Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA.

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** 4100047645

6. **Project Number and Title of Research Project:** 14 - Myocardial Protein Synthesis After Alcohol Intoxication

7. **Start and End Date of Research Project:** 1/1/2009 – 6/30/2010

8. **Name of Principal Investigator for the Research Project:** Thomas Vary, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 89,374

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Fogle	Grad Student	100	$45,899
Stanley	Mass Spectrometrist	45	$43,475

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Vary	PI	5%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Myocardial Protein Synthesis After Alcohol Intoxication	X☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	4/15/2009	$44,692	$0
Myocardial Protein Synthesis After Alcohol Intoxication	X☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	7/15/2009	$302,284	$302,284

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

None; the PI passed away unexpectedly.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female		1		
Unknown				
Total		**1**		

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic		1		
Unknown				
Total		**1**		

	Undergraduate	Masters	Pre-doc	Post-doc
White		1		
Black				
Asian				
Other				
Unknown				
Total		**1**		

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____X____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    The results of this project provide evidence that myofibrillar, mitochondrial, glycolytic, membrane-associated, cytosolic, and plasma proteins in cardiac muscle are altered following chronic administration of ethanol. This work presented, for the first time, a detailed analysis of some of the proteins affected by long-term alcohol consumption. Specifically, this study used a mass spectrometry-based proteomic approach to identify differentially deregulated proteins in the myocardium following feeding rats a diet containing ethanol. 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.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic

plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

PI died unexpectedly in 2010. This report was prepared using his Annual reports.

The long-term goal of this project is to understand the mechanisms by which alcohol consumption induces myofibrillar damage characteristic of alcoholic heart muscle disease. Alcohol abuse is associated with an increased premature mortality partly resulting from the development of an alcohol-induced cardiomyopathy, a condition diagnosed in approximately 35% of those individuals who chronically consume excessive amounts of alcohol. Although the mechanisms leading to alcohol-dependent myocardial dysfunction are multi-factorial, altered expression of myocardial proteins stands out as a central mechanism. Alcohol consumption inhibits rates of protein synthesis in heart at the level of mRNA translation. By understanding the alterations in the process of mRNA translation it is hoped that new strategies could be developed to combat the pathologic derangements in cardiac muscle structure and function associated with chronic alcohol abuse. We delineated two regulatory steps in the process of protein synthesis, the formation of an active eIF4E·eIF4G complex and the process of elongation that are responsible, in part, for the inhibition of protein synthesis during chronic alcohol administration, whereas acute alcohol intoxication only affects the formation of an active eIF4E·eIF4G complex. We hypothesize that the normal signaling pathway through mTOR responsible for maintaining the functioning of these two steps in protein synthesis at rates observed in control animals is severely compromised by alcohol intake. The net effect is manifested through alterations in the expression of myocardial proteins, including contractile proteins. We further hypothesize that provision of amino acids either through acute gavage or meal feeding to rats administered alcohol can stimulate mTOR leading to an acceleration of rates of protein synthesis. The experimental design for the project period will test the hypothesis that inhibition of protein synthesis in response to chronic alcohol feeding shifts myocardial protein expression. Overall, the research design will establish the mechanism by which alcohol reduces myocardial protein synthesis

Considerable attention has focused on cardiovascular events associated with ethanol consumption. Moderate alcohol consumption has been associated with a reduced risk of cardiovascular disease a finding interpreted to be representative of vessel events (i.e. reduced atherogenesis due to alterations in cholesterol metabolism). In contrast, heavy alcohol consumption is detrimental to the heart and is associated with an increased mortality that appears independent of coronary arterial disease. Excessive ethanol consumption can result in a syndrome termed alcoholic heart muscle disease. Alcoholic heart muscle disease is rarely produced by short-term ethanol administration. Instead, the disease occurs in patients in whom the sole causative agent is excessive and prolonged alcohol consumption (>80 g of ethanol (liter of wine) a day for >5 years). Clinical features of the consequences of prolonged and excessive ethanol consumption encompass defects in myocardial contractility. With continued heavy alcohol consumption, alcoholic heart muscle disease progresses with the development of an alcoholic cardiomyopathy. Clinically, this amount of alcohol consumption results in adverse functional and structural damage to the myocardium. The alcoholic cardiomyopathy is classified

as dilated cardiomyopathy. Patients with an alcoholic cardiomyopathy exhibit a worse outcome compared with patients with an idiopathic dilated cardiomyopathy if drinking is not stopped or severely arrested. Alcoholics diagnosed with alcoholic cardiomyopathy exhibit a worse outcome when compared with patients diagnosed with an idiopathic dilative cardiomyopathy. The alcohol-induced changes are consistent with a shift in myocardial protein expression and activity that occurs as the disease progresses. Notably, there is sexual dimorphism in the development of AHMD. The molecular mechanisms underlying variations in protein expression after prolonged alcohol abuse remain unknown. The current study aims to link alcohol-induced alterations in expression of specific proteins with the differences in susceptibility between male and female to myocardial dysfunction using a chronic rat model. Alcoholic heart muscle disease (AHMD), clinically characterized by thinning of the ventricular wall, and myocardial dysfunction.

Alcohol was provided to pathogen-free male and female Sprague-Dawley rats (Charles River Breeding Laboratories, Cambridge, MA) in two separate experiments. They were maintained for 18 weeks on an ethanol-containing diet in which alcohol was provided both in drinking water (10% v/v) and agar. Initially, all rats were provided the agar block without ethanol for one week. Thereafter, rats were randomly assigned to either an Alcohol or Control group. Animals in the Alcohol group were given free access to ethanol-containing agar blocks. The concentration of ethanol in the agar blocks was increased in 10% increments from 10% to 40% over the first four weeks. Ethanol-fed rats remained on the 40% ethanol-agar block diet for the remainder of the experimental protocol. Control agar blocks contained an equal caloric amount of dextrin-maltose. Standard rat chow (Harlan Teklad no. 8604, Madison, MI) provided nutrient intake in both groups. Total energy consumption was similar in both groups. The average ethanol intake corresponded to 23 grams of alcohol per kilogram body weight per day, resulting in a blood alcohol concentration (0.08 %); equivalent to legal intoxication in humans.

We hypothesize that the long-term effects of an alcohol-induced inhibition of protein synthesis should manifest themselves as alterations in cardiac structure and function. Moreover, we would predict there would be a time delay in the manifestation of cardiac dysfunction following identification of derangements in cardiac structure. The studies presented herein show that feeding rats a diet containing alcohol diminishes the myofibrillar protein content of the heart and is associated with changes in cardiac structure that precede the development of functional abnormalities. Evaluation of myocardial function and structure, performed via echocardiography, allowed for the early detection of changes to the heart throughout the alcohol-feeding regimen. Echocardiograms were performed using the Sequoia C256 Echocardiography System (Siemens Medical Solutions, Mountain View, CA) equipped with a 13 MHz transducer. Before the procedure, animals were anesthetized with ketamine (40 mg/kg) with acepromazine (1 mg/kg) intraperitoneally. Once anesthetized, animals were placed on a heating pad and covered with surgical towels to limit heat loss and maintain core body temperature during the procedure. The transducer was placed on the thorax and M-mode recordings were performed by directing the ultrasound beam at the mid-papillary muscle level. The measurements described herein were obtained after visualizing a well-defined, continuous interface of the septal and posterior walls. According to the methods of the American Society of Echocardiography and other investigators in animal models, the end diastolic (EDD) and end-systolic dimensions (ESD), interventricular septum in diastole (IVSD), and posterior wall in diastole (PWD) thickness were obtained with the leading-edge method. All parameters were measured with

electronic calipers. Intra-observer variability was less than 3% for the echocardiographic parameters measured. The LV mass (gm) and relative wall thickness (RWT) were calculated based on the following echocardiogram parameters: LV mass = 1.04 x [(EDD + PWD + IVSD)3 - EDD3], RWT = 2 + PWD/EDD. Cardiac output was calculated by multiplying the stroke volume x heart rate. After 16 weeks of alcohol ingestion, both male and female rats consuming alcohol showed decreases in stroke volume and left ventricular diastolic volume compared with their respective control group. Males receiving alcohol showed additional significant declines in left ventricular mass and end-diastolic dimension compared with their non-drinking male counterparts.

The current investigations use proteomics which allows for comparison of protein expression, post-translational modifications, protein-protein interactions and protein relationships between a perturbed state and a normal state specifically isobaric tag for relative and absolute quantitation (iTRAQ) followed by mass spectrometry, to delineate shifts in protein expression in rat myocardium as a consequence of chronic (18 week) alcohol intoxication. The iTRAQ workflow consists of reduction, alkylation, and trypsin digestion followed by labeling of each sample with one of the iTRAQ reagents (113, 114, 115, 116, 117, 118, 119, or 121). The iTRAQ-labeled samples are then combined into one mixture for subsequent tandem mass spectrometry analysis allowing both protein identification and quantitation. Following peptide identification and subsequent protein quantitation, a search (Paragon algorithm via Protein Pilot) is performed against a protein database (SwissProt, NCBI, etc.) concatenated with a decoy database containing the randomized sequences of the original, normal database. This search of a concatenated normal and randomized database allows estimation of the false discovery rate (FDR) so that a cutoff is created where identifications are only accepted when the local FDR of the lowest ranking protein is less than 5%. Therefore, all higher-ranking proteins will have decreasingly lower probabilities of being false positives.

One of the current problems is the ability to identify trends in differences in the iTRAQ protein dataset from different sample sets. Heretofore, the process is somewhat fragmented. In order to determine whether protein ratios differ significantly across subjects we will model the ratios using a random effects model. The random effects model assumes that individual iTRAQ runs are estimating different protein ratios. It further assumes that the different ratios are being drawn from a larger distribution with some mean and standard deviation. The analysis allows us to estimate this distribution of effects across different iTRAQ runs. Formally, the underlying statistical model of the (natural log) protein ratio is

$$r_i = \theta_i + \varepsilon_i \qquad (1)$$

where i indexes an iTRAQ run, r_i is a (log) protein ratio from run i, θ_i is a location parameter, and ε_i is zero-mean, normally distributed error term; that is, $\varepsilon_i \sim N(0, \sigma^2)$. In addition, the location of the ratio is being drawn from a larger distribution so that $\theta_i = R + u_i$, where $u_i \sim N(0, \tau^2)$. R represents the overall protein ratio and is the final object of inference. An estimate of R gives us our estimate of the overall ratio across iTRAQ runs. The null hypothesis is that $R = 1$, which implies the same protein concentration in each group. By substitution, the model becomes

$$\mathbf{r_i = (R + \mu_i) + \varepsilon_i} \qquad (2)$$

$$= \mathbf{R} + \boldsymbol{\delta_i}$$

where $\delta_i = \varepsilon_i + u_i$ is a zero-mean normally distributed error term that captures both the within-run variation (u_i) and the between-run variation (ε_i). With this formulation of the model, the overall protein ratio can be obtained as a weighted average of individual ratios from each iTRAQ run

$$R^* = \frac{\sum_{i=1}^{k} w_i r_i}{\sum_{i=1}^{k} w_i} \qquad (3)$$

Where the weight for run *i* is defined as:

$$\mathbf{w_i = (o^2 + \tau^2)^{-1}} \qquad (4)$$

Univariate analysis of the proteomic data will include an analysis of average change in the expression of proteins. This will be done by estimating weighted averages of ratios of protein expression across experiments, where the weights will involve the inverse of the within-experiment as well as between-experiment variability as described in equation (4). Separate summaries will be made for each specific protein, beginning with proteins that only appear in a single iTRAQ run, and continuing sequentially through proteins that appear in two, three, or more iTRAQ runs. Applying a random effects model of meta-analysis to multiple iTRAQ datasets has several distinct advantages. This approach allows for both normalization (bias removal) and uncertainty estimation (confidence interval estimation) to be incorporated not only in the separate analysis from each individual iTRAQ run, but also in the overall summary effect of a particular protein across multiple iTRAQ runs. This summary effect, or pooled ratio, is a weighted average of the ratios obtained from each individual iTRAQ run. As a result, ratios that are more precisely estimated, or have less random variation, are given greater weight in the overall pooled estimate.

At the end of the alcohol-feeding regimen, hearts are excised and perfused in vitro with 50 mls of ice-cold saline in attempt to remove extracellular proteins. The hearts were cut in half and subsequently fractionated into myofibrillar, sarcoplasmic, mitochondrial, and nuclear fractions via differential centrifugation. Each enriched fraction was then subjected to iTRAQ analysis to allow for identification of the greatest number of proteins via our proteomic approach. Early interpretation of the proteomic data obtained from the mitochondrial fraction identified variations in specific proteins, including structural proteins or proteins involved in oxidative phosphorylation and electron transport. In particular, Chronic alcohol ingestion was associated with falls in mitochondrial proteins associated with TCA cycle (citrate synthase, isocitrate dehydrogenase, succinyl-CoA synthetase, malate dehydrogenase) as well as enzymes involved in electron transport (ubiquinone, adenine nucleotide translocator, F1-ATPase, H^+ transporting two-sector ATPase) and energy transfer (nucleoside-diphosphate kinase, creatine kinase). No changes were seen in pyruvate dehydrogenase complex, NADH coenzyme Q reductase, or ubiquinol cytochrome c reductase. These alterations will be further investigated to determine their role in inducing damage to the myocardium characteristic of AHMD.

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. 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 level has been reported, although there are individual reports of changes in some but not all subcellular fractions. An increased understanding of the changes of protein profiles in response to ethanol is important in understanding the pathogenesis of alcoholic cardiomyopathy.

In the studies supported by this funding mechanism, the expression of myocardial proteins was analyzed using a proteomic approach with ICAT technology. The time course of 16 weeks of ethanol consumption was selected because there is evidence of 1) thinning of the ventricular wall at this time based on echocardiography and 2) changes in selective protein content. The ICAT proteomic method labels individual peptides, measures the relative amount of peptide peaks, identifies the peptides with tandem mass spectrometry, and then identifies and quantifies proteins through these identified 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. 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-labeled "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.

Previously known changes in some proteins were correctly identified by ICAT analysis of peptides, showing that the use of the ICAT gives results consistent with assessment of proteins via Western blot. Both experimental and control groups were given equally nutritionally adequate diets emphasizing that alcohol induces alterations in specific heart muscle proteins and these changes are evident even when the nutrition is the same across 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. 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 are consistent with observations that mitochondrial respiratory rates and the efficiency of phosphorylation were depressed in rats given 25% alcohol for six months. However, not all proteins identified in this study were decreased upon ethanol exposure. For example, cytochrome b-c1 complex subunit 2, 2,4-dienoyl-CoA reductase, and 28S ribosomal protein S35 were elevated.

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. Reductions in some of these plasma proteins, including albumin, can be a sign of hepatic dysfunction or protein malnutrition. 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.

Hemopexin is the plasma protein with the highest binding affinity to heme among known proteins. 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 membranes 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 induce intracellular 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. As hemopexin is reduced as a result of chronic alcohol intake, this may be a source of reactive oxygen species formation.

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 three enzymes; the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin-protein ligase (E3). The SCF (Skp1, Cullin, and F-box protein) E3 complex, which is one type of ubiquitin ligase, mediates ubiquitination of proteins targeted for degradation by the proteosome. The function of the F-box protein is to interact with target proteins via protein-protein interaction motifs including leucine rich repeats and tryptophan-aspartate (WD) repeats. The latter domains promote binding of phosphorylated proteins to the SCF complex. In addition to the 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.

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. In 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. Antioxidant protein 2 is the member thiol-specific antioxidant gene family that removes H_2O_2, and in doing so protects proteins, DNA, and lipids from oxidative stress. Overexpression of antioxidant protein 2 protects the pancreas from oxidative stress induced by diabetes. Peroxiredoxin, the antioxidant component of the thioredoxin superfamily, have gained recognition as important redox regulating molecules relevant to the mechanisms underlying ischemia-reperfusion injury. In the present study, the expression of antioxidant protein 2 and perioxiredoxen 5 were 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 thioesters. 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 the discussion is directed towards 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

ribonucleoprotein, 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 investigation provide evidence that myofibrillar, mitochondrial, glycolytic, membrane-associated, cytosolic, and plasma proteins in cardiac muscle are altered following chronic administration of ethanol. The identified proteins presented, for the first time, represent a detailed analysis of some of the proteins affected by long-term alcohol consumption. Specifically, this study used a mass spectrometry-based proteomic approach to identify differentially deregulated proteins in the myocardium following feeding rats a diet containing ethanol. 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 on the role of these deregulated proteins may shed new insights into developing novel therapeutic approaches for patients who abuse alcohol.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
____X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
Impact of Chronic Alcohol Ingestion on Cardiac Muscle Protein Expression	Rachel L. Fogle, Christopher Lynch, Mary Palopoli, Gina Deiter, Bruce A. Stanley, Thomas C. Vary	Alcoholism: Clinical and Experimental Research	May 2010	☐Submitted ☐Accepted X Published
Functional proteomic analysis reveals sex-dependent differences in structural and energy-producing myocardial	Fogle RL, Hollenbeak CS, Stanley BA, Vary TC, Kimball SR, Lynch CJ	Physiol Genomics 43(7): p. 346-356	October 2010	☐Submitted ☐Accepted X Published

proteins in rat model of alcoholic cardiomyopathy				

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No___X_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X___

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Thomas C. Vary, Ph.D.	Professor
eRA COMMONS USER NAME TomVary	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Johns Hopkins University, Baltimore, MD	B.A.	1976	Natural Sciences
Lehigh University, Bethlehem, PA	M.S.	1978	Molecular Biology
Pennsylvania State University, Hershey, PA	Ph.D.	1982	Physiology
Pennsylvania State University, Hershey, PA	Postdoc	1982	Cardiac Metabolism

A. POSITIONS AND HONORS

Professional Experience:

1982-1983 British-American Research Fellow, Nuffield Department Clinical Biochemistry, John Radcliffe Hospital, University of Oxford, Oxford, England (Prof. Sir Philip Randle, FRS)

1983-1988 Assistant Professor, Department Physiology and Maryland Institute for Emergency Medical Services Systems, University of Maryland School of Medicine, Baltimore, MD

1985 Visiting Fellow, Nuffield Department Clinical Biochemistry, University of Oxford, Oxford, England (Prof. Sir Philip Randle, FRS)

1988-1990 Assistant Professor, Dept. of Cellular & Molecular Physiology, The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA

1990-1996 Associate Professor, Dept. of Cellular & Molecular Physiology, The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA

1995-1996 Visiting Scientist, Laboratoire d'Etude Metabolisme Azote, Institut Nationale de la Recherche Agronomique, Saint Genes Champanelle, France

1996-2006 Professor, Department of Cellular & Molecular Physiology, The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA

2007-2010 Distinguished University Professor, Department of Cellular & Molecular Physiology, The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA

Honors and Awards:

1978-1982 PHS Predoctoral Traineeship, Department of Physiology, The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA

1982-1983 British-American Research Fellow, American Heart Association and British Heart Foundation

1990-1995 PHS Research Career Development Award, General Medical Sciences NIH

1990-2000; 2007-2010 Editorial Board, *American Journal of Physiology Endocrinology & Metabolism*

1993 Hinkle Society Outstanding Junior Investigator Award

1993-2010 Editorial Board, *Shock*

1993-1996 American Physiological Society, Membership Committee, 2003-2006 –Awards Committee
1992-1994 Reviewer- Merit Review Grants - Bureau of Veterans' Affairs
1997 PHS Special Reviewer - Respiratory and Applied Physiology Study Section
1997-2003 PHS Reviewer - Special Emphasis Panel Trauma and Burns Program
1998-1999 PHS Reviewer - Metabolism Study Section Ad Hoc
2001-2006 PHS Reviewer - Special Emphasis Panel NIAAA (Chairperson - 2003)
2001-2006 PHS Reviewer - NIAAA AA-1 IRG
2003-2005 PHS Reviewer - NIAAA ZAA1 EE IRG
2004-2007 National Board Medical Examinations – US Medical License Examination

B. SELECTED PEER-REVIEWED PUBLICATIONS

1. Dardevet, D., C. Sornet, **T. Vary**, and J. Grizard. Phosphotidylinositol 3-kinase and p70 S6 kinase participate in the regulation of protein turnover in skeletal muscle by insulin and IGF-1. Endocrinology 137: 4087-4094, 1996.
2. Sinaud S., M. Balage, G. Bayle, D. Dardevet, **T. Vary**, S. Kimball, L.S. Jefferson, and J. Grizard. Diazoxide-induced insulin deficiency greatly reduced muscle protein synthesis in rats: Involvement of eIF4E. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E50-E61, 1999.
3. **Vary T.C.**, D. Dardevet, L. Voisin, J. Grizard, C. Buffiere, P. Denis, D. Breuille, and C. Obled. Pentoxifylline improves insulin action limiting skeletal muscle catabolism following infection. J. Endocrinol.163: 15-24, 1999.
4. Fedele, M.J., **T.C. Vary**, and P.A. Farrell. Neutralizing antibody against IGF-I prevents increases in protein synthesis in incubated epitrochlearis muscles from refed, diabetic rats. J. Appl. Physiol. 90: 1166-1173, 2001.
5. Lynch, C.L., B. J. Patson, J. Anthony, A. Vaval, L.S. Jefferson, and **T. C. Vary**. Leucine is a direct acting nutrient that regulates protein synthesis in adipose tissue. Am, J, Physiol.Endo. Metab.283: E503-E513, 2002.
6. Lynch, C.L., S.M. Hutson, B.J. Patson, A. Vaval, and **T.C. Vary**. Tissue specific effects of chronic dietary leucine and norleucine administration on protein synthesis in rat. Am, J, Physiol. Endo. Metab. 283: E824-E835, 2002.
7. Kumar, V, C. Silvis, G. Nystrom, N. Deshpande, **T. C. Vary**, R. A. Frost, and C. H. Lang. Alcohol-induced increases in insulin-like growth factor binding protein-1 are partially mediated by TNF. Alcoholism Clin. Exp. Res. 26: 1574-1583, 2002.
8. **Vary. T. C.**, G. Deiter, and S.R. Kimball. Phosphorylation of eukaryotic initiation factor eIF2Bε in skeletal muscle during sepsis. Am. J. Physiol. Endocrinol. Metabol. 283: E1032-E1039, 2002.
9. Lang, C.H., V. Kumar, X. Liu, R. A. Frost, and **T. C. Vary**. IGF-I induced phosphorylation of S6K1 and 4E-BP1 in heart is impaired by acute alcohol intoxication. Alcohol Clin. Exp. Res. 27: 485-494, 2003.
10. Lynch, C.J., B. Halle, H. Fuji, **T.C. Vary**, R. Wallin, Z. Dahmuni, and S.M. Hutson. Potential role of leucine metabolism in the leucine signaling pathway involving mTOR. Am. J. Physiol. Endo. Metab. 285: E854-E863, 2003.
11. Lang, C.H., **T.C. Vary**, and R.A. Frost. Acute in vivo elevation of IGFBP-1 decreases plasma free IGF-1 and muscle protein synthesis. Endocrinology 144: 3922-3933, 2003.
12. Lloyd, C.E., M.M. Palopoli and **T.C. Vary**. Effect of central administration of IL-1 receptor antagonist on protein synthesis in skeletal muscle, kidney and liver during sepsis. Metabolism. 52: 1218-1225, 2003
13. Lang, C.H., R.A. Frost, **T.C. Vary**, L.S. Jefferson, and S.R. Kimball Leucine-mediated phosphorylation of 4E-BP1, S6K1, eIF4G and mTOR in skeletal muscle is impaired by alcohol. Am. J. Physiol. Endo. Metab. 285: E1205-E1214, 2003.
14. **Vary, T.C**., A.C. Nairn, and C.H. Lang. Restoration of protein synthesis in cardiac and skeletal muscle following withdrawal of alcohol Alcohol Clin. Exp. Res 28: 517-525, 2004.

15. **Vary, T. C**. and C.J. Lynch. Biochemical approaches to nutritional support of skeletal muscle protein metabolism during sepsis. J. Nutr. Res. Rev. 17: 77-88, 2004.
16. Bolster, D.R., **T.C. Vary**, S.R. Kimball, and L.S. Jefferson. Leucine regulates translation initiation in rat skeletal muscle via enhanced eIF4G phosphorylation. J. Nutr. 134: 1704-1710, 2004.
17. **Vary, T.C**. and C.J. Lynch. Nutrient signaling to muscle and adipose tissue by leucine. In: Nutrients and Cell Signaling. (J. Zempleni and K. Dakshinamurti, eds) Marcel Dekker, New York. 2005. pp. 299-327.
18. **Vary, T. C**. and G. Deiter. Long-term alcohol administration inhibits synthesis of both myofibrillar and sarcoplasmic proteins in heart. Metabolism 54: 212-219, 2005.
19. **Vary, T. C.**, G. Deiter, and S. A. Goodman. Acute alcohol intoxication enhances myocardial eIF4G phosphorylation despite reducing mTOR signaling. Am. J. Physiol. Heart Circ 288: H121-H128, 2005**.**
20. **Vary, T. C.** and C. H. Lang. IGF-I activates the eIF4F system in cardiac muscle in vivo. Mol. Cell. Biochem 272:209-220, 2005.
21. Hong-Brown, A. M. Pruznak, R. A. Frost, **T. C. Vary**, and C. H. Lang. Indinavir alters regulation of protein anabolism and catabolism in skeletal muscle. Am. J. Physiol. Endo. Metab. 289: E382-E390, 2005
22. **Vary, T. C.**, S. Goodman, L. E. Kilpatrick, and C. J. Lynch. Nutrient regulation of PKCε is mediated by leucine, not insulin in skeletal muscle. Am. J. Physiol. Endo. Metab. 289: E689-E694, 2005**.**
23. Crozier, S. J., **T. C. Vary**, S. R. Kimball, and L. S. Jefferson. Cellular energy status modulates translational control mechanisms in the ischemic/reperfused rat heart. Am. J. Physiol. Heart Circ. Physiol. 289: H1242-H1250, 2005.
24. Kraweic, B. J., R. A. Frost, **T. C. Vary**, L. S. Jefferson, and C. H. Lang. Unilateral hindlimb immobilization decreases muscle mass in part by proteasome-dependent proteolysis but independent of protein synthesis and translation initiation factors. Am. J. Physiol. Endo. Metab. 289: E969-E980, 2005.
25. Lang, C. H., R. A. Frost, A. D. Sumner, and **T. C. Vary**. Molecular mechanisms responsible for alcohol-induced myopathy in skeletal muscle and heart. Int. J. Biochem. Cell Biol. 37: 2180-2195, 2005.
26. **Vary, T. C.** IGF-I stimulates protein synthesis in skeletal muscle through multiple signaling pathways during sepsis. Am. J. Physiol. Reg. Integr. Comp. Physiol. 290:R313-R321, 2006.
27. **Vary, T. C.** and C.J. Lynch. Meal feeding enhances formation of eIF4F in skeletal muscle: role of increased eIF4E availability and eIF4G phosphorylation. Am. J. Physiol. Endo. Metab. 290: E631-E642, 2006.
28**.** Lynch, C.J., B. Gern, C. Lloyd, S. M. Hutson, R. Eicher, and **T. C. Vary.** Leucine in food mediates some of the postdrandial rise in plasma leptin concentrations. Am. J. Physiol. Endocrinol. Metab. 291: E621-E630, 2006.
29. **Vary, T.C.** and C.J. Lynch. Meal feeding elicits phosphorylation of multiple effector proteins regulating protein synthetic processes in cardiac muscle. J. Nutr. 136: 2284-2290, 2006.
30. **Vary, T.C.**, G. Deiter, and C.H. Lang. Cytokine-triggered decreases in the level of eIF4G phosphorylation in skeletal muscle during sepsis. Shock 26: 631-636, 2006.
31. **Vary, T.C.**, W.W. Souba, and C.J. Lynch. Regulation of pyruvate and amino acid metabolism. In: Topics in Biochemistry: Mitochondria (S. Schaffer and S. Suleiman, eds) Springer-Verlag, New York. pp. 143-184, 2007.
32. **Vary, T.C**., S. R. Kimball, and A. Sumner. Sex-dependent differences in the regulation of myocardial protein synthesis following long-term ethanol consumption. Am. J. Physiol. Reg. Integr. Comp. Physiol. 292: R778-R787, 2007. PMID: 16946086
33. Lang CH, Frost RA, **Vary TC**. Skeletal muscle protein synthesis and degradation exhibit sexual dimorphism after chronic alcohol consumption but not acute intoxication. Am J Physiol Endocrinol Metab. 292(6):E1497-506, 2007. PMID: 17264221

34. **Vary TC**, Anthony JC, Jefferson LS, Kimball SR, Lynch CJ. Rapamycin blunts nutrient stimulation of eIF4G, but not PKCepsilon phosphorylation, in skeletal muscle. Am J Physiol Endocrinol Metab. 293(1):E188-96, 2007. PMID: 17389711
35. Lang CH, Frost RA, **Vary TC**. Regulation of muscle protein synthesis during sepsis and inflammation. Am J Physiol Endocrinol Metab. 293(2):E453-9, 2007. Review. PMID: 17505052
36. **Vary TC**, Lynch CJ. Nutrient signaling components controlling protein synthesis in striated muscle. J Nutr. 137(8):1835-43, 2007. Review. PMID: 17634251
37. **Vary TC**, Deiter G, Lynch CJ. Rapamycin limits formation of active eukaryotic initiation factor 4F complex following meal feeding in rat hearts. J Nutr. 137(8):1857-62, 2007. PMID: 17634255
38. **Vary TC**. Acute oral leucine administration stimulates protein synthesis during chronic sepsis through enhanced association of eukaryotic initiation factor 4G with eukaryotic initiation factor 4E in rats. J Nutr. 137(9):2074-9, 2007. PMID: 17709445
39. She P, Reid TM, Bronson SK, **Vary TC**, Hajnal A, Lynch CJ, Hutson SM. Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. Cell Metab. 6(3):181-94, 2007. PMID: 17767905
40. Hong EG, Jung DY, Ko HJ, Zhang Z, Ma Z, Jun JY, Kim JH, Sumner AD, **Vary TC**, Gardner TW, Bronson SK, Kim JK. Nonobese, insulin-deficient Ins2Akita mice develop type 2 diabetes phenotypes including insulin resistance and cardiac remodeling. Am J Physiol Endocrinol Metab. 293(6):E1687-96, 2007. PMID: 17911348
41. **Vary TC**, Deiter G, Lantry R. Chronic alcohol feeding impairs mTOR(Ser 2448) phosphorylation in rat hearts. Alcohol Clin Exp Res. 32(1):43-51, 2008. PMID: 18028531
42. **Vary TC**, Lang CH. Differential phosphorylation of translation initiation regulators 4EBP1, S6k1, and Erk 1/2 following inhibition of alcohol metabolism in mouse heart. Cardiovasc Toxicol. 8(1):23-32, 2008. PMID: 18317950
43. Karinch AM, Martin JH, **Vary TC**. Acute and chronic ethanol consumption differentially impact pathways limiting hepatic protein synthesis. Am J Physiol Endocrinol Metab. 295(1):E3-9, 2008. PMID: 18334613
44. Lang CH, Frost RA, **Vary TC**. Acute alcohol intoxication increases REDD1 in skeletal muscle. Alcohol Clin Exp Res. 32(5):796-805, 2008. PMID: 18336631
45. **Vary TC**, Lang CH. Assessing effects of alcohol consumption on protein synthesis in striated muscles. Methods Mol Biol. 447:343-55, 2008. PMID: 18369928
46. **Vary TC**, Frost RA, Lang CH. Acute alcohol intoxication increases atrogin-1 and MuRF1 mRNA without increasing proteolysis in skeletal muscle. Am J Physiol Regul Integr Comp Physiol. 294(6):R1777-89, 2008. PMID: 18401005
47. Forsyth S, **Vary TC**. Partial dissociation of TSC2 and mTOR phosphorylation in cardiac and skeletal muscle of rats in vivo. Mol Cell Biochem. 319(1-2):141-51, 2008. PMID: 18670866
48. Pruznak AM, Hong-Brown L, Lantry R, She P, Frost RA, **Vary TC**, Lang CH. Skeletal and cardiac myopathy in HIV-1 transgenic rats. Am J Physiol Endocrinol Metab. 295(4):E964-73, 2008. PMID: 18713959
49. Pruznak AM, Kazi AA, Frost RA, **Vary TC**, Lang CH. Activation of AMP-activated protein kinase by 5-aminoimidazole-4-carboxamide-1-beta-D-ribonucleoside prevents leucine-stimulated protein synthesis in rat skeletal muscle. J Nutr. 138(10):1887-94, 2008. PMID: 18806097
50. Lang CH, Pruznak AM, Nystrom GJ, **Vary TC**. Alcohol-induced decrease in muscle protein synthesis associated with increased binding of mTOR and raptor: Comparable effects in young and mature rats. Nutr Metab (Lond). 20;6:4, 2009. PMID: 19154606

51. Nairizi A, She P, **Vary TC**, Lynch CJ. Leucine supplementation of drinking water does not alter susceptibility to diet-induced obesity in mice. J Nutr. 139(4):715-9, 2009. PMID: 19244380
52. Jiao Q, Pruznak AM, Huber D, **Vary TC**, Lang CH. Castration differentially alters basal and leucine-stimulated tissue protein synthesis in skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab. 297(5):E1222-32, 2009. PMID: 19755668
53. Kilpatrick LE, Sun S, Li H, **Vary TC**, Korchak HM. Regulation of TNF-induced oxygen radical production in human neutrophils: role of delta-PKC. J Leukoc Biol. 87(1):153-64, 2010. PMID: 19801500
54. Lang CH, Lynch CJ, **Vary TC**. Alcohol-induced IGF-I resistance is ameliorated in mice deficient for mitochondrial branched-chain aminotransferase. J Nutr. 140(5):932-8, 2010. PMID: 20237068
55. Lang CH, Frost RA, Bronson SK, Lynch CJ, **Vary TC**. Skeletal muscle protein balance in mTOR heterozygous mice in response to inflammation and leucine. Am J Physiol Endocrinol Metab. 298(6):E1283-94, 2010. PMID: 20388826
56. Fogle RL, Lynch CJ, Palopoli M, Deiter G, Stanley BA, **Vary TC**. Impact of chronic alcohol ingestion on cardiac muscle protein expression. Alcohol Clin Exp Res. 34(7):1226-34, 2010. PMID: 20477769
57. Tuckow AP, **Vary TC**, Kimball SR, Jefferson LS. Ectopic expression of eIF2Bepsilon in rat skeletal muscle rescues the sepsis-induced reduction in guanine nucleotide exchange activity and protein synthesis. Am J Physiol Endocrinol Metab. 299(2):E241-8, 2010. PMID: 20484009
58. Albaugh VL, **Vary TC**, Ilkayeva O, Wenner BR, Maresca KP, Joyal JL, Breazeale S, Elich TD, Lang CH, Lynch CJ. Atypical antipsychotics rapidly and inappropriately switch peripheral fuel utilization to lipids, impairing metabolic flexibility in rodents. Schizophr Bull. 38(1):153-66, 2012. PMID: 20494946
59. Lang CH, Lynch CJ, **Vary TC**. BCATm deficiency ameliorates endotoxin-induced decrease in muscle protein synthesis and improves survival in septic mice. Am J Physiol Regul Integr Comp Physiol. 299(3):R935-44, 2010. PMID: 20554928
60. Bertsch S, Lang CH, **Vary TC**. Inhibition of glycogen synthase kinase 3[beta] activity with lithium in vitro attenuates sepsis-induced changes in muscle protein turnover. Shock 35(3):266-74, 2011. PMID: 20926980
61. Fogle RL, Hollenbeak CS, Stanley BA, **Vary TC**, Kimball SR, Lynch CJ. Functional proteomic analysis reveals sex-dependent differences in structural and energy-producing myocardial proteins in rat model of alcoholic cardiomyopathy. Physiol Genomics 43(7):346-56, 2011. PMID: 21245415
62. She P, Zhang Z, Marchionini D, Diaz WC, Jetton TJ, Kimball SR, **Vary TC**, Lang CH, Lynch CJ. Molecular characterization of skeletal muscle atrophy in the R6/2 mouse model of Huntington's disease. Am J Physiol Endocrinol Metab. 301(1):E49-61, 2011. PMID: 21505144
63. Giordano E, Hillary RA, **Vary TC**, Pegg AE, Sumner AD, Caldarera CM, Zhang XQ, Song J, Wang J, Cheung JY, Shantz LM. Overexpression of ornithine decarboxylase decreases ventricular systolic function during induction of cardiac hypertrophy. Amino Acids 42(2-3):507-18, 2012. PMID:21814794