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ORIGINAL ARTICLE

Effect of alcohol after muscle-damaging resistance exercise on muscular performance recovery and inflammatory capacity in women

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Abstract
Purpose To investigate the effect of acute alcohol consumption on muscular performance recovery, assessed by maximal torque production, and on inflammatory capacity, assessed by lipopolysaccharide (LPS)-stimulated cytokine production, following muscle-damaging resistance exercise in women.
Methods Thirteen recreationally resistance-trained women completed two identical exercise bouts (300 maximal single-leg eccentric leg extensions) followed by alcohol (1.09 g ethanol kg^{-1} fat-free body mass) or placebo ingestion. Blood was collected before (PRE), and 5 (5 h-POST), 24 (24 h-POST), and 48 (48 h-POST) hours after exercise and analyzed for LPS-stimulated cytokine production (TNF-α, IL-1β, IL-6, and IL-8 and IL-10). Maximal torque production (concentric, eccentric, isometric) was measured for each leg at PRE, 24 h-POST, and 48 h-POST.
Results Although the exercise bout increased LPS-stimulated production of TNF-α (%change from PRE: 5 h-POST 109%; 24 h-POST 49%; 48 h-POST 40%) and decreased LPS-stimulated production of IL-8 (5 h-POST −40%; 24 h-POST −50%; 48 h-POST: −43%) and IL-10 (5 h-POST: −37%; 24 h-POST −32%; 48 h-POST −31%), consuming alcohol after exercise did not affect this response. Regardless of drink condition, concentric, eccentric, and isometric torque produced by the exercised leg were lower at 24 h-POST (concentric 106 ± 6 Nm, eccentric 144 ± 9 Nm, isometric 128 ± 8 Nm; M ± SE) compared to PRE (concentric 127 ± 7 Nm, eccentric 175 ± 11 Nm, isometric 148 ± 8 Nm). Eccentric torque production was partially recovered and isometric torque production was fully recovered by 48 h-POST.
Conclusions Alcohol consumed after muscle-damaging resistance exercise does not appear to affect inflammatory capacity or muscular performance recovery in resistance-trained women. Combined with previous findings in men, these results suggest a gender difference regarding effects of alcohol on exercise recovery.

Keywords Muscle damage · Resistance exercise · Binge drinking · Cytokines · Lipopolysaccharide

Abbreviations

5 h-POST	Five hours after exercise time point
24 h-POST	Twenty-four hours after exercise time point
48 h-POST	Forty-eight hours after exercise time point
ALC	Alcohol condition
ANOVA	Analysis of variance
BAC	Blood alcohol concentration
CK	Creatine kinase
IL	Interleukin
LPS	Lipopolysaccharide
NF	Nuclear regulatory factor
PLA	Placebo condition
PRE	Pre-exercise time point
TNF	Tumor necrosis factor
VAS	Visual analog scale

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Introduction

College athletes and students who frequently engage in vigorous exercise report higher rates of excessive alcohol consumption (e.g., binge drinking) than their inactive peers (Ford 2007; Martens et al. 2006; Moore and Werch 2008). Moreover, those who perform resistance exercise are more likely to binge drink than those who perform moderate or vigorous aerobic exercise (Barry and Piazza-Gardner 2012). Among women athletes, nearly half (48%) of those surveyed reported at least one binge drinking episode within the prior 2 weeks (Nelson and Wechsler 2001). Such alcohol use patterns could have important implications for women athletes (competitive and recreational), since in resistance-trained men, drinking a moderate-to-high dose of alcohol (1 g ethanol kg^{-1} body mass) after strenuous eccentric resistance exercise can result in delayed performance recovery in the days following exercise (Barnes et al. 2010a, b). To our knowledge, only two published studies have investigated this effect in women and neither reported an effect of alcohol. However, the first study investigated the effect of alcohol consumption prior to exercise, included only a small muscle mass exercise (biceps curl), and participants were not specifically resistance trained (Clarkson and Reichsman 1990). In the second, a large muscle mass exercise (leg extensions) was utilized, but the participants were not specifically resistance trained and no increase in creatine kinase (CK) activity, an indirect marker of muscle damage, was observed (McLeay et al. 2016). Women who are resistance exercise-trained comprise the female population most likely to combine muscle-damaging resistance exercise with alcohol and it is unknown how post-resistance exercise alcohol consumption could impact recovery of muscular performance.

Resistance exercise can induce substantial muscle damage, especially during the eccentric phase (LaStayo et al. 2003; Stupka et al. 2000). This damage presents a challenge that requires an immune system response to allow for recovery of the tissues involved. When skeletal muscle is damaged, pro-inflammatory monocytes are selectively recruited to, and subsequently transmigrate into, the injured tissue, where they transform into macrophages (Arnold et al. 2007). These macrophages begin removal of debris, an important early step in the tissue repair process, and secrete a range of cytokines (e.g., interleukin (IL)-6, IL-8, IL-10, IL-1β, and tumor necrosis factor (TNF)-α) that mediate tissue inflammation and thus repair (Cannon and St Pierre 1998). The importance of monocytes for tissue repair after muscle damage was demonstrated by Arnold and colleagues (2007) who found that chemical depletion of circulating monocytes prior to skeletal muscle tissue injury prevented regeneration of that tissue. Thus, a reduction in monocyte function (e.g., by consuming alcohol) could potentially impair muscle recovery.

Whole blood lipopolysaccharide (LPS) stimulation in vitro is an established and validated method to assess the inflammatory capacity of leukocytes, especially monocytes (Damsgaard et al. 2009). Using this LPS stimulation model, McFarlin and colleagues (2004) found an increased capacity of leukocytes for production of inflammatory cytokines (IL-1β, TNF-α, and IL-6) 6 h after an acute resistance exercise bout compared to pre-exercise in older women. Furthermore, we have previously found substantial resistance exercise-induced increases in pro-inflammatory cytokine concentrations (TNF-α, IL-1β, and IL-6) and decreased anti-inflammatory IL-10 concentrations in response to LPS stimulation from before exercise to 5 h after exercise in resistance-trained young men and women (Levitt et al. 2016). The results of these studies indicate that resistance exercise enhances the ability of leukocytes, and especially monocytes, to respond to an inflammatory stimulus.

Inflammatory cytokine production is controlled, at least in part, by the translocation of the heterodimeric form of nuclear regulatory factor (NF)-κB into the nucleus and subsequent binding to the promoter region to enhance transcription of cytokine genes (Baeuerle and Henkel 1994). In contrast, alcohol promotes the translocation of a homodimeric form of NF-κB into the nucleus, which suppresses transcription of cytokine genes (Mandrekar et al. 1997). Thus, independent of exercise, exposure to physiologically relevant doses of alcohol alters the monocytic cytokine response to in vitro LPS stimulation. Alcohol-induced changes include decreased production of TNF-α (Mandrekar et al. 2006; Szabo et al. 1996), IL-1β (Mandrekar et al. 2006; Szabo et al. 1996), and IL-6 (Goral et al. 2004) and increased production of IL-10 (Mandrekar et al. 2006; Szabo et al. 1996). Furthermore, incubation with alcohol in vitro decreased LPS-stimulated TNF-α production in blood collected from patients who had experienced non-exercise-related severe physical trauma that causes high levels of pro-inflammatory cytokine production (Verma et al. 1993). Finally, after an episode of binge drinking in human participants, LPS-stimulated production of IL-1β is decreased 2 and 5 h after drinking and production of IL-10 is increased 5 h after drinking (Afshar et al. 2015), indicating a reduction of inflammatory capacity following alcohol. Combined, these findings for LPS-stimulated cytokine production suggest that acute alcohol exposure impairs the inflammatory capacity of monocytes, which could hinder tissue repair following resistance exercise.

Despite the independent findings for the effects of alcohol on muscle recovery following exercise and on LPS-stimulated cytokine production, to our knowledge,

our previous study is the only published investigation of the effect of alcohol ingestion after resistance exercise on LPS-stimulated cytokine production (Levitt et al. 2016). In that study, we observed that alcohol consumed after heavy squat exercise attenuated LPS-stimulated production of IL-6 at 3 h after exercise and IL-8 at 5 h after exercise in young, recreationally resistance-trained men and women. However, more research in this area is needed to investigate whether the effects persist for periods of time longer than 5 h, to determine the effects of different resistance exercise protocols (especially those that result in substantial muscle damage such as eccentrically biased resistance exercise), and to examine potential effects on performance.

Ingestion of alcohol following strenuous eccentric resistance exercise accentuates the decline in muscular force production capability in the days following the exercise bout in resistance-trained men, but has not been investigated in resistance-trained women. Separately, strenuous resistance exercise increases inflammatory capacity, whereas alcohol ingestion decreases inflammatory capacity. However, the combined effect of alcohol ingestion and muscle-damaging exercise on markers of inflammatory capacity has not been investigated. Despite a high prevalence of alcohol use among those who engage in resistance exercise, the importance of inflammation to the repair of damaged muscle tissue following such exercise, and the effect of alcohol ingestion on inflammatory capacity, there is a dearth of literature on the physical and physiological effects of the combination of alcohol and exercise, particularly in women. Therefore, the purpose of this study was to investigate the effect of acute alcohol consumption on muscular performance recovery and on LPS-stimulated cytokine production following strenuous eccentric resistance exercise in women.

Materials and methods

Brief overview

This study employed a single-leg, repeated measure, crossover design to investigate the effects of post-eccentric resistance exercise alcohol ingestion on muscular performance recovery and lipopolysaccharide (LPS)-stimulated cytokine production in women. The exercise protocol and measurement of muscular performance recovery followed the procedures previously used by Barnes and colleagues (2010a, b) to investigate the effect of post-exercise alcohol ingestion on muscle performance recovery in men. The alcohol dosage and administration (Levitt et al. 2016; Vingren et al. 2013) and LPS stimulation and biomarker analysis (Carpenter et al. 2013; Levitt et al. 2016) followed procedures previously used

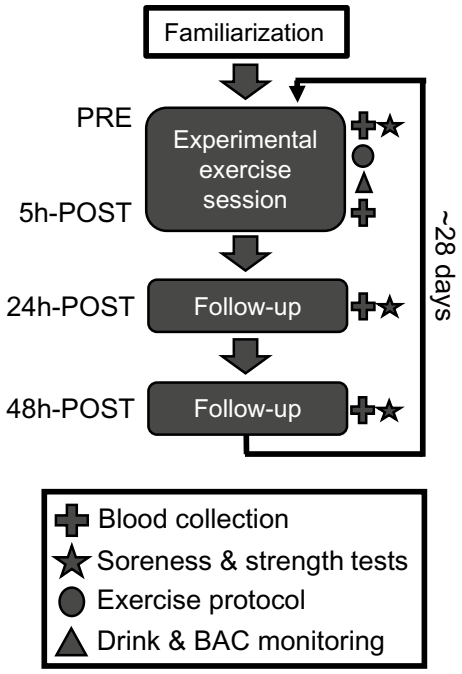

Fig. 1 Overview of study timeline

in our laboratory. For an overview of the study timeline, see Fig. 1. Briefly, 13 recreationally resistance-trained women underwent baseline palpated muscle soreness testing using the visual analog scale (VAS) (Nosaka and Newton 2002) and maximal torque testing (concentric, eccentric, and isometric) on each leg. Then, they completed a high-volume resistance exercise protocol, designed to induce muscle damage, on one leg only (3 sets of 100 repetitions of maximal effort eccentric unilateral leg extensions with 5 min of rest between sets). Ten minutes after exercise, participants ingested a beverage containing either alcohol (ALC) or a non-alcoholic placebo beverage (PLA). Palpated muscle soreness and torque (concentric, eccentric, and isometric) for each leg were measured again 24 and 48 h after the baseline tests. Approximately 28 days later (to control for menstrual cycle phase), participants returned to the laboratory and completed the same testing and exercise protocols, but exercised the contralateral leg, and were assigned the other drink condition. Drink condition and exercising leg orders were counterbalanced and assigned using randomization. Blood was collected before exercise (PRE), and 5 h (5 h-POST), 24 h (24 h-POST), and 48 h after the eccentric exercise protocol (48 h-POST). Blood samples were stimulated with LPS for 24 h, and subsequently analyzed for pro- (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8) and anti-inflammatory (IL-10) cytokines.

Participants

Thirteen recreationally resistance-trained (at least 2 sessions per week for the previous six months, including lower body) women (21–34 years, 162 ± 6 cm, 62.2 ± 7.6 kg, 28.7 ± 8.1% body fat; M ± SD) completed all parts of this study. All study requirements and testing and exercise protocols were thoroughly explained to potential participants before they provided written informed consent to participate. 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 conditions that could confound the results of the study or place them at an elevated risk during the study. Exclusion criteria included: pre-existing heart conditions or abnormalities, blood pressure problems, illness at the time of the study, prior musculoskeletal injury that would limit the range of motion at the knee joint, existing knee problems, and any contraindications to the leg extension exercise. Participants were required to be eumenorrheic and not pregnant or trying to become pregnant. Oral contraceptive use was permitted as long it was consistently used throughout the study duration. Furthermore, potential participants were excluded if their contraceptive use resulted in a planned longer menstrual cycle length or absence of menstrual cycles (e.g., seasonale). Oral contraceptive use was reported by 5 of the 13 subjects. Participants were required to abstain from resistance exercise and vigorous aerobic exercise from 96 h prior to each experimental exercise session until 48 h after the session and from alcohol 48 h prior to each experimental exercise session until 48 h after the session. Additionally, they were required to refrain from any practices that might alter the recovery process and the inflammatory response to the exercise protocol, such as the ingestion of non-steroidal anti-inflammatory drugs, during this time frame.

To be considered for participation in the study, volunteers had to be classified as low- or moderate-alcohol consumers as defined by the US Department of Health and Human Services (National Center for Health Statistics). The "Young Adult Alcohol Problems Screening Test" (Hurlbut and Sher 1992) and the "Alcohol Use Disorders Identification Test" (Babor et al. 2001) questionnaires were used to aid in screening the participants for signs of alcohol misuse. Based upon the screening, participants were neither alcohol dependent nor naïve to alcohol, and they were deemed able to tolerate the amount of alcohol ingestion required for this study.

Procedures

Anthropometric measurements and familiarization

Approximately one week prior to the first exercise session, participants reported to the laboratory for anthropometric measurements and familiarization with the exercise protocol. For anthropometric measurements, participants wore light athletic clothing and no shoes or metals. Following measurements of height and weight, body composition was measured using dual-energy X-ray absorptiometry (Lunar Prodigy General Electric Company, Madison, WI, USA). Then, participants were familiarized with the palpated muscle soreness tests (described below; see "Muscle soreness measurements"). After muscle soreness testing, participants performed a 5-min cycle ergometer warm up. After the warm up, the isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) was adjusted to fit each participant and all settings were recorded to use for subsequent visits. Then, participants were familiarized with the leg extension exercise tests and the eccentric exercise protocol. During familiarization, participants used only light effort (e.g., ~50–60% of maximal effort) to avoid any muscle damage.

Experimental exercise sessions

Each participant completed both drink conditions (ALC and PLA) and thus served as her own control. Participants were "phased" so that the experimental sessions were completed during the early follicular phase of the menstrual cycle (2–7 days after the onset of menses) to control for potential hormonal differences. Thus, the two treatment sessions took place approximately 28 days apart. Drink condition (ALC and PLA) and leg orders were assigned using randomization in a counterbalanced, single-leg crossover design. Participants and investigators (other than the investigator who randomized condition assignment and prepared the beverages and who was otherwise not involved in the data collection procedures) were blind to the condition (ALC and PLA) selected for each experimental session. To prevent participants from anticipating a certain drink condition on either visit, they were told that they could potentially receive the same beverage for both experimental exercise sessions. Participants kept a diet record from the morning of the first experimental exercise session until the 48-h follow-up session. They were required to replicate this diet beginning the morning of the second experimental exercise session to control for any potential effects of diet on inflammatory capacity and/or muscular performance recovery.

On the morning of the first experimental exercise session and the mornings of the 24- and 48-h follow-up visits, participants were administered a standardized meal 2 h prior to their scheduled laboratory arrival time (Ensure Plus®, 8 kcal kg^{-1} body mass). The same standardized meal was administered 2 h prior to the 5 h-POST blood collection to standardize nutrient intake prior to all blood collections. Participants were instructed to consume nothing except their standardized meal and water in the 4 h prior to the experimental exercise session. Additionally, participants verbally attested that they refrained from drinking alcohol (48 h) and engaging in resistance exercise or vigorous aerobic activity (96 h) in the specified time prior to the experimental exercise visit through the 48-h follow-up visit. A breathalyzer test was administered upon laboratory arrival each day to screen for the presence of alcohol. Hydration status was assessed using urine refractometry (Pen-Wrestling Refractometer, ATAGO USA, Bellevue, WA, USA). If participants' urine specific gravity was $\geq$1.020, they were provided with water to drink.

Blood collections and processing

Blood was collected at PRE, 5 h-POST, 24 h-POST, and 48 h-POST using standard sterile venipuncture technique. Blood was collected into evacuated tubes coated with sodium heparin as an anticoagulant and tubes coated with clot activator for serum. Whole blood collected into sodium heparin-coated tubes was stored on a rocker at room temperature until same-day LPS stimulations (described below). Blood collected into tubes with clot activator was allowed to clot for 20 min at room temperature and subsequently centrifuged (1500×*g*, 20 min, 4 °C). Serum was separated into several aliquots and stored at −80 °C until analysis.

Muscle soreness measurements

Following blood collection at PRE, 24 h-POST, and 48 h-POST, participants' vastus lateralis soreness was assessed on both legs (exercised leg and non-exercised leg) at three sites (midpoint between the superior lateral portion of the patella and the anterior superior iliac spine, 5 cm proximal to the midpoint, and 5 cm distal to the midpoint). Force (35 N) was applied to each of the sites using a small probe and a compression gauge (PS-IN103, Prime Scales, Ontario, CA, USA). Participants rated their palpated muscle soreness/pain at each site using the VAS. The scale consisted of a continuous line 10 cm in length where 0 cm indicated "no pain" and 10 cm indicated "worst possible pain."

Muscle performance measurements

After assessment of muscle soreness at PRE, 24 h-POST, and 48 h-POST, participants performed a warm up for 5 min on a cycle ergometer at a standardized work rate of 100 W. After the warm up, peak torque was measured for the knee extension exercise on each leg using the isokinetic dynamometer. To ensure that movement was restricted to the knee joint and to isolate the quadriceps muscle group, straps were placed across the chest, hips, and working leg. Each participant performed five maximal concentric, eccentric, and isometric single leg knee extensions for each leg. Two minutes of passive recovery separated each muscle action test (concentric, eccentric, and isometric). Isometric torque was measured at a 75° knee angle. Concentric and eccentric torques were measured at an angular velocity of $30°s^{-1}$ over a 60° range of motion. The range of 35°–95° was chosen based on pilot testing. Absolute peak torque was recorded for each muscle action.

Eccentric exercise protocol

After torque was assessed at PRE, participants began the eccentric exercise protocol, which was modeled after the exercise protocol employed by Barnes and colleagues (2010a, b). The protocol consisted of 3 sets of 100 repetitions of maximal effort eccentric unilateral knee extensions ($30° \ s^{-1}$ over a 60° range of motion) on the isokinetic dynamometer separated by 5 min of passive rest. Participants were verbally encouraged to resist the downward motion of the lever arm. Torque production over the entire range of motion was monitored throughout the exercise protocol to help ensure maximal effort. A researcher returned the arm of the dynamometer to the starting position (i.e., assisted the concentric portion) after each repetition.

Beverage ingestion and BAC monitoring

Ten minutes after the completion of the eccentric exercise protocol, participants began ingestion of their assigned drink (either ALC or PLA) as previously described (Levitt et al. 2016; Vingren et al. 2013). In the ALC condition, participants received a volume of alcohol equal to 1.09 g ethanol kg^{-1} fat-free body mass (Vodka, The Smirnoff Co., Norwalk, CT, USA). This was equal to 4.4 ± 0.5 standard drinks consumed by each participant. The alcohol was diluted to 15% v/v in an artificially sweetened beverage. In the PLA condition, the volume of alcohol was replaced with water. The beverage volume was split into ten equal portions; one portion was administered each minute over a 10-min ingestion period. Although this ingestion period was shorter than a typical drinking session, the peak BAC

achieved (0.09 ± 0.02 g dL^{-1}, just above the legal driving limit) was reasonable for a standard drinking session. In both conditions, the rim of the cup was coated with a small amount of vodka to reduce the ability of the participants to discern their condition assigned for that session. Due to the well-known physiological and psychological effects of alcohol, participants were generally aware of when they had been assigned the ALC condition, but generally could not distinguish when they had been assigned the PLA condition. To aid in disguising the drink condition and to help prevent anticipation of condition, participants were told that they could potentially receive the same condition twice.

Beginning 40 min after exercise, BAC was measured every 20 min for the first 2 h and every 60 min thereafter using a breathalyzer (AlcoMate AccuCell AL9000, AK GlobalTech, Palisades Park, NJ, USA). In the ALC condition, peak BAC reached 0.09 ± 0.02 g dl^{-1} and occurred 80 ± 20 min after exercise. In the PLA condition, BAC remained at 0.00 g dl^{-1}. The total recovery and BAC monitoring period was 5 h after exercise, at which time BAC was required to be ≤0.03 g dl^{-1} for participants to be released to their ride or escort. Participants were not informed of their BAC at any time during the study.

LPS stimulations

Whole blood LPS stimulation followed procedures previously used in our laboratory (Carpenter et al. 2013; Levitt et al. 2016). Endotoxin-free supplies were used for LPS stimulation and all procedures were performed inside a sterile hood and at room temperature unless otherwise noted. Briefly, sodium heparin-treated whole blood collected at PRE, 5 h-POST, 24 h-POST, and 48 h-POST was diluted 1:10 with sterile phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO, USA) and transferred to a sterile polystyrene culture tube. Then, LPS (LPS-SM ultrapure, InvivoGen, San Diego, CA, USA; 1 mg ml^{-1}) was added to the diluted samples for a final LPS concentration of 15 µg ml^{-1}. Diluted samples containing LPS were cultured for 24 h in a CO_2 incubator (37 °C, 5% CO_2). After incubation, cell-free supernatant was collected and frozen (−80 °C) until analysis.

Creatine kinase (CK) analysis

To indirectly assess muscle damage, serum samples were analyzed in duplicate for CK activity using a commercially available enzymatic assay (Pointe Scientific, Ann Arbor, MI, USA) on an automated chemistry analyzer (Chem-Well-T, Awareness Technology, Inc., Palm City, FL, USA).

Cytokine analysis

Supernatant from LPS stimulation was thawed and diluted (1:10) with assay buffer (MILLIPLEX MAP Assay Buffer, EMD Millipore, Billerica, MA, USA) based on a lot-specific titer test. After dilution, supernatant was prepared in duplicate for the measurement of TNFα, IL-1β, IL-6, IL-8, and IL-10 using a commercially available human high-sensitivity multiplex cytokine bead-based assay (EMD Millipore, Billerica, MA, USA). After verification of calibration and fluidics, prepared samples were acquired on a Luminex-based system (Magpix, Luminex, Austin, TX, USA) and imaged using a CCD camera. Concentrations of each analyte were then calculated from mean fluorescence intensity using MILLIPLEX® Analyst software (EMD Millipore, Billerica, MA, USA) and subsequently corrected for the dilution factor. Intra-assay coefficient of variation ranged from 0.48 to 9.63% for each analyte.

Statistical analysis

Data were initially examined to ensure that the assumptions for parametric statistics were met. Prior to analysis, all data were examined for any potential order-of-visit effects and none was found. Then, to examine differences in palpated muscle soreness, soreness data for each site of the exercised leg and the non-exercised leg were analyzed using a 2 (condition) × 3 (time) ANOVA with repeated measures on both factors. To assess changes in muscle performance, the isometric, concentric, and eccentric peak torque data for the exercised leg and the non-exercised leg were analyzed using a 2 (condition) × 3 (time) ANOVA with repeated measures on both factors. To indirectly assess muscle damage, CK data were analyzed using a 2 (condition) × 4 (time) ANOVA with repeated measures on both factors. Substantial individual differences in cytokine production have been reported (Yaqoob et al. 1999); therefore, cytokine concentrations were normalized to PRE for each individual within each condition. Then, data were analyzed using a 2 (condition) × 4 (time) ANOVA with repeated measures on both factors. For all analyses, the Greenhouse–Geisser correction for the violation of sphericity was applied where appropriate. When ANOVA revealed significant main effects, eta squared (η^2) effect sizes are reported. Fisher's LSD was used for post hoc pairwise comparisons. The alpha level of significance was set to 0.05. All data are presented using mean ± SE.

Results

Soreness

For the exercised leg, a significant ($p < 0.05$) main effect of time was found for palpated muscle soreness at the midpoint on the vastus lateralis ($\eta^2 = 0.40$), 5 cm distal to the midpoint ($\eta^2 = 0.32$), and 5 cm proximal to the midpoint ($\eta^2 = 0.51$) (see Table 1). Soreness at 24 h-POST and 48 h-POST were not significantly different at any of the three sites. No main effect of condition (ALC vs PLA) or condition × time interaction effect was found for palpated muscle soreness on the exercised leg (see Table 1).

For the non-exercised leg, a significant main effect of condition was found for palpated muscle soreness 5 cm proximal to the midpoint of the vastus lateralis ($\eta^2 = 0.11$). Pairwise comparisons indicated that soreness was greater in ALC compared to PLA; however, the mean difference was quite small (see Table 1). No main effect of time or condition × time interaction effect was found for the non-exercised leg.

Table 1 Muscle soreness

	Exercised leg		Non-exercised leg	
	PLA	ALC	PLA	ALC
Midpoint				
PRE	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.3
24 h-POST	2.7 ± 0.7*	2.5 ± 0.6*	0.6 ± 0.3	0.7 ± 0.4
48 h-POST	3.0 ± 1.0*	2.3 ± 0.7*	0.5 ± 0.3	0.6 ± 0.3
Proximal				
PRE	0.7 ± 0.4	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1‡
24 h-POST	4.1 ± 0.8*	3.6 ± 0.5*	0.2 ± 0.1	0.8 ± 0.3‡
48 h-POST	3.6 ± 0.8*	3.4 ± 0.7*	0.3 ± 0.1	0.4 ± 0.1‡
Distal				
PRE	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.4
24 h-POST	2.6 ± 0.6*	2.0 ± 0.4*	0.2 ± 0.1	0.9 ± 0.5
48 h-POST	2.4 ± 0.7*	2.2 ± 0.7*	0.2 ± 0.1	0.6 ± 0.3

Vastus lateralis soreness for the exercised and non-exercised leg at the midpoint between the superior lateral patella and anterior superior iliac spine (midpoint), 5 cm proximal to the midpoint (proximal), and 5 cm distal to the midpoint (distal) in the placebo (PLA) and alcohol (ALC) conditions before exercise (PRE), 24 h after exercise (24 h-POST), and 48 h after exercise (48 h-POST). A compression gauge was applied to each site and participants indicated soreness/pain upon palpation on a continuous 10-cm line where 0 cm indicated "no pain" and 10 cm indicated "worst possible pain." For the exercised leg, * mean at time point significantly ($p < 0.05$) different from PRE. For the non-exercised leg, ‡ mean of ALC significantly different from PLA. Mean ± SE

Muscle performance

For the exercised leg, a significant ($p < 0.05$) main effect of time was found for peak torque production in the concentric ($\eta^2 = 0.25$), eccentric ($\eta^2 = 0.25$), and isometric ($\eta^2 = 0.18$) muscle actions. No condition × time interaction effect or main effect of condition was found. Overall, peak torque was greatly decreased for the exercised leg 24 h after the exercise bout for all muscle actions. Peak concentric torque had not recovered at 48 h after exercise, peak eccentric torque was partially recovered at 48 h after exercise, and peak isometric torque was fully recovered at 48 h after exercise (see Fig. 2).

For the non-exercised leg, no condition × time interaction effect, main effect of condition, or main effect of time was found. This indicates that peak strength for the non-exercised leg did not change as a result of exercise on the contralateral leg, alcohol, or a combination thereof.

Creatine kinase

A significant ($p < 0.05$) main effect of time was found for CK activity ($\eta^2 = 0.19$). No condition × time interaction effect or main effect for condition was found (see Table 2). This increase in CK activity indicates the presence of muscle damage as a result of the exercise bout.

Lipopolysaccharide (LPS)-stimulated cytokines

A significant ($p < 0.05$) main effect of time was found for LPS-stimulated concentrations of interleukin (IL)-10 ($\eta^2 = 0.51$), IL-8 ($\eta^2 = 0.63$), and tumor necrosis factor (TNF)-α ($\eta^2 = 0.18$) (see Fig. 3a–c). IL-6 and IL-1β remained unchanged over time (see Fig. 3d, e). No main effect of condition or condition × time interaction effect was found for any of the cytokines investigated.

Discussion

Despite the popularity of alcohol among individuals who engage in resistance training, this study appears to be the first to investigate the effect of alcohol ingestion on recovery across the days following muscle-damaging exercise in resistance-trained women. A major finding of this study was that despite the successful induction of muscle damage, as indicated by increased creatine kinase (CK) activity, increased palpated soreness, and reduced maximal torque in the exercised leg, consuming alcohol had no effect on muscle performance recovery in recreationally resistance-trained women. This lack of an effect of alcohol on performance recovery is similar to a recently published finding for women who were not specifically

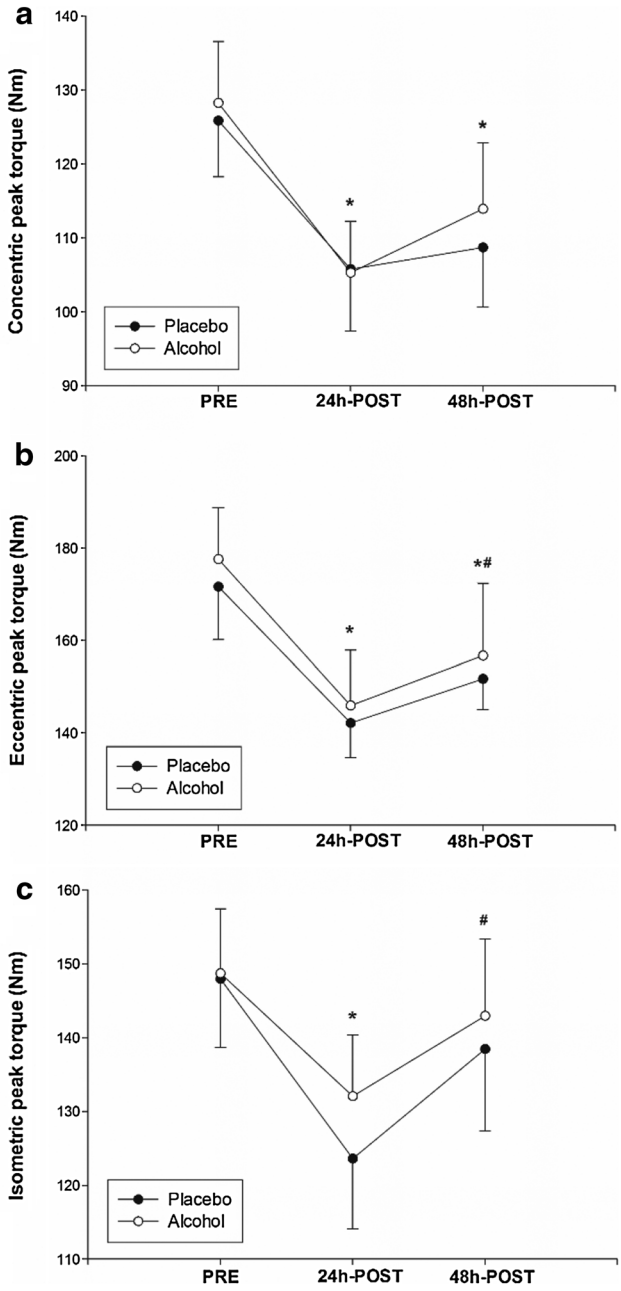

Fig. 2 Concentric (**a**), eccentric (**b**), and isometric (**c**) peak torque for the exercised leg before exercise (PRE), 24 h after exercise (24 h-POST), and 48 h after exercise (48 h-POST). *Asterisk* time point mean significantly ($p < 0.05$) different from PRE, *hash symbol* time point mean significantly different from 24 h-POST. Mean ± SE

Table 2 Creatine kinase activity

	PLA	ALC
PRE	96.5 ± 11.6	110.2 ± 22.6
5 h-POST	127.5 ± 15.7*	155.6 ± 28.0*
24 h-POST	166.8 ± 26.6*	182.5 ± 31.4*
48 h-POST	151.4 ± 24.1*	150.4 ± 21.7*

Serum creatine kinase activity (U L^{-1}) before exercise (PRE), 5 h after exercise (5 h-POST), 24 h after exercise (24 h-POST), and 48 h after exercise (48 h-POST)

* Time point mean significantly ($p < 0.05$) different from PRE. Mean ± SE

resistance trained (McLeay et al. 2016). In that study, no elevated CK activity was observed as a result of the exercise bout. However, our results were unexpected since previous studies in resistance-trained men found that consuming alcohol after the same muscle-damaging exercise protocol resulted in delayed performance recovery for that leg (Barnes et al. 2010a, b). Since no previous study has investigated the effect of consuming alcohol after muscle-damaging resistance exercise on performance recovery in resistance-trained women, this finding is novel and, combined with the previous findings for women who were not specifically resistance trained, demonstrates a likely gender difference with regard to exercise recovery in the context of alcohol ingestion. Another novel finding of this study was that despite changes in inflammatory capacity at 5, 24, and 48 h following the muscle-damaging exercise (increased production of pro-inflammatory tumor necrosis factor (TNF)-α, decreased production of anti-inflammatory interleukin (IL)-10 and chemotactic IL-8), alcohol ingestion did not substantially influence these responses. This is contrary to our previous finding that when consumed after a heavy squat bout, alcohol decreases inflammatory capacity 3 and 5 h after heavy squat exercise in men and women (Levitt et al. 2016). Thus, the effects of the muscle-damaging exercise bout in the current study appeared to overwhelm any potential effects of alcohol at the time points measured.

In contrast to prior findings for resistance-trained men (Barnes et al. 2010a, b), but similar to findings in non-resistance-trained women (McLeay et al. 2016), alcohol ingestion did not prolong the time for performance recovery after muscle-damaging exercise. Although the specific physiological mechanisms underlying acute effects of alcohol on muscle tissue in the context of exercise have not yet been fully elucidated, a possible gender-specific reduction in protein synthesis could help explain the difference in performance recovery between this and previous studies. Recently, we found that in men, but not in women, alcohol prevented an exercise-induced increase in the activation state of components of mTOR signaling pathway, a pathway important for regulation of muscle protein synthesis (Duplanty et al. 2017). Furthermore, evidence exists to suggest that the differences in findings of strength recovery between the current study in women and the previous studies in men could be related to a protective effect of estrogen on muscle tissue. Previous studies using animal models have indicated that estrogen can attenuate the rise

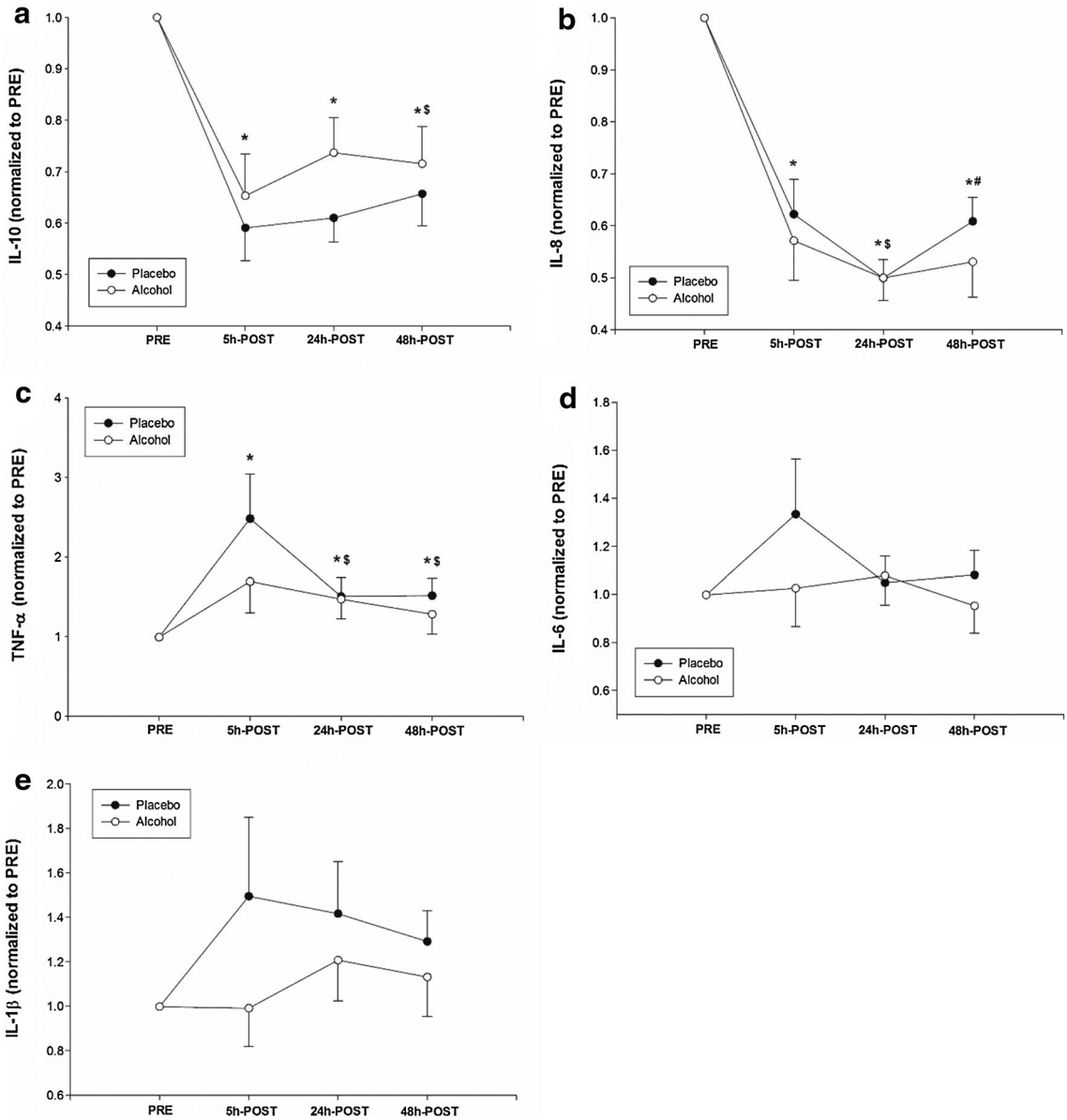

Fig. 3 LPS-stimulated concentrations of IL-10 (**a**), IL-8 (**b**), TNF-α (**c**), IL-6 (**d**) and IL-1β (**e**) normalized to the pre-exercise (PRE) concentrations at 5 h after exercise (5 h-POST), 24 h after exercise (24 h-POST), and 48 h after exercise (48 h-POST). *Asterisk* time point mean significantly ($p < 0.05$) different from PRE, *dollar symbol* time point mean significantly different from 5 h-POST, *hash symbol* time point mean significantly different from 24 h-POST. Mean ± SE

in markers of muscle damage following a damaging bout of exercise (Amelink and Bar 1986; Bar et al. 1988; Tiidus et al. 2001). However, similar research in humans comparing men and women has been less conclusive (Stupka et al. 2000). When comparing strength recovery in women after a damaging bout of exercise that takes place during either the follicular phase or the luteal phase of the menstrual cycle, it appears that women in the follicular phase recover strength more quickly than those in the luteal phase (Markofski and Braun 2014). However, circulating concentrations of estrogens are lowest during the early follicular phase of the menstrual cycle compared to pre-ovulatory (late follicular) and luteal phases (Baird and Fraser 1974). Therefore, the early follicular phase is the menstrual cycle phase where estrogen would be least involved in providing a protective effect to the muscle tissue. Regardless, it is still possible that estrogen and menstrual cycle phase played a role in attenuating the magnitude of muscle damage in this women-only study.

The lack of a greater reduction in strength for the non-exercising leg from alcohol in the current study is consistent with the hypothesis that acute alcohol consumption

does not negatively affect intact muscle tissue but instead interferes with the repair process in damaged muscle tissue (Barnes et al. 2010a). This hypothesis was recently supported by Haugvad and colleagues (2014) who found that consuming alcohol after a traditional resistance exercise bout (i.e., not a protocol designed to induce muscle damage) did not delay muscular performance recovery. Therefore, it appears that a minimum threshold for muscle damage must be reached before alcohol has an effect on performance recovery. Although we found evidence of muscle damage (i.e., prolonged decrease in force production, increased CK activity, and changes in lipopolysaccharide (LPS)-stimulated production of TNF-α, IL-8, and IL-10), the magnitude of muscle damage could potentially have been less than in the previous studies in men (and below the minimum threshold) due to potential difference in the training status of participants and/or the hormonal milieu (e.g., estrogen).

Separately, alcohol and resistance exercise have opposite effects on inflammatory capacity. Specifically, alcohol attenuates production of pro-inflammatory TNF-α (Goral and Kovacs 2005; Mandrekar et al. 2006; Szabo et al. 1996; Verma et al. 1993), IL-1β (Mandrekar et al. 2006; Szabo et al. 1996), IL-6 (Goral et al. 2004; Goral and Kovacs 2005), and IL-8 (Arbabi et al. 1999; Szabo et al. 1999) and augments production of anti-inflammatory IL-10 (Mandrekar et al. 2006; Szabo et al. 1996) in response to LPS stimulation; whereas, resistance exercise increases the production of TNF-α, IL-1β, and IL-6 (Levitt et al. 2016; McFarlin et al. 2004) and decreases the production of IL-10 (Levitt et al. 2016) in response to LPS stimulation. Furthermore, eccentrically biased exercise models designed to induce muscle damage have elicited similar changes in inflammatory capacity to that of resistance exercise. For example, Cannon and colleagues (1991) found that a bout of downhill running induced increases in production of IL-1β and TNF-α in response to LPS stimulation at 24 h after exercise. We have also observed that a strenuous bout of downhill running increases the production of IL-1β in response to LPS stimulation at 4, 24, 48, 72, and 96 h after exercise and decreases the production of IL-10 at 24, 48, and 72 h after exercise (unpublished observations). In the current study, the effect of eccentric exercise appeared to overpower any potential effect of alcohol on inflammatory capacity. However, since alcohol alone decreases inflammatory capacity, and we have found that the effects of exercise appear to overwhelm those of alcohol when administered at a dose of 1.09 g ethanol kg^{-1} fat-free body mass, further investigations should utilize different doses of alcohol and ingest alcohol later in the recovery period to determine if alcohol has an effect on inflammatory capacity following exercise-induced muscle damage under such conditions.

Although there were no statistically significant differences in LPS-stimulated IL-1β or IL-6 concentrations due to alcohol ingestion or the performance of exercise, the mean differences between conditions at 5 h-POST had moderate ($d = 0.54$) and small–moderate ($d = 0.47$) effect sizes, respectively, where the change from PRE was greater in the PLA condition for both cytokines (Fig. 3d, e). IL-1β and IL-6 each play important roles in the early phase of the inflammatory response, which is, in turn, essential for the complex process of muscle repair after damage (Arnold et al. 2007; Cannon and St Pierre 1998). Among other functions, IL-1β can activate the NF-κB transcription factor (Sizemore et al. 1999) and induce production of other pro-inflammatory cytokines, including IL-6. Among the many roles that IL-6 plays, this molecule is particularly important for proliferation of satellite cells which later differentiate into mature myocytes (Serrano et al. 2008). Therefore, although alcohol did not have a statistically significant effect on production of IL-1β or IL-6, the effect could still be meaningful and worthy of further exploration. Blunting the capacity of cells to produce IL-1β and IL-6 (e.g., by consuming alcohol after muscle-damaging exercise) could disrupt key events in the repair of muscle tissue.

In conclusion, it appears that for young recreationally resistance-trained women, consuming alcohol (1.09 g ethanol kg^{-1} fat-free body mass resulting in a BAC of 0.09 ± 0.02 g dL^{-1}) after strenuous eccentric resistance exercise does not affect performance recovery. Although alcohol consumption did not have a statistically significant effect on inflammatory capacity at 5, 24, and 48 h after exercise, consumption resulted in potentially meaningful differences in LPS-stimulated production of IL-1β and IL-6 at 5 h after resistance exercise; if real, such a difference could negatively influence the capacity for muscle recovery. Overall, the effect of the muscle-damaging exercise bout appeared to overpower any potential effect of alcohol on whole blood inflammatory capacity in women. Collectively, the present study and previous reports in the literature indicate a differential performance recovery response to alcohol consumption following strenuous eccentric resistance exercise in recreationally resistance-trained men and women. This difference warrants future investigations into potential mechanisms involved with gender dimorphic responses to post-exercise alcohol consumption.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of, and approved by, the Institutional Review Board of the University of North Texas and in accordance with the 1964 Helsinki declaration and its later amendments. This article does not contain any studies with animals performed by the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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