50 microliter of 5% collagenase solution (Type IV; Sigma, St. Louis, Mo) were added to approximately 50 mg of heart tissue in a 2-mL microcentrifuge tube. After mixing, the samples were incubated at 37°C for 1 hour and then homogenized on ice using a hand-held polytron tissue homogenizer.

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**Table 2.** Hemodynamic Changes in Rats Fed ± Alcohol ± Selenium Deficient ± Vitamin E Deficient Diets for 36 ± 2 Weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>-Se</th>
<th>-VE</th>
<th>-Se/-VE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BW (g)</strong></td>
<td>297.14±11.1</td>
<td>277.33±5.19</td>
<td>282.86±6.42</td>
<td>306.57±5.49</td>
</tr>
<tr>
<td><strong>HR</strong></td>
<td>397.14±10.2</td>
<td>429.17±9.0</td>
<td>372.5±19.9</td>
<td>427.14±8.9†</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>133.14±6.25</td>
<td>152.33±7.31</td>
<td>132.86±11.3</td>
<td>154.57±2.95†</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>99.17±4.21</td>
<td>106.33±4.94</td>
<td>99.17±4.21</td>
<td>113.71±1.11†</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>115.43±4.57</td>
<td>127.17±6.32</td>
<td>112.71±9.90</td>
<td>131.43±2.14†</td>
</tr>
<tr>
<td><strong>SV (ml)</strong></td>
<td>0.30±0.02</td>
<td>0.25±0.02</td>
<td>0.27±0.02</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td><strong>CO (ml/min)</strong></td>
<td>118.50±6.69</td>
<td>106.20±9.38</td>
<td>100.00±7.88</td>
<td>135.57±10.8</td>
</tr>
<tr>
<td><strong>LVEDP (mmHg)</strong></td>
<td>5.14±0.4</td>
<td>3.8±0.73</td>
<td>3.86±0.46</td>
<td>4.14±0.63</td>
</tr>
<tr>
<td><strong>dp/dt (mmHg)</strong></td>
<td>7471.4±503</td>
<td>7720.0±338</td>
<td>6557.1±570</td>
<td>8128.6±193</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± S.E.M., n=7. ETOH, indicates alcohol; MAP, mean arterial blood pressure; CO, cardiac output; SV, stroke volume; dp/dt, rate of left ventricle pressure increase with time; HR, heart rate; Se, selenium; and VE, vitamin E. Normal diet, non-alcohol group vs all other groups: †P<0.05; ‡P<0.01. Normal diet, alcohol group vs all other groups: †P<0.05; ‡P<0.01.

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model PT 10/35; Brinkman Instruments, Westbury, NY). After homogenization, 50 microliter of 2% protease solution was added to each tube, mixed and then incubated at 37°C for 30 minutes. After incubation, 500 microliter of the SDS-ethanol-BHT solution was added to each tube, and extracted twice with 500 μL of hexane containing 0.1% BHT (w/v). The incubation, homogenization, and extraction were carried out in the same tube to avoid loss of tissue sample. Fifty to 100 microliter of extract was applied to HPLC machine with Novapak C(18) columns (4 μm; 300 x 3.9 mm; Water’s Associates, Milford, Mass) for measurement of α-tocopherol concentration. The mobile phases A and B consisted of acetonitrile, tetrahydrofuran, methanol, and 1% ammonium acetate in different proportions. The total run-time, including re-equilibration, was 47 minutes. The HPLC effluent was monitored at 300 for tocopherols.

Morphological Studies
Morphological analysis of left ventricles (2) from each of the 8 experimental groups was performed using both light microscopy and electronic transmission microscopy and interpreted by a cardiac pathologist (T. Yoneyama) blinded to the alcohol and diet treatment.

Statistical Analyses
Hemodynamic results are presented as mean ± SEM. Student Newman-Keuls analysis was used to analyze cardiac performance (HR, MAP, SV, CO, LVEDP and dp/dt). Two-way ANOVA was used to analyze the interactive effects of alcohol and the four prescribed diets. Probability levels of <0.05 were considered significant.

RESULTS
After 36 ± 2 weeks of pair feeding, blood alcohol level (BAL) was determined; the peak BAL occurred between 1 to 2 hours following alcohol gavage. The peak BAL ranged from 175 to 405 mg/dL (Table 1). Since only 1 to 2 per group were studied, no conclusion can be made regarding which of the alcohol-treated groups achieved significantly higher peak BAL following chronic alcohol consumption.

The body weight (BW) of the alcohol,
selenium- and vitamin E-deficient rats were lower than the normal diet, non-alcohol rats \((P<0.05)\) or the normal diet, alcohol rats \((P<0.05)\) (Table 2).

Cardiac function from all 8 treatment groups is summarized in Table 2. Compared to the normal diet, non-alcohol group, the heart rate (HR) of the non-alcohol, selenium-/vitamin E-deficient \((-A/-Se/-V_{E})\) rats are higher \((P<0.05)\) while HR of the alcohol, selenium-/vitamin E-deficient \((A/-Se/-V_{E})\) rats are lower \((P<0.05)\). Similarly, the mean arterial blood pressure (MAP) of the \(-A/-Se/-V_{E}\) rats is higher \((P<0.05)\) while the MAP of \(A/-Se/-V_{E}\) rats is lower \((P<0.05)\) than the normal diet, non-alcohol group. Among all eight treatment groups, only the \(A/-Se/-V_{E}\) rats show a significantly lower thermodilution cardiac output (CO) \((P<0.05)\). The LV filling pressure (LVEDP) is slightly but significantly higher in the \(-A/-Se/-V_{E}\) rats than the normal diet, non-alcohol rats \((P<0.05)\). However, \(A/-Se/-V_{E}\) rats have a lower LVEDP than the normal diet, non-alcohol rats \((P<0.05)\). The rate of change of pressure \((dp/dt)\) is significantly lower in the \(A/-Se/-V_{E}\) rats only. Most importantly, in analyzing the interactive treatment effects of alcohol and the prescribed diets using the two-way ANOVA, only the \(A/-Se/-V_{E}\) group shows significant decrease in CO \((P=0.0007)\), SV \((P=0.03)\) and \(dp/dt\) \((P=0.0001)\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>(-Se/V_{E})</th>
<th>(-Se)</th>
<th>(-V_{E})</th>
<th>Normal</th>
<th>(-Se/V_{E})</th>
<th>(-Se)</th>
<th>(-V_{E})</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se (mcg/gm)</td>
<td>0.81±0.29</td>
<td>0.74±0.19</td>
<td>1.26±0.10</td>
<td>1.20±0.14</td>
<td>1.08±0.22†</td>
<td>0.99±0.17†</td>
<td>1.38±0.09</td>
<td>1.37±0.15</td>
</tr>
<tr>
<td>(V_{E}) (mcg/gm)</td>
<td>10.42±1.1</td>
<td>9.04±0.67</td>
<td>7.55±0.37</td>
<td>9.27±0.52</td>
<td>7.07±0.68†</td>
<td>8.23±0.74</td>
<td>7.14±0.91†</td>
<td>10.29±0.86</td>
</tr>
</tbody>
</table>

\(1^{st}\) P<0.05 vs. alcohol, normal diet group.

Levels of cardiac selenium when compared to the normal diet, normal diet group (Table 3). Levels of cardiac selenium in alcohol, selenium-deficient group and \(A/-Se/-V_{E}\) group were 21% and 28% decreased \((P<0.05)\) when compared with their non-alcohol counterparts (Table 3) suggesting a possible cardiac selenium sparing effect by chronic alcohol.

In the non-alcohol, vitamin E-deficient group, there was a 19% decrease in cardiac vitamin E content when compared to the non-alcohol, normal diet group \((P<0.05)\) (Table 3). However, in the alcohol, vitamin E-deficient and \(A/-Se/-V_{E}\) group, the cardiac vitamin E content was significantly reduced \((31\% \text{ and } 31\%)\) when compared to the alcohol, normal diet group \((P<0.05)\) for both). Unexpectedly, there was a non-significant 20% decrease in cardiac vitamin E content in the alcohol, selenium-deficient group when compared to the alcohol, normal diet group.

Levels of lipid peroxidation were increased in both the alcohol, selenium-deficient group and \(A/-Se/-V_{E}\) group (Table 4). However, they are not significantly different when compared to their non-alcohol, normal diet and alcohol, normal diet counterparts \((P>0.05)\).

Histological study showed thickened endocardium, interstitial fibrosis, and hypertrophy of myocytes and focal regions of necrotic myocytes by light microscopy in the hearts from the \(A/-Se/-V_{E}\) group only (not shown). When the hearts were seen under EM, increased number of mitochondria, some
rounded forms with loss of cristae, scattered vacuolization with mild myofibril disarrangement and rare subsarcolemmal blebs were observed in the alcohol, normal diet group (Figure 1a). For the alcohol, -Vitamin E diet group, the morphological changes were similar to that of alcohol, normal diet group (Figure 1b). In addition to a similar change of mitochondria morphology and number, scattered myofibril lysis were also observed in the alcohol, selenium-deficient group (Figure 1c). In the -A/-Se/-V₆ group, the mitochondria contained slightly more disarrayed cristae but without myofibril lysis (Figure 1d). In the A/-Se/-V₆ group, EM showed severe mitochondrial changes (swollen and fused giant mitochondria with disorganized cristae) (Figure 1e), super-contraction band with loss of myofibril bundles (contraction band necrosis) and replacement fibrosis, dilated sarcoplasmatic reticulum, and infiltration of mononuclear white blood cells (Figure 1f).

DISCUSSION
Since it is unethical to induce alcoholic cardiomyopathy (ACM) by human experimentation, investigators have long tried to establish animal models to study the disease. To date, only a few non-transgenic animal models of ACM have been reported. Although the typical histopathology of ACM has previously been shown in turkeys²² and rats,²³ in either model, the experimental conditions do not mimic human ACM. In the turkey model, alcohol is administered to the birds starting at birth. In the rat model, a catalase inhibitor has to be given concomitantly with alcohol in order to induce the cardiomyopathy. Nevertheless, findings from these two models indicate the involvement of free radicals in the development of ACM.²²,²³ More recently, ACM was induced by alcohol alone in adult chickens,²⁴ in transgenic mice that over expressing alcohol dehydrogenase²⁵ and in rats.²⁶ Nonetheless, no in vivo hemodynamic study confirming the low output failure has been performed in any of these 5 animal models of ACM.²²,²⁶ In the current experiment, the in vivo hemodynamic and histological data are consistent with the findings of ACM suggesting in addition to chronic alcohol, nutritional deficiency involving both selenium and vitamin E is a pre-requisite for the development of alcoholic cardiomyopathy. This novel model may potentially explain the clinical milieu for development of the disease and therefore potentially more pertinent in its use to study other aspects of alcoholic cardiomyopathy. A clear disadvantage of this new experimental approach is the lengthy period of feeding (and hence cost) needed to fully develop the disease.

Previous epidemiologic studies have not shown that alcoholic cardiomyopathy being more prevalent among alcoholic patients who have a vitamin E and selenium deficiency. However, there exists in the literature a frequent association of alcoholism with malnutrition and vitamin deficiencies, with selenium deficiency being more common than
**Figure 1a.** Rat LV, normal diet + alcohol gavage for 38wk (magnified 15,500 x, enlarged 1.6x).

**Figure 1b.** Rat LV, -V<sub>E</sub> diet + alcohol gavage for 38wk (magnified 15,500 x, enlarged 1.6x).

**Figure 1c.** Rat LV, -Se diet + alcohol gavage for 38wk (magnified 15,500 x, enlarged 1.6x).

**Figure 1d.** Rat LV, -Se/-V<sub>E</sub> diet + non-alcohol for 38wk (magnified 15,500 x, enlarged 1.6x).

**Figure 1e.** Rat LV, -Se/-V<sub>E</sub> diet + alcohol gavage for 20wk (magnified 24,250 x, enlarged 1.6x).

**Figure 1f.** Rat LV, -Se/-V<sub>E</sub> diet + alcohol gavage for 38wk (magnified 4,000 x, enlarged 1.6x).
vitamin E deficiency. While a vitamin E deficiency was observed in a subpopulation of alcoholics in Great Britain, leading potentially to cardiomyopathy, a similar finding was not observed in alcoholics from Spain. Epidemiological data from these Spanish investigators showed no correlation between vitamin E deficiency and development of cardiomyopathy. Selenium deficiency, however, does show correlation with cardiomyopathy in both alcoholic and non-alcoholic patients. Since ACM takes many years to develop, the nutritional status of these groups of ACM patients might have been consuming a diet deficient in selenium and vitamin E during the early years of their alcohol drinking history, and the nutrients from diets thirty years ago might have lower contents of selenium and vitamin E.

Serum levels of vitamin E may not reflect accurately the cardiac content and functional importance; myocardial but not serum vitamin E content from cardiomyopathic Syrian hamster is shown to be reduced by about 50% in the late stage as compared to the early stage of the cardiomyopathy, suggesting vitamin E is involved in the progression of the heart failure in the absence of nutritional deficiency involving vitamin E. Vitamin E supplementation has been shown to prevent the transition of hypertrophy to heart failure in chronic pressure overloaded guinea pigs that are not deficient in vitamin E.

Although the amount of alcohol given to the rats in our experiment (5 gm/kg/day) was similar to the amount consumed by human alcoholic cardiomyopathy patients (about 3-4 gm/kg/day), alcohol was administered twice daily by gavage, which does not mimic the continuous drinking pattern of most alcoholic cardiomyopathy patients. In our study, the peak alcohol levels (BAL) obtained by gavage (Table 1) are quite comparable to other chronic alcohol feeding experiments using alcohol liquid diet fed ad libitum. However, it remains to be demonstrated whether chronic ad libitum alcohol feeding can induce alcoholic cardiomyopathy under the otherwise same experimental conditions of Se and \( V_E \) deficiencies.

The mechanism of increased oxidative stress in the development of alcohol-induced organ damage and attenuation by selenium and vitamin E supplementation has been well studied in other organs but not in heart. However, in recent years, increased oxidative stress has been proposed as a potential mechanism to explain the progression of ventricular dysfunction in clinical heart failure. Keith et al. reported that serum marker of lipid peroxidation (malondialdehyde) correlated with both clinical heart failure class and prognosis, suggesting the progression and prognosis of heart failure may be closely linked to an increase in free radical injury to the myocardium. Moreover, clinical or experimental evidence suggests that adriamycin-induced cardiomyopathy, iron-overload (hemochromatosis) cardiomyopathy and diabetic cardiomyopathy are related to free radical-mediated myocardial damage as well. When cultured cardiomyocyte were treated with ethanol, intracellular oxidative stress is increased and the level of intracellular glutathione is reduced.

Although antioxidant enzyme activities were not measured in the present study, lipid peroxidation (LP) in heart tissues was increased in alcohol -Se & \( V_E \) group and alcohol, -Se group. The unexpected increase in LP in the alcohol, -Se group may be due to the fact that conjugated diene is not specific as an index of LP. Alternatively, the unexplained decrease in cardiac vitamin E in this group might have contributed to the increased diene but is not sufficient to induce alcoholic cardiomyopathy. It is also possible that other known cellular,
histological, and metabolic derangement resulted from the direct and/or indirect effects of alcohol may be the mechanism of primary injury instead. Similarly, the progression from cardiac dysfunction to cardiac enlargement/remodeling may be an indirect effect of chronic alcohol via other pathways involving the renin-angiotensin-aldosterone system, natriuretic peptides, endothelin, matrix metalloproteinases, cytokines, nitric oxide synthetases, etc.

In Keshan disease, the dilated cardiomyopathy results from selenium deficiency, cardiac selenium content is reduced by 65 to 85%. Therefore, the extent in the decrease in cardiac selenium shown in this study is unlikely to be sufficient by itself to induce the cardiomyopathic changes, but most likely act as a cofactor.

In humans, elevated left ventricular filling pressure as measured from LVEDP or pulmonary capillary wedge pressure has been shown in both alcoholics with and without dilated cardiomyopathy. In experimental animals, LVEDP has been shown to be the same after chronic alcohol though it becomes elevated with saline infusion.

Though the duration and amount of alcohol given in the current study are quite similar to these reports, we demonstrate a slight, though statistically significant elevated LVEDP in the alcohol, normal diet group. However, the LVEDP in the alcohol, selenium-/vitamin E-deficient group is not different from the non-alcohol, normal diet controls despite a significant reduction in cardiac contractile function coupled with severe histopathology. Since in alcoholic and other dilated cardiomyopathy, LVEDP should be elevated, our finding is somewhat unexpected and this may be due to the fact that the in vivo thermodilution cardiac output technique of measurement is inherently influenced by an uncontrolled preload condition.

Finally, the mechanism of cardiac function depression induced by alcohol and selenium-/vitamin E deficiency has not been answered by the current study. Our histological and LP data do suggest the following factors may be involved in this pathogenesis: (1) Disruption of mitochondrial function—it is known that chronic alcohol exposure leads to mitochondrial swelling and disarrayed cristae resulting in uncoupling of oxidative phosphorylation and decrease in cytosolic ATPase. (2) Acetaldehyde toxicity—acetaldehyde has been shown to increase myocardial lipid peroxidation in a dose-dependent manner and inhibits Na⁺/K⁺-ATPase activity in cardiac plasma membrane. Further studies are needed to identify the molecular pathways involved in the development of ACM.

CONCLUSION
Chronic heavy alcohol consumption plus a diet deficiency in both selenium and vitamin E leads to depression in cardiac contractile function. Together with the non-quantitative histological findings of cardiomyopathy, our data suggest that simultaneous selenium- and vitamin E-deficiency are cofactors necessary in the development of alcoholic cardiomyopathy probably by enhancing the alcohol-induced oxidative stress in the myocardium.

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