Levitt, Danielle E. *The Effect of Post-Resistance Exercise Alcohol Ingestion on LPS-Stimulated Cytokines*. Master of Science (Kinesiology), August 2015, 26 pp., 1 table, 2 figures, 41 numbered references.

The purpose of this study was to examine the effect of post-resistance exercise alcohol ingestion on LPS-stimulated production of IFNγ, TNF-α, IL-1β, IL-6, IL-8, and IL-10. Recreationally resistance-trained men (*n* = 10, 25 ± 3 yr, 177 ± 7 cm, 83.8 ± 15.7 kg, 14.8 ± 8.5% body fat) and women (*n* = 8, 23 ± 2 yr, 161 ± 3 cm, 59.5 ± 6.0 kg, 26.5 ± 3.0% body fat) completed the study. Participants visited the laboratory for an initial visit at which time they were screened, familiarized with procedures, and had their 1-repetition maximum (1RM) back squat tested. Subsequently, participants visited the laboratory 2 more times and completed 2 identical heavy resistance exercise bouts (6 sets of 10 repetitions of 80% 1RM back squat) after which a beverage, either containing alcohol (alcohol condition, ALC; 1.09 g EtOH per kg fat free mass) or water (placebo condition, PLA), was administered. Blood samples were collected before exercise (PRE), and at 3 hours (3h) and 5 hours (5h) after exercise. Samples were stimulated with lipopolysaccharide (LPS) and cultured overnight. Supernatant was collected and analyzed for IFNγ, TNF-α, IL-1β, IL-6, IL-8, and IL-10. A significant (*p* < 0.05) main effect for time was found for IFNγ, TNF-α, and IL-1β (5h greater than PRE) and for IL-10 (5h less than PRE and 3h, 3h less than PRE). An interaction effect was found for IL-8 (ALC less than PLA at 5h) and for IL-6 (ALC greater than PLA at PRE and ALC less than PLA at 3h). For IL-6, ALC was less at 3h than at PRE, and PLA was greater at 3h than at PRE. Overall, the LPS-stimulated cytokine response was pro-inflammatory by 5h. Alcohol consumed after heavy resistance exercise reduced LPS-stimulated
production of IL-6 and IL-8 but not of IFNγ, TNF-α, IL-1β, or IL-10. These data indicate that alcohol affected inflammatory capacity but that the potential effect of alcohol on the production of cytokines in response to LPS was largely overwhelmed by the resistance exercise bout.
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by

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INTRODUCTION

A positive correlation exists between exercise engagement (frequency and volume) and rates of self-reported alcohol intake (frequency and amount) in young men and women (6, 14, 27). Importantly, binge drinking in this population often follows a strenuous exercise bout (6). Drinking moderate to high doses of alcohol (0.8-1 g ethanol per kg body mass) after resistance-exercise-induced muscle damage accentuates performance decrements in men in the days after the exercise bout compared to exercise that is not followed by alcohol consumption (3-5).

Strenuous exercise presents a stressor that often requires an immune system response to allow for the involved tissue and systems to recover and for the body to regain homeostasis. Resistance exercise involving an eccentric phase can induce substantial muscle damage (20, 35). The immune system response to such muscle damage includes the migration of neutrophils and monocytes from circulation into the damaged tissue. Once in the muscle, monocytes transform into macrophages and along with neutrophils begin removal of debris, an important step early step in the tissue repair process (9). When stimulated, these immune cells (neutrophils, monocytes, and macrophages) produce and release a host of cytokines and chemokines that mediate inflammation including: interleukin (IL)-8, interferon (IFN)γ, tumor necrosis factor (TNF)-α, IL-1β, IL-6, and IL-10 (9). Interleukin-8 is a potent chemoattractant, especially for neutrophils (13); IFNγ, TNF-α, IL-1β, and IL-6 promote inflammation and macrophage activation (7, 28); and IL-10 attenuates inflammation (12).

Although many studies have examined circulating cytokine concentrations following an acute bout of resistance exercise (8), only a few have investigated the
inflammatory capacity of leukocytes (e.g., cytokine production in response to an inflammatory stimulus) in whole blood following a bout of resistance exercise (25, 33). Although circulating cytokine concentrations provide important information regarding the systemic inflammatory state they do not necessarily reflect the ability of leukocytes in circulation to respond to an inflammatory stimulus such as muscle damage.

Lipopolysaccharide (LPS) stimulation of whole blood in vitro is a valid method to assess the capacity of leukocytes, especially monocytes, to respond to an inflammatory stimulus as measured by the production of inflammatory cytokines (11). LPS primarily stimulates cytokine production from monocytes, via toll-like receptor 4 and CD14 receptor binding, although LPS can also stimulate other leukocyte subsets (21, 26).

Using this LPS stimulation method, it has been demonstrated that compared to baseline values, an acute bout of resistance exercise acutely increases the capacity of leukocytes to produce IL-1β 2 hours after exercise in older women (33) and IL-6, TNF-α, and IL-1β in trained and untrained older women 6 hours after exercise (25). In healthy young men, strenuous mixed aerobic and anaerobic exercise increases the capacity to produce IL-6 and TNF-α (34). Combined, the findings from these three studies suggest that exercise can acutely increase the inflammatory capacity of leukocytes; however, the effect of heavy resistance exercise on the inflammatory capacity (LPS-stimulated cytokine response) in resistance-trained young men and women does not appear to have been investigated.

In contrast to the limited amount of research investigating the effect of resistance exercise on inflammatory capacity, a substantial amount of literature exists on the effect of alcohol on the inflammatory capacity of leukocytes. Acute alcohol treatment
decreases the inflammatory capacity as evidenced by reduced LPS-stimulated pro-inflammatory cytokines and chemokines (e.g., IL-1β, TNF-α, IL-6, and IL-8) in isolated macrophages collected from rodents 3 and 24 hours after alcohol administration (16, 17) and from isolated human mononuclear cells either incubated with alcohol (1, 37, 40) or collected 18 hours after alcohol ingestion (24, 36). Further, isolated human peripheral blood monocytes treated with ethanol also display an increased LPS-stimulated release production of the anti-inflammatory cytokine IL-10 (37). Thus, it appears that a bout of resistance exercise and acute alcohol administration have opposite effects on inflammatory capacity. Since alcohol consumption after exercise is common (6), it is important to elucidate the combined effect of alcohol consumption and exercise on inflammatory capacity. However, to date, no study appears to have investigated this combined effect. Therefore, the purpose of this study was to examine the effect of post-resistance exercise alcohol ingestion on LPS-stimulated production of IFNγ, TNF-α, IL-1β, IL-6, IL-8, and IL-10. It was hypothesized that after resistance exercise, the LPS-stimulated production of IFNγ, TNF-α, IL-1β, IL-6, and IL-8 would increase, but the increase would be less when alcohol administration followed exercise. It was also hypothesized that after resistance exercise, the LPS-stimulated production of IL-10 would decrease, but the decrease would be less when alcohol consumption followed exercise.
METHODS

Overview of Methodology

Ten men and 8 women, all of whom were recreationally resistance-trained, volunteered for this study. Using a within-subjects crossover design, participants performed two identical acute heavy resistance exercise bouts approximately 28 days apart. This study followed the experimental protocol for resistance exercise and alcohol consumption previously employed by Vingren et al. (41). Each exercise bout consisted of a standardized warm-up followed by a resistance exercise protocol consisting of 6 sets of 10 repetitions of Smith machine back squats with 80% of each participant’s pre-determined 1-repetition maximum (1RM). Ten minutes after the exercise bout, participants consumed either alcohol (alcohol condition; ALC: 1.09 g ethanol per kg fat-free body mass) or water (placebo condition; PLA) diluted in an artificially sweetened, low-calorie beverage. Participants consumed the assigned beverage across 10 minutes. Blood samples were collected before exercise (PRE), 3 hours after exercise (3h), and 5 hours after exercise (5h). Blood samples were treated with LPS and incubated overnight, and cell-free supernatant was analyzed for concentrations of IFNy, TNF-α, IL-1β, IL-6, IL-8, and IL-10. For the second trial, participants were assigned the other drink condition, but all other procedures were unchanged. Treatment order was assigned in a randomized and counter-balanced manner.

Participants

Recreationally resistance-trained (at least 2 resistance exercise sessions per week for the previous 6 months, including the back squat) men \( (n = 10, 25 \pm 3 \text{ yr}, 177 \pm 7 \text{ cm}, 83.8 \pm 15.7 \text{ kg}, 14.8 \pm 8.5\% \text{ body fat}) \) and women \( (n = 8, 23 \pm 2 \text{ yr}, 161 \pm 3 \text{ cm}, \)
59.5 ± 6.0 kg, 26.5 ± 3.0% body fat) were recruited to participate in this study. Participants were informed of the study requirements, restrictions, and risks and benefits, and then provided written informed consent. This study was approved by the University of North Texas Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Participants were screened for medical concerns that might place them at an elevated risk during the study procedures or that could potentially confound results. Exclusion criteria included pre-existing heart or respiratory conditions, blood pressure problems, herniated inter-vertebral discs, and any other orthopedic injuries that would limit the range of motion about the shoulder, elbow, hip, knee, or ankle joint. Women were eumenorrheic and not pregnant or trying to become pregnant. In order to screen participants for signs of alcohol abuse and dependence, each participant completed the “Young Adult Alcohol Problems Screening Test” (18) and the “Alcohol Use Disorders Identification Test” (2) questionnaires. Based upon questionnaire results, participants did not show signs of alcohol dependence and were capable of tolerating the amount of alcohol required for this study.

Procedures

Session 1: Anthropometric Measurements, Familiarization, and 1RM Test

Approximately 1 week before the first experimental session, participants reported to the laboratory for anthropometric measurements, familiarization, and 1RM testing. Height and weight were measured. Then, body composition was determined using dual-energy X-ray absorptiometry (Lunar Prodigy General Electric Company, Madison, WI). Participants performed a standardized dynamic warm-up consisting of heel kicks, lunges, high knees, high kicks, and un-weighted squats. After the warm-up, participants
were familiarized with the Smith machine squat and instructed in proper technique. Once participants demonstrated proper Smith machine squat technique, their 1RM was determined according to the methods used by Kraemer et al. (19). Briefly, participants began by performing 8-10 repetitions at approximately 50% of their estimated 1RM, then 2-5 repetitions at approximately 85% of their estimated 1RM. Then, 4-5 sets of single repetitions with 2 minutes of rest were performed to determine the 1RM.

Before leaving the laboratory, participants were reminded of study requirements for subsequent sessions. Subjects were required to refrain from resistance or high-intensity aerobic exercise for 96 hours prior to each treatment session, from alcohol consumption for 48 hours prior to each experimental treatment session, and from sexual activity for 24 hours prior to each treatment session. Subjects were instructed to limit caffeine intake on treatment days to a maximum of 1 cup of coffee or equivalent (approximately 120 mg of caffeine). No food or beverages other than water were allowed in the 2 hours leading up to each treatment session.

_Sessions 2 and 3: Experimental Treatment Sessions (See Figure 1 for an overview of the experimental timeline)_

**Figure 1.** Timeline of experimental visits.

Upon arrival at the laboratory, participants verbally attested compliance with all study requirements. An Alcomate Accucell breathalyzer (AK, Palisades Park, NJ) was
used to confirm the absence of blood alcohol. Hydration status was determined using urine refractometry. If urine specific gravity was ≥1.020, the subject was provided with water to drink. Participants consumed a standard meal replacement beverage (Ensure Plus®) containing 8 kcal·kg⁻¹ body mass 65 minutes before the resistance exercise protocol began. Once the beverage was consumed, a catheter was inserted using a Teflon coated cannula (Vascular access, Becton-Dickerson, Sandy, UT) into a superficial vein on the participant’s forearm. The catheter was maintained by sterile saline (0.9% sodium chloride inj., USP, Hospira Inc. Lake Forest, IL).

Thirty-five minutes prior to the resistance exercise protocol, the PRE blood sample was collected. Then, participants completed the same dynamic warm-up as they did in the familiarization session. Following the dynamic warm-up, participants performed a single set of 10 Smith machine squats at 50% of the 1RM. The resistance exercise protocol required participants to perform 6 sets of 10 repetitions of Smith machine squats at 80% of 1RM. Two minutes of passive rest separated each set. If participants were unable to complete all 10 repetitions in a given set, they were assisted by the researchers, and the load was reduced for the remaining sets. The load for each set was recorded and replicated on the second experimental treatment session.

After the resistance exercise bout, participants sat quietly for the remainder of the session. Ten minutes after the completion of exercise, participants consumed either alcohol (alcohol condition; ALC) or an equal volume of water (placebo condition; PLA) diluted in an artificially-sweetened, low-calorie beverage following procedures previously used in this laboratory (41). The beverage was divided into 10 equal servings, and participants consumed 1 serving every minute for 10 minutes. The dose of alcohol in the
ALC condition was equal to 1.09 g ethanol (vodka, 40% v/v ethanol, Smirnoff, Norwalk, CT) per kg fat-free body mass diluted to a concentration of 15% v/v ethanol. This dose was estimated to result in a blood alcohol concentration (BAC) of approximately 0.12 g∙dL⁻¹. To prevent anticipation of either beverage condition, participants were informed that they could possibly receive the same condition on both visits. In order to minimize the ability to distinguish the treatment condition, the rim of the cups in both conditions were blotted with a small amount of vodka. Participants were not informed of their BAC or their treatment condition at any time during the study. Since alcohol consumption has well-known physiological and psychological side effects, some participants were aware of having consumed alcohol, but were generally unaware of when they had not received alcohol.

Blood Collection and LPS Stimulation

For LPS stimulation, blood was collected into 2 mL sodium heparin-treated vacuum tubes at PRE, 3h, and 5h and stored at room temperature on a rocker until same-day LPS stimulation. LPS stimulation followed the methods of Carpenter et al. (10). All procedures were performed in a sterile hood using sterile technique and endotoxin-free supplies. Whole blood from PRE, 3h, and 5h was diluted 1:10 with phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO). LPS (LPS-SM ultrapure, InvivoGen, San Diego, CA; 1 mg∙mL⁻¹) was added to diluted samples for a final concentration of 15 µg∙mL⁻¹ LPS and cultured in a CO₂ incubator (37°C, 5% CO₂) for 24 hours. Cell-free supernatant was collected and frozen (-80°C) until analysis.

For determination of BAC, blood samples were collected at PRE and every 20 minutes after exercise into a vacuum tube treated with serum clot activator and
subsequently allowed to clot at room temperature for 20 minutes. Samples were then centrifuged at 1500 × g for 20 minutes at 4 °C, and serum was separated and stored at 4 °C until same-day analysis.

**Cytokine Analysis**

Based upon a lot-specific titer test, supernatant was thawed and diluted (1:5) with assay buffer (MILLIPLEX MAP Assay Buffer, EMD Millipore, Billerica, MA). Samples were then prepared in duplicate using a commercially available human high-sensitivity cytokine assay (HSCYTMAG-60SK, EMD Millipore, Billerica, MA) and acquired using a Luminex-based system (Magpix, Luminex, Austin, TX). The system calibration and fluidics were verified prior to use. Concentrations of IFNγ, TNF-α, IL-1β, IL-6, IL-8, and IL-10 were then calculated using MILLIPLEX® Analyst software (EMD Millipore, Billerica, MA). The intra-assay coefficients of variation ranged from 0.5 % to 6.5 % for each analyte.

Serum samples were analyzed for alcohol concentration using a commercially available enzymatic assay (Pointe Scientific, Canton, MI) and an automated chemistry analyzer (ChemWell-T, Awareness Technology, Inc., Palm City, FL). Results from serum concentrations were converted to BAC following manufacturer instructions. Serum alcohol concentrations are approximately 16% higher than in whole blood; therefore, the alcohol concentration in serum was divided by 1.16 to calculate the alcohol concentration in whole blood.

**Statistical Analysis**

Statistical analyses were conducted using Statistica (Stat Soft, Inc., Tulsa, OK). Data were log-transformed prior to analysis to correct for violation of normality. A 2
(condition) × 3 (time) ANOVA with repeated measures on both factors was used for further analyses. Results from Mauchly’s test indicated that sphericity was violated for time (IFNγ) and for the condition × time interaction (IFNγ, TNF-α, and IL-10). Therefore, the Greenhouse-Geisser correction was applied. Where appropriate, pairwise comparisons were made using Fisher’s Least Significant Difference post hoc test. The α-level of significance was set at $p < 0.05$. Unless otherwise noted, data are presented as mean ± standard deviation.
RESULTS

Blood Alcohol Concentration

There was no presence of alcohol (BAC < 0.01 g∙dL\(^{-1}\)) at PRE for ALC or any time point for PLA. In the ALC condition peak BAC (0.11 ± 0.02 g∙dL\(^{-1}\)) was achieved at 80-120 minutes after exercise (60-100 minutes after the completion of beverage consumption).

LPS-Stimulated cytokines

There was a significant main effect of time for IFN\(_\gamma\) \(\left(F_{(1.8,18.6)} = 56.17, p < 0.001\right)\).

The LPS-stimulated IFN\(_\gamma\) concentration was significantly \(p < 0.05\) greater at 5h than at PRE (1437%) and 3h (1262%) (Table 1). No main effect of condition or condition × time interaction effect was found.

There was a significant main effect of time for TNF-\(\alpha\) \(\left(F_{(2,34)} = 53.41, p < 0.001\right)\).

The LPS-stimulated TNF-\(\alpha\) concentration at 5h was significantly greater than PRE (133%) and 3h (132%) (Table 1). No main effect of condition or condition × time interaction effect was found.

There was a significant main effect of time for IL-1\(\beta\) \(\left(F_{(2,34)} = 34.10, p < 0.001\right)\).

The LPS-stimulated IL-1\(\beta\) concentration at 5h was significantly greater than PRE (71%) and 3h (69%) (Table 1). No main effect of condition or condition × time interaction effect was found.

There was a significant condition × time interaction effect for IL-6 \(\left(F_{(2,34)} = 7.11, p = 0.003\right)\). Post hoc pairwise comparisons found that at PRE, the LPS-stimulated IL-6 concentration was significantly greater in ALC than in PLA, whereas at 3h, the LPS-stimulated IL-6 concentration was significantly lower in ALC than in PLA (Figure 2A). In
ALC, the LPS-stimulated IL-6 concentration was significantly less at 3h than at PRE (-13%), whereas in PLA, the LPS-stimulated IL-6 concentration was significantly greater at 3h than at PRE (20%). By 5h, the interaction effect was no longer found; the LPS-stimulated IL-6 concentration was significantly greater at 5h than at PRE (69%) and 3h (67%).

There was a significant condition × time interaction effect for IL-8 ($F_{(2,34)} = 3.34$, $p = 0.047$). Post hoc pairwise comparisons indicate that at 5h, the LPS-stimulated IL-8 concentration was significantly less in ALC than in PLA (Figure 2B). Within the ALC condition, the LPS-stimulated IL-8 concentration was significantly less at 5h than at PRE (-37%).

There was a significant main effect of time for IL-10 ($F_{(2,34)} = 45.74$, $p < 0.001$). The LPS-stimulated IL-10 concentration was significantly less at 5h than at PRE (-59%) and at 3h (-41%), and the LPS-stimulated IL-10 concentration was significantly less at 3h than at PRE (-30%) (Table 1). No main effect of condition or condition × time interaction effect was found.

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<td>1.6 (0.8)</td>
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<td>26.6 (11.7)</td>
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<tr>
<td>IL-1β</td>
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<td>IL-10</td>
<td>32.6 (23.7)</td>
<td>24.6 (15.8)</td>
<td>20.3 (11.5)</td>
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Table 1. Mean (SD), Absolute LPS-stimulated cytokine concentrations (pg∙mL$^{-1}$) before exercise (PRE), 3 hours after exercise (3h), and 5 hours after exercise (5h) for the Placebo (PLA) and Alcohol (ALC) conditions. *Significantly ($p < 0.05$) different (main effect of time) from PRE; †significantly different (main effect of time) from 3h.
Figure 2. LPS-stimulated concentrations of (A) IL-6 and (B) IL-8. $significantly (p < 0.05) different from PLA at corresponding time point, #significantly different from corresponding PRE, †significantly different from corresponding 3h. Mean ± SE.
DISCUSSION

The current study appears to be the first to investigate the effect of acute alcohol consumption on whole blood inflammatory capacity (as measured by LPS-stimulated production of pro-inflammatory and anti-inflammatory cytokines) following a bout of heavy resistance exercise. The major finding of this study was that alcohol consumption after heavy resistance exercise affected LPS-stimulated production of IL-6 (decreased 3 hours after exercise) and IL-8 (decreased 5 hours after exercise) but not of IFNγ, TNF-α, IL-1β, and IL-10. Furthermore, it was found that the resistance exercise bout induced an increase in LPS-stimulated production of pro-inflammatory cytokines (IFNγ, TNF-α, IL-1β, and IL-6) 5 hours after exercise, regardless of treatment condition. The inflammatory capacity of leukocytes is important for the progression of muscle tissue repair and remodeling following resistance exercise (32, 38). Therefore, the findings of this study provide unique and important physiological insight into the inflammatory process as they demonstrate the acute changes in inflammatory capacity in response to resistance exercise and they demonstrate how alcohol modulates this response in young men and women.

In the absence of exercise, alcohol has been found to reduce LPS-stimulated production of IL-8 in whole blood 4 hours following consumption (36) and in isolated blood monocytes incubated with ethanol for 1 hour (1). Although no previous study appears to have assessed LPS-stimulated IL-8 production in whole blood following an acute bout of resistance exercise, it has been documented that resistance exercise can augment the mRNA expression of IL-8 in muscle tissue (29) suggesting that resistance exercise could result in increased IL-8 production. In the present study, heavy
resistance exercise alone did not affect LPS-stimulated release of IL-8 at 3 or 5 hours after the exercise bout. However, as hypothesized based on the effect of alcohol in absence of exercise, alcohol consumption reduced LPS-stimulated IL-8 release although this effect was only found 5 hours after exercise. IL-8 is a potent neutrophil chemoattractant (13) and neutrophil infiltration peaks at approximately 6 to 24 hours after muscle damage induction (22, 23, 39); reduced IL-8 production could potentially affect neutrophil transmigration into damaged muscle tissue and subsequent phagocytosis.

Previous research has demonstrated opposite effects of a bout of resistance exercise (25) and acute alcohol administration (16, 17) on LPS-stimulated IL-6 release. Resistance exercise results in increased LPS-stimulated IL-6 release 6 hours after the exercise bout (25); whereas, acute alcohol administration results in decreased LPS-stimulated IL-6 production 3 to 24 hours after administration (16, 17). In the current study, LPS-stimulated IL-6 concentrations were greater at 5h than at PRE in both conditions. In addition, LPS-stimulated IL-6 significantly increased from PRE to 3h in PLA, whereas LPS-stimulated IL-6 significantly decreased from PRE to 3h in ALC. Combined these results suggest that alcohol consumption attenuated the LPS-stimulated IL-6 release in response to the exercise bout 3 hours after exercise, but by 5 hours after exercise, the effect of alcohol on LPS-stimulated IL-6 was no longer distinguishable. The results for IL-6 should be interpreted with caution because despite the randomized, counterbalanced, and double blinded design of the study, PRE concentrations were greater for ALC than PLA. Since a difference at PRE between conditions was found only for IL-6 it is difficult to speculate on the underlying cause of
this initial difference and its consequences.

Acute alcohol administration *in vitro* (37) and *in vivo* (24) increases LPS-stimulated release of the anti-inflammatory cytokine IL-10 from isolated human monocytes; however, in those studies, IL-10 production was measured only at 18 hours after *in vivo* alcohol intake. No research appears to have investigated LPS-stimulated IL-10 production within the first 5 hours after alcohol consumption or after resistance exercise. Since the initial local response to muscle damage is primarily pro-inflammatory and only later in the muscle tissue repair process (usually days after damage) shifts to being primarily anti-inflammatory (31, 32, 39), a reduction in LPS-stimulated IL-10 production following resistance exercise would be expected. In the present study, the results showed the expected pattern, as LPS-stimulated IL-10 release was reduced at 3h and further reduced at 5h compared to PRE across conditions. Contrary to the hypothesis, no difference was found between conditions at 3h or 5h after resistance exercise. The effect of alcohol on LPS-stimulated IL-10 production has not been previously investigated during the first several hours after administration; thus, it is possible that the initial inflammatory response to alcohol administration does not increase the capacity to produce IL-10, as observed in the present study.

Alcohol administration alone reduces the inflammatory capacity of leukocytes, especially monocytes, as indicated by reduced LPS-stimulated production of IFNγ, TNF-α, IL-1β, IL-6, and IL-8, along with increased LPS-stimulated release of IL-10 (16, 17, 37). In the present study, differences between ALC and PLA were found only for IL-6 and IL-8 (release was less with alcohol ingestion for each); however, large increases in
IFNγ, TNF-α, IL-1β, and IL-6 were found in response to heavy resistance exercise 5 hours after the exercise bout for both conditions. The release of pro-inflammatory TNF-α, IL-1β, IL-6, and IL-8 from monocytes and macrophages is modulated by p38 mitogen-activated protein kinase (MAPK) and/or ERK1/2 MAPK (1, 15-17, 30). Previous research has demonstrated that alcohol reduces LPS-stimulated release of TNF-α, IL-6, and IL-8, at least partially through a reduced activation of these MAPKs at 3 and at 24 hours after administration (1, 16, 17). The effect of alcohol-induced reduction of p38 and ERK1/2 MAPK activation has not been investigated for IL-1β but could follow a similar pattern. Although this was not measured, a reduced activation of p38 MAPK and ERK1/2 MAPK could explain the reduced production of IL-6 (3 hours after exercise) and IL-8 (5 hours after exercise) with alcohol ingestion found in the present study. The lack of an effect of alcohol on TNF-α and IL-1β release was in contrast to our hypothesis but might be due to the potent effect that was observed for heavy resistance exercise on LPS-stimulated release of these biomarkers.

Regardless of treatment condition, in the present study the heavy resistance exercise bout induced a large increase in LPS-stimulated production of IFNγ, TNF-α, IL-1β, and IL-6 (pro-inflammatory cytokines) 5 hours after exercise and a large decrease in LPS-stimulated production of IL-10 (anti-inflammatory cytokine) at 3 and at 5 hours after exercise compared to PRE. The acute effect of resistance exercise on LPS-stimulated cytokine release has not previously been examined in young resistance trained men and women. In older women, resistance exercise increased LPS-stimulated release of IL-6, TNF-α, and IL-1β in whole blood 6 hours after exercise (25), which is consistent with the findings of the current study. Combined, these findings show that a bout of
resistance exercise can substantially increase inflammatory capacity of circulating leukocytes and suggest an increase in the ability of these leukocytes to respond to resistance exercise-induced muscle damage, an important initial step in the tissue repair process.

The mechanism by which acute alcohol consumption following a bout of resistance exercise differentially affects components of the inflammatory milieu in response to LPS stimulation warrants further examination. Overall, heavy resistance exercise appears to have a more potent effect than acute alcohol consumption at a dose that elicits a BAC of approximately 0.11 g·dL⁻¹ on the inflammatory capacity of leukocytes in whole blood 5 hours after the exercise bout.
CONCLUSION

Findings from this study indicate that consuming alcohol after a bout of heavy resistance exercise reduces the capacity of leukocytes to produce IL-6 at 3 hours after exercise and IL-8 at 5 hours exercise. Further, the increase in capacity to produce IFNγ, TNF-α, IL-1β, and IL-6 at 5 hours after the heavy resistance exercise bout overwhelmed the effect of alcohol on inflammatory capacity in this study. Future research should investigate the importance of the increase in the inflammatory capacity found with heavy resistance exercise on the time course of muscle recovery and the consequences of the alcohol-induced alterations in this inflammatory capacity. The current study utilized only 1 alcohol dose (1.09 g ethanol per kg fat free mass); a range of alcohol doses should be investigated to determine whether an alcohol dose-response exists with regard to LPS-stimulated cytokine production following resistance exercise.
REFERENCES


