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Review

Molecular mechanisms responsible for alcohol-induced myopathy in skeletal muscle and heart

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Abstract

Chronic alcohol abuse has the potential to modulate striated muscle physiology and function. The skeletal muscle alcoholic myopathy is characterized by muscle weakness and difficulties in gait and locomotion, while chronic alcohol consumption ultimately leads to a decrease in cardiac contractility and output. In both tissues a loss of protein mass results in part from a decreased protein synthesis that initially manifests as a defect in translational efficiency. This review focuses on recent developments in understanding the cellular and molecular mechanisms by which alcohol impairs mRNA translation in skeletal and cardiac muscle, including identification of the signaling pathways and biochemical sites negatively impacted. Defective signaling potentially results from resistance to the normal stimulating effects of anabolic hormones (insulin and insulin-like growth factor-I) and nutrients (leucine) as well as increased production of several negative regulators of muscle mass. Overall, the biochemical mechanisms contributing to the pathogenesis of loss of skeletal and cardiac muscle are reviewed.

Keywords: Myopathy; Atrophy; Protein synthesis; Translational control; Alcohol

Contents

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1. Introduction

Alcohol abuse, both chronic use and acute intoxication, exacts a staggering economic cost to society and remains a major public health problem (Kochanek & Smith, 2004). Excessive alcohol consumption is associated not only with increased mortality, but also with premature death and preventable health concerns (Stinson, Williams, Bertolucci, & Noble, 1987). Alcohol continues to play a major role in the admission and mortality of patients in intensive care units and alcohol abuse increases morbidity among hospitalized patients in general (Delgado-Rodriguez, Gomez-Ortega, Mariscal-Ortiz, Palma-Perez, & Sillero-Arenas, 2003; Mostafa & Murthy, 2002).

Among some of the earliest recognized, but least understood, changes produced by sustained and excessive consumption of alcohol are the plethora of ultrastructural, biochemical and physiological alterations in striated muscle—both skeletal and cardiac. A number of excellent reviews are available presenting a historical perspective of this syndrome as well as earlier hypotheses regarding its etiology (Preedy, Keating, & Peters, 1992; Preedy, Salisbury, & Peters, 1994; Preedy et al., 1996, 2001, 2003; Urbano-Marquez & Fernandez-Sola, 2004). For the most part, this early literature will not be reiterated. Instead, the review will focus on recent findings pertaining to the cellular and molecular mechanisms underlying the often-reported cardiac and skeletal muscle myopathy. Specifically, the review will highlight alcohol-induced alterations related to the synthetic side of the protein balance equation because of the paucity of definitive data on the effect of alcohol on protein degradation in muscle (Lang, Kimball, Frost, & Vary, 2001a; Lang, Silvis, Nystrom, & Frost, 2001b).

2. Cardiac myopathy

2.1. Prevalence and clinical significance

Considerable attention has been directed towards an understanding of alcohol-induced effects on cardiovascular function. Moderate alcohol consumption is associated with a reduced risk of cardiovascular disease, a finding interpreted to be representative of blood vessel events (i.e., reduced atherogenesis due to antithrombotic, antioxidant and anti-inflammatory effects as well as alterations in cholesterol metabolism). In contrast, heavy alcohol consumption is detrimental to the heart and associated with an increased mortality that is independent of coronary artery disease (Faris, Henein, & Coats, 2003). Alcoholic heart muscle disease occurs in patients in whom the sole causative agent is excessive and prolonged alcohol consumption

(>80 g of ethanol a day for >10 years), but is rarely produced by short-term ethanol administration. Clinical manifestations of alcoholic heart muscle disease encompass defects in myocardial contractility involving both systolic and diastolic function. This abuse leads to the development of a dilated cardiomyopathy and, ultimately, to a low-output heart failure. Alcoholic patients with advanced heart failure have a relatively poor prognosis, particularly if alcohol consumption continues, with fewer than 25% surviving more than 3 years (Spies et al., 2001).

2.2. Inhibition of protein synthesis

Alcohol-induced defects in protein metabolism may contribute to early changes in myocardial structure and reductions in left ventricular (LV) function through changes in ventricular mass. Combined left and right ventricular weight is significantly reduced in rats fed a nutritionally complete diet containing alcohol for 16 weeks and this alcoholic myopathy results from a decreased protein content (Vary, Lynch, & Lang, 2001). This loss of myocardial protein is due in large part to a marked decrease in protein synthesis that is independent of nutritional status of the alcohol-fed rats. Although the synthesis of total mixed proteins represents the average of the synthesis of myofibrillar proteins and non-myofibrillar (sarcoplasmic) proteins, alcohol feeding has also been shown to specifically decrease the rate of synthesis of myofibrillar proteins (Vary & Deiter, 2005).

Myosin heavy chain and desmin are reduced under these conditions (Patel et al., 1997; Richardson, Patel, & Preedy, 1998). The myocardial content of actin, myosin light chain I and II, tropomyosin, and vimentin showed no reductions with alcohol feeding for 6 weeks (Patel et al., 1997). However, a reduction of both ventricular α-myosin and actin content is observed with prolonged (16 weeks) chronic ethanol consumption (Vary & Deiter, 2005). In contrast, the expression of β-myosin was increased in hearts from alcohol-fed rats (Meehan, Piano, Solaro, & Kennedy, 1999; Vary & Deiter, 2005). The decreased content of actin and myosin does not result from a concomitant reduction in mRNA content, indicating that the loss of the myocardial content of these two proteins in alcohol-fed rats results, in part, from an inhibition of the mRNA translation.

Several potential mechanisms may explain the alcohol-induced inhibition of myocardial protein synthesis. In this regard, alcohol consumption does not lower the plasma availability of total amino acids (Lang et al., 1999b) to a rate-limiting concentration or alter the energy charge of the heart (Vary et al., 2001a). Furthermore, the reduction in protein synthesis did not result from a diminished mRNA content or the abundance of ribosomes (Lang et al., 1999a; Lang, Frost, Kumar, & Vary, 2000a; Vary et al., 2001a; Vary, Nairn, Deiter, & Lang, 2002; Vary, Nairn, & Lang, 2004). Therefore, alcohol appears to decrease cardiac protein synthesis by decreasing translational efficiency.

2.3. Alcohol inhibits translation initiation

Protein synthesis is a multistep, highly regulated process that includes amino acid transport, signal transduction events, transcription, and translation. The process of mRNA translation is composed of three highly regulated stages referred to as initiation, elongation, and termination. Regulatory control of this process can be exerted at the level of either peptide-chain initiation or elongation. The phase of the translation process affected by chronic alcohol feeding has been examined by determining the distribution of ribosomal subunits between free (i.e., ribosomal subunits not associated with polysomes) and the polysome-associated state. Analysis of hearts from chronic alcohol-fed rats reveals no difference in ribosomal subunit distribution indicating a relatively equal inhibition of both translation initiation and elongation (Lang et al., 1999a; Vary et al., 2001a). In contrast, acute ethanol intoxication is associated with an increased myocardial content of free 40S and 60S ribosomal subunits in conjunction with an inhibition in protein synthesis (Lang et al., 2000a; Lang, Frost, Kumar, Wu, & Vary, 2000b). Therefore, acute alcohol intoxication preferentially affects peptide-chain initiation, while long-term ethanol ingestion affects both initiation and elongation.

Two steps in translation initiation have been identified as major regulatory points in the overall control of protein synthesis (Browne & Proud, 2002). The first one is the binding of met-$tRNA_i$ to the 40S ribosomal subunit to form the 43S preinitiation complex. This reaction is facilitated by eukaryotic initiation factor (eIF)-2 and is regulated in part by the activity of eIF2B. The second regulatory step involves the recognition,

unwinding and binding of mRNA to the 43S preinitiation complex, catalyzed by a multi-subunit complex of eukaryotic factors referred to as eIF4F (Sonenberg & Dever, 2003).

One mechanism that may account for the alcohol-induced decrease in protein synthesis is an alteration in amount or activity of specific eIF proteins. However, feeding rats an alcohol-containing diet for 14 weeks does not reduce the abundance of eIF2B protein or eIF2B activity in cardiac muscle (Lang et al., 1999a). Additionally, no change was detected in either total eIF2α or the amount of the inactive phosphorylated form of eIF2β in hearts from alcohol-fed rats. Therefore, inhibition of cardiac protein synthesis following alcohol is not due to a defect in eIF2B-mediated guanine nucleotide exchange or a limitation in the formation of the 43S preinitiation complex (Lang et al., 2000a, 2001a; Vary et al., 2001a; Vary, Lynch, & Lang, 2001b).

The next step in the process of mRNA translation initiation is formation of 48S initiation complex catalyzed by the eIF4F complex (Pain, 1986). eIF4F is a heterotrimeric protein complex composed of (1) eIF4A (a RNA helicase that unwinds secondary structure in 5′-untranslated region of mRNA), (2) eIF4E (a protein that binds directly to the m^7GTP cap structure present at the 5′-end of most eukaryotic mRNAs), and (3) eIF4G (a protein that functions as a scaffold for eIF4E, eIF4A, the mRNA and the ribosome). eIF4G appears to be the nucleus around which the initiation complex forms because it possesses binding sites not only for eIF4E but also for eIF4A and eIF3. eIF4E is available in relatively limited amounts and plays a critical role in determining global rates of mRNA translation. eIF4E is regulated by alterations in either its availability or extent of phosphorylation (Morley & Traugh,1990; Sonenberg & Dever, 2003). Decreasing eIF4E content through transfection with anti-sense RNA causes an inhibition of protein synthesis. However, the myocardial content of eIF4E is not decreased in rats acutely intoxicated or fed an alcohol-containing diet (Lang et al., 1999a, 2000a, 2003a; Vary et al., 2001a, 2004). Therefore, alcohol does not inhibit protein synthesis by limiting the overall cellular content of eIF4E.

Phosphorylation of eIF4E enhances the affinity of the factor for the m^7GTP cap on mRNA as well as for eIF4G and eIF4A, and correlates with enhanced rates of protein synthesis in cultured cells stimulated with various mitogens or oncogenes (Morley & Traugh, 1990). Conversely, reduced phosphorylation of eIF4E correlates with an inhibition of protein synthesis produced by serum depletion (Duncan, Milburn, & Hershey, 1987). The phosphorylation of eIF4E in heart is not affected by ethanol consumption (Lang et al., 2000a, 2001a; Vary et al., 2001a), indicating this is not an important mechanism mediating alcoholic cardiomyopathy.

Translation initiation may also be regulated through the formation of the eIF4E·eIF4G complex (Shah, Anthony, Kimball, & Jefferson, 2000). A strong positive linear relationship between rates of protein synthesis and the amount of eIF4G associated with eIF4E has been reported in hearts under in vivo conditions (Vary et al., 2001b). Moreover, the assembly of the active eIF4E·eIF4G complex is diminished in hearts from rats administered ethanol either acutely or chronically (Lang et al., 1999a, 2000a, 2001a; Vary et al., 2001a, 2004). The relationship between decreased protein synthesis and the reduced amount of the eIF4G·eIF4E complex is consistent with the proposed role of this complex in the regulation of translation initiation.

Formation of the active eIF4E·eIF4G complex is controlled by the (1) amount of eIF4G, (2) availability of eIF4E, and (3) phosphorylation of eIF4G. Diminished eIF4E·eIF4G complex does not result from a reduced myocardial eIF4G protein content following alcohol intoxication (Lang et al., 1999a, 2000a; Vary et al., 2001b, 2004). The availability of eIF4E for binding to eIF4G is regulated, in part, through the association of eIF4E with a family of translational repressor proteins (4E-BPs) (Pause et al., 1994). 4E-BP1 is the predominant form of the 4E-BPs in heart (Kimball & Vary, unpublished data). When eIF4E is bound to 4E-BP1, eIF4E cannot bind to eIF4G. Consequently, less mRNA binds to the ribosome, thereby inhibiting cap-dependent translation of mRNA by physically sequestering eIF4E into an inactive eIF4E·4E-BP1 complex. eIF4E binding to 4E-BP1 is reduced following phosphorylation of 4E-BP1 through a PI3-kinase-dependent pathway involving signaling through AKT/PKB and mTOR (Kimball, Jurasinski, Lawrence, &, Jefferson, 1997). Phosphorylation of 4E-BP releases eIF4E from 4E-BP1 and allows the eIF4E·mRNA complex to bind to eIF4G and, then to the 40S ribosome. In hearts of animals consuming alcohol, there is a decreased phosphorylation of 4E-BP1. This results in increased

formation of the inactive eIF4E·4E-BP1 complex with a reciprocal reduction in abundance of the eIF4E·eIF4G complex (Lang et al., 2000a; Vary et al., 2001a, 2004). Hence, a major portion of the alcohol-induced alteration in the formation of eIF4E·eIF4G complex appears mediated via mechanisms limiting phosphorylation of 4E-BP1. These data indicate that alcohol limits mRNA translation initiation by inhibiting the binding of mRNA to the 43S preinitiation complex, a process mediated by eIF4F.

2.4. Alterations in peptide-chain elongation

Peptide-chain elongation is the programmed assembly of amino acids into a polypeptide as dictated by the sequence of bases in the mRNA and thus contributes to the overall regulation of protein synthesis. This latter phase involves the sequential addition of amino acid residues to the carboxy-terminal end of the nascent peptide, and requires two elongation factors (EFs); eEF1A and eEF2 (Browne & Proud, 2002). During elongation, the aminoacyl-tRNA associates with the A-site on the ribosome forming a ternary complex with eEF1A and GTP. After hydrolysis of GTP, which is catalyzed by eEF1A (formerly known as eEF1α), the eEF1·GDP complex leaves the ribosome, and a peptide bond between the nascent peptide chain on the ribosomal P-site and aminoacyl-tRNA is formed. eEF2 then catalyzes the translocation of the elongated peptidyl-tRNA from the A-site on the ribosome and the ejection of the deacylated tRNA from the P-site.

In response to acute alcohol intoxication the inhibition of translation efficiency occurs secondary to a block in peptide-chain initiation (Lang et al., 2000a, 2000b). As the duration of ethanol exposure increases, the inhibition of myocardial protein synthesis that develops results from an inhibition in both the initiation and elongation phases of translation (Lang et al., 1999a). The protein content of eEF1A and eEF2 is reduced in hearts from rats fed a diet containing alcohol for 16 weeks, but not in those animals fed the alcohol-containing diet for 8 or 12 weeks (Vary et al., 2002, 2004). The decreased eEF protein content is not associated with a concomitant reduction in the mRNA abundance for either eEF1A or eEF2. The phosphorylation state of eEF2 in heart is also not affected by chronic alcohol consumption. Hence, decreases in eEF1A and eEF2 protein may partially explain the inhibition of elongation and translation observed in response to relatively prolonged alcohol consumption.

Because alcohol consumption does not directly alter the myocardial content of eEF1A and eEF2 mRNA, it is likely that the defect in elongation occurs through decreased translation of mRNA encoded for by these two elongation factors. eEF1A and eEF2 mRNAs possess a regulatory sequence containing a 5′-tract of oligopyrimidines (5′-TOP) that controls their translation. The synthesis of these factors correlates in part with alterations in the phosphorylation of ribosomal protein S6 via the S6K1-mTOR-signaling pathway. The constitutive phosphorylation of both S6K1 and S6 is reduced in hearts from animals given alcohol, and would be expected to lower the myocardial content of eEF1A and eEF2. The observation that inhibition of mTOR by rapamycin has only a mild repressive effect on translation of 5′-TOP mRNAs (Stolovich et al., 2002) is consistent with the findings that ethanol requires extended periods (>8 weeks) to lower the cellular content of eEF1A and eEF2 (Vary et al., 2002). Thus, reduced mTOR signaling may diminish the phosphorylation of both 4E-BP1 and S6K1, thereby inhibiting cardiac protein synthesis following alcohol consumption.

Heat shock protein (HSP)-70 is representative of a class of proteins that facilitate protein/protein interactions, acting as a molecular chaperone. HSP70 plays a role in the peptide-chain elongation step of mRNA translation by guiding the growing nascent polypeptide through the channel on the 80S ribosome (Beckmann, Mizzen, & Welch, 1990). Mutants of HSP70 lead to a slow-growing phenotype with a reduced proportion of actively translating ribosomes (Nelson, Ziegelhoffer, Nicolet, Werner-Washbourne, & Craig, 1990). Furthermore, decreased polysome-associated HSP70 may slow peptide-chain elongation during skeletal muscle atrophy (Ku, Yang, Menon, & Thomason, 1995). Members of the HSP70 family are composed of the constitutive 70 kDa heat shock cognate (HSC70) and the inducible HSP70 form. In cardiac muscle, HSC70 is unaffected by chronic alcohol feeding. In contrast, HSP70 is reduced in rats fed a diet containing ethanol for between 6 and 16 weeks (Patel et al., 1997; Vary & Deiter, 2005). The changes in HSP70 protein are the result of a decrease in the abundance of HSP70 mRNA following alcohol feeding in rats and may be suggestive of a greater susceptibility to cardiac derangements.

2.5. Restoration of myocardial protein synthesis

Prognosis improves in patients with alcoholic cardiomyopathy if complete abstinence from alcohol is accomplished. However, it remains unknown how long the adverse effects of alcohol consumption on protein synthesis persist following withdrawal from ethanol. Withdrawal of alcohol from the diet after continued consumption for 6 months restores rates of cardiac protein synthesis to values not different from those of pair-fed control rats (Vary et al., 2004). The restoration of protein synthesis correlates with the reversal of defects in the phosphorylation of 4E-BP1 and S6K1, formation of the active eIF4E·eIF4G complex, and the reduced eEF1A and eEF2 content. Hence, irreversible changes in protein metabolism in cardiac muscle have not occurred, at least at this stage.

2.6. Alterations in ventricular wall thickness and function

Chronic alcohol consumption (>20 years) in humans alters cardiac structure and function, and these changes are dependent upon the duration of alcohol consumption. Short-term exposure to an alcohol-containing diet does not diminish LV mass or wall thickness. In contrast, feeding rats alcohol for 24 weeks reduces the mass and thickness of the LV wall, the interventricular septum, and the posterior wall (Fig. 1). These data indicate that a thinning of ventricular walls is a common feature observed with alcoholic cardiomyopathy and temporally follows the fall in the protein synthetic rate. There is no preferential loss of a portion of the left ventricle myocardium as the ratio of intraventricular septum thickness to posterior wall thickness is unchanged (Table 1). Changes in the ventricular muscle dimension can be expected to eventually modulate cardiac function. After 6 months, cardiac output was reduced which was produced by a secondary reduction in stroke volume because heart rate was unaltered (Table 1). The reduction in stroke volume results from a diminished LV volume with no change in systolic volume. Additional analysis revealed that chronic alcohol exposure decreases the LV systolic and diastolic dimensions (Table 1).

When the duration of alcohol exposure is extended to 1 year, signs of progressive heart disease with evidence of further myocardial derangements were

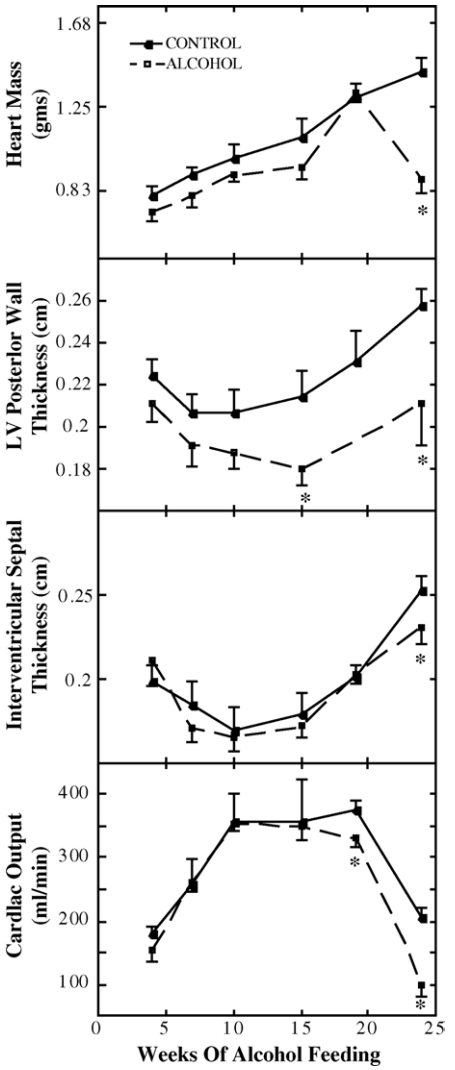

Fig. 1. Changes in cardiac structure and function in rats in response to various durations of consuming an alcohol-containing diet. Panel A, changes in ventricular septal thickness determined by echocardiography; Panel B, changes in ventricular septal thickness determined by echocardiography; Panel C, changes in ventricular septal thickness determined by echocardiography; and Panel D, changes in cardiac output (cardiac output was calculated by multiplying the stroke volume by the heart rate using data presented in Table 1). Values shown are mean ± S.E. for $n = 5$–9 in each group. *$p < 0.05$ vs. pair-fed controls at the same time.

observed (Kim, Beck, Bieniarz, Schumacher, & Piano, 2001; Piano, 2002). In particular, 1 year of chronic alcohol consumption is associated with the development of dilated cardiomyopathy characteristic of symp-

Table 1
Effect of chronic alcohol feeding on cardiac structure and function

Parameter	Condition	Weeks of alcohol feeding					
		4	7	10	16	19	24
Heart rate (beats/min)	Control	441 ± 13	400 ± 29	418 ± 19	398 ± 21	396 ± 5	370 ± 21
	Alcohol	457 ± 14	405 ± 21	418 ± 11	398 ± 13	370 ± 16	383 ± 16
Stroke volume (ml)	Control	0.42 ± 0.04	0.72 ± 0.05	0.85 ± 0.10	1.02 ± 0.12	0.92 ± 0.04	0.69 ± 0.08
	Alcohol	0.33 ± 0.03	0.66 ± 0.04	0.85 ± 0.03	0.88 ± 0.05	0.94 ± 0.07	0.58 ± 0.06*
Diastolic dimension (mm)	Control	0.56 ± 0.02	0.68 ± 0.01	0.72 ± 0.03	0.74 ± 0.04	0.75 ± 0.01	0.67 ± 0.03
	Alcohol	0.52 ± 0.02	0.67 ± 0.02	0.73 ± 0.01	0.74 ± 0.02	0.77 ± 0.02	0.58 ± 0.03*
Systolic dimension (mm)	Control	0.05 ± 0.01	0.17 ± 0.04	0.17 ± 0.02	0.21 ± 0.03	0.21 ± 0.04	0.19 ± 0.02
	Alcohol	0.04 ± 0.01	0.16 ± 0.01	0.17 ± 0.03	0.14 ± 0.02	0.23 ± 0.03	0.12 ± 0.02*
IVS/LVPW	Control	0.88 ± 0.3	0.88 ± 0.04	0.84 ± 0.05	0.86 ± 0.09	0.86 ± 0.06	0.99 ± 0.04
	Alcohol	1.0 ± 0.1	0.94 ± 0.03	0.89 ± 0.05	0.94 ± 0.03	0.90 ± 0.04	1.04 ± 0.11
Fractional shortening (%)	Control	91 ± 1	74 ± 6	84 ± 4	72 ± 4	73 ± 5	72 ± 3
	Alcohol	94 ± 1	76 ± 3	89 ± 5	81 ± 3	70 ± 3	79 ± 3

Values shown are expressed as mean ± S.E. for $n = 5$–9 animals in each group.
* $P < 0.05$ compared to time-matched control values.

tomatic alcohol abusers. This defect is exemplified by alcohol-induced increases in end-diastolic diameter, end-systolic diameter, and LV mass. In addition, the LV pressure-volume relationship is shifted down and to the right, and is characteristic of a dilated ventricle. These observations are consistent with a slow progressive decline in ventricular function that ultimately results in heart failure with prolonged alcohol consumption—a pathology that is preceded by a decrease in myocardial protein synthesis.

3. Skeletal muscle myopathy

3.1. Prevalence and clinical significance

Excessive ethanol consumption results in the erosion of lean body mass (LBM) with the prevalence of chronic alcohol myopathy estimated at between 45 and 70% (Preedy et al., 1994). Regardless of the exact number of afflicted individuals, this myopathy is one of the most prominent muscle diseases and occurs more frequently than many of the hereditary myopathies (Preedy et al., 2001). The myopathy is characterized by proximal muscle weakness and often leads to impaired ambulation, frequent falls and a generalized reduction in quality of life indices. The alcohol-induced atrophy of skeletal muscle is proportional to the total lifetime alcohol ingestion and, under severe conditions, may lead to the erosion of up to 20% of the entire muscle mass (Martin, Ward, Slavin, Levi, & Peters, 1985; Urbano-Marquez et al., 1995). The underlying etiology of the loss of LBM in this patient population remains poorly elucidated, but cannot be explained by disturbances in electrolytes, peripheral neuropathy, oxidative injury, overt hepatic toxicity, inactivity or deficiencies in nutrients, vitamins or minerals (Lang et al., 2001a; Preedy et al., 1992, 1994). Hence, while these factors along with genetic, gender and environmental determinants can modulate the extent and severity of the disease, they do not appear to play a causative role.

3.2. Alterations in protein synthesis

Chronic consumption of an alcohol-containing diet by rats for at least 6 weeks produces a myopathy characterized by decreased skeletal muscle protein content and weight (Preedy & Peters 1988a, 1992). Consistent with these observations is an alcohol-induced decrease in fat-free mass determined noninvasively using whole body electrical conductivity (Lang et al., 1999a). The muscle content of myofibrillar and sarcoplasmic proteins is maintained by the dynamic balance of protein synthesis and protein degradation. However, because there are few if any definitive studies on proteolysis per se, the second part of this review will focus on alcohol-induced derangements in protein synthesis.

A decreased rate of in vivo-determined muscle protein synthesis is a consistent finding in rats fed an ethanol-containing nutritionally complete diet. A similar reduction has been reported in alcoholic patients (Pacy, Preedy, Peters, Read, & Halliday, 1991). This decrement negatively impacts both myofibrillar and sacroplasmic proteins (Preedy & Peters, 1988a, 1998b; Lang et al., 1999a). In some animal studies the decreased protein synthesis is partially dependent upon a reduction in the number of ribosomes (Preedy & Peters, 1988b). This change may result from an increased RNase activity (e.g., total RNase, RNase A and RNase T1L) within the muscle (Reilly, Erylmaz, Amir, Peters, & Preedy, 1998). In contrast, other studies show that the impaired protein synthesis is independent of a change in ribosome number (Lang et al., 1999a). Thus, these data indicate that at least a portion and in some cases all of the chronic alcohol-induced decrease in muscle protein synthesis results from a decline in translational efficiency. Furthermore, acute alcohol intoxication ethanol is uniformly reported to proportionally decrease both muscle protein synthesis and translational efficiency (Reilly et al., 1997; Lang et al., 2000b; Lang, Liu, Nystrom, Wu, & Vary, 2000).

The synthesis of both myofibrillar and sarcoplasmic proteins in skeletal muscle appears equally affected by alcohol (Preedy et al., 1997). This impairment is observed within the first hour after an intoxicating dose of ethanol and a portion of the inhibitory effect persists up to 24 h later (Reilly et al., 1997). Although all muscles are affected to some extent, the alcohol-induced decrease in protein synthesis is most evident in muscles with a predominance of type II fast-twitch fibers (plantaris and gastrocnemius) as opposed to muscle with a predominantly type I slow-twitch fibers (e.g., soleus). This type-II muscle fiber atrophy is also seen in humans who chronically abuse alcohol (Hanid et al., 1981) as well as the wasting observed in other catabolic conditions such as sepsis, diabetes and glucocorticoid excess (Flaim, Copenhaver, & Jefferson, 1980; Lang et al., 2001a; Vary & Kimball, 1992). Furthermore, the sustained consumption of alcohol produces specific reductions in the Iβ, IIx and IIb myosin isoforms in muscle (Reilly, McKoy, Peters, Goldspink, & Preedy, 2000). In the case of the latter two myosin isoforms, their respective mRNAs were not concomitantly reduced, a finding consistent with a decreased translational efficiency.

3.3. Alterations in eIF2/2B system

As described above, the binding of met-tRNA to the 40S subunit to form the 43S preinitiation complex is mediated by eIF2 (Pain, 1986). Similar to results from cardiac muscle, alcohol does not alter either the total content or extent of phosphorylation of the α-subunit of eIF2. The ability of eIF2 to form a ternary complex can also be regulated by eIF2B that catalyzes guanine nucleotide exchange and is required to regenerate the active eIF2·GTP complex. Our studies show a small but significant reduction in the activity of eIF2B (Lang et al., 1999a). However, this impairment cannot be explained by a concomitant reduction in the amount or phosphorylation of eIF2B, or changes in the redox state. Hence, the mechanism by which eIF2B activity is reduced by alcohol and its physiological significance remains to be elucidated.

3.4. Eukaryotic initiation factor-4F formation

A second critical focus of translational regulation involves the binding of the 5′-end of cellular mRNA to the 43S preinitiation complex, a reaction mediated by the cap-binding protein eIF4F (Sonenberg & Dever, 2003). Of the three major proteins that form a functional eIF4F complex, eIF4E is the least abundant in muscle and under many conditions may be rate limiting in the binding of mRNA to ribosomes. Although neither alcohol feeding nor acute ethanol intoxication decreases the relative abundance of total eIF4E protein, both markedly alter the availability of eIF4E as evidenced by the increased inactive eIF4E·4E-BP1 complex and the reciprocal decrease in the amount of the active eIF4E·eIF4G complex. This redistribution of eIF4E is mediated by a decreased phosphorylation of 4E-BP1 (Lang et al., 1999a, 2000b).

The interaction between eIF4E and eIF4G may also be regulated by the posttranslation modification of either protein. While the phosphorylation of eIF4E increases the ability of this initiation factor to bind to the cap mRNA and thereby stimulates protein synthesis, there is no detectable effect of alcohol on eIF4E phosphorylation in skeletal muscle (Lang et al., 1999a, 2000b, 2001). In contrast, constitutive eIF4G phosphorylation – a known positive regulator of translation initiation (Morley, Curtis, & Pain, 1997) – is modestly decreased in muscle in response to acute alcohol

intoxication (Lang, Kumar, Liu, Frost, & Vary, 2003b). Future studies will need to address the role of this change in the alcohol-induced decrease of eIF4F complex formation and protein synthesis.

It is noteworthy that the reduction in muscle protein synthesis and translation initiation in alcohol-fed rats is not a permanent phenomena, but represents a reversible process. When rats are withdrawn from the alcohol-containing diet for 3 days, protein synthesis returns to control values as do all measured indices of translation initiation. This observation is consistent with clinical findings demonstrating at least a partial restoration of biochemical changes as well as muscle strength after 2–12 months of abstinence from drinking alcohol (Peters, Martin, & Ward, 1985).

3.5. Alterations in mTOR and S6K1

In the protein synthetic signal transduction pathway, numerous anabolic stimuli appear to converge at the proline-directed Ser/Thr protein kinase mammalian target of rapamycin (mTOR) (Shah et al., 2000). In this canonical pathway mTOR represents a point of bifurcation whereby the stimulation of mTOR leads to the phosphorylation of 4E-BP1 along one pathway and the phosphorylation of S6K1 along a parallel pathway. Its activity is regulated in part by phosphorylation. The constitutive phosphorylation of mTOR is decreased in muscle from alcohol-treated rats (Lang et al., 2003a, 2003b). In addition, alcohol also decreases the phosphorylation of S6K1 and S6 in skeletal muscle (Kumar, Frost, & Lang, 2002a). It is noteworthy, that none of the above-mentioned alcohol-induced decreases in 4E-BP1, S6K1, S6, eIF4G or mTOR phosphorylation can be explained by a concomitant reduction in the phosphorylation (and activation) of the insulin or IGF-I receptor, IRS-1 or protein kinase B (PKB) in skeletal muscle (Kumar et al., 2002a). Thus, the impaired translational control of protein synthesis produced by alcohol appears mediated by factors that more directly affect mTOR activity.

3.6. Alterations in peptide-chain elongation

The content of eEF1A protein in fast-twitch skeletal muscle is not altered by acute alcohol intoxication or in response to up to 12 weeks of chronic alcohol consumption (Vary et al., 2004). However, eEF1A protein is decreased in muscle from rats maintained on the alcohol-containing diet for 16 weeks. Northern blot analysis revealed no diminution in the steady-state mRNA content for this protein at this late time point, suggesting a reduction in the translation of eEF1A mRNA already present or an accelerated rate of eEF1A degradation in muscle. In contrast, the eEF1A protein content in slow-twitch soleus muscle, which did not demonstrate a decreased protein synthesis in response to alcohol-feeding, remained unchanged. Cessation of alcohol consumption for 72 h is sufficient to restore the muscle content of eEF1A to levels observed in muscle from pair-fed control animals.

There is no effect of either acute alcohol intoxication or chronic alcohol consumption on total eEF2 protein content in skeletal muscle (Vary et al., 2004). The absence of a decrease in eEF2 after 16 weeks of alcohol-feeding is unexpected based on the above mentioned reduction in eEF1A at this time point and implies that these EFs may be regulated by different mechanisms (e.g., selective degradation of eEF1A versus eEF2). Furthermore, there is no change in eEF2 phosphorylation detected in skeletal muscle from rats maintained on the alcohol-containing diet for up to 16 weeks. However, eEF2 phosphorylation is decreased after acute alcohol intoxication, but such a change would be expected to accelerate rather than limit protein synthesis. Collectively, these data suggest that impaired elongation is likely to adversely impact muscle protein synthesis only under conditions where alcohol intake is relatively prolonged. In contrast, the decreased translation efficiency in muscle in response to acute alcohol intoxication and even relatively short-term alcohol ingestion is independent of a change in elongation and, therefore, an unlikely cause for the alcohol-induced myopathy.

4. Potential regulators of muscle protein synthesis

4.1. Insulin

Rates of protein synthesis are modulated by both positive and negative regulators. The most important positive regulators of muscle protein synthesis are hormones and nutrients. A decrease in either the circulating concentration or the responsiveness of tissues to

one of these anabolic stimuli might mediate at least part of the muscle wasting produced by alcohol. Among the many hormones capable of regulating protein balance, the anabolic effects of insulin are debatably the most well recognized. Muscle protein synthesis and translation efficiency are both reduced in insulinopenic diabetes and stimulated by insulin replacement (Kimball et al., 1997; Shah et al., 2000). However, the prevailing circulating concentration of insulin is either unchanged or modestly increased in response to alcohol (Lang et al., 1999a, 2000b; Preedy & Peters, 1988a). The mild hyperinsulinemia observed in response to alcohol represents a compensatory response by the host to the development of insulin resistance. In this regard, acute alcohol intoxication blunts the normally observed insulin-induced increase in S6K1 and S6 phosphorylation in skeletal muscle by approximately half under in vivo conditions (Kumar et al., 2002a; Lang et al., 2004b). In contrast, the ability of insulin to increase the formation of active eIF4F complex (evidenced by an increase in eIF4E·eIF4G, a decrease in eIF4E·4E-BP1, and increased phosphorylation of 4E-BP1) is unaltered. Consistent with these latter results, alcohol did not impair the phosphorylation of the insulin receptor, IRS-1 or PKB (Kumar et al., 2002a). Hence, while alcohol does not impair the ability of insulin to stimulate cap-dependent translation it may modestly decrease the translation of 5′-TOP mRNAs.

4.2. Insulin-like growth factor (IGF)-I

IGF-I stimulates translational control of protein synthesis (Bark, McNurlan, Lang, & Garlick, 1998; Kumar et al., 2002a) and has been administered in a number of catabolic conditions to increase LBM (Lang & Frost, 2002, 2004). However, the importance of IGF-I as a regulator for the alcohol-induced decrease in muscle protein synthesis, is dependent upon the duration of alcohol exposure. That is, there is an almost universal agreement that chronic (>6 weeks) consumption of an alcohol-containing diet decreases the circulating concentration of total IGF-I (Lang, Fan, Lipton, Potter, & McDonough, 1998; Lang et al., 1999a; Sonntag & Boyd, 1988). Moreover, the plasma concentration of free or unbound IGF-I, believed to be the bioactive form of the peptide, is also reduced by alcohol feeding (Lang, Frost, Svanberg, & Vary, 2004a). The reduction in blood IGF-I results from a decreased synthesis and secretion of IGF-I from the liver with little change in the rate of IGF-I clearance (Lang & Frost, 2004). IGF-I is also synthesized by a variety of extrahepatic tissues, including muscle, and the local production may have important autocrine and/or paracrine effects therein (Frost & Lang, 2002). Hence, decreases in the tissue content of IGF-I may regulate muscle protein balance. In this regard, the tissue IGF-I content is preferentially decreased in several fast-twitch skeletal muscles from alcohol-fed rats. Furthermore, the decreased IGF-I is proportional to the reduction in muscle protein synthesis and the formation of the functional eIF4F complex (Lang et al., 2001a). Hence, reduction in IGF-I bioavailability may be responsible for the muscle wasting that characterizes chronic alcohol feeding.

The putative role of IGF-I in the alcohol-induced atrophy is also supported by studies in which a complex composed of IGF-I and IGFBP-3 was injected into rats fed an alcohol-containing diet in order to return the plasma IGF-I concentration to basal control values (Lang et al., 2004a). This binary complex is advantageous because it has a slower rate of removal from the circulation and a prolonged bioavailability compared to unbound IGF-I. Injection of this binary complex for 3 days reversed the alcohol-induced decrease in muscle protein synthesis and translational efficiency. The binary complex also partially reversed the alcohol-induced decrease in the binding of eIF4E to eIF4G, but is independent of a change in the 4E-BP1 phosphorylation (Lang et al., 2004a).

In contrast to chronic alcohol consumption, there is no change or only a slight decrease in the prevailing plasma concentration of IGF-I in response to acute alcohol intoxication (Kumar et al., 2002a, 2002b; Lang et al., 2003a, 2003b). However, alcohol acutely impairs the anabolic effects of IGF-I (Kumar et al., 2002a; Lang et al., 2004b). Ethanol completely prevents the expected increased phosphorylation of S6K1 and S6 in muscle in response to a maximally stimulating dose of IGF-I. This effect is manifested within 1 h after alcohol administration and persists for up to 24 h. A maximal inhibitory effect of alcohol is observed at blood alcohol concentrations of 165 mg/dL, but a partial inhibition is present even with alcohol levels as low as 15 mg/dL. This inhibitory effect is independent of the route of ethanol administration (oral versus intraperitoneal), nutritional state (fed ver-

sus fasted) and gender. In contrast, alcohol produced only relatively minor alterations in the ability of IGF-I to increase 4E-BP1 phosphorylation and the amount of the functional eIF4F complex. Complementary data indicate that IGF-I increases muscle protein synthesis by the same increment in both control and alcohol-treated rats. Comparable changes are also observed in cardiac muscle (Lang et al., 2003a). Hence, changes in eIF4E availability, as opposed to S6K1 activation, appear to be the primary mechanism driving global protein synthesis in response to acute IGF-I stimulation. Hence, IGF-I resistance produced by acute alcohol intoxication may selectively prevent the ability of IGF-I to enhance the translation of 5'-TOP mRNAs encoding specific proteins.

4.3. Growth hormone (GH)

The synthesis of IGF-I is largely controlled by both the plasma concentration of GH and the responsiveness of target tissues to this anabolic hormone. The actions of GH on whole body metabolism are diverse and essential for postnatal somatic growth and accretion of LBM. Regulation of the circulating GH concentration is multifactited involving both the hypothalamus and pituitary as well as the modulatory roles of a staggering number of neurohormones and neurotransmitters. The complexity of the system precludes an extensive description of relevant literature but has been previously reviewed (Muller, Locatelli, & Cocchi, 1999). The majority of the current data suggest that chronic alcohol ingestion decreases the spontaneous GH secretory pattern, although the mechanism is unresolved. A more detailed account of the effects of alcohol on GH secretion has been recently published (Lang & Frost, 2005).

It is also possible that alcohol produces a growth hormone resistance as observed in other catabolic conditions (Rodgers, 1996). Two groups have conducted studies to determine the existence of alcohol-induced GH resistance—unfortunately, the conclusions of these studies do not resolve the question. In one study, Srivastava et al. (2002) used transgenic mice that over express bovine GH. When the diet of the transgenic mice was switched to one containing alcohol, the IGF-I concentration in the blood and the hepatic IGF-I mRNA content were both decreased, compared with transgenic mice fed a control diet. This decrease occurred without a change in the plasma GH concentration and is consistent with the presence of hepatic GH resistance. In contrast, in studies where GH is exogenously administered the increment in plasma IGF-I as well as hepatic and muscle IGF-I protein content is comparable in alcohol-fed and control rats (Lang et al., 2000c). Hence, the ability of alcohol to mediate changes in IGF-I via the induction of GH resistance remains unresolved.

4.4. Anabolic actions of leucine

Nutritional signals, such as those transmitted by amino acids, are of central importance in regulating protein synthesis and maintaining muscle mass. Refeeding amino acids after brief periods of starvation increases mixed muscle protein synthesis by stimulating peptide-chain initiation (Kimball & Jefferson, 2005). The branch-chain amino acid leucine appears largely, if not exclusively, responsible for the anabolic actions of a full complement of amino acids. Orally administered leucine increases muscle protein synthesis to the same extent in control and alcohol-treated rats (Lang et al., 2003a). However, because of the alcohol-induced decrease in basal protein synthesis, the absolute synthetic rate in muscle is still less in alcohol-treated rats receiving leucine than in control animals in the fasted condition. Alcohol partially prevents the leucine-indued redistribution of eIF4E in muscle and blunts the increased phosphorylation of 4E-BP1. Alcohol also abrogates the stimulating effect of leucine on mTOR phosphorylation. Regardless of the exact mechanism, in alcohol-treated rats leucine only increases 4E-BP1 phosphorylation and shifts eIF4E distribution back to values observed in muscle from control rats treated with vehicle. These changes are consistent with the ability of alcohol to blunt the leucine-indued increase in muscle protein synthesis. Similarly, alcohol also blunts the stimulation of muscle protein synthesis in fasted rats refed enterally, but not intravenously, with all amino acids, glucose and lipids (Sneddon et al., 2003).

In contrast to the partial efficacy of leucine at restoring eIF4F function, acute alcohol intoxication completely prevents the ability of this amino acid to stimulate the phosphorylation of S6K1 (both Thr421/Ser424 and Thr389) and ribosomal protein S6. These results cannot be explained by differences in gastrointestinal absorption of leucine because the

prevailing plasma leucine concentration is comparable in control and alcohol-treated rats. Alcohol also acutely prevents the stimulation of S6K1 in response to an enterally administered complete diet (Sneddon et al., 2003). Collectively, these data indicate the presence of a muscle "leucine resistance" in response to acute alcohol intoxication. Whether such pathology exists in animals provided an alcohol-containing diet has not been described.

4.5. Excess glucocorticoids

Alternatively, or in addition to, the negative nitrogen balance observed in response to alcohol could be mediated by overexpression of one or more factors that negatively regulate muscle protein balance. In this regard, acute alcohol intoxication activates the hypothalamic-pituitary-adrenal axis and increases secretion of glucocorticoids. In addition, exogenously administered steroids prevent amino acid-induced hyperphosphorylation of S6K1 and 4E-BP1 as well as the stimulation of protein synthesis in muscle (Shah et al., 2000). However, pretreatment of animals with the glucocorticoid receptor antagonist RU486 failed to prevent or attenuate the alcohol-induced dephosphorylation of either S6K1, S6, 4E-BP1 or mTOR in muscle (Lang et al., 2004b). Therefore, whereas a pharmacological excess of glucocorticoids clearly impairs translation initiation and protein synthesis in muscle, the alcohol-induced defects in leucine signaling to the translational apparatus appear largely independent of elevations in endogenous glucocorticoids in this particular catabolic conditions.

4.6. IGF binding protein (IGFBP)-1

Altering the plasma or tissue concentration of one or more of the six high-affinity IGFBPs can modulate the bioavailability and bioactivity of IGF-I (Lang & Frost, 2002; Rajaram, Baylink, & Mohan, 1997). A more robust stimulation of weight gain and nitrogen retention was noted in rats treated with des-IGF-I and LR^3-IGF-I than native IGF-I (Tomas, Lemmey, Read, & Ballard, 1996). These IGF-I variants have a reduced affinity for the IGFBPs and hence the results suggest that IGFBPs normally restrain the protein anabolic actions of IGF-I. Although alcohol alters the plasma concentration and tissue mRNA content for several IGFBPs (Lang & Frost, 2005), the most consistent and dramatic change is the increased IGFBP-1 observed after either acute ethanol intoxication or chronic alcohol consumption (Kumar et al., 2002b). Although muscle does not synthesize IGFBP-1 per se, IGFBP-1 protein is sequestered or trapped in muscle during catabolic conditions (Frost & Lang, 2004). In cultured human skeletal muscle cells, IGFBP-1 dose-dependently decreases the ability of IGF-I or serum to stimulate protein synthesis (Frost & Lang, 1999). Importantly, the in vivo infusion of IGFBP-1 that produces circulating levels comparable to those seen after alcohol also decreases muscle protein synthesis (Lang, Vary, & Frost, 2003c). This reduction is due in part to a concomitant decrease in the concentration of free IGF-I and S6K1 activation. However, until the development of specific inhibitors of IGFBP-1 synthesis or the performance of studies using IGFBP-1 null mice, the physiological relevance of the alcohol-induced increase in IGFBP-1 remains unresolved.

4.7. Myostatin

Gene targeting studies reveal myostatin to be a negative regulator of LBM. Mice carrying a deletion of the myostatin gene exhibit increased skeletal muscle size (McPherron, Lawler, & Lee, 1997) whereas a loss of muscle mass is seen in adult mice that overexpress myostatin (Zimmers et al., 2002). The addition of myostatin to cultured myocytes also decreases protein synthesis (Taylor et al., 2001), and an inverse correlation between myostatin mRNA content and protein mass in gastrocnemius is seen after thermal injury and glucocorticoid excess (Lang et al., 2001b). Chronic alcohol feeding increases myostatin mRNA content (Lang et al., 2004a), but acute alcohol intoxication fails to produce a detectable increase in muscle myostatin (Lang & Frost, unpublished observation). Therefore, although the results of this single study are consistent with a possible role in chronic alcohol-induced atrophy, the physiological importance of this change remains to be determined.

5. Direct versus indirect effect of alcohol

The ability of ethanol to impair protein synthesis may be mediated directly or via acetaldehyde or acetate

(e.g., active metabolites) generated by the oxidative metabolism of ethanol. Inhibition of ADH with 4-methylpyrazol (MP) did not prevent the decrease in muscle protein synthesis produced in vivo by acute alcohol intoxication (Preedy et al., 1992). Further, none of the alcohol-induced effects on either basal or IGF-I–stimulated S6K1/S6 phosphorylation are prevented by 4-MP (Lang et al., 2004b). Hence, it is unlikely ethanol metabolism is required for alcohol to modulate protein synthesis. However, these results do not differentiate between an alcohol effect on muscle per se versus the release of a secondary mediator from a distant organ that circulates and affects muscle in an indirect manner.

Different experimental approaches have been used to resolve these two possibilities. First, when ethanol is added to cultured human skeletal muscle cells protein synthesis is inhibited dose- and time-dependently (Hong-Brown, Frost, & Lang, 2001). Prolonged exposure (e.g., 3 days) of myocytes to ethanol also blunted the protein synthetic response toward insulin and IGF-I. Hence, alcohol is capable of directly impairing protein synthesis in cultured muscle cells. Secondly, the direct effects of alcohol have also been assessed using the isolated perfused hindlimb muscle preparation. In this study, alcohol was included in the perfusate at a concentration comparable to that observed in vivo, and the hindlimb musculature perfused for the same time period as in vivo studies (Lang et al., 2004b). The exposure of muscle under these conditions qualitatively recapitulates the same defects in basal and IGF-I stimulation of S6 phosphorylation as were observed in vivo suggesting that alcohol can directly decrease the translational control of muscle protein synthesis.

6. Conclusions and future directions

The importance of the studies outlined herein ultimately lies in the development of new therapeutic approaches to combat problems associated with alcoholism at the cellular and molecular level. It is important to determine whether limiting the loss of protein preserves structure and function, especially in the heart. One approach would be to supplement the diet with nutrients that stimulate the biochemical sites in the protein synthetic pathway inhibited by alcohol and follow cardiac changes longitudinally using noninvasive techniques. Additional studies designed to further explore the molecular mechanisms responsible for the defective signaling are also needed. Aside from providing specific information regarding the role of a signaling pathway on protein synthesis, it is anticipated that such pathways will be involved in other key steps responsible for maintenance of normal muscle structure and function (e.g., calcium handling). Presently, proteonomics with respect to alcohol-induced alterations in muscle proteins is rudimentary and newer technologies must be applied. Genes encoding proteins involved in sarcomere structure, enzymes involved in carbohydrate or fat metabolism, and proteins involved in cytoskeleton, cell–cell and cell–matrix interactions need to be examined. Another point of interest will be to identify mRNAs that have 5′-TOP tracts or have high degree of secondary structure in their 5′-UTR that implies translational regulation by ribosomal protein S6 or 4E-BP1, respectively. Another question that may be pursued is whether transcriptional control is altered by alcohol, as many of the signaling pathways affected by ethanol may modulate gene transcription. Finally, there is a paucity of data related to the effect of alcohol on muscle protein degradation and the potential regulatory role of mTOR in this process. Hence, systematic investigation of this side of the protein balance equation is warranted. While great strides have been made in recent years regrading understanding the cellular and molecular defects produced by alcohol that lead to cardiac and skeletal myopathy, continued research is necessary to elucidate those modulators that are of physiological importance so that rationale therapies and interventions can be formulated.

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