Skeletal muscle protein synthesis and degradation exhibit sexual dimorphism after chronic alcohol consumption but not acute intoxication

Charles H. Lang, Robert A. Frost, and Thomas C. Vary
Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Submitted 12 November 2006; accepted in final form 24 January 2007

Lang CH, Frost RA, Vary TC. Skeletal muscle protein synthesis and degradation exhibit sexual dimorphism after chronic alcohol consumption but not acute intoxication. Am J Physiol Endocrinol Metab 292:E1497–E1506, 2007. First published January 30, 2007; doi:10.1152/ajpendo.00603.2006.—Epidemiological evidence suggests alcoholic myopathy is more severe in females than males, but comparable animal studies are lacking that make elucidating the biochemical locus for this defect problematic. The present study determined whether skeletal muscle protein synthesis and markers of degradation exhibit a sexual dimorphic response to either chronic alcohol consumption or acute intoxication. Male and female rats were fed an alcohol-containing diet, pair-fed for 26 wk (chronic), or received an intraperitoneal injection of alcohol (acute). In males, chronic alcohol decreased gastrocnemius protein synthesis by 20%. This reduction was associated with a twofold increase in the inactive eukaryotic initiation factor (eIF) 4E·4E-binding protein 1 (4E-BP1) complex and a 60% reduction in the active eIF4E·eIF4G complex. This redistribution of eIF4E was associated with decreased phosphorylation of both 4E-BP1 and eIF4G (50–55%). The phosphorylation of ribosomal protein S6 was also reduced 60% in alcohol-consuming male rats. In contrast, neither rates of protein synthesis nor indexes of translation initiation in muscle were altered in alcohol-fed female rats despite blood alcohol levels comparable to males. Chronic alcohol ingestion did not alter atrogin-1 or muscle RING finger-1 mRNA content (biomarkers of muscle proteolysis) in males but increased their expression in females 50–100%. Acute alcohol intoxication produced a comparable decrease in muscle protein synthesis and translation initiation in both male and female rats. Our data demonstrate a sexual dimorphism for muscle protein synthesis, translation initiation, and proteolysis in response to chronic, but not acute, alcohol intoxication; however, they do not support evidence indicating females are more sensitive toward the development of alcoholic skeletal muscle myopathy.

ribosomal protein S6; 4E-binding protein 1; eukaryotic initiation factor 4G; insulin-like growth factor I; proteolysis

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

WHEREAS LIGHT TO MODERATE alcohol consumption may exert beneficial effects in some organ systems (36), chronic alcohol abuse leads to diffuse organ pathology, including proximal muscle wasting (16, 30). The severity of the muscle atrophy is proportional to the duration and amount of alcohol consumed and leads to decreased muscle strength (39). Although the etiology of this alcohol myopathy is multifactorial, at least one defect pertains to the diminished synthetic rate of structural and myofibrillar proteins (29, 31, 42). A decrease in protein synthesis is also observed in fast-twitch muscles in response to acute alcohol intoxication (15, 19, 34). Alcohol reduces translational efficiency in skeletal muscle by limiting peptide-chain initiation (19, 20, 37). A decreased activity of the proline-directed serine (Ser)/threonine (Thr) protein kinase mammalian target of rapamycin (mTOR) appears to be a primary locus for this impairment. This is evidenced by the alcohol-induced dephosphorylation of mTOR per se as well as of two mTOR substrates [the 4E-binding protein (BP1) and the ribosomal S6 kinase (S6K1) (18, 19, 42) that control selected aspects of mRNA translation (8).

Epidemiological evidence suggests that the prevalence of alcoholism and binge drinking is increasing in women in the United States (43). Furthermore, many of the adverse consequences of alcohol consumption, including alcoholic cardiac and skeletal muscle myopathy, develop more rapidly and require lower levels of alcohol exposure in females compared with males (9, 12, 27, 29, 38, 39). These observations cannot be explained by differences in body weight, fat distribution, body water, differential rates of ethanol oxidation, or other potentially confounding variables. Hence, although it is generally accepted that females have a greater sensitivity to alcohol-induced muscle damage than males, the underlying metabolic and pathophysiological mechanisms leading to this sex difference have been largely neglected. Recently, it has been reported that female animals had a greater alcohol-induced decrease in the RNA-to-protein ratio in some muscles (e.g., plantaris) but not others (e.g., gastrocnemius and soleus) compared with males, suggesting a decreased capacity for protein synthesis in particular types of skeletal muscles (11); however, protein synthesis was not directly quantitated. Therefore, the purpose of the present study was to determine whether chronic alcohol consumption produced a sexual dimorphic response related to muscle protein synthesis per se and to assess whether alcohol-induced changes in the signal transduction pathways regulating protein synthesis were compromised in both male and female rats. In addition, we determined whether a comparable decrement in the muscle protein synthetic response in male and female rats occurred after acute alcohol intoxication (e.g., binge drinking). Finally, tissue protein content represents a balance between rates of synthesis and degradation. Therefore, we also determined whether there was a differential response between male and female rats in response to alcohol with regard to several muscle-specific E3 ligases known to regulate the ubiquitin-proteasome degradation of proteins.

MATERIALS AND METHODS

Chronic alcohol feeding. Age-matched, pathogen-free male and female Sprague-Dawley rats (Charles River Breeding Laboratories)
were housed at a constant temperature, exposed to a 12:12-h light-dark cycle, and maintained on standard rodent chow (no. 2018; 18% protein rodent diet; Harlan, Madison, WI) and water ad libitum for at least 1 wk before experiments were performed. Thereafter, rats were maintained for 26 wk on an ethanol-containing diet in which alcohol was provided both in drinking water and previously described agar blocks (2, 20, 21, 42). The duration of alcohol feeding was selected based on previous results indicating that alcoholic cardiomyopathy was more severe at this time point than at early times between 12 and 20 wk (16). Initially, all rats (both male and female) were provided the agar block without ethanol for 2 days. Male and female animals were then randomly assigned to either an alcohol or control group. Animals in the alcohol group were given free access to ethanol-containing agar blocks. The concentration of ethanol in the agar was increased in 10% increments from 10 to 40% over the first 4 wk. Ethanol-fed rats remained on the 40% ethanol-agar block diet for the remainder of the experimental protocol. Control agar blocks contained an equal caloric amount of dextrose-maltose. Standard rat chow (Teklad no. 8604; Harlan) provided nutrient intake in both groups. Control rats were provided the same amount of solid food as freely consumed by the sex-matched alcohol-fed group. Rats had free access to food and/or alcohol until 7:00 A.M. on the morning of the experiments. Total energy consumption does not differ between alcohol- and pair-fed animals of the same sex (20). Over the final 4 wk of the feeding protocol, total ethanol intake was significantly greater in female rats compared with male animals (30 ± 1 vs. 27 ± 1 g ethanol·kg body wt⁻¹·day⁻¹, P < 0.05; see Ref. 41), which is consistent with previous reports indicating greater ethanol consumption by female rats (1). All four experimental groups were run concurrently. This and all experiments described herein were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine and adhered to National Institutes of Health (NIH) guidelines for use of experimental animals.

**Acute alcohol intoxication.** Age-matched overnight-fasted male and female rats were divided randomly into two groups; the alcohol-treated group was injected intraperitoneally with ethanol (75 mmol/kg body wt; 20% wt/vol in saline), and the control group was injected with an equal volume of 0.9% sterile saline. This dose of ethanol was selected because it significantly decreases muscle protein synthesis (15, 19, 34). The inhibition of muscle protein synthesis is independent of the route of alcohol administration (e.g., ip injection vs. oral gavage; see Ref. 19). Rats were wrapped loosely in a porous towel at 30, 60, and 90 min, and a small (∼0.2 ml) blood sample was collected from a tail snip for determination of alcohol concentration. Rats were then anesthetized 2.5 h after receiving either ethanol or saline with an intraperitoneal injection of pentobarbital sodium (100 mg/kg), protein synthesis was determined, and blood and tissues were collected at this time.

**Muscle protein synthesis.** In vivo protein synthesis in gastrocnemius was determined in both chronic alcohol-fed animals and animals injected intraperitoneally with ethanol using the flooding-dose technique (5, 15, 20). Briefly, animals were anesthetized (Nembutal; 100 mg/kg body wt), and a catheter was surgically placed in the carotid artery. An initial 1 ml of blood was removed for measuring the plasma concentrations of ethanol and various hormones. Subsequently, a bolus injection of L-[2,3,4,5,6-³H]phenylalanine (Phe; 150 mM, 30 µCi/ml; 1 ml/100 g body wt) was injected via the jugular vein. At 2, 6, and 10 min after Phe injection, blood samples (1 ml) were drawn for measurement of Phe concentration and radioactivity. Immediately after the final blood sample, a portion of the gastrocnemius muscle was frozen between aluminum blocks precooled to the temperature of liquid nitrogen, and another portion of muscle was homogenized directly. Blood was centrifuged, and plasma was collected. All tissue and plasma samples were stored at −80°C until analyzed. The frozen muscle was powdered under liquid nitrogen, and a portion was used to estimate the rate of incorporation of [³H]Phe into protein, exactly as previously described (5).

**Immunoprecipitation and Western blotting.** The tissue preparation was the same as previously described by our laboratory (15, 17, 19). Briefly, muscle (either gastrocnemius or soleus) was homogenized with a 1.5 ratio of ice-cold homogenization buffer (in mM: 20 HEPS, pH 7.4, 2 EGTA, 50 NaF, 100 KCl, 0.2 EDTA, 50 β-glycerophosphate, 1 dithiothreitol, 0.1 phenylmethylsulfonyl fluoride, 1 benzamidine, and 0.5 sodium vanadate) using a Polytron homogenizer and clarified by centrifugation (10,000 g). The gastrocnemius and soleus muscles were sampled because they are representative muscles with a predominance of fast-twitch and slow-twitch fibers, respectively. The supernatant was aliquoted into microcentrifuge tubes, and an equal volume of 2× Laemmli sample buffer was added. The samples were boiled before being used for Western blot analysis. The samples were subjected to electrophoresis on either a 6% polyacrylamide gel for mTOR, 7.5% gel for S6K1, or a 15% polyacrylamide gel for phosphorylated ribosomal protein S6 and 4E-BP1. Proteins were electrophoretically transferred to nitrocellulose membranes. The blots were incubated with either primary antibodies to total S6K1 (no. 20; Santa Cruz Biotechnology, Santa Cruz, CA), phospho-specific S6K1 (Thr389; Cell Signaling), total 4E-BP1 (Bethyl Laboratories, Montgomery, TX), phospho-specific 4E-BP1 (Thr754; Cell Signaling), total and phosphorylated (Ser248 and Ser244) mTOR (Bethyl Laboratories), total and phosphorylated (Ser1108) eukaryotic initiation factor (eIF)-4G (Cell Signaling), or total and phosphorylated S6 (Ser235/Ser236) (Cell Signaling). The blots were washed with 1× TBS including 0.1% Tween-20 and incubated with secondary antibody (horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit) at room temperature. The blots were developed with enhanced chemiluminescence (ECL) Western blotting reagents as per the manufacturer’s (Amersham) instructions. The blots were exposed to X-ray film in a cassette equipped with a DuPont Lightning Plus intensifying screen. After development, the film was scanned (ScanMaker IV; Microtek, Carson, CA) and analyzed using NIH Image 1.6 software. For this and all subsequent analyses, samples from all four experimental groups were run concurrently.

The 4E-BP1-eIF4E and eIF4G-eIF4E complexes were quantified as described previously (15, 17, 19). Briefly, eIF4E was immunoprecipitated from aliquots of supernatants using an anti-eIF4E monoclonal antibody (Drs. Jefferson and Kimball, Pennsylvania State University, Hershey, PA). Antibody-antigen complexes were collected using magnetic beads and subjected to electrophoresis using a 7.5% or a 15% polyacrylamide gel. Proteins were then electrophoretically transferred to a nitrocellulose membrane. The blots were incubated with a mouse anti-human eIF4E antibody, a rabbit anti-rat 4E-BP1 antibody, or a goat anti-eIF4G antibody. The phosphorylated forms of 4E-BP1 were measured after immunoprecipitation of 4E-BP1 from the tissue homogenates after centrifugation. The various phosphorylated forms of 4E-BP1 were separated by SDS-PAGE and analyzed by protein immunoblotting. The blots were then developed with ECL, and the autoradiographs were scanned for analysis as described above.

**Northern blot analysis of E3 ubiquitin ligases.** Total RNA was isolated from frozen gastrocnemius and soleus using TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s protocol. Samples (25 µg) of total RNA were electrophoresed under denaturing conditions in 1.0% agarose/6% formaldehyde gels using 1× HEPES running buffer. Northern blotting occurred via capillary transfer to Nytran SuperCharge membranes (Schleicher & Schuell, Keene, NH). Rat oligonucleotides were synthesized (IDT, Coralville, IA) and radioactively labeled using TdT (Promega, Madison, WI). A 18S oligonucleotide was labeled by the same method and used for normalization of RNA loading. Northern blots were hybridized using ULTRAhyb (Ambion, Austin, TX). All membranes were initially washed two times in 2× saline-sodium citrate (SSC)-0.1% SDS at 42°C and one time in 0.2× saline-sodium citrate (SSC)-0.1% SDS at 65°C.
Sex- and alcohol-induced changes in protein synthesis

Plasma concentrations of alcohol and hormones. The plasma insulin concentration was measured using a commercial RIA for rat insulin (Linco Research, St. Charles, MO). Additionally, the plasma concentrations of insulin-like growth factor (IGF)-I, estradiol, and testosterone were determined using commercial RIA kits (DSLabs, Webster, TX). The limit of detection for these latter two RIAs was 2.2 pg/ml and 0.08 ng/ml, respectively. The plasma alcohol concentration was determined by a rapid analyzer (Analog Instruments, Lunenburg, MA).

Statistical analysis. Experimental data for each condition are summarized as means ± SE, where the number of animals for each treatment group is indicated in the legends to Figs. 1–8 and Tables 1 and 2. Statistical evaluation of the data was performed using ANOVA followed by the Student-Newman-Keuls test to determine treatment effect. Differences between the groups were considered significant when P < 0.05.

RESULTS

Plasma alcohol concentration. In chronic alcohol-fed rats, the plasma alcohol concentration was not significantly different between male (87 ± 23 mg/dl) and female (110 ± 23 mg/dl) animals at the time of death.

Muscle protein content and synthesis. After the alcohol-containing diet was consumed for 26 wk, the gastrocnemius muscle weight was significantly reduced by 11% in male rats compared with values from pair-fed male control animals (Table 1). As a result of this change, alcohol-fed male rats exhibited a comparable decrease in the total protein per muscle. In contrast, chronic alcohol consumption did not alter the weight or muscle protein content of the gastrocnemius in female rats (Table 1).

Muscle protein synthesis, expressed as nanomoles Phe incorporated per gram wet weight muscle per hour, was 20% lower in chronic alcohol-fed male rats compared with pair-fed male control animals (Fig. 1). This reduction in protein synthesis occurred despite equal caloric and nitrogen intake between the pair-fed control and alcohol-fed groups. Hence, the alcohol-induced derangements in protein synthesis and translation initiation described subsequently appear to be independent of nutritional status. Although the basal rate of gastrocnemius protein synthesis tended to be greater in control females vs. control males (24%), this change did not achieve statistical significance. Alcohol feeding did not significantly reduce muscle protein synthesis in female rats, in contrast to the decrement seen in male rats (Fig. 1). Qualitatively similar alcohol- and sex-induced changes in gastrocnemius protein synthesis were also observed when the synthetic rate was normalized to the muscle protein concentration [male controls = 1.03 ± 0.04, male + alcohol = 0.79 ± 0.05 (P < 0.05 compared with male control values), female controls = 1.44 ± 0.09 (P < 0.05 compared with male control values), female + alcohol = 1.28 ± 0.05 (P < 0.05 compared with male control values) nmol Phe·mg protein~1·h~1], except protein synthesis in the gastrocnemius from female rats was now statistically greater than that seen in male rats.

To determine whether a change in the number of ribosomes or the efficiency of mRNA translation was responsible for the alcohol-induced changes in muscle protein synthesis, the RNA content of muscle was quantified. There was no statistically significant sex- or alcohol-induced change in the total RNA per muscle protein (Table 1). These data suggest that the alcohol-induced decrease in muscle protein synthesis seen in male rats (Fig. 1) resulted from a corresponding change in the efficiency of translation (e.g., nmol Phe·mg RNA~1·h~1) and not a change in the number of ribosomes.

Regulation of eIF4F complex. The mechanistic interactions between sex and alcohol were investigated by analysis of known regulatory steps in the control of translation initiation (16, 18). Our previous studies indicated that alcohol feeding does not alter the eIF2/2B system, which controls the binding of met-tRNAi to the 4OS ribosomal subunit and the formation of the 43S preinitiation complex, in skeletal muscle (17). Therefore, we focused on elucidating the alcohol-induced changes in a second critical locus of translational regulation involving the binding of the 5′-end of cellular mRNA to the 43S preinitiation complex, a reaction mediated by the cap-binding protein complex eIF4F (28). This is a heterotrimeric

Table 1. Sex-related differences in gastrocnemius wet weight, protein content, and RNA concentration in rats fed an alcohol-containing diet for 26 wk

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alcohol</td>
</tr>
<tr>
<td>Gastrocnemius wt, g</td>
<td>7.20 ± 0.26†</td>
<td>6.44 ± 0.25†</td>
</tr>
<tr>
<td>Total muscle protein, g protein/muscle</td>
<td>1.43 ± 0.10*</td>
<td>1.24 ± 0.06*</td>
</tr>
<tr>
<td>RNA concentration, mg RNA/g muscle</td>
<td>1.19 ± 0.05</td>
<td>1.16 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE, where the sample size was 8, 17, 8, and 18 for the 4 groups, respectively. Values with different superscripts for a particular parameter are significantly different from each other, P < 0.05. Values with the same superscript are not significantly different.
complex composed of eIF4E, eIF4G, and eIF4A, of which the former protein is considered rate limiting in skeletal muscle. There was no significant difference in the total amount of eIF4E in gastrocnemius from male and female rats or in rats fed an alcohol-containing diet (Fig. 2A). However, there were marked sex-specific effects in the distribution of eIF4E between active and inactive complexes in response to alcohol consumption. The amount of the inactive 4E-BP1:eIF4E complex was increased more than twofold in male alcohol-fed rats (Fig. 2, B and D). In contrast, the association of eIF4E with 4E-BP1 in female rats was not altered by alcohol feeding. Moreover, there was no difference in the amount of this inactive complex in gastrocnemius from male and female rats under basal conditions.

The amount of the active eIF4G:eIF4E complex was decreased 60% in gastrocnemius from alcohol-fed male rats compared with values from pair-fed control animals (Fig. 2, C and E). In contrast, an alcohol-induced decrease in the binding of eIF4G with eIF4E was not seen in female rats, although there was a 30% reduction in the basal amount of the active eIF4G:eIF4E complex in female rats compared with basal control values from male rats.

**Phosphorylation of 4E-BP1, eIF4G, eIF4E, and S6.** The binding of eIF4E to eIF4G is controlled in part by a family of cap-dependent translational repressors, and, in skeletal muscle, the most prominent member of the family is 4E-BP1 (10). Hyperphosphorylation of 4E-BP1 liberates it from eIF4E and allows binding of eIF4E with eIF4G and the stimulation of protein synthesis. The amount of the hyperphosphorylated γ-isofrom of 4E-BP1 was comparable in gastrocnemius from male and female rats under basal conditions (Fig. 3). However, a sex-specific response to alcohol feeding was observed in which 4E-BP1 phosphorylation was decreased only in male rats.

The interaction between eIF4E and eIF4G may also be regulated, in part, by the phosphorylation of eIF4G, which is enhanced by mitogen stimulation and inhibited by the mTOR inhibitor rapamycin (8, 32, 33). Although there was no sex-related difference in the extent of constitutive eIF4G phosphorylation, chronic alcohol consumption reduced eIF4G phosphorylation by nearly 70% in gastrocnemius from male, but not female, rats (Fig. 4, A and C). This change was independent of a change in the total amount of eIF4G (Fig. 4B).

Increased phosphorylation of eIF4E per se enhances binding of the mRNA to eIF4E (32). However, we failed to detect a statistically significant effect of chronic alcohol on eIF4E phosphorylation in gastrocnemius from either male [control = 100 ± 8 arbitrary units (AU) vs. alcohol = 107 ± 11 AU] or female [control = 97 ± 8 AU vs. alcohol = 104 ± 9 AU] rats. Therefore, differential phosphorylation of this initiation factor by alcohol does not appear to modulate the sexual dimorphic response in muscle protein synthesis between male and female rats seen either under basal conditions or after alcohol feeding.

Similar to the above-mentioned changes in the phosphorylation of 4E-BP1 and eIF4G, the phosphorylation of the ribosomal protein S6 was markedly decreased in gastrocnemius from alcohol-fed male rats but not in female animals (Fig. 5, A and C). There was no statistical difference in the constitutive phosphorylation of S6 between male and female rats. Finally, there were not sex- or alcohol-induced changes in total S6 content in muscle (Fig. 5B). In the current study, we did not assess the phosphorylation state and activation of the upstream kinase S6K1 because constitutive phosphorylation of this protein is essentially undetectable in muscle under basal conditions (17).

**Translation initiation in soleus muscle.** In contrast to the type II fiber-rich gastrocnemius muscle, the soleus is composed of predominantly type I fibers. Hence, we also assessed selected endpoints in this muscle. Immunoblot analysis indicated there was no alcohol-induced decrease in 4E-BP1, eIF4G, or S6 phosphorylation nor any alteration in the distribution of eIF4E between active and inactive complexes in the soleus for either male or female rats (data not shown). Because of the lack of any detectable alcohol-induced change in soleus muscle, the absolute rate of protein synthesis was not determined.
Hormone concentrations. There was no consistent sex- or alcohol-induced change in the plasma insulin concentration at the time point examined (Table 2). Chronic alcohol consumption also did not alter the plasma testosterone concentration in male rats or the estradiol concentration in either male or female rats (Table 2).

IGF-I content. In contrast to the other hormones analyzed, the plasma IGF-I concentration was decreased by 23% in alcohol-fed male rats compared with pair-fed male control values (Fig. 6A). In contrast, the blood-borne IGF-I concentration was not altered by alcohol feeding in female rats, although the basal IGF-I concentration was lower in female animals compared with basal male values. Similarly, alcohol-fed male rats had a 30% reduction in muscle IGF-I mRNA content when compared with values in pair-fed control rats (Fig. 6, B and C). Again, the constitutive expression of IGF-I mRNA was 30% lower in muscle from female rats compared with male rats under similar conditions. In contrast to the response seen in males, alcohol feeding did not further decrease in muscle IGF-I mRNA content in female animals.

Muscle proteolysis. Muscle protein content represents a balance between protein synthesis and degradation, and a number of stimuli decrease lean body mass by increasing muscle protein breakdown (23, 35). Because in vivo and in situ quantitation of proteolysis is technically difficult, especially in heavier more mature animals, the current study assessed the mRNA content of MAFbx (aka atrogin-1) and MuRF-1. These are both muscle-specific ubiquitin E3 ligases central to the muscle wasting observed in other catabolic conditions (3).

Chronic alcohol consumption in male rats did not alter muscle mRNA content for atrogin-1 or MuRF1 (Fig. 7). In contrast, the expression of both mRNAs was significantly increased (80 and 30%, respectively) in gastrocnemius from alcohol-fed female rats compared with time-matched pair-fed female control animals. In contrast, there was no alcohol or sex difference in the atrogin-1 or MuRF-1 mRNA content in soleus muscle among the various groups (data not shown).

Acute alcohol intoxication. There was no significant difference in the blood alcohol concentration between male and female rats at any of the time points assessed (i.e., 0.5 h: males = 487 ± 41 mg/dl vs. females = 510 ± 35 mg/dl; 1 h: males = 423 ± 44 mg/dl vs. females = 459 ± 61 mg/dl; 1.5 h: males = 344 ± 33 mg/dl vs. females = 374 ± 39 mg/dl; and 2.5 h: males = 254 ± 19 mg/dl vs. females = 248 ± 27 mg/dl). The plasma estradiol concentration was determined only in female rats because of the exceedingly low estradiol concentration detected in control male rats from the previous study (see data in Table 2 for chronic alcohol-fed rats). In contrast to chronic alcohol consumption, acute alcohol intoxication significantly reduced the plasma estradiol concentration (control = 26.5 ± 2.9 pg/ml vs. alcohol = 16.4 ± 2.7 pg/ml; P < 0.05) in female rats. Likewise, acute alcohol intoxication decreased the plasma testosterone concentration in male rats (control = 3.1 ± 0.4 ng/ml vs. alcohol = 1.8 ± 0.3 ng/ml; P < 0.05). Finally, there was no sex- or alcohol-induced change in the plasma insulin concentration in response to acute alcohol intoxication (data not shown).

In contrast to the sexual dimorphic response of muscle protein synthesis described above for chronic alcohol ingestion, acute alcohol intoxication decreased muscle protein syn-
thesis to a comparable extent in both male and female rats (Fig. 8A). Moreover, with regard to various indexes of translation initiation, both male and female rats responded in a similar manner (Fig. 8, B–F). Specifically, acute alcohol intoxication increased the amount of eIF4E bound to the translation repressor 4E-BP1 and decreased the phosphorylation of 4E-BP1 and eIF4G to a comparable extent in male and female rats. Finally, although acute alcohol intoxication decreased the amount of eIF4E bound to eIF4G and the phosphorylation of ribosomal protein S6 in both sexes, the decrease was significantly greater in female compared with male rats.

Acute alcohol intoxication did not significantly alter the atrogin-1 or MuRF-1 mRNA content of gastrocnemius from either male or female rats at the time point examined (data not shown).

**DISCUSSION**

Epidemiological evidence suggests that females may be more sensitive to alcohol-induced myopathy than males, although the data are more convincing for cardiac as opposed to skeletal muscle myopathy (29, 38). Few attempts have been made to determine the cellular mechanisms for this sexual dimorphism in muscle protein balance (11). In contrast to human studies, the results of the current investigation suggest that female rats are less susceptible to the inhibitory effects of chronic alcohol consumption on muscle protein synthesis and translation initiation than males. Our findings in male rats fed an alcohol-containing diet for 26 wk corroborate early results obtained after 12–16 wk of feeding (20, 42). At these earlier time points, gastrocnemius protein synthesis was decreased...
sex- and alcohol-induced changes in protein synthesis

E1503

Fig. 7. Effect of chronic alcohol consumption on the mRNA content for atrogin-1 and muscle RING finger (MuRF-1) in gastrocnemius. Gastrocnemius was collected after 26 wk of alcohol feeding or from pair-fed control animals. A: representative Northern blots for atrogin-1 and MuRF-1 in gastrocnemius from the 4 treatment groups; 18S RNA is also shown for each group as a loading control. Bars indicate densitometric analysis of atrogin-1 (B) and MuRF-1 (C) mRNA abundance, where the value from the male pair-fed control rats was set at 1.0 AU after being normalized to 18S mRNA. For bar graphs, values are means ± SE, where n = 8, 17, 8, and 18, respectively. Values with different letters are significantly different from each other, P < 0.05. Values with the same letter are not significantly different. Groups with 2 letters (ab) are not statistically different from groups with either the letter a or b alone.

~40% in alcohol-fed rats compared with isocaloric isonitrogenous-fed control rats. Collectively, this sustained reduction indicates that the alcoholic myopathy is not a transient response but persists for the duration of alcohol consumption and is a primary response for the decrease in lean body mass (20). However, the apparent reduction in the magnitude of the alcohol-induced decrease in gastrocnemius protein synthesis between 12 and 16 wk (e.g., 40%) and 26 wk (e.g., 20%) was unexpected given that, at least in humans, the extent of alcoholic myopathy is proportional to the lifetime alcohol exposure (39). Hence, we cannot exclude the possibility that some degree of alcohol tolerance may have developed in male rats or that the magnitude of the alcohol-induced decrease in muscle protein synthesis would be further attenuated if alcohol feeding was prolonged for >26 wk.

In contrast to the response of male rats, no decrease in gastrocnemius protein synthesis or protein content was seen in alcohol-fed female rats. However, because only a single time point was assessed in the current study, we cannot exclude the possibility that female rats would have demonstrated a decreased rate of protein synthesis if studied at either an earlier or later time point. In this regard, protein synthesis in the gastrocnemius was comparably reduced in both male and female rats in response to acute alcohol intoxication. Furthermore, the possibility remains that muscle protein synthesis would be decreased in female rats of other strains or in animals fed a different type of alcohol-containing diet. Although the mechanisms underlying this sexual dimorphic response are not known, we can eliminate as likely mediators differences in alcohol intake, which was actually higher in female compared with male rats, and the prevailing blood alcohol concentration, which was not different between groups. Furthermore, we found no evidence that alcohol feeding induced CYP1A1/2 protein in gastrocnemius from either male or female rats (unpublished observation). Finally, the dissimilar response in muscle protein synthesis between female rats and humans may suggest that an interaction exists between alcohol and some secondary factor (e.g., nutrition) in the human population that is absent in our tightly controlled animal study (26). Alternatively, the lack of an alcohol-induced decrease in muscle protein synthesis in female rats may represent a species-specific response. Because of the above-mentioned uncertainties, the clinical relevance of our findings remains to be elucidated. This alcohol-induced decrease in protein synthesis is largely, if not exclusively, the result of a reduction in translation efficiency and not a reduction in ribosome number and confirms our previous report in male rats (20). These data are internally consistent with the impaired phosphorylation of various mediators of peptide-chain initiation in alcohol-fed male rats. For example, in male animals, alcohol feeding markedly reduced the phosphorylation of the ribosomal protein S6. Furthermore, alcohol also reduced phosphorylation of 4E-BP1 and eIF4G. These findings are noteworthy because such decreases are likely responsible for the redistribution of eIF4E from the active eIF4E-eIF4G to the inactive eIF4E-4E-BP1 complex observed in alcohol-fed male rats (10). An alcohol-induced decrease in the binding of eIF4E with eIF4G and the subsequent reduction in the functional eIF4E complex limits protein synthesis at the step involving binding of mRNA to the 43S preinitiation complex (16). In contrast to these changes observed in male rats, we detected no significant change in the signal transduction pathway for protein synthesis in alcohol-fed female rats.

Differences in the hormonal milieu between male and female rats have been posited to modulate sexual dimorphic responses in other organ systems (1, 9, 11, 12, 25, 29, 41). In this regard, we have previously reported that long-term alcohol feeding decreases the plasma and muscle content of the anabolic hormone IGF-I and that these changes are proportional to the amount of active eIF4E-eIF4G and the rate of protein synthesis in muscle of male rats (18). This association was confirmed in the present study in male rats. In contrast to males, there was no change in either the plasma IGF-I concentration or the muscle IGF-I mRNA content in female rats.
Although the relatively lower plasma IGF-I concentration in females vs. males has been previously described (40), we are not aware of data directly comparing sex-specific differences in muscle IGF-I. Our results show that the IGF-I mRNA content is considerably lower in muscle from female vs. male rats and that the IGF-I content does not correlate with absolute rates of muscle protein synthesis or the phosphorylation of various proteins known to regulate translation initiation under basal postabsorptive conditions.

Insulin also enhances muscle protein synthesis by stimulating translation initiation (13). However, the single-point determination of plasma insulin indicates no difference among the four groups, suggesting that a differential insulin response is an unlikely mediator of the sexual dimorphism. Testosterone is a potent anabolic agent also capable of increasing protein synthesis and the accretion of lean body mass (6). Unlike some studies that report an alcohol-induced decrease in testosterone (25), our chronic alcohol feeding protocol did not alter the plasma testosterone concentration in male rats. Hence, we cannot attribute the alcohol-induced decrease in protein synthesis in this group to a drop in testosterone. Finally, estrogen may provide protection against some of the toxic effects of alcohol. Because of the marked differences in the plasma concentration of this hormone between male and female rats, this mechanism remains a possible explanation for the observed sex-specific response of skeletal muscle to chronic alcohol consumption. Additional studies are required to determine whether the absence of testosterone and/or the presence of estradiol in females mediates the sex-related differences.

In contrast to liver, where alcohol feeding slows protein degradation via alterations in the activity of the ubiquitin-proteasome pathway (4), there is a paucity of data pertaining to alcohol-induced changes in muscle proteolysis. In one study, the fractional rate of myofibrillar breakdown as estimated by the difference between muscle protein content and protein synthesis was reported to be decreased by chronic alcohol consumption (31). However, the tissue proteasome activity was not significantly altered after 6 wk of alcohol feeding (14). Historically, the ubiquitination of proteins in the N end-rule pathway by the ubiquitin-conjugating enzyme E214k and the ubiquitin ligase E3 was considered the primary regulatory pathway for muscle loss in wasting states (23, 35). However, a host of diverse atrophic stimuli, such as immobilization, denervation, diabetes, dexamethasone, and nutrient deprivation, strongly increase the gene expression of muscle-specific atrogin-1 and MuRF1 (3, 22). Furthermore, a reduced loss of muscle mass is evident in atrogin-1 and MuRF1 null mice after muscle denervation (3). Hence, atrogin-1 and MuRF1 have collectively been referred to as atrophy-related genes or “atrogenes.” We could not detect any consistent change in atrogene expression in muscle from alcohol-fed male rats compared with values from pair-fed male control animals. In contrast, the
expression of both atrogin-1 and MuRF1 was elevated in the gastrocnemius muscle from female rats consuming the alcohol-containing diet for 26 wk. Collectively, these data again demonstrate a sex-specific response to alcohol feeding and suggest that, although there is no change in the rate of muscle protein degradation in males, alcohol may accelerate proteolysis in female animals. Despite the apparent increased atrogin-1 and MuRF1 mRNA expression in alcohol-fed female rats, there was no detectable change in the mass or protein content of the gastrocnemius. This discrepancy may suggest either that these atrogens are not the rate-controlling step in muscle proteolysis and/or that changes in the mRNA content for atrogin-1 and MuRF1 mRNA do not reflect changes in their protein content.

In summary, our data reveal a sexual dimorphic response to chronic alcohol feeding with female rats being less sensitive to the alcohol-induced decrease in protein synthesis and translation initiation. Conversely, alcohol feeding increased atrogin-1 and MuRF1 mRNA content only in the gastrocnemius from female rats, with male animals showing alcohol tolerance, suggesting a possible sexual dimorphic response for muscle proteolysis. In contradistinction, acute alcohol intoxication markedly decreased muscle protein synthesis and translation initiation in both male and female rats with no discernable sex-specific pattern. Because the blood alcohol concentrations were severalfold greater in both male and female rats injected intraperitoneally with alcohol compared with values from chronic alcohol-fed rats, it is possible that females have a higher threshold for the suppressive effects of alcohol on muscle protein synthesis. However, once this threshold is surpassed, alcohol decreases protein synthesis via similar mechanisms in both male and female rats.

ACKNOWLEDGMENTS

We are grateful to Gina Deiter, Heather Hubler, Marcia Pratt, Anne Pruznak, and Daunta Huber for excellent technical assistance. We gratefully acknowledge Dr. Leonard S. Jeffress for kindly providing several antibodies used in this study.

GRANTS

This work was supported by National Institute on Alcohol Abuse and Alcoholism Grants AA-12814 (T. C. Vary) and AA-11290 (C. H. Lang) and the Commonwealth of Pennsylvania Department of Health Tobacco Settlement Award (T. C. Vary).

REFERENCES


