Acute and chronic effects of alcohol exposure on skeletal muscle c-myc, p53, and Bcl-2 mRNA expression

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Nakahara, Tatsuo, Kijiro Hashimoto, Makoto Hirano, Michael Koll, Colin R. Martin, and Victor R. Preedy. Acute and chronic effects of alcohol exposure on skeletal muscle c-myc, p53, and Bcl-2 mRNA expression. Am J Physiol Endocrinol Metab 285: E1273–E1281, 2003.—Skeletal muscle atrophy is a common feature in alcoholism in men (due to the greater prevalence of alcoholism in this gender), women appear to be particularly susceptible. There is also some evidence to suggest that malnutrition exacerbates the effects of alcohol on muscle. However, the mechanisms responsible for the myopathy remain elusive, and some studies suggest that acetaldehyde, rather than alcohol, is the principal pathogenic perturbant. Previous reports on rats dosed acutely with ethanol (<24 h) have suggested that increased proto-oncogene expression (i.e., c-myc) may be a causative process, possibly via activating preapoptotic or transcriptional pathways. We hypothesized that 1) increases in c-myc mRNA levels also occur in muscle exposed chronically to alcohol, 2) muscle of female rats is more sensitive than that from male rats, 3) raising acetaldehyde will also increase c-myc, 4) prior starvation will cause further increases in c-myc mRNA expression in response to ethanol, and 5) other genes involved in apoptosis (i.e., p53 and Bcl-2) would also be affected by alcohol. To test this, we measured c-myc mRNA levels in skeletal muscle of rats dosed either chronically (6–7 wk; ethanol as 35% of total dietary energy) or acutely (2.5 h; ethanol as 75 mmol/kg body wt) except the study that examined gender susceptibility in male and female rats. At the end of the studies, rats were killed, and c-myc, p53, and Bcl-2 mRNA was analyzed in skeletal muscle by RT-PCR with an endogenous internal standard, GAPDH. The results showed that 1) in male rats fed ethanol chronically, there were no increases in c-myc mRNA; 2) increases, however, occurred in c-myc mRNA in muscle from female rats fed ethanol chronically; 3) raising endogenous acetaldehyde with cyanamide increased c-myc mRNA in acute studies; 4) starvation per se increased c-myc mRNA levels and at 1 day potentiated the acute effects of ethanol, indicative of a sensitization response; 5) the only effect seen with p53 mRNA levels was a decrease in muscle of rats starved for 1 day compared with fed rats, and there was no statistically significant effect on Bcl-2 mRNA in any of the experimental conditions. The increases in c-myc may well represent a preapoptotic effect, or even a nonspecific cellular stress response to alcohol and/or acetaldehyde. These data are important in our understanding of a common muscle pathology induced by alcohol.

EXCESSIVE EXPOSURE TO ALCOHOL causes damage to skeletal muscle, leading to the development of a specific disease entity called alcoholic myopathy (44, 45, 49, 51). It is one of the most common skeletal muscle disorders, with a prevalence of 2,000 cases per 100,000 population (44, 45, 49, 51). Although principally occurring in men (due to the greater prevalence of alcoholism in this gender), women appear to be particularly susceptible (62), and there is some evidence to suggest that malnutrition may also exacerbate this disease (16, 19). Predominant features of alcoholic myopathy include difficulties in gait, cramps, impaired muscle strength, and reduced whole body lean tissue mass (44, 45, 49, 51). These pathologies are also accompanied by reductions in the relative amounts of specific contractile proteins within the muscle itself, such as myosin, desmin, actin, and troponin (53). However, the sequence of events between alcohol exposure and skeletal muscle damage is unknown, although recent evidence suggests that changes may be initiated at the molecular level (41). In particular, studies in intact rats have shown that acute ethanol dosage increases the levels of the proto-oncogene c-myc (protein and mRNA) (41). The importance of this relates to the fact that proto-oncogenes have also been implicated in the molecular control of cellular adaptations in mature muscle (64). Such increases trigger other genes to activate cellular or molecular cascades, such as ribosome biogenesis (4, 18, 28, 41). Furthermore, the increase in c-myc may also be related to apoptosis (6). More recently, however, the suggestion has been made that activation of c-myc...
can induce DNA damage, which could also occur in alcohol-exposed tissue (63).

However, there are four outstanding issues. First, it is possible that in the chronic situation, adaptive mechanisms within muscle would lead to an amelioration of the increases in c-myc mRNA due to alcohol. Second, greater increases would be expected to occur in female rats, reflecting the greater sensitivity of women seen in the clinical setting. Third, the putative role of acetaldehyde on c-myc has hitherto not been addressed. A failure to investigate this in past studies may be due to the fact that the administration of acetaldehyde in vivo is fraught with practical difficulties due to its extreme reactivity. However, a number of groups have resolved this by cyanamide predosing, which inhibits acetaldehyde dehydrogenase and markedly raises endogenous acetaldehyde (30, 31). Recently, we have adopted such a protocol to investigate the putative effects of acetaldehyde in alcohol-related pathology (2, 26, 33, 42, 46, 59). Finally, our previous studies on c-myc mRNA in alcohol-exposed muscle were carried out without reference to other regulator genes involved in apoptosis (41). For example, other studies have analyzed a cohort of complementary or related mRNAs or their products, such as c-myc, p53, and Bcl-2 in skeletal muscle (15), as well as saponin-exposed lymphocytes (21), uveal melanoma (71), and gastric (71) and breast cancer (27). In general, as represented by p53 and Bcl-2.

Or genes involved in apoptosis and/or cellular damage were observing a generalized response of c-myc per se, whereas Bcl-2 protects against these processes (9, 55). It was thus important to determine whether we (10), whereas Bcl-2 protects against these processes (9, 55). Finally, our previous studies on c-myc mRNA in alcohol-exposed muscle were carried out without reference to other regulator genes involved in apoptosis (10). For example, other studies have analyzed a cohort of complementary or related mRNAs or their products, such as c-myc, p53, and Bcl-2 in skeletal muscle (15), as well as saponin-exposed lymphocytes (21), uveal melanoma (71), and gastric (71) and breast cancer (27).

In simple terms, p53 promotes cell arrest or apoptosis (10), whereas Bcl-2 protects against these processes (9, 55). It was thus important to determine whether we were observing a generalized response of c-myc per se, or genes involved in apoptosis and/or cellular damage in general, as represented by p53 and Bcl-2.

To resolve some of these issues, we measured c-myc, p53, and Bcl-2 mRNA levels in skeletal muscle of rats dosed with ethanol. We used well-characterized protocols for investigating either the long- or short-term effects of alcohol (48). We compared the responses of male and female rats to chronic ethanol feeding and then investigated the acute effects of alcohol in the presence of cyanamide or starvation.

**METHODS**

Male or female Wistar rats were obtained from accredited commercial suppliers at ~60 g body wt. Three different groups of rats were obtained for the three separate studies. Rats were maintained and studied according to Home Office Guidelines and a specific project licence. Muscles from these animals had already been used to measure other parameters not related to this present study (for example, see Refs. 2 and 17) to minimize the use of animals according to Home Office guidelines. Rats were maintained in a temperature- and humidity-controlled animal house for ~1 wk until they weighed ~0.1–0.15 kg (the rats were 6–7 wk old when treatments with alcohol commenced). We selected young rats, as they are more susceptible to alcohol because of their higher rate of protein turnover. Myopathy as defined by fiber atrophy or muscle loss is observable at 2 wk in younger animals compared with 12 wk in mature rats (47, 50, 67). In the present study, muscle weights of ethanol-fed rats were lower than those of their matched controls after 6–7 wk, which is indicative of alcoholic myopathy (data not shown but see also Ref. 17). The attributes of our model have been summarized previously (52).

Batches of rats were ranked on the basis of weight and then divided into the appropriate number of groups of equal mean body weight in each experiment. The studies were as follows.

**Study 1: comparison of the effect of chronic alcohol exposure for 6 wk in male and female rats.** Rats were divided into the following groups: control male (n = 10); control female (n = 8); ethanol male (n = 10); ethanol female (n = 8).

Rats were subjected to a Lieber-DeCarli alcohol-feeding regimen in which treated rats were fed a nutritionally complete liquid diet containing 35% of total calories as ethanol ad libitum (48). Details of the diets are given here, and see also Tables 1 and 2. There was no restriction on the amount of alcohol consumed by these rats. Controls were pair-fed the same diet in which ethanol was replaced by isocaloric glucose (48). After 6–7 wk (specifically, 44 and 45 days for female rats and 46–48 days for males), mean body weights of female control and ethanol-fed animals were 0.184 and 0.175 kg, respectively, whereas corresponding male rats weighed 0.215 and 0.199 kg, respectively (17). Mean total cumulative intakes of liquid diets for female and male rats were 2.2 and 2.4 liters, respectively. Mean daily intakes of diets were 49 and 52 ml/day for female and male rats, respectively (although consideration should be given to the fact that intakes increased as rats became accustomed to the diets and grew). Rats were killed by decapitation, and representative skeletal muscle (hindlimb musculature) was dissected on ice into the various regions. These were frozen immediately in liquid nitrogen and stored at ~70°C until analysis.

**Study 2: effects of acute ethanol exposure and of raising endogenous acetaldehyde with cyanamide.** Rats were divided into the following groups: saline + saline (n = 7); cyanamide + saline (n = 7); saline + ethanol (n = 7); cyanamide + ethanol (n = 7).

The dosage used was 75 mmol/kg body wt for alcohol and 0.5 mmol/kg body wt for cyanamide. Controls were injected with identical volume of 0.15 mM NaCl. The experimental procedure involved intraperitoneal injection (0.5 ml/100 g

| Table 1. Recipe for control and alcohol-containing liquid diets |
|-----------------------------------------|-------------|-------------|-------------|
| **Content, g** | **Total kcal** | **Total kJ** |
| **Alcohol Diet** | | |
| Water | 126 | 0 | 0 |
| F-resubin | 600 | 586 | 2,448 |
| Added glucose | 0.0 | 0.0 | 0.0 |
| Casein | 15.0 | 60 | 251 |
| Orovite (sucrose) | 2.46 | 9 | 39 |
| Alcohol (61.8 ml) | 49.08 | 348 | 1,457 |
| Total | 1,063 | 1,003 | 4,194 |
| **Control Diet** | | |
| Water | 126 | 0 | 0 |
| F-resubin | 600 | 586 | 2,448 |
| Added glucose | 93 | 349 | 1,458 |
| Casein | 15.0 | 60 | 251 |
| Orovite (sucrose) | 2.46 | 9 | 39 |
| Alcohol | 0 | 0 | 0 |
| Total | 1,106 | 1,004 | 4,196 |

Values show differences between control and alcohol-containing liquid diets. Liquid diet was made up using F-resubin (Fresenius Kabi, Mano Park, UK), glucose and casein (both Merck BDH, Lutterworth, UK), Orovite 7 (Boots Chemist), 99.9% alcohol (Hospital Pharmacy), and water.

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body wt) “pretreatment” (30 min) with either saline or cyanamide, followed by intraperitoneal injection (1 ml/100 g body wt) “treatment” (150 min) with either saline or ethanol. Rats were then killed after a total 2.5 h of exposure to ethanol.

Study 3: effects of starvation and acute superimposition of alcohol. Rats were either treated as in the fed state or starved 1 or 2 days before use (i.e., total food deprivation 24 and 48 h, respectively).

Liquid diets. Fresh liquid diets used for the chronic ethanol feeding were prepared on a daily basis according to the recipe described in Table 1. A food blender was used to thoroughly mix the ingredients. To prevent the possibility of ethanol precipitating the protein in the alcohol diet, absolute ethanol was the last ingredient to be added, carefully, and contents were then thoroughly stirred during the addition. The diets were freshly prepared each day and presented to the animals between 9:00 AM and 12:00 noon.

The compositions of the diets have been given previously. Control and alcohol-containing diets were isocaloric, isonitrogenous, and isoenergetic (Tables 1 and 2). We do not have the blood alcohol levels from this particular set of rats, but in another study in which almost identical proportions of ethanol (35% of dietary energy) were used, we reported that there was no gender difference in either the consumption of ethanol per unit body weight (13 g ethanol·kg body wt⁻¹·day⁻¹) or the blood ethanol levels (∼40 mmol/l) (22). Similar blood ethanol levels were obtained in male rats in our model (34).

Method for c-myc, p53, Bcl-2, and GAPDH. Total RNA was prepared from the muscle (hindlimb musculature) by standard methods described previously (11). The levels of c-myc, p53, and Bcl-2 mRNAs were quantified by RT-PCR with an endogenous internal standard, GAPDH, as previously described (36). RT was performed on 1 μg of total RNA for 90 min at 42°C in a 5-μl reaction mixture containing (in mmol/l) 25 Tris·HCl (pH 8.3), 50 KCl, 5 MgCl₂, 2 dithiothreitol, and 1 of each deoxynucleotide, 10 U AMV reverse transcriptase (Roche Molecular Biochemicals, Mannheim, Germany), 10 U ribonuclease inhibitor (Roche Molecular Biochemicals), and 0.8 μg oligo(dT)₁₅ primer (Roche Molecular Biochemicals). The RT was terminated by heating the sample at 95°C for 2 min (36).

The multiplexed PCR was carried out in a 20-μl reaction mixture containing 10 mmol/l Tris·HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 2% (vol/vol) dimethyl sulfoxide, 0.2 mmol/l each of deoxynucleotide, 0.1 μmol/l each of 5’ and 3’ GAPDH primers, 1 μmol/l each of 5’ and 3’ c-myc-, p53-, and Bcl-2-specific primers, 25 ng of reverse-transcribed total RNA, and 0.5 U Taq DNA polymerase (Roche Molecular Biochemicals). The PCR amplification was performed for 28 (c-myc), 30 (p53), or 34 (Bcl-2) cycles, consisting of denaturation (94°C, 45 s), annealing (60°C or 63°C for Bcl-2, 45 s), and extension (72°C, 75 s). After eight cycles (c-myc or p53) or twelve cycles (Bcl-2), 0.1 μl of each GAPDH primer pair was added to the reaction mixture, and PCR cycles were further continued for 20 (c-myc) or 22 (p53 or Bcl-2) cycles (36). The primer sequences used for amplification of the coding regions are summarized in Table 3. The PCR products were analyzed on a 10% polyacrylamide gel by electrophoresis. Gels were stained with ethidium bromide, visualized with UV transillumination, photographed, and submitted to image analysis. Quantitative image analysis of the PCR fragments was performed using the NIH Image program (Scion, Frederick, MD). The mRNA levels were calculated as the ratios of optical density of the PCR products to those of the GAPDH PCR product. Representative gels are shown in Fig. 1.

### Table 2. Composition of control and alcohol-containing liquid diets

<table>
<thead>
<tr>
<th></th>
<th>Liquid Control (Glucose)</th>
<th>Liquid Alcohol Diet, per kg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ</td>
<td>3,816*</td>
<td>3,812?</td>
<td></td>
</tr>
<tr>
<td>Fat/oil, g</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Protein, g</td>
<td>34</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>142</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>83</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>0</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Water, g</td>
<td>779</td>
<td>779</td>
<td></td>
</tr>
<tr>
<td>Vitamin A, µg</td>
<td>243</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>136</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>Vitamin E, µg</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Vitamin K, µg</td>
<td>26.9</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td>Vitamin B1, mg</td>
<td>1.16</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Vitamin B2, mg</td>
<td>1.46</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>Vitamin B6, mg</td>
<td>12.9</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12, mg</td>
<td>1.54</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>vitamin B12, mg</td>
<td>1.07</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid, mg</td>
<td>1.88</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>Biotin, mg</td>
<td>53.7</td>
<td>53.7</td>
<td></td>
</tr>
<tr>
<td>Folic acid, µg</td>
<td>54</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid, mg</td>
<td>51.1</td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>322</td>
<td>322</td>
<td></td>
</tr>
<tr>
<td>Iron, mg</td>
<td>540</td>
<td>540</td>
<td></td>
</tr>
<tr>
<td>Iodine, µg</td>
<td>57.1</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Values show differences between control and alcohol liquid diets for selective nutrients. Caloric values were calculated as follows: ethanol, 7.1 kcal/g; carbohydrate, 3.8 kcal/g; protein, 4.0 kcal/g; fat, 9.0 kcal/g; and 4.18 kJ was assumed to be 1.0 kcal. * 912 kJ; † 911 kJ.</td>
<td></td>
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</tbody>
</table>

### Table 3. Oligonucleotide primers, product length, annealing temperatures, and number of PCR cycles

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Direction</th>
<th>Sequence</th>
<th>PCR Product, bp</th>
<th>Annealing Temp, °C</th>
<th>Cycles</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>5’-CAGGAAATGCATGCTGCAC-3’</td>
<td>429</td>
<td>60, 63</td>
<td>20, 22</td>
<td>X02231</td>
</tr>
<tr>
<td>c-myc</td>
<td>Reverse</td>
<td>5’-GATGGCTGTTGGAATGCAAGG-3’</td>
<td>274</td>
<td>60</td>
<td>28</td>
<td>Y00396</td>
</tr>
<tr>
<td>p53</td>
<td>Reverse</td>
<td>5’-TTCGCAAGCTTGGAGACACAG-3’</td>
<td>270</td>
<td>60</td>
<td>30</td>
<td>X13058</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Reverse</td>
<td>5’-CGACTGGAGAGAGATGGCA-3’</td>
<td>274</td>
<td>63</td>
<td>34</td>
<td>L14680</td>
</tr>
</tbody>
</table>
from gels were compiled as histograms and displayed in Figs. 2–4.

Statistical analysis. All data are expressed as means ± SE (n = 6–10). Statistical analyses are described in the legend to each figure. Posteriori comparisons were conducted on essential groupings only after careful scrutiny of graphic and descriptive representation of the data to reduce the total number of multiple comparisons made to the absolute minimum, and α was allowed to remain at P = 0.05. This conservative approach to posteriori analysis was taken to protect the analysis from an elevated risk of type I statistical error (12). In all studies, significance was indicated when P ≤ 0.05.

RESULTS

Study 1: comparison of the effect of chronic alcohol exposure for 6 wk in male and female rats. Comparative levels of c-myc mRNA as a function of gender and treatment are shown in Fig. 2. There was no evidence of a main effect of gender [P = nonsignificant (NS)] or treatment (P = NS). A statistically significant interaction was observed between gender and treatment (P < 0.05). Posteriori analysis revealed female ethanol-treated rats to have significantly higher c-myc mRNA
levels than female control rats ($P < 0.05$), whereas there were no significant differences in c-myc mRNA levels between male control and male ethanol-treated rats ($P = NS$). Control males were observed to have significantly higher levels of c-myc mRNA than female control animals ($P < 0.05$; Fig. 2).

For both p53 and Bcl-2 mRNA levels, there was no evidence of a main effect of gender ($P = NS$), treatment ($P = NS$), or interaction ($P = NS$; Fig. 2). There were no other significant differences between groups as determined by post hoc analysis of p53 and Bcl-2 mRNA ($P = NS$; Fig. 2).

Study 2: effects of acute ethanol exposure and of raising endogenous acetaldehyde with cyanamide. Comparative levels of c-myc mRNA as a function of cyanamide pretreatment and ethanol treatment are shown in Fig. 3. Two-way ANOVA showed that statistically significant main effects of pretreatment ($P < 0.01$) and treatment ($P < 0.01$) were observed. A statistically significant interaction was observed between pretreatment and treatment ($P < 0.01$). Posteriori analysis revealed the cyanamide + ethanol group to have significantly higher c-myc mRNA levels than the saline + saline and saline + ethanol groups (both $P < 0.01$).

For p53 and Bcl-2 mRNA, there were no statistically significant main effects of pretreatment ($P = NS$), treatment, or interaction ($P = NS$; Fig. 3). There were no other significant differences between groups as determined by post hoc analysis of p53 and Bcl-2 ($P = NS$; Fig. 3).

Study 3: effects of starvation and acute superimposition of alcohol. Comparative levels of c-myc mRNA as a function of treatment and starvation are shown in Fig. 4. Statistically significant main effects of treatment ($P = 0.05$) and starvation ($P < 0.05$) were observed, with treatment and starvation associated with increased c-myc mRNA levels. No statistically significant interaction was observed between treatment and starvation ($P = NS$). Posteriori analysis revealed the treatment group starved for 1 day to have significantly higher c-myc mRNA levels than the starved 1 day control group ($P = 0.05$). The starved 2 days control group was observed to have significantly higher c-myc mRNA levels than the fed control group ($P < 0.05$; Fig. 4).

For p53 mRNA, no statistically significant main effects of ethanol treatment and interaction were observed ($P = NS$), but there was a significant effect of starvation ($P < 0.01$). Posteriori analysis revealed control rats starved 1 day to have significantly lower p53 mRNA levels than the fed control group ($P < 0.01$; Fig. 4).

There was no evidence of a statistically significant interaction between treatment, starvation, and their interaction for Bcl-2 mRNA ($P = NS$). There were no other significant differences between groups as determined by post hoc analysis of Bcl-2 data ($P = NS$; Fig. 4).

DISCUSSION

The focus of these studies was the investigation of c-myc, p53, and Bcl-2 mRNA expression in skeletal muscle exposed to ethanol in vivo. We showed that 1) in male rats fed ethanol chronically, there were no increases in c-myc mRNA; increases however, occurred in c-myc mRNA in muscle from female rats fed ethanol chronically; 2) raising endogenous acetaldehyde in male rats with cyanamide increased c-myc mRNA in acute studies; and 3) 2 days of starvation in male rats
increased c-myc mRNA levels and at 1 day potentiated the acute effect of ethanol, indicative of a sensitization response (although this effect of alcohol was not observed after 2 days of starvation). In contrast, the only effect seen in starved rats treated with saline was a decrease in muscle p53 mRNA levels. Neither chronic nor acute ethanol treatment was effective in modulating the levels of p53 or Bcl-2 mRNA.

**Gender differences in alcohol toxicity and implications for muscle.** In the studies presented here, we show that chronic ethanol feeding has no measurable effect on c-myc mRNA levels in male rats. On the other hand, in female rats, c-myc mRNA levels increased, even though the treatment protocols in male and female rats were comparable. This is entirely compatible with previously reported clinical and animal studies (22, 62). In clinical studies, female alcoholics drink less ethanol, yet the prevalence of myopathy is similar in both male and female alcoholics (62). We have also shown that skeletal muscle in female rats is more sensitive to alcohol than that in their male counterparts (22). One explanation for the gender susceptibility pertains to the hormonal response to alcohol. For example, in female rats, alcohol induces the release of corticosterone compared with male rats (54), and corticosterone is a well-known catabolic perturbant in skeletal muscle (38).

We are unable to explain why the c-myc value in muscle of control female rats was lower than that in control male rats. Although the precise molecular events in the regulation of muscle growth are unknown, we do know that they involve a number of regulatory processes, involving physiological and biochemical factors such as stretch, endocrine signals, and molecular events of transcription and translation (for example see Refs. 37 and 61), and perhaps there are gender differences in these regulatory processes.

**The role of acetaldehyde.** Upregulation of acetaldehyde dehydrogenase effectively eliminates some of the cytotoxic effects of acetaldehyde (3). In relation to the present studies, this suggests, but does not prove, that acetaldehyde per se could be a potent stimulator of c-myc mRNA. Increasing acetaldehyde with cyanamide also elevated c-myc mRNA in the present acute studies. Furthermore, acetaldehyde also increases oncogene expression in fat-storing cells in vitro (7). Presently, there are no mechanistic studies into how this might occur in skeletal muscle. However, in fat-storing cells, increases in c-myc mRNA occur via activation of protein kinase C (7).

c-myc, p53, and Bcl-2 change in alcohol exposure and other muscle pathologies. The cellular transcription factor p53 is involved in “growth arrest” and apoptosis (5). It does this via repression or activation of other genes downstream and is stimulated by either DNA damage or cellular stress events (5, 39). However, there is also evidence that the p53 protein is involved in development and differentiation of cells (43). Although there are a number of studies on p53 gene expression in cardiac (70), vascular (20), and intestinal (14) tissue, there is a general paucity of data pertaining to skeletal muscle. Some studies have proposed that upregulation of p53 may be involved in muscle disorders or myopathies, but there is no consensus that there is coordinate upregulation of p53 with closely related genes and/or proteins such as Bcl-2 and c-myc. In juvenile dermatomyositis, analysis of immunoreac-

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**Fig. 4.** Effects of starvation and acute superimposition of alcohol on muscle c-myc, p53, and Bcl-2 mRNA levels in male rats (study 3). Rats were either treated as in the fed state or starved 1 or 2 days before use. Rats were then divided into the following groups (nutritional state + treatment with an exposure time of 2.5 h): fed + saline; fed + ethanol; starved 1 day + saline; starved 1 day + ethanol; starved 2 days + saline; starved 2 days + ethanol. At end of study, muscle was dissected for analysis of c-myc, p53, and Bcl-2 mRNA relative to GAPDH mRNA. Data are means ± SE and were analyzed with a between-subjects 2 × 3 two-way ANOVA, with treatment (ethanol, no ethanol) and starvation status (fed, 1 day starved, 2 day starved) as independent factors. Otherwise, data analysis procedure was identical to that of studies 1 and 2. Results of ANOVA for c-myc were as follows: ethanol, P = 0.05; starvation, P < 0.05; interaction, NS. Results of ANOVA for p53 were as follows: ethanol, NS; starvation, P < 0.01; interaction, NS. Results of ANOVA for Bcl-2 were as follows: ethanol, NS; starvation, P < 0.01; interaction, NS. Post hoc analyses are displayed over histograms.
tive proteins in muscle shows that there is upregulation of Bcl-2, but not of c-myc or p53 (15). Furthermore, p53 mRNA or protein is not affected in reperfusion injury (13), atrophic muscle caused by nerve injury (25), myopathy due to steroid exposure (29), or alcohol-induced muscle damage (studies 1–3 in this work). Because chronic ethanol feeding reduces muscle weights in this set of rats (see Ref. 17), we must conclude that, in general, p53 is not upregulated in this defined myopathic condition and is even downregulated in starvation. The situation for Bcl-2 is similarly contradictory, in that in myopathic conditions muscle Bcl-2 protein is unaffected in cancer cachexia (23, 72); reduced in brachial plexus injury (25), cardiac cachexia (65, 66), and muscle dystrophy (56); and increased in sporadic amyotrophic lateral sclerosis (58).

Muscle exposed acutely to alcohol demonstrated an increase in c-myc mRNA, as shown in the present study by use of RT-PCR and in previous experiments by Northern blotting (41). A similar effect has been shown in a variety of other tissues exposed to alcohol, such as embryos (28) and liver (32). Cytotoxic agents also increase tissue c-myc mRNA (1, 4, 35). Many of these changes have been ascribed to signaling changes in growth, differentiation, or apoptosis. However, at present, the significance of the increased c-myc mRNA in alcohol-exposed skeletal muscle remains elusive, and studies are needed to determine whether there is a corresponding increase in protein in the present conditions. As argued previously, changes in c-myc may be a reaction to cellular stress (41). Cell stress responses are closely linked with elevation in c-myc protein and/or mRNA (57, 68). This linkage arises as the c-myc protein complex binds to sites in the HSP70 gene promoter region (60). On the other hand, we can confidently preclude apoptosis as being the final step, as there is no evidence of cell death in muscle exposed either acutely or chronically to alcohol (40). However, stages leading to apoptosis may be activated. Interestingly, c-myc mRNA has been shown to increase in hypertrophying muscles (69). In alcoholic muscle disease, there is a tendency for protein synthesis to fall and muscle mass to decrease (44, 45, 49, 51), which is quite the opposite of the hypertrophic response. Thus increases in c-myc may even be a compensatory adaptation to overcome the catabolic stimuli of alcohol. These explanations all require further elucidation, although it is interesting to note that, in isolated perfused hearts, alcohol does not increase apoptosis (24), although it does in primary cardiomyocytes (8).

Conclusions. Alcohol acutely increases c-myc mRNA in skeletal muscle, possibly reflecting a preapoptotic effect, a compensatory stimulus to induce hypertrophy to counteract the catabolic effects of alcohol, or even a nonspecific cellular stress response to alcohol and/or acetaldehyde. Contrary to expectations, neither p53 nor Bcl-2 mRNA levels were affected by alcohol, even in the presence of cyanamide predosing or starvation. These data are important in our understanding of a common muscle pathology induced by alcohol.

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