THESIS

CAFFEINE AUGMENTS THE LACTATE AND INTERLEUKIN-6 RESPONSE TO MODERATE-INTENSITY EXERCISE IN MALES BUT NOT FEMALES

Submitted by

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ABSTRACT

CAFFEINE AUGMENTS THE LACTATE AND INTERLEUKIN-6 RESPONSE TO MODERATE-INTENSITY EXERCISE IN MALES BUT NOT FEMALES

The release of interleukin (IL)-6 from contracting skeletal muscle is thought to contribute to some of the health benefits bestowed by exercise. This IL-6 response appears proportional to exercise volume. Unfortunately, high volumes of exercise are not feasible for all people. Caffeine augments the magnitude of increase in circulating concentration of IL-6 in response to high-intensity and long-duration exercise, in males. Caffeine is also known to increase circulating concentrations of lactate during exercise. One of the mechanisms thought to contribute to IL-6 release from exercising skeletal muscle is lactate production. We hypothesized that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of lactate and IL-6 in a study population comprising both males and females. 15 healthy adults (9 males and 6 females, aged 26±7 years, (mean ± SD)) completed 30-minutes of moderate-intensity cycle ergometer exercise, equivalent to the ventilatory threshold, after ingesting either caffeine (6 mg/kg) or placebo. Arterialized-venous blood was collected throughout each of the exercise sessions. Compared with placebo, caffeine increased end-exercise circulating concentrations of lactate (5.72±3.95 *vs*. 7.14±4.66 mmol/L, $P<0.001$) but not end-exercise IL-6 (1.84±0.97 *vs*. 2.37±1.04 pg/mL, $P=0.139$). However, when females were excluded from the analysis, caffeine augmented ($P=0.04$) the magnitude of increase of end-exercise IL-6 concentration (1.80±0.86 *vs*. 2.57±1.21 pg/mL); this effect was further exaggerated after 30-minutes of inactive recovery (3.81±2.32 *vs*. 5.06±3.22 pg/mL). Noteworthy, caffeine

evoked greater end-exercise lactate concentrations in data sets containing only males (P=0.02) and only females (P=0.002) but did not influence the IL-6 response in females (P=0.94). Our preliminary data imply that in males unable/unwilling to perform high-intensity and/or long-duration exercise, caffeine may potentially enhance the IL-6 mediated health benefits of relatively short, moderate-intensity exercise.

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1. REVIEW OF LITERATURE

Problem

Exercise represents an effective nonpharmacologic lifestyle intervention to improve health. In diseased populations, evidence suggests exercise can have an additive effect to medical treatment [1]. Both the American College of Sports Medicine (ACSM) and the Centers for Disease Control (CDC) recommend that adults perform at least 30-minutes of moderate-intensity aerobic exercise five days a week or 20-minutes of vigorous-intensity aerobic activity three days a week [2]. The implementation of regular exercise has the potential to evoke many health benefits for disease-free and clinical populations, with exercise at higher intensities inducing greater benefits than exercise at lower intensities. However, for various reasons, many adults are unable or unwilling to meet the aforementioned guidelines [3], implying a need to maximize the health benefits from single exercise sessions. One potential target for accomplishing this need lies in the manipulation of the interleukin (IL)-6 response to exercise, as IL-6 has the potential to mediate many of the health benefits observed in response to exercise.

Interleukin-6

IL-6 Binding and Signaling

IL-6 is a pleiotropic cytokine; it affects the activity of multiple cell types. Basal circulating concentrations of IL-6 range between 1-5 pg/mL but under inflammatory states, such as sepsis, these concentrations can increase 1,000-fold [4]. Of recent relevance, IL-6 has been implicated as a marker of the 'cytokine storm' due to the increased circulating concentrations of IL-6 seen in severe cases of coronavirus disease 2019 (Covid-19) [5]. The high concentrations of IL-6 in diseases presenting with inflammation have led elevated concentrations of circulating IL-6 to be commonly used as a biomarker of increased inflammation.

IL-6 binds to a membrane-bound IL-6 receptor (IL-6R), and the complex of IL-6 and IL-6R associates with the ubiquitously expressed transmembrane protein gp130 [6,7]. The binding can initiate several intracellular signaling pathways. The main pathways stimulated by IL-6 binding are the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and rat sarcoma proto oncogene (ras)/mitogen-activated protein kinase and phosphoinositide-3 kinase pathways [6]. These pathways are the major modulators of the intracellular effects of IL-6.

Intracellular signaling cannot be initiated by IL-6 independently of its receptor. IL-6 only exhibits a measurable affinity to IL-6R, and only the complex of IL-6 and IL-6R bind to gp130 and initiate intracellular signaling [6]. However, IL-6 can operate via two signaling mechanisms giving rise to some of its varying, and sometimes opposing, physiological actions. These distinct signaling mechanisms are classical signaling and trans-signaling.

Classical signaling entails IL-6 binding to a membrane-bound IL-6R (Figure 1). The complex of IL-6 and IL-6R then associates with gp130 and initiates the intracellular signaling

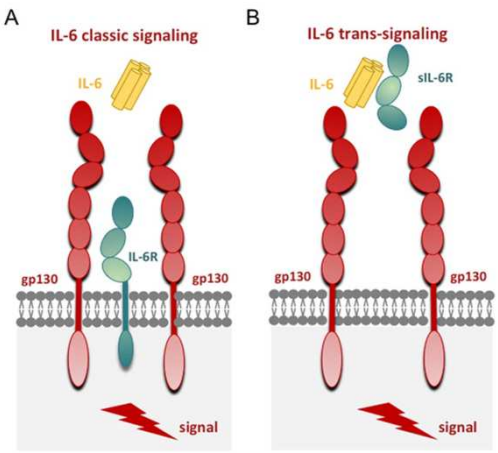

Figure 1: From Rose-John, 2017: Illustration of IL-6 binding in classical signaling (A) and trans-signaling (B) [9].

cascade [8]. All cells express gp130, but only a few cells, such as hepatocytes and some leukocytes express membrane-bound IL-6R [6,8]. Most IL-6R expressing cells express far more gp130 than IL-6R molecules meaning circulating IL-6 alone will only engage a few gp130 molecules [6]. Accordingly, the actions of IL-6 via classical signaling are restricted to certain cell types and are limited by the number of receptors on those cells.

In trans-signaling, IL-6 binds to a circulating soluble IL-6 receptor (sIL-6R) to form the IL-6-sIL-6 complex (Figure 1). The IL-6-sIL-6 complex can bind to gp130 and initiate the intracellular signaling cascade. This can occur independently of whether a cell expresses IL-6R greatly increasing the range of IL-6 target tissues and activities [8,9].

The distinction of signaling type is important because it can account for the varying actions of IL-6. Activation of gp130 via trans-signaling mediates the pro-inflammatory effects of IL-6 while activation of gp130 via classical signaling mediates the anti-inflammatory and protective effects of IL-6. The diverse actions of IL-6 have led to it being ascribed a controversial role in health and disease.

IL-6 in Health and Disease

Metabolism

High plasma concentrations of IL-6 are implicated in disease states presenting low-grade chronic inflammation. Individuals with obesity have higher plasma IL-6 concentrations than their healthy counterparts [10,11]. Additionally, plasma IL-6 concentrations are also positively correlated with body mass index (BMI) [10], waist to hip ratio [10,11], and visceral adiposity [12] and inversely correlated with insulin sensitivity [10,13]. High plasma concentrations of IL-6 have also been shown in those with cancer and/or cancer cachexia [14]. These observations have led to speculation that IL-6 is implicated in metabolic dysregulation and the development of disease. However, the results of IL-6 administration suggest that these data are purely correlational.

The acute effects of IL-6 indicate it does not directly cause impaired glucose or lipid metabolism. Infusion of recombinant human IL-6 (rhIL-6) does not impair glucose uptake in healthy individuals [15] and it reduces circulating insulin concentration and increases lipolysis in humans with type 2 diabetes, independent of changes in catecholamines [16]. These data suggest that it is not IL-6 per se, causing metabolic dysregulation.

Further, it appears that IL-6 may be having a beneficial effect on metabolism. Evidence for this has been accumulated in animals, *in vitro*, and *in vivo*. Mice lacking the gene encoding for IL-6 develop mature-onset obesity which can be partially reversed by IL-6 injection [17]. Incubation with 120 ng/mL of IL-6 increased glucose transport, glucose oxidation, and glucose incorporation into glycogen in human skeletal muscle [18]. When humans with type 2 diabetes and/or obesity were given tocilizumab (an IL-6 receptor antagonist) it led to a 26% reduction in active glucagon-like peptide-1 (GLP-1) in response to meal ingestion and exercise, indicating regulation of GLP-1 is dependent on IL-6 [19]. GLP-1 has a variety of actions including inhibiting gastric emptying and improving pancreatic β-cell function, which is why GLP-1 based treatment is associated with weight loss and reduced hypoglycemia. Additionally, rhIL-6 infusion delayed gastric emptying, reduced postprandial glucose and insulin concentrations, and the insulin:glucagon ratio in healthy males, and in males with type 2 diabetes delayed gastric emptying and reduced insulin secretion [20].

These data provide some clarity as to the role of IL-6 in health and disease. Observational data indicate that individuals with diseases presenting with chronic inflammation have higher concentrations of IL-6 compared to their healthy counterparts. However, IL-6 plays an important role in metabolism and acute increases in IL-6 are beneficial to improving metabolic function. Thus, IL-6 may be an indicator but not the cause of disease.

Pro and Anti-Inflammatory Effects

Individuals suffering from diseases presenting with low-grade chronic inflammation have higher plasma concentrations of IL-6 than their healthy counterparts [10,11]. Historically, that has been interpreted to imply that IL-6 causes inflammation. However, based on the observation that acute increases in IL-6 have anti-inflammatory actions, the current thinking is that in cases of chronic inflammation, high circulating concentrations of IL-6 may be a symptom rather than a cause.

In healthy male subjects, both cycle ergometer exercise (3 hours at a workload corresponding to 75% maximal oxygen uptake (VO_{2max})) and sedentary infusion of rhIL-6 to simulate exercise-induced increases in IL-6 attenuated the production of the inflammatory cytokine, tumor necrosis factor (TNF)-α, following ingestion of a bolus of an endotoxin designed to elicit low-grade inflammation [21]. These data indicate a negative feedback loop between TNF-α and IL-6 whereby TNF-α stimulates IL-6 production and IL-6 inhibits TNF-α production.

Further evidence for IL-6 as an anti-inflammatory cytokine is illustrated via its induction of other anti-inflammatory cytokines. Infusion of rhIL-6 increases circulating concentrations of the anti-inflammatory cytokines IL-1ra, and IL-10 in healthy males [22]. Notably, TNF-α concentrations were not affected by rhIL-6 infusion suggesting that IL-6 is able to induce the release of anti-inflammatory cytokines independently of stimulation by TNF-α production. The lack of effect on circulating TNF-α seems paradoxical, given that IL-6 attenuates the production of TNF-α. One explanation is that IL-6 inhibits dynamic TNF-α production and/or release, meaning that IL-6 can suppress the rise of TNF-α in pro-inflammatory states but does not greatly affect basal plasma concentrations. The authors also postulate that multi-day treatment with IL-6

could decrease basal plasma concentrations of TNF-α, especially in those with pro-inflammatory conditions such as obesity or type 2 diabetes [22] suggesting an additive effect of IL-6 (i.e., a single increase in IL-6 does not decrease basal levels of TNF-α but multiple increases could).

While conditions of chronic inflammation present with increased concentrations of IL-6, the acute effects of IL-6 appear to be anti-inflammatory. Acute increases in IL-6 appear to be beneficial as they suppress pro-inflammatory cytokines and stimulate other anti-inflammatory cytokines. Collectively, these observations imply that IL-6 should be classified as anti-

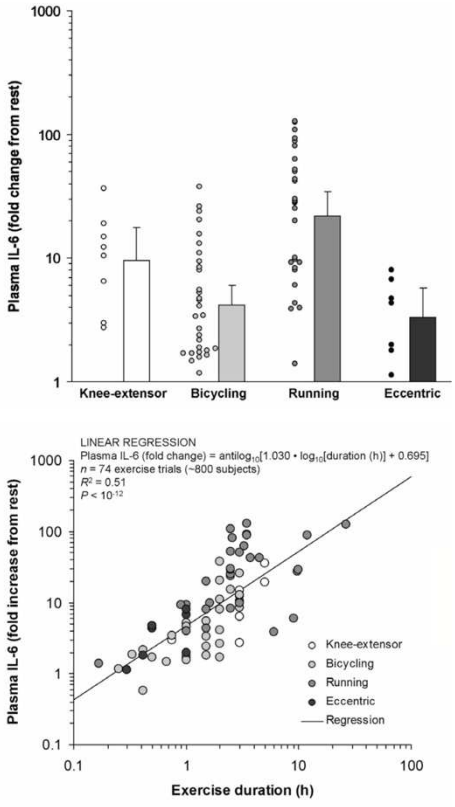

Figure 2: From Fischer, 2006: Plasma IL-6 change in response to various types of exercise (Top). Plasma IL-6 increase correlation with duration (Bottom) [23].

inflammatory and the high concentrations of IL-6 present in inflammatory conditions likely indicate rather than cause inflammation.

IL-6 and Exercise

Following exercise, there is an acute increase in plasma IL-6 concentrations peaking at the end of exercise or early during recovery. Increases of up to 100-fold have been observed, although smaller increases are more common [23]. The increase is accounted for by the production/release of IL-6 from exercising skeletal muscle [24]. The amount of muscle mass recruited is an important component when discussing exercise-induced increases in plasma IL-6 concentrations, with higher amounts of muscle mass eliciting larger increases in IL-6 release (Figure 2) [23].

In addition to exercise modality (which can determine the volume of active muscle), exercise duration and intensity are important determinants of the magnitude of exercise-induced IL-6 increase, with high-intensity and long-duration evoking the greatest increases. Of these, duration is the most important factor in determining the post-exercise IL-6 amplitude, with more than 50% of the post-exercise variation in IL-6 being explained by the exercise duration (Figure 2) [23]. Exercise-induced increases in IL-6 were originally thought to be related to muscle damage, but eccentric exercise is not associated with more marked increases in IL-6 compared to non-eccentric exercise (Figure 2) [23]. These observations suggest that the IL-6 response to exercise is not merely a response to muscle damage but induced by multiple factors brought on by long-duration exercise.

Role of IL-6 in Adaptations to Exercise

Regular exercise results in many beneficial health adaptations that can contribute to positive health outcomes for both disease-free and clinical populations. IL-6 contributes to many beneficial health outcomes derived from exercise, including decreasing inflammation [21,22],

decreasing visceral adiposity [25], promoting muscle hypertrophy and attenuation of sarcopenia [26], and improving glucose tolerance and insulin sensitivity [27,28].

Some of the most striking evidence for the importance of IL-6 in exercise-induced improvements in health comes from its role in decreasing visceral adiposity. Adults with abdominal obesity were placed on a 12-week exercise training program and given either placebo or the IL-6 receptor antagonist tocilizumab. Individuals given a placebo showed an 8% reduction in visceral adipose mass, but in the group taking tocilizumab, visceral adipose mass was increased by ~10% [25]. Additionally, total cholesterol and low-density lipoprotein (LDL) cholesterol increased in the group given tocilizumab and did not change in the placebo group, indicating that increases in total and LDL cholesterol from IL-6 receptor antagonism cannot be rescued by exercise [25]. The fact that blocking the actions of IL-6 abrogates the favorable effects of exercise training pertaining to visceral adipose and LDL-cholesterol demonstrates the important contribution of IL-6 to some of the important health benefits derived from exercise. It also indicates the critical role of skeletal muscle IL-6 in exercise-induced adaptations.

Many individuals, including those suffering from disease, can benefit from the transient post-exercise increase in IL-6. Unfortunately, the types of exercise (long-duration and/or high-intensity) that elicit the greatest increase in IL-6 are often unachievable and/or untenable for those who would benefit most. A way to increase the IL-6 response to more moderate-intensity and/or short-duration exercise could greatly benefit those unable to perform the aforementioned difficult exercise. In this regard, caffeine may be worthy of consideration.

Caffeine

Caffeine Information/Background/Metabolism

Caffeine is the world's most consumed psychoactive substance [29]. The National Health and Nutrition Examination Survey (NHANES) found that between 2001-2010, 89% of American

adults consumed caffeine on any given day and the mean intake for adult consumers was 211 mg/day [30]. Caffeine is often consumed in beverages such as coffee, tea, or sodas. The amount of caffeine ingested in a single serving varies dramatically depending on the mode of administration (e.g., beverage vs. capsule) and the preparation.

Caffeine metabolism primarily occurs in the liver, with 95% of caffeine being metabolized by the cytochrome P450 1A2 (CYP1A2) enzyme [31,32]. After oral administration, caffeine appears in blood within minutes, and peak plasma concentrations occur at times (T_{max}) between 30 to 120 minutes [33–35]. The average half-life of caffeine is generally reported to be between 4 and 6 hours in humans, but factors such as pregnancy, diet, and/or variation in the CYP1A2 gene can cause the half-life of caffeine to differ between individuals and fall outside this range [29,33,36,37].

While generally considered safe, high amounts of caffeine consumption have resulted in adverse health effects. The acute toxic level for caffeine ingestion is not well established, but for adults is approximately 10 g/day (~ 100 cups of coffee) [32]. Caffeine intoxication (also referred to as "caffeinism") is a recognized clinical syndrome and results from repeated excessive caffeine consumption. The symptoms of caffeine intoxication include anxiety, agitation, restlessness, insomnia, gastrointestinal disturbances, tremors, tachycardia, psychomotor agitation, and in extreme cases, death [32]. In lethal cases of caffeine intoxication, the concentration of caffeine in the blood is often found to be over 60 mg/L [32]. For context, the World Anti-Doping Agency (WADA) encourages athletes to maintain a urine caffeine concentration below 12 μg/mL, which corresponds to a dosage of 10 mg/kg body mass orally over several hours [29]. Thus, while it is not impossible for someone to overdose on caffeine, these situations are rare, and caffeine consumption is generally thought to be safe.

Caffeine as an ergogenic aid

Caffeine is not classified as a "controlled" substance by either the International Olympic Committee (IOC) or WADA [29]. The ergogenic properties of caffeine have long been of interest in exercise science, and caffeine remains a popular ergogenic aid for competitive athletes. Caffeine ingestion improves endurance exercise performance [38–40], but its effect on short-duration high-intensity exercise is less clear [29,41]. A typical ergogenic dose of caffeine is between 3 and 6 mg/kg body weight, as the ergogenic effect decreases outside of this range [40,42].

Caffeine's mechanisms of action are threefold: intracellular calcium mobilization via direct interaction with calcium channels in the sarcoplasmic reticulum, inhibition of phosphodiesterase, and adenosine receptor antagonism [43]. Interaction with calcium channels and inhibition of phosphodiesterase require concentrations of caffeine that would be toxic *in vivo,* so the most likely ergogenic mechanism is through adenosine receptor antagonism, the main targets of which being the A_1 and A_{2A} receptors [29,43,44]. The antagonism of these receptors increases neurotransmitter release, motor unit firing rates, and pain suppression; the downstream effects of which are thought to lead to improved exercise performance [29].

While caffeine targets the central nervous system (CNS), the downstream effects can be seen in the periphery. Caffeine supplementation increases ventilation, circulating epinephrine, and both blood glucose and blood lactate concentrations [41,42,45–50]. Caffeine ingestion does not affect oxygen uptake (VO_2), and there are mixed results regarding whether or not caffeine ingestion influences heart rate, respiratory exchange ratio (RER), lipolysis, and fat oxidation [29,41,42,48]. Supplementation with caffeine exerts a suppressive effect on the rating of perceived exertion (RPE) [42] which could factor into the improved endurance exercise performance seen after caffeine administration.

Caffeine and IL-6

Caffeine augments the circulating concentration of IL-6 in response to long-duration and/or high-intensity exercise in males [51–56]. To our knowledge, only one study has examined the effect of caffeine on the IL-6 response to maximal exercise in both males and females. The subjects were considered active (had been training (aerobic and resistance) for longer than 12 months) and only details regarding the age range (18-30 years), body mass index (BMI; 20 – 30 kg/m^2), and male to female ratio (8 males and 16 females) were reported. Participants ingested 6 mg/kg of caffeine 30-minutes prior to a maximal treadmill test and blood samples were collected at baseline, pre-exercise, and five-minutes post-exercise. Caffeine supplementation of 6 mg/kg increased circulating concentrations of IL-6 in response to a maximal treadmill test [57]. These results indicate that caffeine can increase the IL-6 response to maximal exercise in males and females. However, the participants' training status might not be representative of the general population, and certainly not representative of a clinical population. Additionally, maximal exercise may not be feasible for many individuals, including those who would benefit most from increases in IL-6. There exists a need for more testing examining the effect of caffeine on the IL-6 response to submaximal exercise in cohorts comprising males and females.

Lactate

Lactate Not Just Waste

Lactate is produced when the enzyme lactate dehydrogenase reduces pyruvate. This reaction oxidizes NADH to NAD^+, which is beneficial for NAD^+ regeneration and helps maintain the NAD^+:NADH ratio within cells. Originally thought to be a waste product of anaerobic metabolism, it is now well known that lactate is always produced irrespective of the presence/absence of oxygen and plays an integral role in metabolism.

Lactate can be shuttled both through a variety of monocarboxylate transporters (MCT), both within cells and in circulation, where it can be taken up by other cells [58,59]. Thus, lactate

can exert autocrine, paracrine, and endocrine effects and fulfill at least three major purposes; 1) as a major energy source, 2) as the major gluconeogenic precursor, 3) as a signaling molecule [58,59]. One example of lactate as an energy source is the Cori cycle. Lactate is shuttled out of skeletal muscle into circulation, taken up by the liver, where it undergoes gluconeogenesis, and the newly formed glucose is transported out of the liver into circulation. In addition to undergoing gluconeogenesis, lactate can be taken up by multiple tissues, including the brain, heart, and skeletal muscle, and used as an energy source.

Lactate and Exercise

Vigorous exercise elicits elevated circulating lactate concentrations. This is due to a disproportionate increase in the lactate rate of appearance (R_a) compared to the rate of disappearance (R_d) from plasma. Increased plasma lactate can result from increased lactate production, inadequate lactate clearance, or both [60]. In response to incremental exercise, it is common to see a point of inflection where plasma lactate concentrations increase at an elevated rate. That point has been described as the 'lactate' or 'anaerobic' threshold or the onset of blood lactate accumulation (OBLA) and is often used as a determinant of exercise ability and in the prescription of exercise intensities to healthy, clinical, and athletic populations.

Lactate and IL-6

Lactate production is one of the mechanisms thought to induce IL-6 release from skeletal muscle. Evidence for this has been accumulated in several species (Figure 3). In mice, intramuscular injection of lactate increased IL-6 mRNA expression and serum IL-6 concentration, and inhibition of lactate-dependent protease activity lowered IL-6 secretion following swimming exercise [61]. Electrical pulse stimulation of human muscle cells resulted in a correlated increase in lactate and IL-6 concentrations in media [61]. *In vivo* end-exercise lactate concentrations positively correlate with post-exercise IL-6 concentrations [61–63]. Of note, most

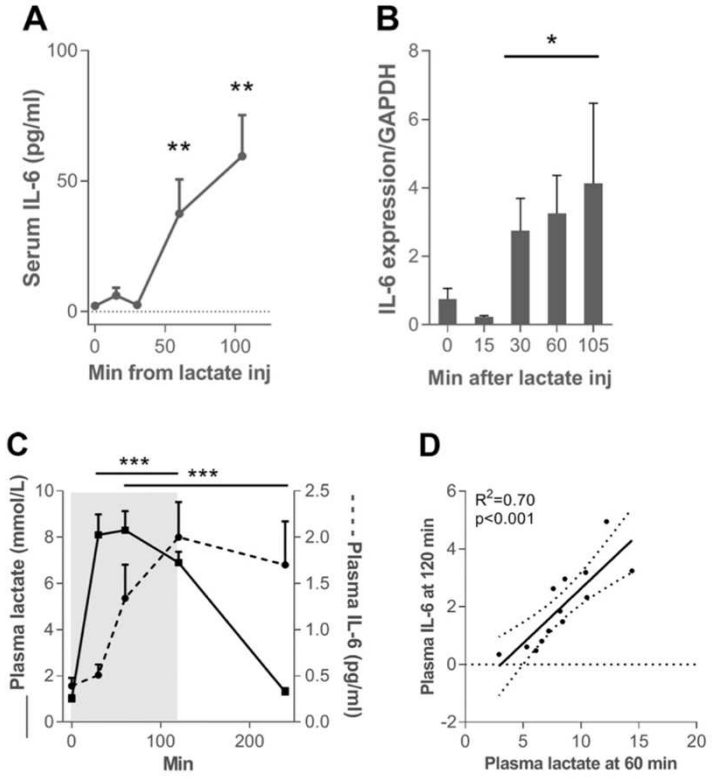

Figure 3: From Hojman et al. 2019. – A and B: Serum IL-6 concentration (A) and IL-6 mRNA expression in muscle (B) after lactate injection into the tibialis anterior of female mice. C and D: Plasma lactate and IL-6 concentrations during and following 120-minutes of high-intensity interval-based cycling intervention in thirteen healthy males (C) and correlations between plasma IL-6 concentrations at 12- minutes and plasma lactate concentration at 60-minutes (D) [61].

human participants were male (total participants in three studies n= 36, male: female 31:5). Taken together, these data indicate that increased lactate production potentially stimulates IL-6 release from skeletal muscle.

Sex Differences

Females Are Not "Males but Different"

We know that biological sex differences contribute to legitimate physiological dissimilarities between males and females. Biologically the male is not the "default human." One specific example relates to iron status. Data from NHANES 1988-1994 indicate that 11% of United States females between the ages of 16-49 experience iron deficiency (diminished iron stores), and 5% experience iron deficiency anemia (diminished iron stores and anemia) compared to less than 1% of males for both symptoms, [64] and those discrepancies likely hold true today. Iron deficiency can impact several physiological outcomes, including muscle strength [65], fatigue [66,67], and processes such as immune function [68,69]. If this one factor can affect so many physiological processes, it is reasonable to speculate that iron status and/or other sex differences could exist regarding IL-6 release from skeletal muscle.

Caffeine increases circulating concentrations of IL-6 in response to high-intensity exercise in males [51–56]. Of note, there is a lack of information surrounding the effects of caffeine on IL-6 in females. We found one study that examined the effects of caffeine on the IL-6 response to strenuous exercise in a cohort comprising trained males and females [57]. Importantly, in this study, individuals were described as "active" (> 12 months aerobic and resistance training), but few subject characteristics were reported, and the exercise stimulus was a maximal treadmill test. Due to the lack of information surrounding the effects of caffeine on the IL-6 response to sub-maximal exercise in both males and females, further testing is needed.

The paucity of studies describing the effects of caffeine on the female IL-6 response to exercise represents a significant gap in current knowledge. Given that caffeine elicits similar ergogenic effects in females and males [70,71], it might be reasonable to speculate that it similarly impacts the IL-6 response to exercise. However, in a healthy population, males have been observed to have higher circulating concentrations of IL-6 at rest than females [12]. While these observations could be due to multiple factors, including lean mass, fat mass, and visceral fat, these data suggest a potential for sex differences regarding the IL-6 response to caffeine and/or exercise. Since IL-6 is integral to many of the beneficial health effects derived from exercise, it is essential to understand if caffeine can exert similar effects on IL-6 release in both males and females. Thus, there is a need to examine the impact of caffeine on the IL-6 response to exercise at varying intensities in cohorts comprising males and females

Conclusion

Exercise elicits many positive health outcomes, some of which may be mediated through the release of IL-6 from skeletal muscle. The amount of IL-6 released from skeletal muscle is proportionate to exercise volume. Unfortunately, high-intensity and/or long-duration exercise may be unfeasible for many individuals, especially those suffering from a disease who would greatly benefit from these health improvements. Caffeine augments the magnitude of increase of circulating concentrations of IL-6 in response to exercise. Most of these studies have been conducted in males only. Caffeine also increases circulating concentrations of lactate during exercise. Lactate production is one of the mechanisms thought to contribute to IL-6 release from skeletal muscle. Accordingly, the purpose of this study was to establish proof of concept that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of both lactate and IL-6 in a study population comprising males and females. We hypothesized that caffeine, ingested prior to moderate-intensity exercise, would lead to greater

circulating concentrations of lactate and IL-6 in a study population comprising both males and females.

2. INTRODUCTION

Exercise is an effective nonpharmacologic intervention to improve health and in diseased populations can have an additive benefit to medical treatment [1]. Higher intensities and/or longer durations of exercise evoke the most health benefits. Unfortunately, for various reasons many individuals are unable or unwilling to exercise at these intensities implying the need to maximize the health benefits from single exercise sessions. One target lies in manipulating the IL-6 response to exercise.

IL-6 is a pleiotropic cytokine generally found at basal circulating concentrations of between 1-5 pg/mL [4]. In response to exercise, circulating IL-6 concentrations increase as the result of IL-6 release from skeletal muscle [23,24]. These increases appear important, as IL-6 contributes to many beneficial health outcomes derived from exercise, including decreasing inflammation [21,22], decreasing visceral adiposity [25], promoting muscle hypertrophy and attenuation of sarcopenia [26], and improving glucose tolerance and insulin sensitivity [27,28]. The IL-6 response to exercise appears proportional to exercise volume [23]. However, high volumes of exercise are not feasible for all people, especially clinical populations who could benefit most from the health benefits bestowed by IL-6. Thus, there exists a need to manipulate the IL-6 response to exercise at more moderate intensities. In this regard, caffeine may merit examination.

The active ingredient in coffee, caffeine is a popular ergogenic aid for competitive athletes, and its ingestion prior to exercise is generally considered safe. Caffeine augments the increase in circulating concentrations of IL-6 in response to high-intensity and/or long-duration exercise in males [51–56]. One study found that caffeine augments the increase in circulating concentrations of IL-6 in trained males and females in response to a maximal exercise test [57].

Given that sex differences result in dissimilarities in physiology between males and females, there exists a need to examine the effects of caffeine on the IL-6 response to exercise at moderate intensities in both males and females.

Caffeine also increases the lactate response to both maximal and submaximal exercise in males and females [41,42,45–49]. Lactate is thought to be one of the mechanisms that contribute to IL-6 release from skeletal muscle. In rodents, intramuscular injection of lactate increased IL-6 mRNA expression and serum IL-6 concentration, while inhibition of lactate-dependent protease activity lowered IL-6 secretion following swimming exercise [61]. Electrical pulse stimulation of human muscle cells resulted in a correlated increase in lactate and IL-6 concentrations in media [61]. *In vivo* in humans circulating end-exercise lactate and post-exercise IL-6 concentrations are positively correlated [61–63]. These data suggest that increased lactate concentrations can stimulate IL-6 release from skeletal muscle. Accordingly, the purpose of this study was to establish proof of concept that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of both lactate and IL-6 in a study population comprising males and females. We hypothesized that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of lactate and IL-6 in a study population comprising both males and females.

3. METHODS

This study was conceived prior to the Covid-19 pandemic and was initiated several months after institutional implementation of Covid-19 related regulatory guidelines. To avoid the burden of repeated laboratory-based exercise in a potentially vulnerable clinical population, males and females considered healthy, but otherwise untrained or recreationally active, were invited to participate in this proof-of-concept study. The project was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Colorado State University (Protocol #20-10002H). Written informed consent was provided by all participants. Inclusion criteria consisted of age between 18 and 75 years old, and willingness to complete several 30-minute bouts of moderate-intensity cycle ergometer exercise. Exclusion criteria included previous diagnosis of cancer, tobacco use, any recurring injury limiting exercise, pregnancy or breast-feeding, a contra-indication to exercise identified during a graded exercise test incorporating 12-lead electrocardiogram assessment, contra-indication to caffeine including unusual heart rhythm, unmedicated high blood pressure, and/or use of medications known to interact with caffeine (such as Quinolones, Theophylline, Duloxetine, Ephedra or Guarana, Rasagiline or Tizanidine).

Protocol Overview

This was a double-blind, randomized, repeated measures, placebo-controlled, crossover study. Following screening and assessment of baseline physiological characteristics, participants reported to the laboratory on two separate occasions to perform 30-minutes of moderate-intensity stationary cycle ergometer exercise. One hour prior to exercise, participants ingested caffeine or placebo. Blood was sampled for the determination of lactate and IL-6 concentration. Indirect calorimetry was used to quantify gas exchange and metabolic rate during exercise.

Screening and Baseline Physiological Characteristics

Prior to study enrollment, potential participants completed a detailed electronic medical history questionnaire. Responses requiring additional queries were addressed either in-person, via telephone, or video conference. Body size and composition were assessed using dual-energy X-ray absorptiometry (DEXA; Hologic, DiscoveryW, QDR Series, Bedford, MA, USA), and a physician's digital scale and stadiometer, as previously described [72,73]. A supervised exercise stress test consisting of a 12-lead electrocardiogram and indirect calorimetry (Parvo Medics, Sandy, Utah, USA) to determine peak oxygen uptake (VO_{2peak}).

Procedure

Following initial screening, subjects returned to the lab on two separate occasions separated by at least 72 hours. Participants arrived at the laboratory approximately one hour after consuming a standardized breakfast (Ensure Original Nutrition Shake, Abbott Laboratories, Lake Bluff, Illinois, USA; 220 kcal, 16% protein, 24% fat, 60% carbohydrate) and having abstained from caffeine for the previous 48 hours to allow for sufficient washout from previous caffeine consumption, consistent with other studies [51,52]. On arrival, a venous catheter was placed into a dorsal hand vein and the hand and forearm were wrapped in a heated blanket in order to sample arterialized venous blood.

Following baseline measurements of heart rate and blood pressure participants ingested 6 mg/kg of caffeine or placebo in the form of pre-prepared capsules. Approximately 25mL of arterialized venous blood was sampled prior to caffeine/placebo ingestion (minute 0) and then 60, 95, and 125 minutes after. Blood intended for IL-6 analysis was immediately transferred into chilled tubes coated with ethylenediaminetetraacetic acid (EDTA) and blood intended for lactate analysis was transferred into chilled tubes coated with sodium fluoride and potassium oxalate.

Samples were placed on ice for up to 30 minutes before isolation of plasma via chilled (4 °C) centrifugation for 10 minutes. 1 mL aliquots of plasma were stored at -80 °C until analysis.

Exercise began 60 minutes after caffeine/placebo ingestion to allow for full absorption of caffeine [44,51–54] and consisted of a five-minute warmup of near load-less cycling followed by 30-minutes of cycling at a work rate corresponding to ventilatory threshold (T_{VE}) on an electronically braked cycle ergometer (Corvial Cpet, Lode BV, Groningen, Netherlands). Expired gasses were collected during minutes 5-10 and 25-30 (Parvo Medics, Sandy, Utah, USA).

Determination of Ventilatory Threshold

T_{VE} was determined by five independent investigators using expired gas data collected during the VO_{2peak} test. Criteria for T_{VE} were the oxygen uptake (VO_2) corresponding to a systematic increase in V_E/VO_2 and in end-tidal partial pressure of O_2 ($P_{ET}O_2$) with no concomitant rise in V_E/VCO_2 or a decrease in end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) [60,74,75]. The work rate corresponding to this VO_2 was considered the T_{VE} and was selected as the work rate at which the participants would exercise.

Determination of lactate and IL-6

Plasma was thawed and analyzed for lactate and IL-6. Lactate analysis was completed in duplicate via an automated analyzer (YSI 2900, Xylem Inc., Rye Brook, New York, USA). IL-6 analysis was undertaken using an enzyme-linked immunosorbent assay (ELISA; HS600C, R&D Systems Inc., Minneapolis, Minnesota, USA) according to the manufacturer's instructions; samples were analyzed in duplicate.

Statistical Analysis

Statistical analysis was completed using commercially available software (SigmaStat 3.0, Systat Software Inc., San Jose, CA, USA). Differences in plasma lactate and IL-6 concentrations

were calculated using two-way analysis of variance (ANOVA; treatment x time) with repeated measures (time) for the group and then for males and females separately. Tukey tests were employed to further investigate identified main effects. Relations between plasma lactate and IL-6 concentrations and time were explored using Pearson Product Moment correlations. All data, unless otherwise stated, are expressed as mean ± standard deviation. The criterion for significance is an alpha value of <0.05.

4. RESULTS

Participants

The progress of all participants throughout the trial (from screening and enrollment through to completion) is presented in Figure 4. 20 Adults volunteered to participate in this study. Five participants withdrew or were excluded. Reasons for withdrawal/exclusion included failure to respond to scheduling requests following initial screening (n=2), contra-indications to exercise identified on the electrocardiogram stress test (n=1), tobacco use determined by medical history questionnaire (n=1), and exposure to Covid-19 combined with failure to reschedule final laboratory visit following quarantine (n=1). Baseline physiological characteristics for the 15 remaining participants are presented in Table 1 and were unremarkable. When differentiated by sex, several potentially relevant differences (P<0.05) between males and females were noted, including lean mass, VO_{2peak}, VO_2 at T_{VE}, and power at T_{VE}.

Cardio-Pulmonary Response to Exercise

The cardio-pulmonary responses to the 30-minute bouts of stationary cycle ergometer exercise, with and without caffeine, are presented in Table 2. Between minutes 5 and 10, the exercise intensity was equivalent to 60 ± 9% of VO_{2peak}, however, during the final five minutes of the exercise, the VO_2 had increased to 65 ± 13% VO_{2peak}, despite no adjustment of the power output. Consistent with the small increases in VO_2, heart rate was also greater at end-exercise compared with minute 10. In contrast, RER was lower during the final five minutes of exercise compared with minutes 5-10. Caffeine did not appear to influence any of these respiratory responses with the exception of greater ventilation at both time points. The only time x caffeine *vs*. placebo interaction was for heart rate, as evidenced by a greater increase with caffeine across the 30-minutes.

Blood pressure and RPE were determined at minutes 15 and 30. While systolic and mean arterial pressure were greater during exercise than at rest ($P<0.001$), neither systolic, diastolic nor mean arterial pressure changed during exercise (all $P>0.06$), nor were they influenced by caffeine (all $P>0.3$; data not shown). RPE was greater ($P < 0.001$) at minute 30 (Placebo: 14 ± 2 *vs*. Caffeine: 14 ± 2) compared with 15 (Placebo: 13 ± 1 *vs*. Caffeine: 13 ± 1) but was unaffected by caffeine ($P=0.29$). Importantly, all participants were able to complete all exercise sessions, indicating that the prescribed intensity was manageable.

Circulating Lactate and IL-6 Responses to Caffeine and Exercise

Circulating lactate concentrations are presented in Figure 5A. Resting lactate concentration was unaffected by placebo or caffeine. At end-exercise, lactate concentration was appreciably increased above rest ($P<0.001$), and the magnitude of increase was greatest in the caffeine condition ($P<0.001$). 30-Minutes post-exercise, lactate had returned to resting concentrations and was no longer greater with caffeine.

Circulating IL-6 concentrations are presented in Figure 5B. Data from two of the 15 participants were excluded from the final analysis due to technical issues. Resting IL-6 concentration was unaffected by placebo or caffeine. At end-exercise, IL-6 concentration was appreciably increased above rest ($P<0.001$), but the magnitude of increase was unaffected by caffeine. IL-6 concentration continued to increase during recovery and was greatest 30-minutes post-exercise compared with all other measurements (all comparisons $P<0.04$). Again, the magnitude of increase was unaffected by caffeine. End-exercise lactate correlated with plasma IL-6 30-minutes after the cessation of exercise in both placebo ($r=0.850$; $P<0.001$; Figure 6A) and caffeine ($r=0.808$, $P< 0.001$; Figure 6B) conditions.

Individual participant end-exercise lactate and post-exercise IL-6 concentrations are displayed in Figures 5C and 5D respectively. On inspection, it appeared end-exercise lactate

concentration was greater with caffeine compared with placebo in 13 of the 15 participants, and post-exercise IL-6 concentration was greater in caffeine compared with placebo in 10 of 13 participants. Previous studies describing the acute interaction between caffeine, exercise, and IL-6 were completed in males only [51–56]. Only one study examined the effect of caffeine in a cohort comprising females but included trained females in response to a maximal treadmill exercise test [57]. Accordingly, in Figures 5C and 5D, the individual participant responses were differentiated by sex. Closer inspection of these panels revealed that, compared with placebo, caffeine resulted in greater end-exercise lactate concentration in 8 of the 9 males and 5 of the 6 females, and greater post-exercise IL-concentration in 6 of the 7 males and 4 of the 6 females. Our experimental design was not statistically powered to examine differences between males and females, however when the entire analysis was repeated in males only, there were main effects of time (P<0.001), caffeine *vs*. placebo (P=0.043), and a time x caffeine *vs*. placebo interaction (P=0.022) for lactate, and main effects of time (P<0.001), and a time x caffeine *vs*. placebo interaction (P=0.044) for IL-6. Caffeine evoked a greater increase in circulating IL-6 at the end of exercise (1.80±0.86 *vs*. 2.57±1.21 pg/mL; P=0.043) and this effect was exaggerated following 30-minutes of inactive recovery (3.81±2.32 *vs*. 5.06±3.22 pg/mL: P=0.002; Figure 5E). The main effect of caffeine *vs*. placebo for IL-6 failed to attain statistical significance (P=0.056). When the same analysis was repeated in females only, there were main effects of time (P<0.001), caffeine *vs*. placebo (P=0.018), and a time x caffeine *vs*. placebo interaction (P=0.002) for lactate, and a main effect of time for IL-6 (P<0.001). There were no main effects of caffeine vs. placebo (P=0.704) and no time x caffeine *vs*. placebo interaction for IL-6 (P=0.942) in females only (Figure 5F).

Table 1: Subject Characteristics. * Indicates different from female.

	Group	Males	Females	P-Value
Number enrolled	15	9	6	
Age (yr)	26 ± 7	27 ± 9	25 ± 3	1.000
Height (cm)	171.0 ± 7.0	174.6 ± 5.3*	166.8 ± 8.1	0.042
Weight (kg)	77.1 ± 15.2	83.4 ± 16.0*	67. 6 ± 7.5	0.044
BMI (kg/m^2)	25.8 ± 4.0	26.9 ± 4.2	24.1 ± 3.3	0.201
Fat Mass (kg)	20.5 ± 6.4	20.0 ± 7.3	21.1 ± 5.5	0.753
Lean Mass (kg)	54.2 ± 11.3	60.8 ± 9.6*	44.3 ± 3.8	0.002
% Fat	26.5 ± 5.9	23.5 ± 4.5*	30.9 ± 5.1	0.010
VO_{2peak} (ml/kg/min)	40.4 ± 7.8	44.0 ± 7.5*	35.0 ± 4.6	0.021
VO_{2peak} (L/min)	3.07 ± 0.77	3.56 ± 0.53*	2.33 ± 0.31	<0.001
VO_2 at Ventilatory Threshold (L/min)	1.55 ± 0.39	1.75 ± 0.35*	1.25 ± 0.22	0.008
Power at Ventilatory Threshold (Watts)	127 ± 28	144 ± 20*	102 ± 18	<0.001

Table 2: Cardiorespiratory responses during exercise. a Indicates HR reported at minute 10 and minute 30. * Indicates main effect of time. # Indicates main effect of condition. & Indicates time x condition interaction.

	Placebo		Caffeine				
	Minutes 5-10	Minutes 25-30	Minutes 5-10	Minutes 25-30	Time	Condition	Interaction
HR (beats/min)[a]	148 ± 23	155 ± 21*	149 ± 24	159 ± 23*&	P < 0.001	P = 0.526	P = 0.049
VE (L/min)	59.50 ± 16.46	64.77 ± 19.53*	63.27 ± 17.50	69.18 ± 20.66*#	P = 0.001	P = 0.012	P = 0.673
RER	0.98 ± 0.05	0.92 ± 0.03*	0.97 ± 0.05	0.92 ± 0.04*	P < 0.001	P = 0.360	P = 0.242
VO_2 (L/min)	1.80 ± 0.38	1.95 ± 0.51*	1.86 ± 0.41	1.97 ± 0.42*	P < 0.001	P = 0.115	P = 0.437
VCO_2 (L/min)	1.76 ± 0.39	1.76 ± 0.44	1.80 ± 0.41	1.81 ± 0.40	P = 0.817	P = 0.070	P = 0.950

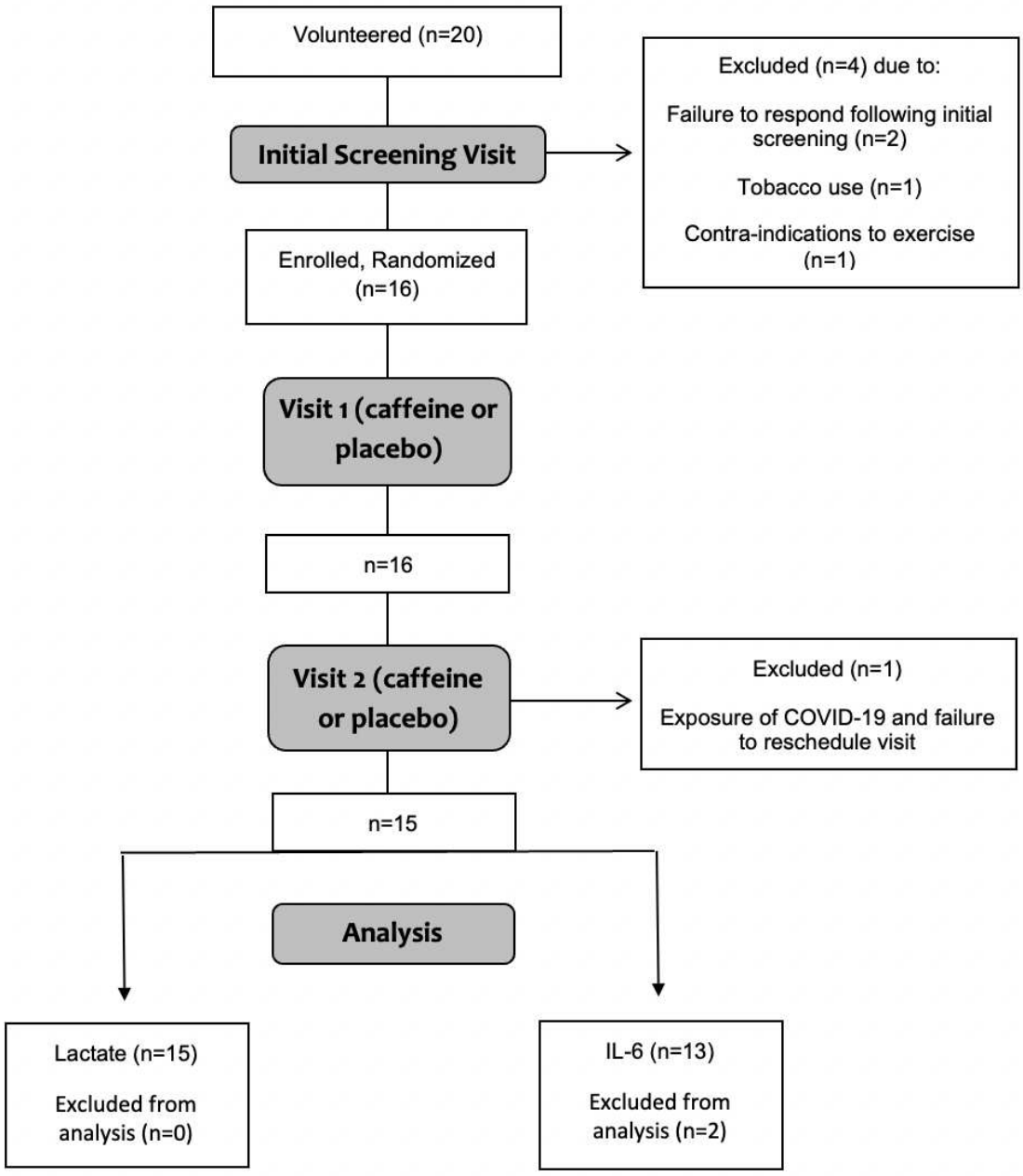

Figure 4: Consolidated Standards of Reporting Trials (CONSORT) diagram depicting participant enrollment. 20 participants were enrolled in the study. 15 completed the trials. 15 were analyzed for lactate. 13 were analyzed for IL-6.

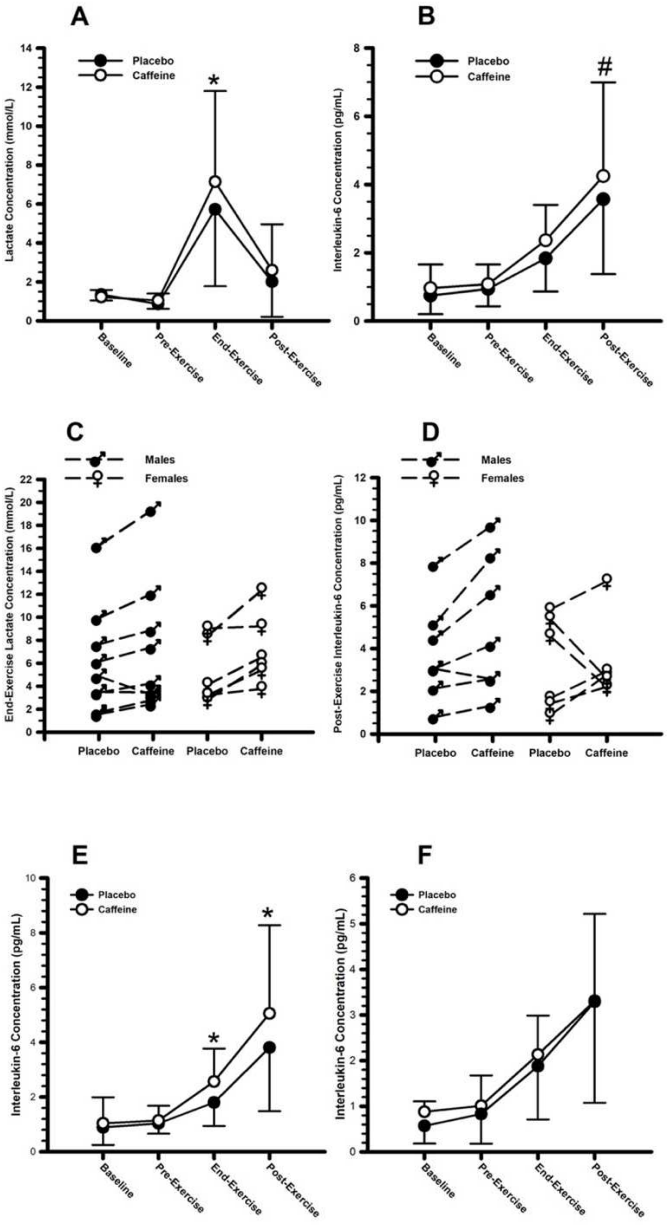

Figure 5: (A) Circulating lactate concentrations over time. (B). Circulating IL-6 concentrations over time. (C) Individual end-exercise circulating lactate concentrations separated as males and females. (D) Individual post-exercise circulating IL-6 concentrations. (E) Circulating IL-6 concentrations over time in males (F). Circulating IL-6 concentrations in females * Indicates different from placebo $P<0.05$. # Indicates different from other timepoints $P<0.001$.

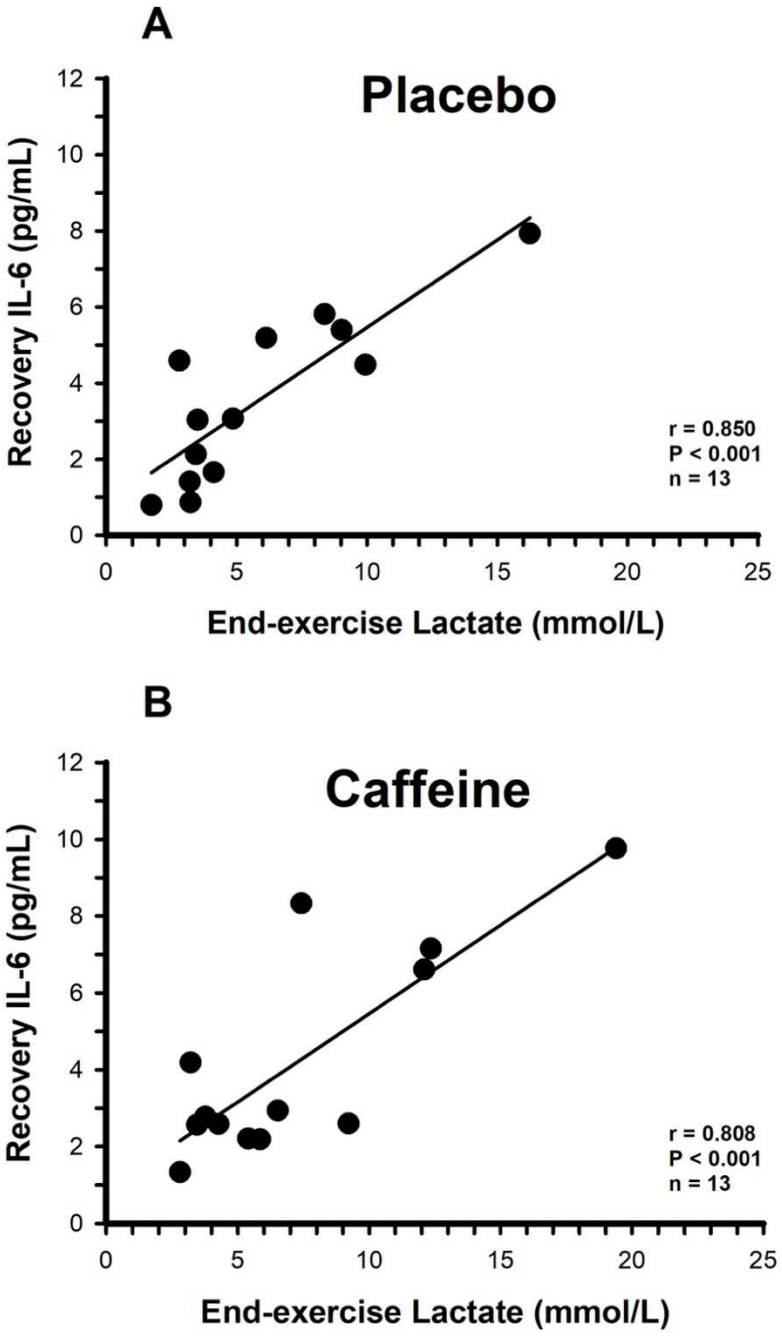

Figure 6: Correlation between circulating end-exercise lactate concentrations and circulating post-exercise IL-6 concentrations in placebo (A) and caffeine (B) trials.

5. DISCUSSION

The purpose of this study was to establish proof of concept that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of both lactate and IL-6 in a study population comprising males and females. We hypothesized that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of lactate and IL-6 in a study population comprising both males and females. Caffeine increased end-exercise circulating concentrations of lactate but not IL-6 in males and females. However, when females were excluded from analysis, caffeine augmented the increase of end-exercise circulating concentrations of IL-6; this effect was further exaggerated after 30-minutes of inactive recovery. It is noteworthy that caffeine evoked greater increases in end-exercise lactate concentrations in both males and females but did not influence the IL-6 response in females.

Lactate

Originally conceived of as a dead-end metabolite, lactate is now known to be an important metabolic intermediate. The role of lactate is threefold, as a major energy source, a gluconeogenic precursor, and an important signaling molecule [58,59]. Both maximal and submaximal exercise elicit increased circulating lactate concentrations. This can be due to a disproportionate increase in lactate Ra compared to lactate Rd. We found that circulating lactate concentrations increased in response to 30-minutes of moderate-intensity cycle ergometer exercise, with the highest lactate concentrations occurring at the end of the exercise in both males (6.62 ± 5.06 mmol/L) and females (6.16 ± 3.02 mmol/L). Circulating lactate

concentrations were not different when comparing any other time points indicating that 30-minutes of inactive recovery allowed circulating lactate to return to pre-exercise concentrations.

Caffeine augmented the exercise-induced increase in circulating lactate concentrations in both males (7.12 ± 5.66 vs. 6.12 ± 4.68 mmol/L caffeine vs. placebo; $P<0.001$) and females (7.19 ± 3.09 vs. 5.13 ± 2.81 mmol/L, caffeine vs. placebo; $P<0.001$). This is in line with previous research showing that caffeine increases circulating lactate concentrations in response to submaximal exercise [42,45,49,50]. Of note, circulating lactate concentrations following 30-minutes of inactive recovery trended toward being significantly higher with caffeine in both males ($P=0.062$) and females ($P=0.063$). We speculate this is due to increased circulating catecholamine concentrations. While we did not measure circulating catecholamines, caffeine is known to elicit increases in circulating catecholamine concentrations [29,38]. Catecholamines bind to beta-2 adrenergic receptors and can increase the rate of glycolysis. Increasing the rate of glycolysis is highly likely to lead to increased lactate production.

IL-6

Historically, the IL-6 response to exercise has pertained to immune function and inflammation. However, it is now known that IL-6 is released from skeletal muscle and is involved in many of the health benefits derived from exercise. Thus, the release of IL-6 from skeletal muscle is a potentially necessary part of the beneficial adaptations to exercise including decreasing inflammation [21,22], decreasing visceral adiposity [25], promoting muscle hypertrophy and attenuation of sarcopenia [26], and improving glucose tolerance and insulin sensitivity [27,28].

We found that exercise elicited increased circulating IL-6 concentrations with concentrations peaking after 30-minutes of inactive recovery. End-exercise IL-6 concentrations trended towards being higher than basal levels of IL-6 ($P=0.051$). The magnitude of this increase was unaffected by caffeine. These data indicate that submaximal, moderate-intensity exercise

can elicit increases in IL-6. As exercise-induced increases in IL-6 are accounted for by IL-6 release from skeletal muscle [24]. Thus, it is reasonable to speculate that moderate-intensity cycle ergometer exercise was able to evoke an increase in skeletal muscle IL-6 production, it was unaffected by caffeine.

However, when the analysis was repeated in males, caffeine augmented the increases in circulating concentrations of IL-6 at the end of exercise. This effect was further exacerbated following 30-minutes of inactive recovery. Previous results have shown that caffeine can augment the IL-6 response to high-intensity and/or long-duration exercise, in males [51–56]. We have now shown that caffeine is able to elicit similar increases in IL-6, in response to sub-maximal exercise lasting only 30 minutes. These data imply that for males unwilling or unable to complete high-intensity, and/or long-duration exercise, caffeine might enhance the IL-6 mediated health benefits from moderate-intensity relatively short duration exercise.

Caffeine did not augment the increase in circulating concentrations in a cohort comprising only females. A previous study found that caffeine was able to increase circulating IL-6 concentrations in a cohort containing males and females in response to a maximal exercise test [57]. These differences can most likely be attributed to our study being underpowered to detect a difference with caffeine compared to placebo. Additionally, they used active (>12 months aerobic and resistance training) females who performed a maximal exercise test. Intensity and modality are two important factors determining IL-6 release from skeletal muscle in response to exercise [23]. While duration is the most important determinate for the IL-6 response to exercise, their test duration was not reported, and we speculate that our duration was not sufficient to overcome the lower intensity and recruitment of muscle mass.

In vivo, human data shows that circulating end-exercise lactate and post-exercise IL-6 concentrations are positively correlated [61–63]. In agreement with these results, we found that end-exercise lactate concentrations were positively correlated with post-exercise IL-6 concentrations in both caffeine and placebo trials. These data show that even in response to relatively short non-fatiguing exercise, there still exists a correlation between exercise-induced increases in circulating lactate and IL-6. Thus, we speculate there exists a relationship between lactate and IL-6, independent of exercise intensity and duration.

Cardiorespiratory Response

There are mixed results regarding the effect of caffeine on the cardiorespiratory responses to exercise. Caffeine is shown to increase ventilation in response during submaximal exercise [42,48]. This is consistent with our results as we found that caffeine increased ventilation when compared to placebo. However, this was the only cardiorespiratory measurement increased by caffeine. We found caffeine had no effect on heart rate, blood pressure, VO_2, VCO_2, and RER. This is not entirely surprising as previous studies have shown that caffeine had no effect on VO_2 and mixed results as to whether it influences heart rate and RER [29,41,42,48]. Not only do our results add to the current literature, but they indicate that caffeine supplementation, from a cardiorespiratory standpoint, is safe at moderate exercise intensities.

Perceived Exertion

Caffeine has been found to exert a suppressive effect on RPE [42]. The next logical thought is that individuals can exercise at harder intensities and/or for longer durations, without the same levels of perceived discomfort. Our data do not reflect this suppressive effect. RPE increased with time, but we found that there was no effect of caffeine on RPE. We speculate that the exercise was not sufficiently long or intense for caffeine to evoke a noticeable decrease in perceived exertion. It should be noted that our RPE only increased by 1 on the Borg scale (13 to

14), a change that indicates participants were exercising at an intensity between "somewhat hard" and "hard". Either way, our data indicate that for more moderate, short-duration exercise, caffeine may not decrease perceived exertion. That being noted, caffeine supplementation is often not administered in a double-blind manner that it was in this study. If the suppressive effect of caffeine on RPE is due to a placebo effect, we do not discourage individuals from using caffeine to exercise and acknowledge the potential benefit of individuals exercising at higher intensities or for longer durations with lower perceived discomfort.

Limitations

There are several limitations to the current investigation. First, we were underpowered to detect a change in IL-6 in females. We have initiated further testing on an additional five participants (one male and four females) to remedy this issue. Increasing and matching the group size will further determine if caffeine exerts a different effect on males and females. At the time of writing this thesis, those data have not been collected and analyzed. Second, we did not screen for caffeine "responders" and "non-responders." We felt this unnecessary as previous literature has extensively detailed that there are no caffeine "responders" and "non-responders" but varying physiological responses to various caffeine doses and stimuli [31,76–78]. Third, despite not changing the exercise intensity, VO_2 (and consequently % VO_{2peak}) and ventilation increased during exercise indicating that subjects potentially were exercising above T_{VE}. However, even with the increase, the exercise intensity could be considered low to moderate-intensity (60% VO_{2peak} and 65% VO_{2peak} respectively) [42] and the wide range of fitness levels included in our study populations could have resulted in an exaggerated response. Additionally, RPE only increased 1 point suggesting that exertion did not drastically increase over the course of exercise. We investigated a relatively young and healthy population. This rationale was due to the Covid-19 pandemic and the hesitance to burden potentially vulnerable diseased or clinical populations.

Our participants did display varying levels of aerobic fitness evidenced by the range in VO_{2peak} (25.8 ml/kg/min to 53.4 ml/kg/min). This heterogeneity provides more ecological validity, as it is slightly more indicative of the fitness spectrum present in the general population. Nonetheless, future studies should aim to address the effect of caffeine on the lactate and IL-6 response to moderate-intensity exercise in clinical populations.

Conclusion

We hypothesized that caffeine, ingested prior to moderate-intensity exercise, would lead to greater circulating concentrations of lactate and IL-6 in a study population comprising both males and females. Caffeine increased end-exercise circulating concentrations of lactate but not IL-6 in males and females. However, when females were excluded from analysis, caffeine augmented the increase of end-exercise circulating concentrations of IL-6; this effect was further exaggerated after 30-minutes of inactive recovery. It is noteworthy, that caffeine evoked greater increases in end-exercise lactate concentrations in both males and females but did not influence the IL-6 response in females. These findings suggest that in males unable/unwilling to perform high-intensity and/or long-duration exercise, caffeine may potentially enhance the IL-6 mediated health benefits of relatively short, moderate-intensity exercise.

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