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Alcohol Consumption Mitigates Apoptosis and MTOR Signaling in Myocardium

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Abstract

Background—Epidemiologic studies have shown that individuals who consume low to moderate alcohol have a lower risk of developing cardiovascular disease compared to abstainers. Although experimental studies confirmed this observation, the effect of alcohol on ischemic myocardium is still unclear. We developed a clinically relevant animal model of chronic myocardial ischemia to investigate the effects of moderate alcohol consumption on the myocardium.

Study Design—Fourteen Yorkshire swine underwent placement of an ameroid constrictor to induce chronic myocardial ischemia. Postoperatively, one group was supplemented with 90mL 50% ethanol daily (ETOH n=7), and one group was supplemented with 80g of sucrose daily to normalize caloric intake between groups (SUC n=7). After 7 weeks, all animals underwent sternotomy, and harvest of the chronically ischemic myocardium and non-ischemic myocardium. Tissues were analyzed for protein expression and stained for apoptosis quantification.

Results—In the ischemic myocardium, alcohol down-regulated pro-apoptotic proteins TNF α , FOXO3, BAD and caspase 9, up-regulated pro-survival proteins AMPK, pAMPK and pFOXO3, and down regulated MTOR signaling by down regulating MTOR, pMTOR and up-regulating Deptor. In the non-ischemic myocardium, alcohol up-regulated pro-survival proteins AKT, pAKT, pBCL2, AMPK, pBAD, pFOXO3 and down regulated MTOR signaling by down regulating pMTOR and up-regulating Deptor. Alcohol also decreased cell death as measured by TUNEL staining in the ischemic and non-ischemic myocardium.

Conclusions—Alcohol consumption down regulates apoptosis and promotes cell survival in the ischemic and non-ischemic myocardium. Alcohol also modulates MTOR signaling, which

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regulates senescence and apoptosis. Perhaps MTOR and apoptosis regulation is another mechanism by which moderate ethanol consumption is cardioprotective.

Introduction

Cardiovascular disease remains the leading cause of mortality in the United States and it is estimated that it accounts for one in three deaths among adults.¹ Given the significant burden of cardiovascular disease on the overall morbidity and mortality in the United States, there is growing urgency to identify at-risk individuals to encourage healthy lifestyle modification and preventative measures to curb the development of cardiovascular disease. Over the last 40 years, epidemiologic studies have demonstrated that low to moderate consumption of alcohol (1–2 drinks daily or 15–30g ethanol) reduces the risk of developing adverse cardiovascular events compared to abstainers. However, high alcohol intake (>3–4 drinks daily or >45–60g ethanol) increases the risk of cardiovascular disease.² This J-shaped relationship between alcohol consumption and cardiovascular risk has been extensively studied in subsequent animal models and the epidemiologic observations have been confirmed.

In a swine model of metabolic syndrome, our lab has shown that daily moderate alcohol consumption (45g ethanol daily) normalizes endothelial dysfunction, reduces oxidative stress, improves myocardial perfusion and alters insulin signaling in peripheral tissues.^{3–5} In another swine model of daily moderate alcohol consumption, we have shown that alcohol improves myocardial insulin signaling, myocardial perfusion, angiogenesis and endothelial dysfunction in chronically ischemic myocardium.^{6, 7}

Although it is now well established that low-moderate doses of alcohol consumption is cardioprotective, the effects of alcohol on cardiomyocyte survival and apoptosis is unclear. Apoptosis of myocytes in the setting of myocardial ischemia can lead to long-term reduction in cardiac contractility and function because cardiomyocytes are terminally differentiated and do not regenerate.⁸ We developed a clinically relevant animal model of daily moderate alcohol consumption and chronic myocardial ischemia to evaluate the effect of alcohol on cardiomyocyte survival and apoptosis.

Methods

ANIMAL MODEL

Fourteen intact male Yorkshire miniswine (Parsons Research, Amherst, MA) were fed 500g/day of regular chow (Sinclair Research, Columbia, MO) daily and underwent ameroid constrictor placement to the left circumflex artery to simulate conditions of chronic myocardial ischemia as described previously⁹. Postoperatively, one group was supplemented with 90 ml of ethanol daily (50%/V, EtOH, n = 7) and the control group was supplemented with 80g of sucrose of equal caloric value to the alcohol (SUC, n = 7). After 7 weeks of diet supplementation, all animals were anesthetized and the heart was exposed via median sternotomy. The animals were euthanized by exsanguination and samples from the ischemic myocardium (in the distribution of the left circumflex coronary artery) and the non-ischemic normally perfused myocardium (in the distribution of the left anterior descending coronary

artery) were collected. Tissue samples were rapidly frozen in liquid nitrogen for histologic and protein expression analysis.

Animals were weighed at the time of harvest. All animals were observed to ensure complete consumption of food and supplement, had unlimited access to water, and were housed in a warm, non-stressful environment for the duration of the experiment.

The Institutional Animal Care and Use Committee of the Rhode Island Hospital approved all experiments. Animals were cared for in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 5377-3 1996).

SURGICAL INTERVENTIONS

Anesthesia—Anesthesia was induced with an intramuscular injection of telazol (4.4 mg/kg). Animals were endotracheally intubated, mechanically ventilated at 12 – 20 breaths per minute, and general anesthesia was maintained with a gas mixture of oxygen at 1.5 – 2 liters/min and isoflurane at 0.75 – 3.0% concentration.

Ameroid Constrictor Placement—Animals were given a single dose of intravenous enrofloxacin 5mg/kg for antibiotic prophylaxis and general anesthesia was induced and maintained. Animals were prepped and draped in the usual sterile fashion. The heart was exposed through a left mini-thoracotomy and pericardiotomy. The left atrial appendage was retracted, and the left circumflex artery was dissected at the take off of the left main coronary artery. The ameroid constrictor was placed around the left circumflex artery (Research Instruments SW, Escondido, CA). The pericardium was loosely re-approximated followed by a layered closure of the surgical incision. Post-operative pain was controlled with a single dose of intramuscular Buprenorphine (0.03 mg/kg) and a 72 hour Fentanyl patch (4µg/kg). All animals received 325mg of aspirin daily starting 1 day pre-operatively and continuing for a total of 5 days for prophylaxis against thrombo-embolic events. All animals continued perioperative antibiotics: enrofloxacin 68mg orally daily for 5 days.

Cardiac Harvest—Under general anesthesia, the heart was exposed via median sternotomy. Animals were euthanized by exsanguination and chronically ischemic myocardial samples from the left circumflex territory and non-ischemic myocardial samples from the left anterior descending territory were collected for further analysis.

PROTEIN EXPRESSION

Frozen tissue samples were homogenized and in a radio-immunoprecipitation assay solution (Boston BioProducts, Ashland, MA) supplemented with protease and phosphatase inhibitors. Forty micrograms of whole-tissue lysates were fractionated by SDS-PAGE 4–12% Bis-Tris gels (NuPage Novex Mini Gel, Invitrogen, Carlsbad, CA) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were incubated overnight at 4°C with primary antibodies at dilutions recommended by the manufacturer against tumor necrosis factor α (TNF α), phosphorylated (phosphorylated B-cell CLL/ Lymphoma 2) pBCL2 (Ser70), 5'adenosine monophosphate-activated protein kinase

(AMPK), phosphorylated AMPK (Thr 172), BCL2 associated death promoter (BAD), phosphorylated BAD (Ser 112), cysteine aspartic acid specific protease 9 (Caspase 9), Cleaved Caspase 3, mammalian target of rapamycin (MTOR), phosphorylated MTOR (Ser2481), protein kinase B (AKT), phosphorylated AKT (Thr 308), forkhead box protein 3 (FOXO3), phosphorylated FOXO3 (Ser 318/321) (all from Cell Signaling, Danvers, MA), and Deptor (Sigma-Aldrich St. Louis, MO). Membranes were incubated with the appropriate horseradish peroxidase-linked secondary antibody for one hour at room temperature (Jackson ImmunoResearch, West Grove, PA). Immune complexes were visualized with enhanced chemiluminescence and images were captured with a digital camera system (G-Box, Syngene, Cambridge, England). Band densitometry was quantified as arbitrary light units using Image-J software (National Institutes of Health, Bethesda, MD). All membranes were probed with GAPDH to correct for loading error.

TUNEL Staining

Frozen myocardium from the non-ischemic and ischemic territory was sectioned (10- μ m-thickness). Apoptotic cells were identified using the commercially available TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling) ApopTag detection kit (Millipore) according to the manufacturer's instructions. Images were captured at X20 magnification using Aperio ScanScope technology in three random fields from each animal. The percentage of TUNEL-positive cardiomyocytes was measured using Image J software in a blinded fashion.

DATA ANALYSIS

All results are reported as mean $\pm$ standard error of the mean. A Student's T-test was used to compare the means of all studies using GraphPad Prism 5.0 Software (GraphPad Software Inc., San Diego, CA).

Results

ANIMAL MODEL

All animals included in the analysis survived the entire experiment. None of the animals developed wound infection or dehiscence of the mini-thoracotomy site. One animal in the SUC group died shortly after placement of the ameroid constrictor due to persistent ST elevations and ventricular fibrillation arrest. One animal from the ETOH group died on post-operative day 6 and necropsy revealed a perforated gastric ulcer. The two animals that did not survive were excluded from the analysis. We previously reported that there was no difference in cardiac output, contractility or compliance but there was improved perfusion in the ETOH group with demand pacing at 150 beats per minute.⁶

APOPTOSIS-SURVIVAL PROTEIN EXPRESSION IN ISCHEMIC MYOCARDIUM

Alcohol supplementation significantly down-regulated pro-apoptosis proteins BAD, FOXO3 and Caspase 9. Alcohol supplementation also down-regulated TNF α expression, however this did not reach statistical significance. Alcohol up-regulated pro-survival proteins AMPK and pAMPK. Alcohol also increased the expression of the inhibited form of pro-apoptosis

protein FOXO3, and down regulated of MTOR signaling by down-regulating MTOR, pMTOR and increasing Deptor expression (Table 1).

APOPTOSIS-SURVIVAL PROTEIN EXPRESSION IN NON-ISCHEMIC MYOCARDIUM

Alcohol supplementation significantly up-regulated pro-survival proteins AKT, pAKT, pBCL2 and AMPK. Alcohol also up-regulated the inhibited form of BAD and FOXO3, which are pro-apoptosis proteins. Alcohol supplementation down-regulated pro-apoptosis protein Caspase 9 and up-regulated pro-apoptosis protein FOXO3. Alcohol supplementation inhibited MTOR signaling by down-regulating pMTOR and increasing expression of Deptor, which promotes MTOR degradation (Table 2).

TUNEL STAINING

Cell death was measured by TUNEL staining to detect DNA fragmentation by labeling the terminal end of nucleic acids. In the chronically ischemic myocardium, there was an increase in cell death compared to the non-ischemic myocardium. Alcohol supplementation significantly decreased the number of TUNEL positive cells in both the ischemic and non-ischemic myocardium compared to the control (Figure 1 and 2).

Discussion

In this model of daily moderate alcohol consumption and chronic myocardial ischemia, we demonstrate that alcohol promotes survival signaling and down regulates apoptosis and MTOR signaling in both chronically ischemic myocardium and in normally perfused non-ischemic myocardium. The biochemical evidence of decreased apoptosis signaling in the myocardium was corroborated by histologic TUNEL staining showing that alcohol decreased cell death in both the ischemic and non-ischemic myocardium.

Apoptosis is a process of programmed cell death in which cells undergo cytoplasmic shrinkage, nuclear condensation, membrane blebbing and cell fragmentation into neat membrane-bound apoptotic bodies. Macrophages phagocytose the apoptotic bodies without evoking an inflammatory response.¹⁰ Apoptosis is regulated by death receptor (extrinsic) and mitochondrial (intrinsic) pathways. In the extrinsic pathway, TNF α binds its membrane receptor, which triggers the formation of a death-inducing signal complex. The death complex activates down stream caspases including caspase 3. Once activated, caspases are cleaved and activate down stream caspases and commits the cell to apoptosis. The intrinsic pathway is activated under conditions of cell stress such as ischemia, hypoxia, oxidative stress, starvation, DNA damage, and toxin exposure. In the intrinsic pathway, the outer mitochondrial membrane is permeabilized, which results in the release of apoptogenes such as cytochrome c into the cytoplasm. Cytochrome c in turn promotes the assembly of the apoptosome and caspase 9 activation.

There are multiple inhibitors that can rescue the cell from apoptosis. AKT is a serine-threonine kinase in the insulin signaling cascade that also promotes cell survival by preventing mitochondrial permeability transition pore opening, which allows for the release of cytochrome c resulting in cell death.¹¹ AKT also activates the reperfusion injury salvage kinase pathway, which as been shown to promote myocardiocyte survival and reduce infarct

size after myocardial ischemia.¹¹ Bcl-2 blocks the release of cytochrome c through the mitochondrial outer membrane and inhibits cytochrome c mediated caspase activation.¹² Dephosphorylated BAD forms a heterodimer with Bcl-2 and neutralizes anti-apoptotic Bcl-2 activity. However, when BAD is phosphorylated by AKT, BAD becomes inactive and cannot form heterodimers and inactivate Bcl-2.¹² FOXO3 belongs to the forkhead box family of transcription factors, which induces the expression of pro-apoptosis proteins and down regulates the expression of pro-survival proteins. AKT phosphorylates FOXO3, which inactivates and sequesters FOXO3 in the cytoplasm.¹³ In our study, alcohol supplementation decreased the expression of TNF α , BAD, FOXO3, caspase 9 and increased expression of pFOXO3 in the ischemic myocardium, which all result in a reduction of apoptosis signaling. Although alcohol supplementation increased expression of the pro-apoptotic protein FOXO3 in the non-ischemic myocardium, there was also a significant up-regulation of the inhibited form of FOXO3, pFOXO3. There was also a significant up-regulation of pro-survival proteins AKT, pAKT, pBCL2, AMPK, down-regulation of pro-apoptosis protein caspase 9, and up-regulation of the inhibited form of pro-apoptosis protein pBAD. Therefore, the overall, alcohol inhibits apoptosis signaling and promotes pro-survival signaling in the non-ischemic myocardium.

AMP-activated protein kinase acts as an intra-cellular energy sensor during times of hypoxia, glucose starvation or increased energy consumption, the AMP to ATP ratio increases and activates AMPK, which promotes catabolic pathways to generate ATP.¹⁴ Like AKT, AMPK also activates the reperfusion injury salvage kinase pathway and has been shown to promote cell survival and cardioprotection by increasing ATP generation.¹⁵ MTOR is a central regulator of protein synthesis and cell growth. In a well-fed state, insulin signaling is activated and AKT phosphorylates and activates MTOR. However, under conditions of cell stress, AMPK activation inhibits MTOR in order to minimize the energy consuming process of cell growth and proliferation. Deptor is an MTOR binding protein that inhibits MTOR activity. Our results demonstrate that moderate daily alcohol consumption up-regulated AMPK, which in turn resulted in MTOR down regulation. Alcohol supplementation suppressed MTOR signaling in the ischemic myocardium as evidenced by the increase in the expression of AMPK, pAMPK, Deptor and decreased expression of MTOR and pMTOR. In the non-ischemic territory, alcohol up-regulated pro-survival proteins AKT, pAKT and AMPK and inhibited MTOR signaling by down-regulating pMTOR and increasing Deptor expression.

The protein expression profile indicating decreased apoptosis and increased survival correlated with the histologic results in our study demonstrating that there was indeed less cell death in the ischemic and non-ischemic myocardium in animals treated with daily alcohol. In the ischemic territory, there was more cell death overall, which is expected because the tissue has been exposed to chronic ischemia for several weeks.

Clinical studies have demonstrated that low-moderate alcohol consumption can reduce the risk of developing coronary artery disease by approximately 25% compared to abstainers.¹⁶ Alcohol has been shown to attenuate ischemia-reperfusion injury and improve outcomes and survival after myocardial infarction.^{2, 17} Animal studies have revealed that moderate alcohol consumption protects against ischemic insult by “ethanol preconditioning”, which renders

the myocardium more resistant to ischemia reperfusion injury.^{18, 19} In this study, alcohol reduced apoptosis signaling, decreased cell death and improved cell survival in both the ischemic and non-ischemic myocardium. Previous studies conducted by our group have demonstrated that alcohol supplementation improves insulin signaling and endothelial dysfunction and increases myocardial perfusion and angiogenesis.^{3, 6, 7} Perhaps the improved insulin signaling optimizes cell energy utilization and the increased blood supply to the ischemic myocardium reduces the ischemic insult, resulting in an overall reduction in cell stress and cell death. Our group has also demonstrated that alcohol supplementation reduces oxidative stress in ischemic myocardium.³ The reduction in oxidative stress may also reduce the stimulus for intrinsic apoptosis and cell stress. The results of this study are consistent with previous studies supporting the cardioprotective effects of low-moderate alcohol consumption, and provide further mechanistic insight for alcohol-mediated cardioprotection.

Limitations

There are several limitations of the current study. Firstly, the study was limited to 7 weeks of alcohol supplementation and animals were all harvested at one time point. It is possible alcohol modulates apoptosis and survival signaling differently at varying time points. Secondly, only one dose of alcohol was given in this study. It is well established that alcohol has a dose dependent effect on coronary artery disease. In this study, we chose to study a moderate dose of alcohol consumption. It would have been useful to repeat this experiment using multiple doses of alcohol to determine the dose response of alcohol on apoptosis and survival signaling. Lastly, although this is a well-established and validated porcine model of chronic myocardial ischemia, animal models do not completely replicate human disease.

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Abbreviations

SUC	sucrose group
ETOH	ethanol group
TNFα	tumor necrosis factor α
BCL2	phosphorylated B-cell CLL/Lymphoma 2
AMPK	5'adenosine monophosphate-activated protein kinase
BAD	BCL2 associated death promoter
Caspase	cysteine aspartic acid specific protease
MTOR	mammalian target of rapamycin

AKT	protein kinase B
FOXO3	forkhead box protein O3
TUNEL	terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling

References

1. Roger VL, Go AS, Lloyd-Jones DM, et al. American Heart Association Statistics C, Stroke Statistics S. Heart disease and stroke statistics--2012 update: A report from the American Heart Association. *Circulation*. 2012; 125:e2–e220. [PubMed: 22179539]
2. Krenz MKR. Moderate ethanol ingestion and cardiovascular protection: From epidemiologic associations to cellular mechanisms. *J Mol Cell Cardiol*. 2012; 52:93–104. [PubMed: 22041278]
3. Chu LM, Lassaletta AD, Robich MP, et al. Effects of red wine and vodka on collateral-dependent perfusion and cardiovascular function in hypercholesterolemic swine. *Circulation*. 2012; 126:S65–S72. [PubMed: 22965995]
4. Elmadhun NY, Lassaletta AD, Chu LM, et al. Vodka and wine consumption in a swine model of metabolic syndrome alters insulin signaling pathways in the liver and skeletal muscle. *Surgery*. 2012; 152:414–422. [PubMed: 22938901]
5. Lassaletta AD, Chu LM, Elmadhun NY, et al. Cardioprotective effects of red wine and vodka in a model of endothelial dysfunction. *J surg Res*. 2012; 178:586–592. [PubMed: 22748601]
6. Lassaletta AD, Elmadhun NY, Liu Y, et al. Ethanol promotes arteriogenesis and restores perfusion to chronically ischemic myocardium. *Circulation*. 2013; 128:S136–S143. [PubMed: 24030397]
7. Elmadhun NY, Lassaletta AD, Burgess T, et al. Alcohol consumption improves insulin signaling in the myocardium. *Surgery*. 2013; 154:320–327. [PubMed: 23889958]
8. Hajnoczky G, Buzas CJ, Pacher P, et al. Alcohol and mitochondria in cardiac apoptosis: Mechanisms and visualization. *Alcohol Clin Experiment Res*. 2005; 29:693–701.
9. Lassaletta AD, Chu LM, Robich MP, et al. Overfed Ossabaw swine with early stage metabolic syndrome have normal coronary collateral development in response to chronic ischemia. *Basic Res Cardiol*. 2012; 107:243. [PubMed: 22231675]
10. Whelan RS, Kaplinskiy V, Kitsis RN. Cell death in the pathogenesis of heart disease: Mechanisms and significance. *Ann Rev Physiol*. 2010; 72:19–44. [PubMed: 20148665]
11. Perrelli MG, Pagliaro P, Penna C. Ischemia/reperfusion injury and cardioprotective mechanisms: Role of mitochondria and reactive oxygen species. *World J Cardiol*. 2011; 3:186–200. [PubMed: 21772945]
12. Konstantinidis K, Whelan RS, Kitsis RN. Mechanisms of cell death in heart disease. *Arterioscler Thromb Vasc Biol*. 2012; 32:1552–1562.
13. Lam EW, Francis RE, Petkovic M. Foxo transcription factors: Key regulators of cell fate. *Biochem Soc Trans*. 2006; 34:722–726.
14. Towler MC, Hardie DG. Amp-activated protein kinase in metabolic control and insulin signaling. *Circ Res*. 2007; 100:328–341. [PubMed: 17307971]
15. Terai K, Hiramoto Y, Masaki M, et al. Amp-activated protein kinase protects cardiomyocytes against hypoxic injury through attenuation of endoplasmic reticulum stress. *Mol Cell Biol*. 2005; 25:9554–9575. [PubMed: 16227605]
16. Collins MA, Neafsey EJ, Mukamal KJ, et al. Alcohol in moderation, cardioprotection, and neuroprotection: Epidemiological considerations and mechanistic studies. *Alcohol Clin Experiment Res*. 2009; 33:206–219.
17. Miyamae M, Kaneda K, Domae N, Figueredo VM. Cardioprotection by regular ethanol consumption: Potential mechanisms and clinical application. *Current Drug Abuse Rev*. 2010; 3:39–48.
18. Miyamae M, Diamond I, Weiner MW, et al. Regular alcohol consumption mimics cardiac preconditioning by protecting against ischemia-reperfusion injury. *Proc Nat Acad Sciences*. 1997; 94:3235–3239.

19. Di Castelnuovo A, Costanzo S, Donati MB, et al. Prevention of cardiovascular risk by moderate alcohol consumption: Epidemiologic evidence and plausible mechanisms. *Int Emerg Med*. 2010; 5:291–297.

Figure 1.

Ischemic territory. TUNEL staining in the chronically ischemic myocardium. Cell-death specific staining of frozen sections of the chronically ischemic left ventricle. Brown staining indicates cell death. *** $p < 0.001$. ETOH, ethanol group; SUC, sucrose group; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling.

Figure 2.

Normally perfused territory. TUNEL staining in the normally perfused, non-ischemic myocardium. Cell-death specific staining of frozen sections of the normally perfused, non-ischemic left ventricle. Brown staining indicates cell death. *** $p < 0.001$. ETOH, ethanol group; SUC, sucrose group; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling.

Table 1

Apoptosis and Survival Signaling in the Ischemic Myocardium.

Protein target	SUC	ETOH	p Value
TNF α	1.0 $\pm$ 0.154	0.62 $\pm$ 0.135	0.095
AKT	1.0 $\pm$ 0.109	0.88 $\pm$ 0.080	0.37
pAKT (Thr 308)	1.0 $\pm$ 0.114	0.77 $\pm$ 0.061	0.1
pBCL-2	1.0 $\pm$ 0.122	0.98 $\pm$ 0.052	0.87
AMPK	1.0 $\pm$ 0.161	1.59 $\pm$ 0.154	0.02
pAMPK (Thr 172)	1.0 $\pm$ 0.370	2.28 $\pm$ 0.162	0.01
BAD	1.0 $\pm$ 0.102	0.45 $\pm$ 0.124	0.01
pBAD (Ser112)	1.0 $\pm$ 0.163	0.84 $\pm$ 0.099	0.45
FOXO3	1.0 $\pm$ 0.074	0.72 $\pm$ 0.100	0.05
pFOXO3 (Ser 318/321)	1.0 $\pm$ 0.131	2.48 $\pm$ 0.232	0.0001
CASPASE 9	1.0 $\pm$ 0.147	0.66 $\pm$ 0.041	0.05
CLEAVED CASPASE 3	1.0 $\pm$ 0.079	1.06 $\pm$ 0.084	0.62
MTOR	1.0 $\pm$ 0.099	0.62 $\pm$ 0.060	0.01
pMTOR (Ser 2481)	1.0 $\pm$ 0.140	0.70 $\pm$ 0.091	0.03
Deptor	1.0 $\pm$ 0.145	1.41 $\pm$ 0.080	0.03

Western blot analysis for expression of apoptosis and survival proteins in the chronically ischemic left ventricle in the territory of the left circumflex coronary artery. Levels represent fold change $\pm$ standard error of the mean compared to SUC.

ETOH, ethanol group; SUC, sucrose group.

Table 2

Apoptosis and Survival Signaling in the Non-Ischemic Myocardium.

Protein target	SUC	ETOH	p Value
TNF α	1.00 $\pm$ 0.064	1.01 $\pm$ 0.032	0.85
AKT	1.00 $\pm$ 0.018	1.09 $\pm$ 0.012	0.001
pAKT (T308)	1.00 $\pm$ 0.057	1.16 $\pm$ 0.027	0.03
pBCL2 (S70)	1.00 $\pm$ 0.050	1.29 $\pm$ 0.053	0.002
AMPK	1.00 $\pm$ 0.046	1.21 $\pm$ 0.054	0.01
pAMPK (T172)	1.00 $\pm$ 0.034	0.99 $\pm$ 0.023	0.98
BAD	1.00 $\pm$ 0.174	1.12 $\pm$ 0.040	0.5
pBAD (S112)	1.00 $\pm$ 0.090	1.31 $\pm$ 0.078	0.02
FOXO3	1.00 $\pm$ 0.062	1.37 $\pm$ 0.072	0.002
pFOXO3 (S318/321)	1.00 $\pm$ 0.019	1.09 $\pm$ 0.031	0.03
CASPASE 9	1.00 $\pm$ 0.090	0.70 $\pm$ 0.052	0.01
Cleaved CASPASE 3	1.00 $\pm$ 0.071	1.17 $\pm$ 0.093	0.18
MTOR	1.00 $\pm$ 0.053	1.03 $\pm$ 0.055	0.68
pMTOR (S2481)	1.00 $\pm$ 0.029	0.89 $\pm$ 0.026	0.01
Deptor	1.00 $\pm$ 0.041	1.35 $\pm$ 0.042	<0.01

Western blot analysis for the expression of apoptosis and survival proteins in the non-ischemic normally perfused left ventricle in the territory of the left anterior descending coronary artery. Levels represent fold change $\pm$ standard error of the mean compared to SUC.

ETOH, ethanol group; SUC, sucrose group.