Inhibition of muscle protein synthesis by alcohol is associated with modulation of eIF2B and eIF4E

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Lang, Charles H., Duanqing Wu, Robert A. Frost, Leonard S. Jefferson, Scot R. Kimball, and Thomas C. Vary. Inhibition of muscle protein synthesis by alcohol is associated with modulation of eIF2B and eIF4E. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E268–E276, 1999.—The present study examined potential mechanisms for the inhibition of protein synthesis in skeletal muscle after chronic alcohol consumption. Rats were maintained on an alcohol-containing diet for 14 wk; control animals were pair fed. Alcohol-induced myopathy was confirmed by a reduction in lean body mass as well as a decrease in the weight of the gastrocnemius and psoas muscles normalized for tibial length. No alcohol-induced decrease in total RNA content (an estimate of ribosomal RNA) was detected in any muscle examined, suggesting that alcohol reduced translational efficiency but not the capacity for protein synthesis. To identify mechanisms responsible for regulating translational efficiency, we analyzed several eukaryotic initiation factors (eIF). There was no difference in the muscle content of either total eIF2α or the amount of eIF2α in the phosphorylated form between alcohol-fed and control rats. Similarly, the relative amount of eIF2α in muscle was also not different. In contrast, alcohol decreased eIF2B activity in psoas (fast-twitch) but not in soleus or heart (slow-twitch) muscles. Alcohol feeding also dramatically influenced the distribution of eIF4E in the gastrocnemius (fast-twitch) muscle. Compared with control values, muscle from alcohol-fed rats demonstrated 1) an increased binding of the translational repressor 4E-binding protein 1 (4E-BP1) with eIF4E, 2) a decrease in the phosphorylated-γ-form of 4E-BP1, and 3) a decrease in eIF4G associated with eIF4E. In summary, these data suggest that chronic alcohol consumption impairs translation initiation in muscle by altering multiple regulatory sites, including eIF2B activity and eIF4E availability.

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MATERIALS AND METHODS

Experimental protocol. Specific pathogen-free, male Sprague-Dawley rats (Charles River Breeding Laboratories, Cambridge, MA) were maintained on an ethanol-containing agar block diet for 14 wk. Initially all rats were provided agar without alcohol for 2 days. Thereafter, half of the animals were fed agar containing 10% alcohol, while the others were provided with agar containing an equal caloric amount of dextrin-maltose. The ethanol content of the agar was increased to 20% and finally to 30% at 1-wk intervals. Rats were maintained on the 30% ethanol-agar block for the remainder of the experimental protocol. Animals were also supplemented with ethanol-containing (10%) drinking water. In addition, the alcohol-fed rats were permitted free access to solid chow (Purina, St. Louis, MO). Over the final 10 wk of the experiment, the alcohol-fed rats were permitted free access to ethanol-containing (10%) drinking water. In addition, the alcohol-fed rats were permitted free access to solid chow (Purina, St. Louis, MO). Over the final 10 wk of the experimental protocol, alcohol-fed rats had an average total energy consumption (food + alcohol) of 291 ± 18 kcal·kg⁻¹·day⁻¹, which is sufficient to meet the nutritional requirements of growing rats (35). Ethanol intake during this same period was 19 ± 3 g·kg⁻¹·day⁻¹. Blood ethanol levels determined at the time of death were 137 ± 34 mg/dl and are comparable to those observed in intoxicated human subjects (39). Ethanol consumption decreases voluntary food consumption (27). Therefore, control animals were provided the same amount of agar (without alcohol, but substituted isocaloric with dextrin-maltose), solid chow, and alcohol-free water. Total energy intake in control rats was 286 ± 21 kcal·kg⁻¹·day⁻¹ during the final 10 wk of the experiment. Thus total caloric intake was not different between the two groups. Additional details of this protocol have been previously described (2, 25).

The morning of the experiment, rats were anesthetized with pentobarbital sodium (60 mg/kg) and total body electrical conductivity was measured noninvasively to estimate body composition (EM-SCAN, Springfield, IL). Thereafter, a laparotomy was performed and a heparinized blood sample was collected from the inferior vena cava. Selected tissues (e.g., gastrocnemius, psoas, soleus, and heart) were then rapidly dissected free of connective tissue and rinsed of blood. The soleus and heart were chosen as representative of muscles with a high proportion of slow-twitch and the gastrocnemius and psoas as representative of muscles composed of mixed fast-twitch fibers. Because of the limited quantity of muscle available, not all assays could be performed on all tissues. However, because of their similar fiber-type composition, it was assumed that both gastrocnemius and psoas respond in a comparable manner to alcohol feeding (43).

A portion of soleus, psoas, and heart was used directly for either analysis of ribosomal subunits or determining eukaryotic initiation factor (eIF) 2B activity. The remainder of each muscle sample was frozen in liquid nitrogen-cooled clamps. Frozen muscle samples were powdered under liquid nitrogen and stored at −70°C. After excision of muscles, the left tibia was removed and freed of connective tissue, and its maximal length was measured. Expressing muscle mass per tibial length has been previously reported as a valid method to normalize mass when body weight differs between experimental groups of rats (48).

Determination of total RNA. Total RNA was measured from homogenates of muscle samples (44). Briefly, 0.3 g of fresh muscle was homogenized in 5 vol of ice-cold 10% TCA. The homogenate was centrifuged at 9,000 g for 11 min at 4°C. The supernatant was discarded, and 6% perchloric acid (PCA) was added to the remaining pellet. The sample was centrifuged at 9,000 g for 6 min, the supernatant was discarded, and the procedure was repeated. Next, 0.3 N KOH was added to the pellet and the samples were incubated for 1 h at 50°C. Samples were then mixed with 4 N PCA and centrifuged at 9,000 g for 11 min. The concentration of RNA was determined by absorbance at 260 nm corrected by the absorbance at 232 nm (7). Total RNA was expressed as milligram RNA per gram wet weight of tissue.

Isolation of ribosomal subunits. Fresh muscle tissue (psoas, soleus, and heart) was used to isolate 40S and 60S ribosomal subunits by sucrose density gradient centrifugation, as described previously (12). Briefly, muscles were homogenized in a motor-driven glass-on-glass homogenizer in 4 vol of homogenization buffer (14 mM triethanolamine (pH 7.0), 2 mM magnesium acetate, 250 mM KCl), 0.5 mM dithiothreitol (DTT), 0.08 mM EDTA, 5 mM EGTA, 250 mM sucrose, and 1 mg nagarse (protease, type XXVI) (Sigma, St. Louis, MO). The homogenate was centrifuged at 10,000 g for 15 min, and the supernatant was recovered. Aliquots of the samples (0.7 ml), to which 0.1 vol of 10% (wt/vol) Triton X-100 and deoxycholate solution had been added, were then layered onto 0.44–2.0 M exponential sucrose gradients. The samples were centrifuged at 167,000 g in a SW41 rotor (Beckman Instruments) for 20 h to resolve the 40S and 60S ribosomal subunits. The absorbance of the gradients was monitored at 254 nm, and fractions were collected with a density gradient fractionator (Instrumentation Specialties, Lincoln, NE). These data provide information related to changes in peptide-chain initiation relative to changes in elongation-termination but do not directly quantitate either process.

Amount of eIF2 and eIF2B in muscle. The relative amounts of the α-subunit of eIF2 (eIF2α), the phosphorylated form of eIF2α, and the e-subunit of eIF2B (eIF2Bε) in various muscles were estimated by protein immunoblot analysis, as described previously (14, 16, 45). eIF2 and eIF2B were chosen because changes in the expression and/or activity of these initiation factors correlate with alterations in protein synthesis (5). eIF2 consists of three subunits of which the α-subunit appears important in regulating protein synthesis (48). Likewise, eIF2B is a multimeric protein consisting of five subunits, with the e-subunit being the catalytic subunit (47). Previous studies have established that the expression of the e-subunit is representative of other subunits (46). Therefore, the relative abundance of eIF2Bε was taken as representative of the eIF2B holoenzyme. Briefly, muscle was homogenized in 7 vol of buffer composed of (in mM) 20 Tris (pH 7.4), 250 sucrose, 100 KCl, 0.2 EDTA, 1 DTT, 50 NaF, 50 β-glycerophosphate, 1 phenylmethylsulfonyl fluoride (PMSF), 1 benzamidine, and 0.5 sodium vanadate. The samples were mixed with 2× Laemmli SDS buffer (60°C), boiled for 3 min, and centrifuged. Equal amounts of protein (~160 µg) from muscle homogenates were electrophoresed at 60 mA in a 12.5% polyacrylamide gel. After electrophoresis, proteins in the gel were transferred to nitrocellulose. After being blocked for 30 min with nonfat milk (5% wt/vol) in 25 mM Tris (pH 7.6)-0.9% saline containing 0.01% Tween 20 (Tris-NaCl-Tween), the membranes were washed extensively in Tris-NaCl-Tween. The nitrocellulose was incubated for 1 h at room temperature with an antibody specific for either eIF2α (46), ser-51-phosphorylated eIF2α (from Dr. Gary S. Krause, Wayne State University), or eIF2βε (19). Antibodies were visualized with an enhanced chemiluminescence procedure with the secondary antibody linked to horseradish peroxidase (Amersham). The blots were exposed to X-ray film in a cassette equipped withDu Pont Lightning Plus intensifying screen. After development, the film was scanned (Microtek ScanMaker IV) and quantitated with National Institutes of Health Image 1.6 software.
Determination of eIF2B activity. eIF2B activity in muscle was measured in postmitochondrial supernatants with a [\(^{3}\text{H}\)]GDP·GDP exchange assay, as previously described (17). Fresh tissue was homogenized in 4 vol of buffer consisting of (in mM) 20 triethanolamine (pH 7.0), 2 magnesium acetate, 150 KCl, 0.5 DTT, 0.1 EDTA, 250 sucrose, 5 EGTA, and 50 \(\beta\)-glycerolphosphate. The homogenate was then centrifuged at 15,000 \(g\) for 15 min at 4°C. The supernatant was assayed immediately for eIF2B activity, as described previously (17). Briefly, aliquots of the reaction mixture were analyzed for eIF2B activity by measuring the decrease in eIF2·[\(^{3}\text{H}\)]GDP immediately for eIF2B activity, as described previously (17).

Phosphorylation state of 4E-BP1. The various phosphorylated forms of 4E-BP1 were measured after immunoprecipitation of 4E-BP1 from muscle homogenates after centrifugation at 10,000 \(g\) (18). 4E-BP1 was immunoprecipitated as described in Quantification of 4E-binding protein 1·eIF4E and eIF4G·eIF4E complexes for immunoprecipitation of eIF4E. The immunoprecipitates were solubilized with the SDS sample buffer. The various phosphorylated forms of 4E-BP1 were separated by electrophoresis and quantitated by protein immunoblot analysis as described previously (18).

Tissue ATP content and plasma insulin levels. An aliquot of powdered gastrocnemius was extracted in cold PCA, neutralized, and used for the determination of ATP and creatine phosphate (CP) by standard fluorometric methods. Plasma insulin concentrations were determined by RIA (DPC, Los Angeles, CA).

Statistics. Values are presented as means ± SE. The number of rats per group is indicated in the legends to the Figs. 1–6 and Tables 1–2. Data were analyzed by Student's \(t\)-test to determine treatment effect. Statistical significance was set at \(P < 0.05\).

RESULTS

 Whole body composition and muscle weights. The body weight of the alcohol-fed rats was reduced 19% after 14 wk of feeding, compared with pair-fed control animals (427 ± 11 vs. 526 ± 12 g, respectively). The average weight gain during the experimental period was reduced by 24% in alcohol-fed rats, despite having the same caloric intake (Table 1). Estimates of body composition indicated that alcohol feeding lessened the accretion of LBM without significantly altering the amount of body fat (Table 1). As a result of these changes, the percentage of LBM normalized to body weight decreased and the relative percentage of fat increased in alcohol-fed rats. When LBM was normalized to tibial length (TL), which was 3.1% shorter in alcohol-fed rats \((P < 0.05)\), the LBM-to-TL ratio remained significantly lower (22%) in the alcohol-consuming animals.

Figure 1A illustrates that, compared with pair-fed control animals, alcohol feeding decreased the weight of the gastrocnemius (22%), psoas (20%), and heart (10%); no change was detected in the weight of the soleus muscle. When muscle mass was normalized for tibial length (Fig. 1B), the gastrocnemius and psoas were still significantly smaller (18–20%) in the alcohol-fed rats than in pair-fed control animals. However, the alcohol-induced decrease in ventricular mass no longer achieved statistical significance.

In the gastrocnemius, alcohol feeding caused an 11% decrease in the protein concentration, compared with

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<th>Table 1. Alcohol-induced alterations in body composition</th>
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<td>BW Gain, g</td>
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</tr>
<tr>
<td>Control</td>
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<tr>
<td>Alcohol</td>
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Values are means ± SE; \(n = 10\) rats/group. BW, body weight; LBM, lean body mass; TL, tibial length. * \(P < 0.05\) vs. values from control animals.
control values (132 ± 4 vs. 150 ± 7 mg protein/g wet wt; *P < 0.05). The protein content of other muscles was not determined due to a lack of significant amounts of tissue. There was no difference in the wet weight-to-dry weight ratios for muscles from control and alcohol-fed rats regardless of the fiber-type composition (data not shown).

Total RNA content. A decreased rate of protein synthesis may result from alterations in either the number of ribosomes or the efficiency of translation. Ribosomal RNA accounts for as much as 80% of the total RNA in muscle. Hence, changes in the total tissue RNA content presumably reflect changes in ribosomal RNA. The total RNA content in muscles from control and alcohol-fed rats is presented in Fig. 2. No alcohol-induced changes in the RNA content of psoas, gastrocnemius (data not shown), soleus, or heart were detected.

Ribosomal subunits. We examined the potential role of decreases in peptide-chain initiation and/or elongation-termination in inhibiting protein synthesis in alcohol-fed rats. The relative rate of peptide-chain initiation vs. elongation-termination can be assessed by isolating nonpolysome-associated 40S and 60S ribosomal subunits. Figure 3 illustrates that alcohol-feeding resulted in small (15–25%), but statistically significant, decreases in RNA content of fractions containing the free 40S and 60S subunits isolated from the psoas (fast-twitch) and soleus (slow-twitch) muscle. In contrast, the amount of free 40S and 60S subunits in heart was unaltered by alcohol consumption. In general, the distribution of 40S and 60S ribosomal subunits between polysome and nonpolysome fractions is indicative of the balance between the rates of initiation and elongation-termination. That is, when the rate of elongation-termination is decreased relative to peptide-chain initiation, free ribosomal subunits are binding to mRNA at a faster rate (initiation) than they are moving along mRNA (elongation) and exiting (termination). The net result of this defect is a reduction in the abundance of free 40S and 60S ribosomal subunits (22).
Relative amounts of eIF2, eIF2B, and eIF2B activity. One possible mechanism for the alcohol-induced decrease in protein synthesis is via alterations in the amount and/or activity of specific eIF proteins. There was no significant difference in either total eIF2α or the amount of eIF2α in the phosphorylated (inactive) form in any of the muscles between control and alcohol-fed rats (Table 2). Similarly, alcohol feeding did not significantly alter the relative amount of eIF2Bε in the soleus, psoas, or heart, although eIF2Bε content in the psoas did tend to be decreased in alcohol-fed rats (~10%; Table 2).

The effect of chronic alcohol feeding on eIF2B activity was measured in postmitochondrial supernatants of muscles from control and experimental rats. eIF2B activity was decreased 37% in psoas from alcohol-fed rats, compared with pair-fed control values (Fig. 4). In contrast, there was no difference in eIF2B activity in the soleus and heart of alcohol-consuming and control animals.

Regulation of eIF4E. Another potential mechanism for decreasing muscle protein synthesis involves the reduced ability of eIF4E to initiate translation. A decrease in the availability of eIF4E can occur when the translational repressor 4E-BP1 binds with eIF4E forming an inactive complex. Figure 5A illustrates that the amount of 4E-BP1 associated with eIF4E was increased 42% in gastrocnemius obtained from alcohol-fed rats. In contrast, there was no alcohol-induced change in 4E-BP1 binding with eIF4E in soleus muscle (control, 1,524 ± 207 arbitrary units (AU); alcohol, 1,609 ± 197AU).

4E-BP1 has at least five potential phosphorylation sites, which are resolved into three bands by SDS-PAGE. These forms have been identified as α (least phosphorylated and fastest migrating), β (intermediate), and γ (most phosphorylated and slowest migrating). The total amount of all three phosphorylated forms did not differ between control and alcohol-fed rats (2,538 ± 77 vs. 2,589 ± 108 AU, respectively), indicating that the total amount of 4E-BP1 was not altered. However, phosphorylation of 4E-BP1 in the γ-form results in a decreased association of the BP with eIF4E and an increase in translation (41). In the current study, the amount of 4E-BP1 in the γ-form was decreased 42% in alcohol-fed rats, compared with control values (Fig. 5B). This is particularly important because, on the basis of previous studies, a decrease in 4E-BP1 in the γ-form would be expected to maintain the integrity of the eIF4E·4E-BP1 complex and thereby decrease initiation (41).

Table 2. Effect of chronic alcohol feeding on relative amount of total and phosphorylated eIF2α and eIF2Bε content

<table>
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<tr>
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<th>Psoas</th>
<th>Soleus</th>
<th>Heart</th>
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<tr>
<td>eIF2α</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>20,832 ± 2,568</td>
<td>29,513 ± 1,365</td>
<td>43,514 ± 3,865</td>
</tr>
<tr>
<td>Alcohol</td>
<td>25,493 ± 1,530</td>
<td>30,648 ± 1,571</td>
<td>33,602 ± 3,797</td>
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<tr>
<td>eIF2α (P)</td>
<td></td>
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<tr>
<td>Control</td>
<td>15,796 ± 695</td>
<td>35,111 ± 1,305</td>
<td>30,105 ± 1,295</td>
</tr>
<tr>
<td>Alcohol</td>
<td>15,154 ± 480</td>
<td>31,282 ± 1,384</td>
<td>25,723 ± 2,274</td>
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<tr>
<td>eIF2Bε</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5,061 ± 303</td>
<td>5,410 ± 374</td>
<td>6,407 ± 433</td>
</tr>
<tr>
<td>Alcohol</td>
<td>5,464 ± 333</td>
<td>5,443 ± 450</td>
<td>5,922 ± 373</td>
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Values are means ± SE; n = 9 samples/group. Relative amount of total amount of eukaryotic initiation factors (eIF)2α and eIF2α phosphorylated (P) and amount of eIF2Bε was determined by densitometric analysis of immunoblots.

Fig. 4. Eukaryotic initiation factor 2B (eIF2B) activity in control and alcohol-consuming rats. Muscles were obtained from either pair-fed control animals (open bars) or alcohol-consuming rats (solid bars). Values are means ± SE; n = 9/group. *P < 0.05 vs. control values.

Fig. 5. Effect of chronic alcohol consumption on amount of eIF4E associated with 4E-binding protein 1 (4E-BP1; A) and 4E-BP1 phosphorylation state (B) in gastrocnemius. Amount of 4E-BP1 in γ-form is expressed as percentage of total of all phosphorylated and nonphosphorylated forms. Values are means ± SE; n = 5 animals/group. *P < 0.05 vs. control values.
In a similar manner, eIF4E immunoprecipitates were used to measure the association of eIF4E with eIF4G. The gastrocnemius isolated from alcohol-fed rats demonstrated a 47% decrease in the amount of eIF4G that immunoprecipitated with eIF4E (Fig. 6A). This decrease was not the result of a reduction in the amount of eIF4E in the immunoprecipitate between the two groups (control = 10,202 ± 245 AU vs. alcohol = 10,210 ± 560 AU). In contrast, in soleus there was no alcohol-induced change in eIF4E binding to eIF4G (control, 846 ± 55 AU; alcohol, 821 ± 102 AU).

The aforementioned data suggest that the alcohol-induced decrease in translation in skeletal muscle results in part from a decreased formation of the active eIF4E·eIF4G complex. To further define the mechanism through which alcohol inhibits translation, the phosphorylation of eIF4E was examined (Fig. 6B). In gastrocnemius from control animals, 54% of the total eIF4E was in the phosphorylated state. Chronic alcohol consumption did not significantly alter the phosphorylation status of eIF4E.

ATP-CP content and plasma insulin. There was no difference in the ATP concentration in gastrocnemius between control and alcohol-fed rats (6.91 ± 0.13 vs. 7.02 ± 0.21 µmol/g wet wt, respectively). Likewise, there was no difference in CP levels between the two groups (21.4 ± 0.9 vs. 20.7 ± 1.1 µmol/g wet wt). These data suggest that a generalized energy deficit is not responsible for the alcohol-induced decrease in muscle protein synthesis.

Plasma insulin levels did not differ significantly between control and alcohol-fed rats (23 ± 3 vs. 21 ± 2 µU/ml, respectively).

**DISCUSSION**

Early work by other investigators has definitively shown that chronic alcohol consumption in rats decreases the rate of protein synthesis in skeletal muscle (33). Moreover, this impairment appears to be largely localized to muscles containing a relatively high percentage of fast-twitch glycolytic fibers (e.g., gastrocnemius and psoas) as opposed to those classified as slow-twitch oxidative muscles (e.g., soleus) (32). Quantitatively greater decreases in muscle protein synthesis have also been reported in fast-twitch muscles in response to acute ethanol intoxication (37). The reason why alcohol preferentially affects fast-twitch muscle is not known but is consistent with the response observed in other catabolic states, including diabetes, fasting, sepsis, and glucocorticoid administration (5, 22).

Inhibition of protein synthesis can occur by a decrease in the number of ribosomes and/or a reduction in translational efficiency. In the present study, we assessed the possibility that ribosomal number was decreased. Because 80–90% of the cellular RNA is ribosomal, changes in muscle RNA content primarily reflect changes in the abundance of ribosomes. Previous studies have reported that chronic alcohol feeding significantly decreases both translational efficiency and the total RNA content in the gastrocnemius (33) and that the latter change is associated with an increase in RNA catabolism (36). In contrast, we were unable to confirm a significant decrease in total RNA content in any muscle examined from alcohol-consuming rats. The reason for this difference between our study and earlier work is unclear. Our data suggest that the alcohol-induced decrease in muscle protein synthesis results primarily from an impairment in translational efficiency and not from a decrease in the capacity of protein synthesis. An impairment in translational efficiency has also been reported in other catabolic conditions, including infection and diabetes (14, 44).

The alcohol-induced impairment in translational efficiency may result from an impairment in either peptide-chain initiation and/or elongation-termination (5). In the present study, analysis of the distribution of ribosomal subunits between free subunits and polysomes was used to estimate the rate of peptide-chain initiation relative to elongation. We anticipated that alcohol feeding would increase the number of nonpolysome-associated 40S and 60S ribosomes in fast-twitch muscle, but not slow-twitch muscle, as observed in other catabolic conditions, such as infection, diabetes, and starvation (10, 14, 44). However, in contradistinction to these previous studies, our present investigation indicated a moderate reduction in the accumulation of free 40S and 60S ribosomal subunits in both the psoas and soleus of alcohol-fed rats. No change was detected in heart. The
decreased abundance of free 40S and 60S subunits in alcohol-fed rats indicates a decrease in elongation-termination relative to peptide-chain initiation. However, the inhibition of protein synthesis after alcohol consumption cannot be interpreted unambiguously as being solely due to a decrease in elongation. Most likely our results are consistent with an alcohol-induced inhibition of both processes, but with the decrease in elongation being of greater magnitude than the decrease in initiation. More direct determination of initiation, such as measuring incorporation of initiator methionyl-tRNA (met-tRNA\textsuperscript{met}) into the 40S initiation complex, was not performed in the current study.

Because other catabolic conditions decrease peptide-chain initiation, we also investigated the effects of alcohol feeding on selective elements of the elf2 system. Elf2 represents a major regulatory control point for initiation of protein synthesis in skeletal muscle (31). The first step in initiation is the formation of a ternary complex comprising elf2, GTP, and met-tRNA\textsuperscript{met}. Elf2 mediates the binding of met-tRNA\textsuperscript{met} to the 40S ribosomal subunit to form the 43S preinitiation complex. A decrease in elf2 activity could result from a reduction in the tissue content of elf2 protein. In several nonmuscle tissues, the amount of elf2 protein is linearly related to rates of protein synthesis (21). However, in the present study, there was no significant difference in the elf2 content (as assessed by Western blot analysis of elf2) in any muscle examined in response to chronic alcohol feeding.

Alternatively, a decrease in the activity of another elf, elf2b, can decrease elf2 availability (47). Elf2b is a guanine nucleotide exchange factor required for exchange of GDP for GTP on elf2. Hence, a decrease in elf2b activity would ultimately decrease the amount of elf2-GTP that is available to bind to tRNA\textsuperscript{met}, thereby limiting translation initiation and protein synthesis. Under several physiological conditions, the rate of protein synthesis is directly proportional to elf2b activity in muscle (10, 14). Our results indicate that chronic alcohol feeding decreases elf2b activity in psoas muscle by almost 40%. In contrast, elf2b activity was not altered in soleus. These results suggest that muscles composed primarily of fast-twitch fibers are relatively more sensitive to the effects of alcohol than those composed primarily of slow-twitch fibers. This fiber-type specificity has been previously demonstrated in other catabolic conditions, such as infection, uncontrolled diabetes, prolonged fasting, or after administration of glucocorticoids (10, 17), all of which are associated with muscle wasting. Therefore, a decrease in elf2b-mediated guanine nucleotide exchange appears to be at least partially responsible for the alcohol-induced decrease in initiation.

Several mechanisms are known to regulate elf2b activity (47). The first major mechanism involves the phosphorylation of the \(\alpha\)-subunit of elf2, which increases the affinity of elf2 for elf2b (31). The formation of the highly stable elf2b(\(P\)\(\alpha\))-elf2b complex effectively sequesters available elf2b, under conditions where the cellular content of elf2b exceeds that of elf2b. When elf2b is bound to elf2, guanine nucleotide exchange activity of elf2b does not occur. Therefore, because essentially all of the elf2b is present in the inactive form, peptide-chain initiation is effectively inhibited. Although the extent of elf2\(\alpha\) phosphorylation has been demonstrated to be inversely proportional to the rate of protein synthesis under selective in vitro conditions (26, 40), no change in elf2\(\alpha\)(P) was observed in muscle obtained from alcohol-fed rats. Hence, alcohol-induced decreases in elf2b activity appear to be independent of the phosphorylation state of elf2\(\alpha\). This finding is consistent with the lack of a change in elf2\(\alpha\) phosphorylation in skeletal muscle from either 48-h-fasted, diabetic, or septic rats, conditions resulting in an inhibition of translation initiation (13, 14, 43). Second, alcohol consumption could decrease elf2b activity via decreasing the availability of elf2b present in muscle. However, this mechanism does not appear operational because the amount of elf2b protein, as assessed by Western blot analysis of elf2b, was not significantly altered by alcohol. This failure of chronic alcohol consumption to lower elf2b protein is in contrast to the strong correlation between the reduction in elf2\(\alpha\) protein (and mRNA expression) and protein synthesis in skeletal muscle from septic rats (45, 46). Third, elf2b activity can also be regulated allosterically by changes in the cellular redox potential. Increases in the NAPD(P)-to-NAD(P)H ratio would be expected to inhibit elf2b activity (14). Although alcohol markedly alters the redox potential in liver (24), the NAD\(^{+}\)-to-NADH ratio in skeletal muscle appears to be largely unaffected (15). Hence, an alcohol-induced alteration in the redox potential is unlikely to be an important regulator of muscle elf2b activity in our experimental model. Finally, phosphorylation of the \(\epsilon\)-subunit of elf2b under in vitro conditions can regulate the activity of the holoenzyme (20). This potential mechanism was not investigated in the current study and cannot be excluded as a regulator of alcohol-induced changes in elf2b activity at this time.

A second regulatory step in peptide-chain initiation involves the binding of mRNA to the 43S preinitiation complex, which is mediated by elf4f (5). One of the protein components of the elf4f complex, elf4e, binds directly to the m\(^7\)GTP cap structure present at the 5’-end of all eukaryotic mRNA and plays a critical role in maintaining protein synthesis (38). During translation initiation, the elf4e-mRNA complex binds to elf4g and elf4a to form the active elf4f complex (38). One mechanism for modulating the formation of the elf4f complex is by regulating the relative distribution of elf4e between inactive and active complexes with other proteins. Elf4e binds with a small, acid- and heat-labile protein termed 4e-BP1 (PHAS-I) in rat skeletal muscle to form an inactive complex (28). In the present study, the amount of 4e-BP1 associated with elf4e was markedly increased in skeletal muscle by chronic alcohol feeding. We also observed a concomitant decrease in the amount of elf4e bound to elf4g in alcohol-fed rats. These data strongly suggest that alco-
hol feeding decreases initiation, at least in part, by an impairment in eIF4E function secondary to decreased eIF4E availability. Similar changes in eIF4E have been previously demonstrated in other conditions associated with decreases in muscle protein synthesis (9, 18).

The interaction between 4E-BP1 and eIF4E is regulated in part by the extent of phosphorylation of 4E-BP1. Phosphorylation of 4E-BP1 releases eIF4E from the 4E-BP1·eIF4E complex, thereby permitting the eIF4E·mRNA complex to bind first to eIF4G and then ultimately to the 4OS ribosome (41). Previous studies have reported that diabetes-induced decreases in muscle protein synthesis are associated with a decrease in the percentage of 4E-BP1 in the phosphorylated γ-form (18). Similarly, data from our study indicate that there is a marked reduction in the amount of phosphorylated 4E-BP1 in muscle from alcohol-fed rats.

In addition, changes in the phosphorylation state of eIF4E can also influence eIF4E availability. Although both phosphorylated and nonphosphorylated eIF4E binds to the mRNA cap structure, phosphorylation of eIF4E enhances the affinity of the factor for the m7GTP cap by several fold (30). In addition, in vitro studies have demonstrated that increases in phosphorylation are proportional to increases in translation (41). However, chronic alcohol consumption failed to significantly alter the phosphorylation state of eIF4E. Hence, alcohol feeding interferes with initiation via changes in the phosphorylation state of 4E-BP1 but not eIF4E.

A marked reduction in insulin would be expected to decrease eIF2β activity and eIF4E availability as well as decrease muscle protein synthesis (16–19). However, the alcohol-induced changes in these initiation factors do not appear to be mediated by insulin, because there was no significant difference in the circulating levels of this hormone between alcohol-fed and control animals. Changes in the insulin-like growth factor I (IGF-I) concentration within the physiological range are also known to produce proportional changes in the rate of protein synthesis (1, 6). Although there is no information on the ability of IGF-I to modulate either eIF2β activity or eIF4E availability in skeletal muscle, it is possible that IGF-I and insulin have similar effects based on their similar intracellular signal transduction pathways. Therefore, decreased circulating IGF-I levels (25) might be partially responsible for the observed changes in initiation factors and the reduction in translation initiation in muscle from alcohol-fed rats.

In summary, our data strongly suggest that the alcohol-induced decrease in protein synthesis in fast-twitch muscles results from reductions in both elongation-termination and peptide-chain initiation. Moreover, the impairment in translational initiation results, at least in part, from a decrease in eIF2β activity. This diminished activity is independent of changes in eIF2α content, eIF2α phosphorylation, and eIF2α content. In addition, alcohol-fed rats also appear to have an impairment in eIF4F function as evidenced by the increase in 4E-BP1 associated with eIF4E, the decrease in 4E-BP1 in the γ-form, and the decreased amount of eIF4G bound to eIF4E. Hence, chronic alcohol feeding apparently alters a variety of key regulatory steps in translation initiation that would be expected to impair protein synthesis in skeletal muscle.

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