Acute alcohol administration inhibits the refeeding response after starvation in rat skeletal muscle

A. A. Sneddon, M. Koll, M. C. Wallace, J. Jones, J. P. Miell, P. J. Garlick, and V. R. Preedy. Acute alcohol administration inhibits the refeeding response after starvation in rat skeletal muscle. Am J Physiol Endocrinol Metab 284: E874–E882, 2003. First published October 15, 2002; 10.1152/ajpendo.00209.2002. This study determined whether an acute alcohol dose could inhibit the refeeding response in starved muscle. Rats starved for 24 h were pretreated with alcohol or saline before refeeding by intragastric or intravenous infusion of enteral diet (ENT), total parenteral nutrition (TPN), or saline. Refeeding by TPN or ENT stimulated increases in the fractional rate of protein synthesis (k_s) in skeletal muscle. Alcohol prevented the increase in k_s when refeeding occurred intragastrically (TPN or ENT) (P < 0.001) but not intravenously (TPN). Upon intragastric refeeding, alcohol inhibited the increase in both eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and p70 S6 kinase (p70s6k) phosphorylation in plantaris but caused only partial inhibition in soleus muscle (ENT only). When rats were refed intravenously, alcohol had no effect on the increased 4E-BP1 or p70s6k phosphorylation in either muscle. Plasma insulin levels were augmented by alcohol. Alcohol-related changes in plasma amino acid concentrations were similar irrespective of the route of feeding, whereas IGF-I levels showed differential changes. This is the first study to demonstrate that acute alcohol ingestion impedes the starved-to-fed response in skeletal muscle.

fasting; myopathy; protein synthesis; ethanol; feeding

THE CONSUMPTION OF ALCOHOL induces a variety of pathogenic responses in many different tissues, including skeletal muscle (7, 29, 39, 40). Studies have shown that up to 60% of chronic alcoholics exhibit associated alcoholic myopathy (characterized by muscle weakness and wasting) (15, 34, 35). Alcoholic myopathy affects virtually all muscle groups with a selective atrophy of type II (especially IIb, fast, glycolytic) fibers relative to type I (slow, oxidative) fibers (15, 30). Although the precise mechanism of the pathogenicity is unknown, a number of pathways have been proposed, including an increased generation of reactive oxygen species and disrupted membrane function (1, 14). In skeletal muscle, as in other tissues, alcohol has been shown to promote a reduction in the fractional rate of protein synthesis (k_s) (17, 23). There appears to be very little effect of alcohol on the rate of protein degradation, at least with acute alcohol treatment (10). The reduction in k_s is greater in type II fiber-rich muscles such as plantaris compared with type I fiber-rich muscles such as soleus (24). The effect on k_s has been shown to be due, in part, to a reduction in total RNA levels (16, 25) in addition to a reduction in the translational efficiency of the mRNA (11, 12). Each of these effects appears more prominent in the plantaris compared with the soleus muscle (12, 26). The impairment of the initiation of translation in skeletal muscle has been shown, at least after chronic alcohol consumption, to occur at several sites, including eukaryotic initiation factor (eIF)2B activity and eIF4E availability (12).

Although impaired dietary intake is a frequent event in alcohol misusers (3), a number of studies have demonstrated that the myopathy occurs independently of malnutrition (6). However, exacerbation of the alcohol-induced type II fiber atrophy is found in animals on protein-deficient diets (5). Furthermore, it is also possible that alcohol interferes with the fasting-feeding transition. Therefore, the purpose of the present study was to determine whether acute alcohol ingestion inhibits the refeeding response after fasting and whether this response is similar in muscles with varying fiber types. This was addressed by measuring the k_s in skeletal muscle of rats that had been starved for 24 h and then pretreated with alcohol before being refed. In addition, the activation of both eIF4E-binding protein-1 (4E-BP1) and ribosomal protein p70 S6 kinase (p70s6k), key factors involved in the control of protein translation, were also measured in muscles of differing fiber type predominance to determine the effect of alcohol on these pathways.

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MATERIALS AND METHODS

Animals. Male Wistar rats weighing between 85 and 95 g were obtained from Harlan Olac (Bicester, Oxford, UK) and kept in a temperature-controlled and humidified animal house on a 12:12-h light-dark cycle. These animals were selected after 1 wk, when they weighed ~100 g. Animals were weighed, ranked, and divided into the groups described in Table 1.

In the present experimental design, we were primarily concerned with the hypothesis that alcohol interferes with the fasting-feeding response. To test this, we effectively compared refed with fasted animals. We did not introduce a fed animal group, since we have described in detail the synthesis rates of muscle proteins in fed (ad libitum-fed controls), starved, and refed animals (either chow solid diet or enteral or parenteral feeds) (15, 21). In addition, we employed a standard regimen of acute ethanol dosage, namely 75 mmol/kg body wt (22). With this regimen, pathophysiological plasma alcohol concentrations are achieved, for example, ~450 mg/dl at 20 min, which drops steadily to ~250 mg/dl after 2.5 h (27). The study was originally conducted as two original experiments. Thus the data are presented as two experimental sets: group A is a control for the first experiment (enteral vs. parenteral diet), and group E is a control for the second experiment (different routes (intraperitoneal vs. intragastric)). Groups A and E differ only in the infusion rate (5 vs. 2.3 ml/h, respectively).

Total parenteral nutrition and enteral nutrition. The total parenteral nutrition (TPN) solution contained Synthamin 17 without electrolyte (Baxter Healthcare, Thetford, Norfolk, UK), glucose, and 20% Ivelipid (Baxter Healthcare). The amino acid solution contained undiluted entera (Fresenius, Runcorn, UK), glucose, and 20% Ivelipid (Baxter Healthcare). The intravenous infusions were carried out for 1 h via the lateral tail vein by use of a Portex tube. The enteral nutrition (ENT) solution contained undiluted entera (Fresenius, Runcorn, Cheshire, UK) and was carried out using a pediatric nasogastric cannula (size 7) inserted via the esophagus into the stomach.

Treatment and protein synthesis measurement. Rats were starved for 24 h, and 2.5 h before being killed, they received an intraperitoneal pretreatment injection (1 ml/100 g body wt) with either saline or alcohol. This was followed 90 min later by a treatment period during which rats were administered either an intraperitoneal or intragastric infusion of water (control intragastric studies), enteral diet (ENT), TPN, or saline (intravenous studies). For treatments with the ENT diet, the infusion rate was 5 ml/h (groups A–D), whereas for the TPN diet the infusion rate was 2.3 ml/h (groups E–J). During the treatment period, the rats were lightly wrapped in a J-cloth. The doses given were 75 mmol/kg body wt for alcohol, and the control groups were injected with an identically equal volume of 0.15 mol/l NaCl. Fifty minutes after the initiation of the treatment period, rats were also injected in the tail vein with a flooding dose of radiolabeled 1-[^3H]phenylalanine (100 μCi/100 g body wt; Amersham) combined with 150 mmol/100 g body wt unlabeled phenylalanine to measure protein synthesis (8). After 10 min, the J-cloth was removed, the animals were killed by decapitation, and the gastrocnemius muscles were dissected, blotted dry, weighed, immediately frozen in liquid nitrogen, and stored at ~70°C until analysis. Phenylalanine specific activity was measured (9) in these muscles, and the k0 (expressed as percent protein synthesized/day) was calculated using the following relationship: k0 = 100 × Sb/Sa × t, where Sb and Sa are the specific radioactivities of free and protein-bound phenylalanine, respectively, and t is the incorporation time in days (9).

Plasma amino acid analysis. Blood samples were taken after decapitation by means of heparinized funnels and tubes, and the plasma was stored at ~70°C after centrifugation. Approximately 100 μl of plasma were weighed, and norleucine (4 nmol) was added. This was then transferred to a Vissipin concentrator (molecular weight cut-off, 5,000; Viva-heat, Stonehouse, Gloucestershire, UK) and lyophilized for 60 min in TSB plus 5% (vol/vol) acetic acid at 8,000 rpm for 15 min at 4°C. The filtrate was then derivatized and amino acid analysis carried out as described (2).

Plasma rat IGF-I RIA. Plasma rat IGF-I levels were analyzed using a DSL-2900 RIA kit (Diagnostic Systems Laboratories). The procedure for this test followed standard RIA protocols utilizing rat IGF-I standards (0, 150, 450, 800, 1,500, and 4,500 ng/ml rat IGF-I), 125I-labeled rat IGF-I (<5.0 μCi, <185 kBq) and goat anti-rat IGF-I antiserum. The sensitivity of the assay was 21 ng/ml.

Plasma rat insulin RIA. The plasma insulin levels were analyzed using a rat insulin RIA kit (Linco Research, St. Charles, MO). The procedure for this test followed standard RIA protocols utilizing rat insulin standards (0.1, 0.2, 0.5, 1, 2, 5, and 10 ng/ml), 125I-labeled rat insulin (<5.0 μCi, <185 kBq), and guinea pig anti-rat insulin antibody. The sensitivity of the assay was 0.1 ng/ml.

Western immunoblotting. Samples of plantaris or soleus muscle (n = 4 per treatment) were rapidly crushed between aluminum blocks, which had been precooled to ~80°C, and then homogenized on ice for 30 s in 20 mM Tris, pH 7.0, 0.27 M sucrose, 5 mM EDTA, 1% (vol/vol) Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium β-glycero-phosphate, 5 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM DTT, 22 μM leupeptin, 5 μM aprotinin, 16 μM trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane (E-64), 40 μM bestatin, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, and 15 μM pepstatin A. The protein concentrations were assayed (bicinchoninic acid assay kit, Pierce, Rockford, IL), and 150 μg (for 4E-BP1) and 20 μg (for p70[S6K]) of total protein from each treatment group were combined with Laemmli sample buffer and subjected to SDS-PAGE analysis. Gels were electrotransferred onto Immobilon-P membrane (Millipore, Bedford, MA) and blocked for 1 h at room temperature in TBST (Tris-buffered saline (TBS), pH 7.6, with 0.1% (vol/vol) Tween 20) containing 5% (wt/vol) dried milk (Marvel, Stafford, UK). Primary antibody [anti-phospho-4E-BP1 (Thr37,46); Cell Signaling Technology, Beverly, MA], p70[S6K] (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-phospho-p70[S6K] (Thr38,44; Upstate Biotechnology, Lake Placid, NY) were added in 5% (wt/vol) Marvel for 1 h at room temperature. Membranes were washed (6× for 5 min) in TBST and then incubated for 50 min in secondary antibody conjugated to horseradish peroxidase (Chemicon International, Harrow, UK). After a further six washes, the membranes were left in TBS, and chemilu-
minescent detection was carried out (Pierce). Specificity of the bands was assessed by the inclusion of an appropriate positive control from the manufacturer.

Statistical analysis. Generally, for statistical comparison between groups, data were analyzed using general analysis of variance (ANOVA). The 4E-BP1 data were analyzed in pairs with the Student’s two-tailed t-test, and the IGF-I and insulin data were analyzed by one-way ANOVA and post hoc t-tests (insulin values were log transformed before analysis). Statistical significance was set at the 5% probability level. Data are expressed as means ± SE (n = 3–9).

RESULTS

In the present studies, we used parenteral solutions without electrolytes. In doing so, we were mindful of the fact that various electrolytes can alter protein metabolism or affect skeletal muscle (20). For example, decreases in plasma and muscle potassium concentrations are associated with reduced rates of protein synthesis (20). However, the introduction of electrolytes into the infusion formulations, together with all the amino acids, glucose, and lipids in these solutions and ethanol in the circulation, would have increased blood osmolarity further. Indeed, in hyperosmolar conditions, there is an inhibition of endocytosis in the liver, which is exacerbated by ethanol exposure (4). In our previous publications on tissue protein synthesis in vivo (19, 21), we also used parenteral solutions (naymely, Synthamin 17) without electrolytes, and the present experiment is thus consistent with this approach.

Protein synthesis measurements. The k_s was measured in gastrocnemius muscle of animals from each of the treatment groups (n = 4–8). Refeeding in all cases (TPN or ENT, intragastric; TPN, intravenous) stimulated a significant increase (P < 0.05), in k_s in skeletal muscle after 24 h of starvation (Fig. 1). Alcohol pre-treatment alone had no effect on k_s. However, pretreatment with alcohol before refeeding prevented the increase in k_s (P < 0.001) when refeeding occurred intragastrically (TPN or ENT) but not intravenously (TPN) (Fig. 1).

Effect of acute alcohol treatment after fasting on the activation of 4E-BP1 and p70^{S6K} proteins in plantaris and soleus muscles. The translational repressor 4E-BP1 and the 70-kDa p70^{S6K} play key roles in regulating protein synthesis in skeletal muscle. The activity of p70^{S6K} is regulated by phosphorylation at several sites, including Thr^{412}, which correlates with p70^{S6K} activity and increased translation in vivo (38). 4E-BP1 is also regulated by phosphorylation, which results in increased translational initiation (18). 4E-BP1 phosphorylation leads to reduced electrophoretic mobility, and typically three bands, α, β, and γ, are resolved. The more highly phosphorylated form of the protein, γ, does not bind eIF4E (13). For skeletal muscle, the γ- and β-forms appear to predominate, whereas the unphosphorylated, α-form is very often expressed at low levels or is absent (36). Bands corresponding to the α-, β-, and γ-forms were detected in plantaris muscle, whereas only β- and γ-forms were detected in soleus muscle (Figs. 2–4, A and C). p70^{S6K} is activated by insulin and growth factors through phosphorylation at multiple sites in the protein (reviewed in Ref. 31). Phosphorylation of two of these sites (Thr^{252} and Thr^{412}) has been shown to contribute the most to activation of p70^{S6K} (38). An anti-phospho-p70^{S6K} (Thr^{412}) antibody was used to estimate the extent of p70^{S6K} activation. As a loading control, the Western blot was probed with an antibody that recognizes both phosphorylated and non-phosphorylated p70^{S6K} protein (Figs. 2–4, B and D). Levels of p70^{S6K} activity (as measured by Thr^{412} phosphorylation) after starvation always appeared higher in soleus than in plantaris muscle (compare Figs. 2–4, B and D).

The effect of alcohol pretreatment alone on starved muscle resulted in consistently decreased activation of both 4E-BP1 and p70^{S6K} in both plantaris and soleus muscles (Figs. 2 and 3). However, this effect was not significant at the 5% level. When refeeding was administered to starved rats by intragastric TPN in plantaris muscle, this resulted in activation of 4E-BP1 and p70^{S6K}, as evidenced by an increase in the abundance of the α-form of 4E-BP1 and a large increase in p70^{S6K} Thr^{412} phosphorylation (P < 0.05; Fig. 2, A and B). Similar activation of both 4E-BP1 (P < 0.05) and p70^{S6K} (P < 0.05) by refeeding by intragastric TPN also occurred in soleus muscle (Fig. 2, C and D). When alcohol was given before refeeding by intragastric TPN, this prevented the activation of both 4E-BP1 (P < 0.05) and p70^{S6K} (P < 0.05) in both muscles (Fig. 2). When refeeding occurred by intragastric ENT, these results were essentially identical for plantaris muscle in that alcohol pretreatment prevented the increase in activation of both 4E-BP1 (P < 0.05) and p70^{S6K} (P < 0.05).
However, in soleus muscle, alcohol pretreatment upon refeeding by intragastric ENT had a much lesser effect. Activation of 4E-BP1 was not prevented by the alcohol pretreatment (Fig. 3C), whereas p70S6K activation was significantly reduced but to levels above those of starvation (Fig. 3D). In contrast, when refeeding occurred by intravenous TPN, alcohol had no effect on either 4E-BP1 or p70S6K phosphorylation in either plantaris (Fig. 4, A and B) or soleus muscle (Fig. 4, C and D).

Plasma amino acid analysis after refeeding: effect of acute alcohol treatment. Amino acid analysis was carried out on the plasma from the animal groups fed TPN (both intravenously and intragastrically) pretreated with and without alcohol. As can be seen in Table 2, alcohol pretreatment alone on starved animals resulted in a significant reduction in serine, alanine, threonine, and methionine concentrations concomitant with an increase in leucine and isoleucine levels. Feeding starved animals with TPN intragastrically resulted in significant increases in histidine, alanine, arginine, proline, lysine, and methionine levels compared with the water-treated controls. Alcohol pretreatment before refeeding with TPN intragastrically resulted in significantly elevated concentrations of glycine, threonine, and all branched-chain amino acids, together with a significant augmentation in the level of histidine compared with saline pretreatment and refeeding (Table 2). Refeeding starved animals with TPN intravenously resulted in significantly increased concentrations of glycine, threonine, and all branched-chain amino acids, together with a significant augmentation in the level of histidine compared with saline pretreatment and refeeding (Table 2). Refeeding starved animals with TPN intravenously caused significant increases in the same amino acids (except for lysine) as when TPN was fed intragastrically, with an additional significant increase in valine levels. Alcohol pretreatment before refeeding with TPN intravenously resulted in similar
changes in amino acid levels compared with alcohol pretreatment before refeeding with TPN intragastrically (Table 2). Namely, there were significant elevations in the concentrations of glycine and threonine, together with the isoleucine and leucine branched-chain amino acids, and a significant augmentation in the levels of histidine and valine compared with saline pretreatment and refeeding.

**Plasma IGF-I and insulin concentrations: effect of acute alcohol.** The plasma concentrations of IGF-I and insulin were measured in animals from each of the treatment groups \((n = 4–9)\). When starved rats were refed intragastrically with either ENT or TPN diet, there were elevations in the plasma level of IGF-I that were significant in the case of TPN (from 482 ± 131 to 857 ± 46 ng/ml; Table 2). In both cases, alcohol pretreatment before refeeding inhibited the increases in plasma IGF-I and resulted in a significantly reduced level of IGF-I compared with the fed controls. Additionally, in the ENT case, the level of IGF-I with the alcohol pretreatment was significantly below the starved level (Table 3). Conversely, when refeeding occurred intravenously with TPN, there was no significant increase in plasma IGF-I levels. Alcohol pretreatment reduced IGF-I levels to below refed levels (Table 3). Plasma concentrations of insulin were significantly elevated when animals were refed intragastrically with ENT, but, although elevated, there were no significant increases of insulin concentrations with the other two refeeding regimens (Table 4). In all three cases, insulin levels were significantly increased over starved levels by alcohol pretreatment. With intrave-
nous refeeding of TPN, there was also a significant increase compared with the refed state (Table 4).  

**DISCUSSION**

In this study, we have investigated the effect of acute alcohol administration on the refeeding response. Refeeding was carried out using two different diets (TPN and ENT) and by two different routes of administration (intragastric and intravenous). The results show that a single bolus injection of alcohol prevents the increase in the \( k_s \) in skeletal muscle normally seen upon refeeding. This effect was dependent on the route of refeeding but independent of diet composition. When refeeding occurred by intragastric infusion of either TPN or ENT diet, \( k_s \) was inhibited by alcohol pretreatment. However, when given intravenously (TPN diet), \( k_s \) was not inhibited by alcohol. This is the first study to demonstrate that alcohol impedes the refeeding response in skeletal muscle.

To investigate further the contrasting results of alcohol on \( k_s \), we measured the levels of amino acids in plasma upon refeeding in the presence and absence of alcohol. Refeeding by the TPN diet invoked essentially similar increases in amino acid concentrations independent of the route of administration (intragastric vs. intravenous). Similarly, the response to alcohol pretreatment was essentially identical, independent of whether the diet was administered intragastrically or intravenously (Table 2): both treatments resulted in increased concentrations of several amino acids, with a
particular large increase in the concentrations of the branched-chain amino acids. We conclude from these results that the inhibitory effect of alcohol on $k_s$ is unlikely to occur as a result of altered uptake of amino acids by the gut when the diet is fed intragastrically. Additionally, we find that the branched-chain amino acids are upregulated in both groups, suggesting that the acute alcohol treatment either prevents their uptake or, alternatively, promotes their release from a tissue(s). Evidence in favor of the latter conclusion comes from the finding that alcohol pretreatment alone, in the absence of refeeding, stimulates a significant increase in plasma isoleucine and leucine levels (Table 2).

We then investigated whether acute alcohol treatment induced changes in plasma concentrations of rat IGF-I or insulin in each of the treatment groups. In general, we found that, as expected, plasma insulin levels were increased upon refeeding but that alcohol pretreatment further augmented these levels. Because there were no clear differences due to the alcohol between intragastric and intravenous refeeding, it is thought unlikely that the differences in $k_s$ were directly mediated by an effect of alcohol on plasma insulin levels. The effect of refeeding on plasma IGF-I levels did exhibit a difference due to the route of refeeding. When refeeding occurred intragastrically, IGF-I levels increased, and these increases were inhibited by alcohol. Conversely, when refeeding occurred intravenously, there was no increase in plasma IGF-I levels, and alcohol reduced the starved levels still further. A potential mechanism, therefore, for the action of alcohol may be proposed if we assume that the increase in $k_s$ that occurs with intragastric refeeding is mediated by plasma IGF-I, whereas the increase with intravenous refeeding is not. Alcohol may, therefore, inhibit $k_s$ when refeeding occurs intragastrically by preventing the increase in IGF-I but would not inhibit $k_s$ by intravenous refeeding since it is not IGF-I mediated. The inhibitory effects of alcohol on two downstream targets of IGF-I signaling, 4E-BP1 and p70S6K (31), during intragastric but not intravenous refeeding would be consistent with this theory (see below). Additionally, an interaction between alcohol and IGF-I has been previously noted. Studies by Sonntag and Boyd (32) showed that feeding alcohol decreased plasma levels of IGF-I, albeit in chronically alcohol-fed animals, which did not appear to occur as a result of reduced growth.

### Table 2. Plasma amino acid concentrations from TPN IG and TPN IV treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Asp</th>
<th>Glu</th>
<th>Ser</th>
<th>Gly</th>
<th>Gin</th>
<th>His</th>
<th>Thr</th>
<th>Ala</th>
<th>Asp</th>
<th>Arg</th>
<th>Pro</th>
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<th>Lys</th>
<th>Met</th>
<th>Ile</th>
<th>Leu</th>
<th>Val</th>
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<tbody>
<tr>
<td>A</td>
<td>SAL IP WATER IG</td>
<td>10.0±1.3</td>
<td>10.8±14.6</td>
<td>119.0±4.0</td>
<td>7.7±1.1</td>
<td>9.0±0.6</td>
<td>10.7±0.7</td>
<td>13.0±2.1</td>
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<tr>
<td>B</td>
<td>ALC IP WATER IG</td>
<td>10.5±1.0</td>
<td>10.8±14.6</td>
<td>119.0±4.0</td>
<td>7.7±1.1</td>
<td>9.0±0.6</td>
<td>10.7±0.7</td>
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<tr>
<td>C</td>
<td>SAL IP ENT IG</td>
<td>10.5±1.0</td>
<td>10.8±14.6</td>
<td>119.0±4.0</td>
<td>7.7±1.1</td>
<td>9.0±0.6</td>
<td>10.7±0.7</td>
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<tr>
<td>D</td>
<td>ALC IP ENT IG</td>
<td>10.5±1.0</td>
<td>10.8±14.6</td>
<td>119.0±4.0</td>
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<td>9.0±0.6</td>
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<tr>
<td>E</td>
<td>SAL IP WATER IG</td>
<td>10.5±1.0</td>
<td>10.8±14.6</td>
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<tr>
<td>F</td>
<td>SAL IP TPN IV</td>
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<tr>
<td>G</td>
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<td>10.8±14.6</td>
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Values are means ± SE in mmol/l plasma; $n = 3–8$. SAL, saline; ALC, alcohol; TPN, total parenteral nutrition; ENT, enteral nutrition.

### Table 3. Plasma IGF-I concentrations: effect of refeeding and alcohol

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>IGF-I</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>SAL IP WATER IG</td>
<td>602±83</td>
</tr>
<tr>
<td>B</td>
<td>ALC IP WATER IG</td>
<td>505±71</td>
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<tr>
<td>C</td>
<td>SAL IP ENT IG</td>
<td>810±35</td>
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<tr>
<td>D</td>
<td>ALC IP ENT IG</td>
<td>302±52.4</td>
</tr>
<tr>
<td>E</td>
<td>SAL IP WATER IG</td>
<td>482±131</td>
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<tr>
<td>F</td>
<td>SAL IP TPN IV</td>
<td>857±46.0</td>
</tr>
<tr>
<td>G</td>
<td>ALC IP TPN IG</td>
<td>326±70.6</td>
</tr>
<tr>
<td>H</td>
<td>SAL IP SAL IV</td>
<td>508±97.0</td>
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<tr>
<td>I</td>
<td>ALC IP TPN IG</td>
<td>610±112</td>
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<tr>
<td>J</td>
<td>ALC IP TPN IV</td>
<td>410±56.0</td>
</tr>
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</table>

Values are means ± SE in ng/ml; $n = 4–9$. $^aP < 0.01$, $^bP < 0.05$ compared with starved group; $^cP < 0.05$, $^dP < 0.001$ compared with refeed control group.

### Table 4. Plasma insulin concentrations: effect of refeeding and alcohol

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Insulin</th>
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<tbody>
<tr>
<td>A</td>
<td>SAL IP WATER IG</td>
<td>0.136±0.009</td>
</tr>
<tr>
<td>B</td>
<td>ALC IP WATER IG</td>
<td>0.178±0.019</td>
</tr>
<tr>
<td>C</td>
<td>SAL IP ENT IG</td>
<td>0.704±0.206$^a$</td>
</tr>
<tr>
<td>D</td>
<td>ALC IP ENT IG</td>
<td>1.545±0.564$^a$</td>
</tr>
<tr>
<td>E</td>
<td>SAL IP WATER IG</td>
<td>0.140±0.019</td>
</tr>
<tr>
<td>F</td>
<td>SAL IP TPN IG</td>
<td>0.207±0.024</td>
</tr>
<tr>
<td>G</td>
<td>ALC IP TPN IG</td>
<td>0.468±0.109$^a$</td>
</tr>
<tr>
<td>H</td>
<td>SAL IP SAL IV</td>
<td>0.173±0.021</td>
</tr>
<tr>
<td>I</td>
<td>ALC IP TPN IG</td>
<td>0.392±0.114</td>
</tr>
<tr>
<td>J</td>
<td>SAL IP TPN IV</td>
<td>1.741±0.543$^a$</td>
</tr>
</tbody>
</table>

Values are means ± SE in ng/ml; $n = 3–9$. $^aP < 0.05$, $^bP < 0.01$ compared with starved control group; $^cP < 0.05$ compared with refeed control group.
hormone levels (33). In addition, it has also been demonstrated that alcohol can inhibit IGF-I signaling, which appears to occur at the level of the IGF-I receptor (28). However, it is equally plausible that the effects of alcohol may not be directly mediated by altered levels of circulating growth factors but instead could be mediated by changes in the expression of autocrine factors in the muscle itself. Some evidence for this already exists (11).

We subsequently investigated the effect of alcohol and refeeding on markers of protein translation to confirm our findings on $k_s$ and determine which signaling pathways were involved and to establish whether this response exhibited fiber type specificity. Phosphorylation of both 4E-BP1 and p70S6K was measured, as together these proteins regulate translational initiation and, ultimately, protein synthesis rates. The basal levels of phosphorylation, and hence activation, of both 4E-BP1 and p70S6K were higher in soleus vs. plantaris muscle in accordance with the higher rates of $k_s$ in soleus (8). Alcohol treatment alone inhibited these basal phosphorylation levels in soleus muscle (Figs. 2 and 3). This finding is inconsistent with the greater effect of alcohol on the atrophy of a predominantly fast-twitch fiber muscle such as plantaris compared with the predominantly slow-twitch fiber soleus muscle (24). Refeeding almost invariably resulted in increased activation of 4E-BP1 and p70S6K in both muscle types. The activation of both 4E-BP1 and p70S6K was prevented in plantaris muscle by acute alcohol treatment before intragastric refeeding, mimicking the results on $k_s$ and identifying the pathway through which these effects are likely mediated. This is the first demonstration that acute alcohol treatment can inhibit p70S6K phosphorylation in skeletal muscle, although a demonstration that acute alcohol treatment can inhibit reduced phosphorylation of this kinase in heart muscle (37). Therefore, it may be that inhibition of p70S6K activity and decreased availability of eIF4E together form the main mechanism through which alcohol inhibits protein synthesis in muscle tissue.

In soleus muscle, it was also noted that, when refeeding occurred by ENT intragastrically, the effect of alcohol on soleus muscle appeared less inhibitory compared with plantaris muscle: 4E-BP1 phosphorylation was not significantly inhibited by alcohol at all, whereas p70S6K activation was inhibited but to a lesser extent than in the faster muscle (Fig. 3). This finding is again consistent with the lesser effect of alcohol on $k_s$ (and myopathy) in soleus compared with plantaris muscle (24).

The regulation of 4E-BP1 and p70S6K activities has been shown to be under both nutritional and hormonal control (for review see Ref. 31). The finding that plasma amino acid concentrations (at least those present 1 h after refeeding) appear very similar in both the alcohol-treated intragastrically and intravenously fed groups, yet 4E-BP1 and p70S6K levels (and $k_s$) are inhibited by intragastric but not intravenous refeeding, suggests that alcohol is unlikely to be mediating its effects through altered absorption of amino acids at the level of the gut. As discussed above, it may be that such changes involve alcohol-induced modulation of plasma hormone levels. Taken as a whole, this study has demonstrated that the consumption of an acute dose of alcohol completely inhibits the refeeding response in fasted animals. It should be noted, however, that this observed acute or “naive” response to ethanol exposure would have to be sustained over prolonged exposure for a suppressed refeeding synthetic response to translate into muscle myopathy, as this occurs as a consequence of repeated and prolonged episodes of ethanol ingestion (15). Nevertheless, this is an important finding in the etiology of alcohol-induced myopathy; furthermore, we have identified a step, namely, the fasting-feeding transition, which should allow further insight into its mechanism of action.

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REFERENCES


