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Impact of Chronic Alcohol Ingestion on Cardiac Muscle Protein Expression

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Abstract

Background—Chronic alcohol abuse contributes not only to an increased risk of health-related complications, but also to a premature mortality in adults. Myocardial dysfunction, including the development of a syndrome referred to as alcoholic cardiomyopathy, appears to be a major contributing factor. One mechanism to account for the pathogenesis of alcoholic cardiomyopathy involves alterations in protein expression secondary to an inhibition of protein synthesis. However, the full extent to which myocardial proteins are affected by chronic alcohol consumption remains unresolved.

Methods—The purpose of this study was to examine the effect of chronic alcohol consumption on the expression of cardiac proteins. Male rats were maintained for 16 weeks on a 40% ethanol-containing diet in which alcohol was provided both in drinking water and agar blocks. Control animals were pair-fed to consume the same caloric intake. Heart homogenates from control- and ethanol-fed rats were labeled with the cleavable isotope coded affinity tags (ICAT™). Following the reaction with the ICAT™ reagent, we applied one-dimensional gel electrophoresis with in-gel trypsin digestion of proteins and subsequent MALDI-TOF-TOF mass spectrometric techniques for identification of peptides. Differences in the expression of cardiac proteins from control- and ethanol-fed rats were determined by mass spectrometry approaches.

Results—Initial proteomic analysis identified and quantified hundreds of cardiac proteins. Major decreases in the expression of specific myocardial proteins were observed. Proteins were grouped depending on their contribution to multiple activities of cardiac function and metabolism, including mitochondrial-, glycolytic-, myofibrillar-, membrane-associated, and plasma proteins. Another group contained identified proteins that could not be properly categorized under the aforementioned classification system.

Conclusions—Based on the changes in proteins, we speculate modulation of cardiac muscle protein expression represents a fundamental alteration induced by chronic alcohol consumption, consistent with changes in myocardial wall thickness measured under the same conditions.

Keywords

Ethanol; Proteomics; Heart; Myofibrillar; Mass Spectrometry; ICAT™

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Heart disease, as well as cirrhosis, represents an important etiology of mortality in chronic alcoholics. Excessive ethanol consumption can result in a syndrome referred to as alcoholic cardiomyopathy. Alcoholic cardiomyopathy is rarely produced by short-term ethanol administration. However, it is observed in those patients who excessively consume alcohol for prolonged periods (greater than 80 g of ethanol a day for longer than 10 years). The clinical feature of this syndrome is a defect in myocardial contractility as assessed by a reduction in ejection fraction, with the degree of cardiac dysfunction proportional to the duration and severity of alcohol consumption (Urbano-Marquez, 1989). Patients diagnosed with alcoholic cardiomyopathy, who continue to drink alcohol, suffer deterioration in their condition leading to congestive heart failure and eventually death ensues.

The major pathologic features revealed through biopsy or postmortem examination include dilation of both ventricles of the heart, thinning of the ventricular wall with fibrosis, and endocardial fibroelastic thickening, interstitial edema, and focal areas of necrosis within the ventricular wall (Bulloch et al., 1972; Ferrans et al., 1975; Hibbs et al., 1965). Microscopic examination of biopsy specimens obtained from humans reveals myocyte degeneration, loss of striations, and myofilament dissolution, consistent with alterations in structural and myofibrillar proteins (Alexander, 1966a,b; Bulloch et al., 1972; Ferrans et al., 1975; Hibbs et al., 1965). Addition of ethanol to the medium reduces the number and uniformity of the myofibrils of myocytes in culture (Adickes et al., 1990). The process of alterations in ethanol-induced cardiac structure and function is referred to as alcoholic heart muscle disease.

The molecular basis for this disease is probably multifactorial. One explanation for reduced contractility and derangements in myofibrillar architecture is that the integrity of cellular proteins may be compromised by prolonged ethanol intake. Early work indicated that chronic ethanol consumption led to a decreased association of actin with myosin heavy chain isoform in vitro (Rubin et al., 1976), and it was suggested that persistent changes in some myofibrillar proteins may have occurred. We have provided evidence that long-term exposure of rats to a diet containing ethanol results in lower cellular content of both actin and α-myosin heavy chain isoform and an increase in the β-myosin heavy chain isoform (Vary and Deiter, 2005) (Table 1). Similarly, both Patel and colleagues (2000) and Figueredo and colleagues (1998) showed decreases in actin and α-myosin heavy chain isoform, whereas Piano et al. showed chronic ethanol consumption induces an increase in the β-myosin heavy chain isoform (Meehan et al., 1999). Table 1 provides a summary of the known changes in myocardial proteins in animals fed a diet containing ethanol. The aim of this study was to analyze cardiac muscle protein expression after chronic alcohol consumption compared with control pair-fed rats using a mass spectrometry-based proteomic approach using Cleavable ICAT™ reagents (isotope coded affinity tags).

MATERIALS AND METHODS

Chronic Alcohol Feeding

All experimental protocols involving animals were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine and adhered to the National Institutes of Health guidelines for the use of experimental animals. Pathogen-free, male Sprague-Dawley rats (Charles River Breeding Laboratories, Cambridge, MA) were maintained for 16 weeks on an ethanol-containing diet in which alcohol was provided both in drinking water and previously described agar blocks (Bautista, 1997; Lang et al., 1999a,b,c; Vary et al., 2001). Initially, all rats were provided the agar block without ethanol for 2 days. Thereafter, the animals were randomly assigned to either an alcohol or control group. Animals in the alcohol group were given free access to ethanol-containing agar blocks. Control rats consumed ethanol-free agar blocks containing an

isocaloric amount of dextrin-maltose. The concentration of ethanol in the agar blocks was increased in 10% increments from 10% to 40% over the first 4 weeks (Lang et al., 1999a,b,c; Vary et al., 2001). Ethanol-fed rats remained on the 40% ethanol-agar block diet for the remainder of the experimental protocol. Averages for ethanol consumption and plasma ethanol concentration at the time of heart excision were 17 ± 2 g/kg body wt and 21 ± 3 mM, respectively. Standard rat chow (Harlan Teklad no. 8604, Madison, MI) furnished the nutrient intake in both groups. Control rats were provided the same amount of solid chow as consumed by the alcohol-fed group the previous 24-h period (Lang et al., 1999a,b,c; Vary et al., 2001). Total energy consumption was the same in both groups (Lang et al., 2004). After 16 weeks on these feeding protocols, hearts were excised and frozen between clamps precooled to the temperature of liquid nitrogen.

Proteomics

The ICAT approach incorporates a stable isotope into 1 of the 2 samples being compared in vitro obviating the need to analyze by mass spectrometry the control and experimental samples separately. Equivalent amounts of cardiac tissue powder from 4 control and 4 alcohol-treated rats were combined according to treatment group and homogenized in ice cold buffer containing 2% sodium dodecyl sulfate and 3.5 mmol/l Tris(2-carboxyethyl)phosphine HCl (Tris HCl) at a final concentration of 20–25 mg/ml. This resulted in 2 tissue homogenates representing the control (Pair-fed) and alcohol-treated groups. A 200 μg protein aliquot of each homogenate was then diluted to 1 mg/ml in buffer containing 50 mM Tris HCL, 1% SDS, and 1 mM TCEP, boiled for 10 min, allowed to cool for 2 min at room temperature, vortexed, and then centrifuged at 14,000 × *g*. The denatured and reduced cys-containing proteins were then labeled with Cleavable ICAT™ reagent (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's protocol. The reagent contains a biotin affinity tag attached with a cleavable linker to either a C12 (light) or C13 containing (heavy) tag linked to cysteine reactive groups. The control protein samples were labeled with “light” (12C) ICAT™ reagent, while the ethanol-fed group was labeled with chemically identical but “heavy” (13C) ICAT™ reagent. After the labeling protocols, the light- and heavy-labeled samples were combined and separated by gel electrophoresis on 10% acrylamide SDS–PAGE gels. The gels were removed from the cassette and stained. The lane containing the stained Cleavable ICAT-labeled cardiac proteins was cut into 10 pieces. The manufacturer's protocol was followed for extracting peptides from the gel. The pieces were washed to remove residual SDS and dried in a centrifugal lyophilization. Peptides remaining in the gel were trypsinized in the gel and were extracted using a solvent consisting of 50% acetonitrile and 0.1% trifloroacetic acid. Each of the extracts was then drawn into a syringe and applied to an Avidin affinity column (Applied Biosystems) to separate labeled from unlabeled proteins. Labeled proteins were collected, and the biotin tag on the Cleavable ICAT™ reagent was then removed with the provided cleaving reagents. The cleaved samples were then dried and resuspended in ultrapure water 3 times by centrifugal lyophilization to remove residual acetylnitrile.

Mass Spectrometry

The resulting labeled peptides from each gel slice were further separated on an Eksigent – ProBot system LC-Tempo nanoflow and MALDI spotting system using a Chromolith CapRod C18 column (150 μm × 0.1 mm; Merck KGaA, Darmstadt, Germany). Each chromatography run yielded ~370 MALDI spots on stainless steel MALDI target plates. Thirteen calibration spots were also added to each of the 15 resulting target plates.

MALDI-TOF was employed to identify heavy and light peptide pairs in each spot and to provide relative quantification. Plate alignment, updated plate calibration, and MS/MS default calibration were performed for each spotted plate as they were inserted into an

Applied Biosystems 4700 Proteomics Analyzer MALDI-TOF/TOF. After these calibrations, MS spectra from 400 laser shots were acquired for each spot, and then a data-dependent MS/MS spectra were acquired from the largest MS peak representing each of the unique peptide peaks observed across the 400 target spots across the entire plate. MS/MS data from all SCX fractions was analyzed by the GPS Explorer™ software to search databases (SwissProt Database) constructed in our Mass Spec/Proteomics Core Facility. GPS Explorer™ software uses a 3-dimensional LC/MS reconstruct algorithm to locate and accurately determine experimental:control (heavy:light) peak ratios in complex proteomic samples. ICAT™-derivatized peptide pairs that differ by exactly 9.03 Da were identified and quantified by the Applied Biosystems GPS Explorer™ and 4000 series software. When the sizes of peaks differing by 9.03 Da differed by more than 30%, the larger of the 2 peaks was subsequently selected for CID fragmentation and tandem MS/MS analysis. In this way, the relative size of the peaks in the first MS dimension provided the quantitative information, and the second MS/MS analysis provided the peptide identification information.

The Mascot algorithm 2.0 was then exploited to compare the data with the NCBInr human database specified tryptic digestion using a peptide mass tolerance of 100 ppm and a fragment ion (MS/MS) tolerance of 0.1 Da. Protein identifications were accepted if they could be established at greater than 95% probability as specified by the Mascot algorithm and contained at least 1 identified peptide. When several accession numbers in the database matched the same set of peptides identified, the average quantification values of the entries are reported. Only 1 protein was reported as identified when several possible homologous proteins corresponded to the observed peptide spectra.

RESULTS

Chronic alcohol feeding resulted in a lower heart weight compared with pair-fed controls, partially attributed to a 25% loss in cardiac protein per heart (Vary et al., 2001). The expression of individual proteins in hearts from rats fed a diet containing ethanol was compared with pair-fed control rats using mass spectrometry-based proteomic analysis. Proteomic analysis was performed on heart tissue labeled with either light (Control) or heavy (Alcohol) Cleavable ICAT™ reagent, an isotope-coded affinity tag that forms covalent links to cysteine residues. Those proteins were then mixed and separated by 1-dimensional polyacryl-amide gel electrophoresis. Separated proteins were digested with trypsin using an in-gel protocol and then extracted. The tagged proteins were isolated by avidin chromatography, and after removing the biotin module, the labeled peptides were separated by liquid chromatography with fractions spotted on MALDI plates for determination of ICAT™ pairs, relative quantification of the reporter ICAT™ ions, and identification of the most abundant of the peptide pairs by MALDI-TOF/TOF. The mass spectro-metric analyses involved simultaneous profiling of multiple specimens to help eliminate artifactual variations resulting from differential protein losses during purification or separation. Peptides of interest were selected if the following criteria were satisfied: (i) contained at least 1 ICAT™-modified cysteine, (ii) at least 20% change in ICAT™ MS ion intensity following ethanol consumption, (iii) confidence interval ≥ 95%, and (iv) each peptide assigned to only 1 protein without redundancy.

Manual inspection of the initial data sets revealed differences in the relative expression of cardiac proteins between control and ethanol-fed rats. An ICAT™ ratio of 1 indicates no change after feeding a diet containing ethanol, whereas an ICAT™ ratio greater than 1 indicates a rise in the tissue content, and a value less than 1 indicates a reduction in the amount of that protein. Proteins whose peptide ICAT™ heavy:light ratios were not significantly different than 1 were excluded from further interpretation and are not listed in subsequent tables as this would indicate that no difference between control and ethanol-fed

rats was observed. The differentially expressed proteins were grouped into the following categories: membrane, plasma, sarcolemmal, mitochondrial, myofibrillar, and cytosolic. Their identity and the magnitude of difference observed in rats fed a diet containing ethanol are shown in Tables 2–7. A total of 75 proteins met the above criteria. The tables list myocardial proteins identified by this technique, the accession number of each protein, and the ratio of ICAT heavy:light derived from control and ethanol-fed rats.

Of particular interest was the analysis of proteins in the myofibrillar category. The relative ICAT™ ratio for α-myosin heavy chain isoform (0.61) and actin (0.61) indicates a ~39% lowering of the protein content (Table 2). With regard to validation of the technique, we previously used these exact same heart samples for analysis of select myofibrillar proteins by Western blot analysis. The reductions in the myofibrillar proteins obtained by ICAT™ analysis are consistent with the values obtained by Western blot techniques (Vary and Deiter, 2005). In that study, we reported reductions in the myofibrillar content of the contractile proteins α-myosin heavy chain isoform (–45%) and actin (–25%) (Vary and Deiter, 2005) in rats fed a diet containing ethanol for 16 weeks. Similarly, consistent with the lack of effect of alcohol treatment on the expression of troponin T and I (Table 2) as measured using Cleavable ICAT™ technique, no difference was detectable using Western blots reported previously (Vary and Deiter, 2005).

Numerous mitochondrial proteins possessed lower expression in the ethanol-fed group compared with the pair-fed controls. For example, many of the mitochondrial dehydrogenases were reduced approximately 30–40% (Table 3). Chronic alcohol ingestion was also associated with decreases in mitochondrial proteins associated with TCA cycle (citrate synthase, isocitrate dehydrogenase, succinyl-CoA synthetase, and malate dehydrogenase), electron transport (ubiquinone, adenine nucleotide translocator, and ATP synthase), energy transfer (nucleoside-diphosphate kinase, creatine kinase), and protein folding, protein targeting to membranes, protein renaturation, and control of protein–protein interactions. On the other hand, no changes were seen in pyruvate dehydrogenase complex, NADH coenzyme Q reductase, or ubiquinol cytochrome c reductase. An increase (+29%) was observed in 2,4-dienoyl-CoA reductase, a protein encoding an accessory enzyme that is involved in beta-oxidation and the metabolism of unsaturated fatty acids. Surprisingly, the mitochondrial ribosomal protein S35 was increased almost 15-fold. The results indicate myocardial mitochondrial proteins are adversely affected in chronic alcohol ingestion.

In addition to the mitochondrial energy producing enzymes, the expression of glycolytic enzymes was reduced by approximately 45–65% (Table 4) with the exception of aldolase, which was increased 1.5-fold. Aldolase activity is increased ~60% in hearts from animals given ethanol for 25 weeks (Sarfesai and Provido, 1978) again showing this technique matches Western blot analysis.

Only 4 membrane-associated proteins were differentially expressed in hearts from rats fed a diet containing ethanol (Table 5); namely integral membrane protein TMP21 (–98%), T-cell receptor alpha chain variable region (–53%), parathyroid hormone 2 receptor (–52%), and low-density lipoprotein receptor-related protein (–59%).

Sarcoplasmic proteins showed a wide range of proteins whose expression was differentially affected by ethanol ingestion; 18 were down-regulated and 10 were up-regulated (Table 6). As expected, the sarcoplasmic proteins that demonstrate altered expression following ethanol ingestion perform a wide range of cellular and extracellular functions. Two of the proteins are members of extracellular connective tissue, fibrillin-2 (–52%) and versican core protein precursor (–31%). Proteins involved in fatty acid metabolism (fatty acid transport protein (–31%) and long-chain fatty acyl-CoA ligase (–36%)) are affected by feeding rats a

diet containing ethanol. Several are DNA-binding proteins implicated in gene transcription (HOX11 (–83%) and zinc finger protein (–29%)). Many of the up-regulated proteins are involved in signal transduction: tyrosine kinase (~2.1-fold increase) and mitogen-activated protein kinase phosphatase 2 (~17.5-fold increase).

The hearts used in this study were frozen in situ with blood contained within the vasculature included in the tissue. Of the plasma proteins, albumin is the most prominent. ICATs™ analysis of the heart tissue revealed a 45% decrease in the amount of albumin in the heart (Table 7). Similarly, the myocardial content of alpha proteinase inhibitor III, transferrin precursor, hemoglobin beta III-1/III-2, Ig kappa chain V region, and hemopexin is reduced. One explanation would be that the blood volume is reduced by a similar percentage. This seems unlikely as other blood proteins such as gamma-2A immunoglobulin heavy chain were unaffected by feeding rats a diet containing ethanol, and CD166 antigen precursor is greatly increased. Supporting the idea that albumin may be specifically decreased by chronic alcohol ingestion, albumin synthesis by the isolated perfused rat liver was significantly reduced by acute alcohol exposure (Kirsch et al., 1973). Reductions in some of these plasma proteins, including albumin, can be a sign of hepatic dysfunction or protein malnutrition. Therefore, the reductions in some of these proteins may reflect protein malnutrition caused by alcohol. However, in these studies there is no sign of protein malnutrition, and hence the decrease in albumin most likely represents a failure of the liver to synthesize albumin. 2,3 bisphosphoglycerate mutase is found in red blood cells. However, a loss of its expression has been linked to muscle dysfunction because its substrate/product regulates hemoglobin affinity. The loss of the RBC gene may be related to alcohol related anemia because the main function of bisphosphoglycerate mutase is the synthesis of 2,3-BPG to shift the equilibrium of hemoglobin toward deoxy-state.

DISCUSSION

The development of alcoholic heart muscle disease is a complex process involving derangements in numerous pathways. The characteristic feature of alcoholic heart muscle disease is a thinning of the ventricle wall (Alexander, 1966a; Hibbs et al., 1965; Urbano-Marquez et al., 1989). Remodeling of the ventricular wall requires coordinated changes in multiple cellular compartments. Alcohol-induced cardiomyopathy remains poorly understood despite contributing to about one-half of all cases of heart failure. Heretofore, no systematic analysis of the protein expression has been reported, although there are individual reports of changes in some but not all subcellular fractions (Aistrup et al., 2006; Meehan et al., 1999; Patel et al., 1997; Vary and Deiter, 2005; Vary et al., 2002, 2004). An increased knowledge of the changes in protein profiles in response to ethanol is important in understanding the pathogenesis of alcoholic cardiomyopathy.

The analysis of myocardial peptides and hence proteins was performed using a proteomic approach with ICAT™ technology. The time course of 16 weeks of ethanol consumption was selected because there is evidence of (i) thinning of the ventricular wall at this time based on echocardiography (Lang et al., 2005) and (ii) changes in selective protein expression (Vary and Deiter, 2005). The ICAT proteomics measures peptides and then completes its protein profiling based on peptide species. Individual MS/MS spectra were searched against a human sequence database, and a variety of recently developed, publicly available software applications were used to sort, filter, analyze, and compare the results (Von-Haller et al., 2003). Although differences in peptide levels could reflect dissimilarity in protein isoforms, multiple peptides covering various locations in the protein sequence contributed to the identification for each of the proteins listed. The observed ratio between the signal intensities for the unfragmented isotopically “light” and “heavy” forms of the same peptide yields the relative abundances of that peptide, and hence the protein from

which it was derived, in the original samples. The changes in the protein identified by ICAT analysis of peptides show that the use of the ICAT ratio gives results consistent with assessment of proteins via Western blot (compare Table 1 with Table 2). Both groups were given equal nutritionally adequate diets emphasizing that alcohol induces alterations in specific heart muscle proteins when the nutrition is the same in both groups. We distinguished significant changes in heart proteins in animals fed alcohol compared to pair-fed controls. Chronic ethanol consumption (> 15 weeks) depresses actin and myosin content (Meehan et al., 1999; Vary and Deiter, 2005). In the present studies, proteins associated with the contractile elements (myosin alkali light chain 3, myosin alkali light chain 4, heavy chain myosin, actin) were uniformly reduced following feeding a diet containing ethanol.

The decrease in mitochondrial proteins associated with energy metabolism is consistent with observations that mitochondrial respiratory rates and the efficiency of phosphorylation were depressed in rats given 25% alcohol for 6 months (Sarfesai and Provido, 1978). In man, lipid accumulation and decreased staining reactions for several oxidative enzymes in cardiac mitochondria were observed in chronic alcohol abusers. Electron microscopic studies have shown the presence of swelling and rupture of mitochondria and the deposition of lipid droplets in the myocardial cell (Alexander, 1966a; Hibbs et al., 1965). In contrast, not all proteins identified were decreased. Cytochrome b–c1 complex subunit 2, 2,4-dienoyl-CoA reductase, and 28S ribosomal protein S35 were elevated.

Hemopexin is the plasma protein with the highest binding affinity to heme among known proteins (Tolosano and Altruda, 2004). It is mainly expressed in liver and belongs to acute phase reactants. Heme is potentially highly toxic because of its ability to intercalate into lipid membrane and to produce hydroxyl radicals. The binding strength between heme and hemopexin and the presence of a specific heme–hemopexin receptor able to catabolize the complex and to induce intra-cellular antioxidant activities suggest that hemopexin is the major vehicle for the transportation of heme in the plasma, thus preventing heme-mediated oxidative stress and heme-bound iron loss.

Selective protein degradation by the ubiquitin-proteosome pathway has emerged as a powerful regulatory mechanism in a wide variety of cellular processes. Ubiquitin conjugation requires the sequential activity of 3 enzymes; ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin-protein ligase (E3). There are a small number of similar E1 isoforms without apparent functional specificity. The specific selection of target proteins is accomplished by the E2 and E3 proteins. F-box proteins contain the F-box motif, a protein structural motif of about 50 amino acids that functions as a site for protein–protein interactions. The SCF (Skp1, Cullin, and F-box protein) E3 complex mediates ubiquitination of proteins targeted for degradation by the proteasome. F-box protein interacts directly with the SCF protein Skp1. The function of the F-box protein is to interact with target proteins via protein–protein interaction motifs including leucine-rich repeats and WD repeats. The latter domains promote binding of phosphorylated proteins to the SCF complex. In addition to ubiquitin-dependent proteolytic pathway, feeding rats a diet containing ethanol affected proteins associated with the lysosomal degradative pathway. In contrast to F-box protein, alcohol ingestion was associated with a 60% increase in P2B/LAMP-1, a lysosomal protein. LAMP-1 is composed of a large luminal portion, which is separated by a proline-rich hinge region in 2 disulfide-containing domains, a single transmembrane-spanning segment and a short cytoplasmic tail of 11 amino acids (Viitala et al., 1988). In LAMP-1-deficient mice, lysosomal properties, such as enzyme activities, lysosomal pH, osmotic stability, density, shape, and subcellular distribution, were not changed in comparison with controls (Andrejewski et al., 1999). Western blot analyses of LAMP-1-deficient and heterozygote tissues revealed an up-regulation of the LAMP-2

protein pointing to a compensatory effect of LAMP-2 in response to the LAMP-1 deficiency.

One potential mechanism to account for the altered expression of proteins is the generation of reactive oxygen species in hearts from rats fed a diet containing ethanol. With this regard, there are redundant enzymatic systems to control the concentration of reactive species. To this end, chronic alcohol intoxication was associated with reductions in peroxiredoxin 5, antioxidant protein 2, and glutathione transferase 5. Anti-oxidant protein 2 is the member of thiol-specific antioxidant gene family that removes H2O2, and in doing so protects proteins, DFNA, and lipids from oxidative stress. Overexpression of antioxidant protein 2 protects the pancreas from oxidative stress induced by diabetes (Yamamoto et al., 2008). Peroxiredoxin, the antioxidant components of the thioredoxin super-family, have gained recognition as important redox regulating molecules relevant to the mechanisms underlying ischemiareperfusion injury. In this study, the expression of antioxidant protein 2 and perioxiredoxin 5 was reduced by 30%. Likewise, the glutathione-S-transferases catalyze the reaction of the major low-molecular mass thiol, glutathione, with reactive oxygen species to form thioethers. The production of increased reactive oxidative defenses could result in the accumulation of reactive oxygen species and cause oxidative stress in the myocardium. Elevated reactive oxygen species affect function of myocardial cells through oxidation of other molecules including DNA, lipids, and proteins.

While much of other discussion is directed toward proteins whose ICAT analysis suggests a decrease in protein content, other peptides suggest proteins are elevated. In particular, proteins associated primarily with the cytosolic fraction appear elevated (CD122, 60 kDa SS-A/Ro ribonucleo-protein, lysosome-associated membrane glycoprotein 1, heat shock 70-kDa protein 14, hepatocyte growth factor-regulated tyrosine kinase substrate, hemogen, suppression of tumorigenicity 5, tyrosine-protein kinase receptor UFO, ribonuclease 1, mitogen-activated protein kinase phosphatase 2, and ras-association domain-containing protein 10).

In summary, the results of the present investigations provide evidence that myofibrillar, sarcoplasmic, membrane-associated, and mitochondrial proteins in cardiac muscle are reduced following chronic administration of ethanol. The identified proteins presented, for the first time, represent a detailed analysis of the proteins affected by long-term alcohol consumption. This study used a mass spectrometry-based proteomic approach to identify differentially deregulated proteins in the myocardium following feeding rats a diet containing ethanol. In this context, the majority of cardiac proteins identified were not modulated by alcohol. Chronic alcohol appears to have selective effects on particular proteins, and the effects were not directly ascribed to overt malnutrition. This may explain some of the functional and morphological characteristics observed in alcohol-induced heart muscle disease, including reduced contractility. Further investigations into the role of these dysregulated proteins may shed new insights into developing novel therapeutic approaches for patients who abuse alcohol.

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Table 1

Changes in Myocardial Proteins With Chronic Alcohol Intoxication Identified by Western Blot Techniques

Protein	Change	Weeks of ethanol consumption	Reference
α-heavy chain myosin	↓15%	6	(Patel et al., 2000)
	↔	13	(Meehan et al., 1999)
	↓45%	15	(Vary and Deiter, 2005)
	↓45%	26	(Vary et al., 2007)
	↓45%	28	(Figueredo et al., 1998)
β-heavy chain myosin	↑32%	13	(Meehan et al., 1999)
	↑1146%	15	(Vary and Deiter, 2005)
	↑143%	26	(Vary et al., 2007)
Actin	↔	6	(Patel et al., 2000)
	↓25%	15	(Vary and Deiter, 2005)
	↓38%	26	(Vary et al., 2007)
SERCA2a	↔	28	(Figueredo et al., 1998)
Phospholamban	↔	28	(Figueredo et al., 1998)
Troponin I	↑40%	15	(Vary and Deiter, 2005)
Troponin C	↔	6	(Patel et al., 2000)
	↔	15	(Vary and Deiter, 2005)
Troponin T	↔	6	(Patel et al., 2000)
	↔	15	(Vary and Deiter, 2005)
eEF2	↓13%	15	(Vary and Deiter, 2005)
HSC70	↔	15	(Vary and Deiter, 2005)
Grp78	↔	15	(Vary and Deiter, 2005)
REDD 1	↔	26	(Vary et al., 2008)
4EBP1	↔	26	(Vary et al., 2007)

Table 2

Effect of Chronic Alcohol Consumption on Myofibrillar Proteins

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
Myosin heavy chain, skeletal muscle	MYSS	P02562	X05958	0.61
		A02985		
Actin, alpha skeletal muscle	ACTA1	P68135	V00872	0.61
	ACTA	ATRB		
		A92182		
Myosin light chain 3	Myl3	P16409	NP_036738.1	0.73
	Mlc1v	MORT3V	NM_012606	
Myosin light chain 4	MYL4	P17209	X51531	0.73
		MORT4E		

Table 3

Effect of Chronic Alcohol Consumption on Mitochondrial Proteins

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
ATP synthase subunit epsilon	ATP5E	P29418	NP_620799.1	0.23
		B44300		
Aspartate aminotransferase	Got2	P05202	NP_034455.1	0.69
	AATM	S01174	uc009mzi.1	
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7	NDUFA7	O95182	NP_004992.2	0.26
	NDUA7	JE0380	uc002mjm.1	
Succinyl-CoA synthetase alpha subunit	Suclg1	P70631	U75394	0.27
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	Ndufa5	Q63362	NP_037117.1	0.37
	NDUA5		NM_012985	
	NUFM			
ADP/ATP translocase 1	Slc25a4	Q05962	NP_445967.1	0.40
	Ant1	I60173	NM_053515	
Citrate synthase	Cs	Q8VHF5	NP_570111.1	0.41
	CISY		NM_130755	
ATP synthase subunit β	Atp5f1	P19511	NP_599192.1	0.49
	Atp5f	A35340	NM_134365	
Isocitrate dehydrogenase [NADP], mitochondrial precursor	Idh2	P54071	Q9EQK1	0.60
Long-chain specific acyl-CoA dehydrogenase	Acadl	P51174	NP_031407.2	0.63
			uc007bir.1	
60-kDa heat shock protein	Hspd1	P63038	AAH16400	0.65
	Hsp60	HHMS60	NP_034607.3	
		P63039	NP_071565.2	
		HHRT60	NM_022229	
		S13089		
Stress-70 protein	Hspa9	P48721	NP_001094128.1	0.65
	Grp75	I56581	S78556	
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	Sdha	Q920L2	NP_569112.1	0.72
	DHSA	Q8K2B3	NM_130428	
			NP_075770.1	
			uc007rfa.1	
Creatine kinase, sarcomeric mitochondrial	KCRS	P09605	X59736	0.67
	S-MtCK	S17188		
Propionyl-CoA carboxylase beta chain	PCCB	P07633	M14634	0.69
		A25516		
Long-chain specific acyl-CoA dehydrogenase	ACADL	P15650	NP_036951.1	0.73
	LCAD	A34252	NM_012819	
Malate dehydrogenase	MDHM	P04636	NP_112413.2.	0.77
	Mdh2		NM_031151	
	Mor1			

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
Pyruvate dehydrogenase E1 component subunit alpha, somatic form	ODPA	P26284	Z12158	1.04
	Pdha1	DERTPA		
Cytochrome b-c1 complex subunit 2	Uqcrc2	P32551	NP_001006971.1	1.21
	QCR2		NM_001006970	
2,4-dienoyl-CoA reductase	Decr1	Q64591	NP_476545.1	1.29
	Decr	S11021	BC059120	
28S ribosomal protein S35	Mrps35	Q8BJZ4	NP_663548.2	15.6
	RT35		uc009ess.1	

Table 4

Effect of Chronic Alcohol Consumption on Glycolytic Proteins

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
Alpha enolase	ENOA	P04764	NM_012554	0.32
	NNE	A23126		
Beta-enolase	ENO3	P13929	NP_001967.2	0.38
	ENOB	S06756	uc002gab.2	
Bisphosphoglycerate mutase	BPGM	P07952	NP_001075738.1	0.4
	PMGE	PMRBBM		
		A24973		
Triosephosphate isomerase	TIM	P48500	NP_075211.2	0.46
	Tpi1		NM_022922	
Pyruvate kinase isozymes M1/M2	Pkm2	P11980	NP_445749.1	0.51
	Pykm	B26186	NM_053297	
Glycogen phosphorylase, muscle form	Pygm	P09812	L10669	0.54
	S34624			
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	P04797	NP_058704.1	0.54
	G3P	DERTG	NM_017008	
	Gapd			
Fructose-bisphosphate aldolase A	ALDOA	P05065	NP_036627.1	0.56
			NM_012495	
L-lactate dehydrogenase A chain	LDHA	P04642	NP_058721.1	0.67
	LDH1	A23083	NM_017025	

Table 5

Effect of Chronic Alcohol Consumption on Membrane Proteins

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
Transmembrane emp24-like trafficking protein 10	Tmed10	Q63584	NP_445919.1	0.02
	Tmp21			
	TMEDA			
T-cell receptor alpha chain	TRA	Q569B0	RGD1359684	0.47
		Q561R6		
		Q3SZN6		
Parathyroid hormone 2 receptor	Pth2r	Q91V95	NP_644676.1	0.48
	Pthr2		uc007bhu.1	
Low-density lipoprotein receptor-related protein 2	Lrp2	P98158	NP_110454.1	0.41
		T42737		

Table 6

Effect of Chronic Alcohol Consumption on Cytosolic Proteins

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
HOX11	TLX1	P43345	NP_005512.1	0.17
		P31314		
		S70632		
DNA repair protein RAD50	Rad50	Q9JIL8	NP_071582.1	0.27
			NM_022246	
Glutathione S-transferase Mu 2	Gstm2	P15626	NP_032209.1	0.32
		B34159	uc008qxw.1	
Dehydrogenase/reductase SDR family member 4	DHRS4	Q8SPU8	NP_777247.1	0.40
	NDRD			
Lactotransferrin	Ltf	P08071	J03298	0.43
	TRFL	A28438		
Fibrillin-2	Fbn2	Q9WUH9	NP_114014.1	0.48
F-box/LRR-repeat protein 17	Fbxl17	Q9QZN1	XP_930228.2.	0.49
	Fbl17			
	Fbx13			
	Fbxo13			
Putative uncharacterized protein unknown (Protein MGC: 6038)	Nnt	Q922E1	BC008518	0.49
Malate dehydrogenase, cytoplasmic	Mdh1	O88989	NP_150238.1	0.52
	Mdh		NM_033235	
Poly [ADP-ribose] polymerase 1	Parp1	P27008	NP_037195.1	0.60
	Adprt	S21163	NM_013063	
Long-chain-fatty-acid–CoA ligase 1	Acsl1	P18163	NP_036952.1	0.64
	Acs2	A36275	NM_012820	
	Acsl2			
	Facl2			
Versican core protein	Vcan	Q9ERB4	AF062402	0.69
	Cspg2			
Leukemia inhibitory factor	Lif	O88211	NP_071532.2	0.69
		JE0224		
Fatty acid transport protein	Cd36	O35754	AF111268	0.69
Peroxiredoxin	Prdx6	O08709	NP_031479.1	0.70
	Aop2			
	Ltw4			
	Prdx5			
	Prdx6	O35244	NP_446028.1	
	Aipla2			
	Aop2			
	Tsa			
Zinc finger protein	Znf292	Q63753	L23077	0.71

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
		A47651		
Peripheral-type benzodiazepine receptor-associated protein 1	Bzrap1	Q9JIR0	AF199337	0.71
	Rbp1			
D-dopachrome decarboxylase	Ddt	P80254	NP_077045.1	0.78
	DOPD	S68237	NM_024131	
60-kDa SS-A/Ro ribonucleoprotein	Trove2	O08848	NP_038863.1	1.53
	Ssa2		uc007cxd.1	
	RO60			
Lysosome-associated membrane glycoprotein 1	Lamp1	P11438	NP_034814.2	1.61
		A60534	uc009kxa.1	
Heat shock 70-kDa protein 14	Hspa14	Q99M31	NP_056580.2	1.61
	Hsp70-4		uc008ief.1	
Hepatocyte growth factor-regulated tyrosine kinase substrate	Hgs	Q9JJ50	NP_062260.2	1.76
	Hrs			
	Hrs2			
Hemogen	Rp59	Q6AZ54	NP_579828.1	1.84
	HEMGN		BC078739	
Suppression of tumorigenicity 5	St5	Q924W7	NP_001001326.1	1.84
	Dennd2b			
Tyrosine-protein kinase receptor UFO	Axl	Q00993	X63535	2.13
	Ark	S23065		
	Ufo			
Ribonuclease 1	Ear11	Q9R134	AF171641	2.18
	R1			
Mitogen-activated protein kinase phosphatase 2	MKP-2	Q99M70	AY028781	17.5
Ras association domain-containing protein 10	Rassf10	Q8BL43	NP_780488.2	59
	RASFA		uc009jhb.1	

Table 7

Effect of Chronic Alcohol Consumption on Plasma Proteins

Protein	Official symbols	Uniprot/PIR AC	Alternate databank	ICAT ratio
Hemoglobin subunit beta-1	Hbb	P02091	NP_150237.1	0.36
	HBB1	S04588		
Ig kappa chain V region	KV5AA	P01643	PL0262	0.51
		KVMS73		
Transferrin precursor	TRFE	P12346	S33761	0.53
Albumin	ALBU	P02770	NM_134326	0.55
	ALB			
Hemoglobin subunit beta-2	HBB2	P11517	NP_001104739.1	0.61
		A25747	X05080	
Alpha-1-inhibitor 3	A1I3	P14046	NP_001033064.1	0.7
	Mug1	A29952	NM_001037975	
Hemopexin	Hpx	P20059	NM_053318	0.71
	HEMO	OQRT		
Gamma-2A immunoglobulin heavy chain	Igg-2a	P20760	M13804	1.11
		PS0019		
CD166 antigen	Alcam	O35112	NP_113941.1	112
			NM_031753	