BIOL3550 Research Lab Report

Jaida Lewis, Emily Clark, Denzel Marican

Dr. Mark Rakobowchuk

Effects of Acute Caffeine Intake on Energy Efficiency and Fat Oxidation During Low-Intensity, Steady-State Cycling Exercise

Abstract:

Introduction: The purpose of this study was to examine the effects of caffeine on energy efficiency and fat oxidation during low-intensity, steady-state cycling exercise. Based on previous research, it is predicted that caffeine will enhance endurance exercise performance through an increase in fat oxidation and energy efficiency.

Methods: Five male and five female participants (ages 18-22) completed two 30-minute low-intensity cycling bouts on a stationary bike. The exercise bouts were completed during two separate visits, and each participant was tested under both the caffeine and non-caffeine (control) conditions.

Results: There is not enough evidence to support the initial prediction that caffeine increased energy efficiency and fat oxidation in participants during low-intensity, steady-state cycling. The results of the analysis concluded there was no statistical difference in energy efficiency ($p=0.3625$) and no statistical difference in RQ values ($p= 0.8347$) between the caffeine and non-caffeine conditions, respectively. Variables of interest such as $\dot{V}O_2$ and $\dot{V}CO_2$ also remained unchanged between conditions. Additionally, self-reported measures of energy levels were higher post-exercise during the non-caffeine condition (on average).

Conclusion: While the results of the study did not support the prediction that caffeine enhances endurance exercise performance through increased energy efficiency and fat oxidation, potential limitations and confounds of the present study should prompt future research that aims to further determine caffeine's effectiveness during endurance exercise.

1. Introduction

Caffeine is a widely-used, mild central nervous system stimulant that blocks adenosine receptors and has been shown to improve a number of metabolic and neuromuscular factors during exercise as well as increases metabolic fat oxidation (Lutsch et al., 2020). Additionally, caffeine is shown to have other performance benefits during low-to-high-intensity exercise such as increased time to exhaustion, enhanced neuromuscular function, and skeletal muscle contraction capabilities (Lutsch et al., 2020).

Caffeine is present in almost all commercially consumed beverages, ranging from coffee to soda and tea. Fulgoni et al. (2015) found that 89% of men and women in the US reportedly consumed caffeine on a regular basis, with an average consumption of 200 mg/day. Caffeine has increasingly become a common supplement among endurance athletes as well. Desbrow and Leveritt (2007) found that during an endurance event, a large proportion (73%) of endurance athletes believed that caffeine aided in their performance and 84% believed it improved their concentration. Additionally, numerous studies have concluded that caffeine consumption increases performance during endurance events (Hodgson et al., 2013; Bello et al., 2019; Stadheim et al., 2021). Southward et al. (2018) also found that moderate doses of caffeine can enhance athletic performance as it can lead to a small improvement in endurance, which can make a substantial difference in competitions where athletes are typically separated by small margins.

Similarly, a study done by Graham et al. (1994) found that acute caffeine ingestion enhanced performance during endurance exercise activity. While more research is still needed on the body's physiological response after caffeine ingestion, caffeine was found to be associated with muscle glycogen sparing and elevated plasma epinephrine levels, which may affect active

muscle groups during endurance exercise (Graham et al., 1994). Although research has shown that caffeine does not improve maximal oxygen capacity ($\dot{V}O_2$ max) directly, it could permit the athlete to train at a greater power output and/or to train longer (Graham, 2001). Additionally, caffeine has also been shown to increase speed and/or power output in simulated race conditions that last as little as 60 seconds or as long as 2 hours (Graham, 2001). Hopker et al. (2010) found that gross energy efficiency (GE) increased after high-intensity exercise training, supporting the idea that energy efficiency is a key variable in increasing exercise performance. If acute caffeine consumption substantially increases GE, we theorize this may be the pathway with which exercise performance is enhanced.

This study aims to measure GE in participants during low-intensity cycling exercise under caffeine and non-caffeine (control) conditions. If an individual has a greater GE during exercise after acute caffeine intake, this can be associated with an enhancement in exercise performance (Hopker et al., 2010).

Another question our study aims to answer is whether caffeine enhances performance through changing substrate usage or through other pathways. Although many studies have shown that caffeine has a beneficial effect on athletic performance, there has been debate around whether the improvement in performance is caused in part by caffeine consumption altering substrate use during exercise. Hodgson et al. (2013) and Turley et al. (2008) both reasoned that the respiratory exchange ratio (RER) was unchanged between caffeine and placebo treatments during exercise. In contrast, other studies have described how caffeine consumption enhanced performance by increasing lipolysis from adipose tissues and lipid oxidation during exercise, which enabled glycogen sparing for later use during prolonged exercise bouts (Ryu et al., 2001; Spriet et al., 1992). Ruiz-Moreno et al. (2022) theorized that acute caffeine ingestion before

exercise is an effective supplementation strategy to enhance fat oxidation as well as increase exercise intensity during steady-state aerobic exercise. Spriet & Randell (2020) also determined that an increase in fat oxidation is important for low-to-moderate intensity exercises, such as cycling and walking, because it provides energy for the contraction of skeletal muscles. If more fat is broken down during exercise after caffeine consumption, then there should be a significant difference in RER and VO_2 levels between caffeine and non-caffeine conditions during steady-state exercise.

To determine the effect of caffeine consumption on substrate use, we must also maintain what is known as a "steady state" in our participants. In a steady state, $\dot{V}O_2$ is maintained at a steady level for a prolonged time period. This is important for measuring the change in RER between conditions as intense exercise above steady-state thresholds can result in RER values above 1.0. An RER above 1.0 is caused by the body using anaerobic respiration, which causes more carbon dioxide to be produced than oxygen consumed (Ferretti et al., 2017). This increase during exercise is attributed to the bicarbonate buffering system in the blood (Niekamp et al., 2012). As we are only measuring $\dot{V}O_2$ levels from the mouth, we do not want the acid buffering system affecting RER as we cannot measure that variable within this study. This is why we selected low-intensity exercise for our participants as it allows us to maintain a steady state where VO_2 represents the entire rate of metabolic energy liberation (Ferretti et al., 2017).

Overall, we aim to determine the effects that caffeine has on energy efficiency, which will be calculated using the participant's rate of work (in Watts) and energy expenditure (in J/s). Fat oxidation through respiratory exchange ratio (RER), also known as respiratory quotient (RQ), will also be measured. Other variables will be measured during exercise such as the volume of oxygen consumed ($\dot{V}O_2$), the volume of carbon dioxide produced ($\dot{V}CO_2$), ventilation

(VE), and heart rate (HR). The RER value (essentially CO_2 production/O_2 intake) is widely employed under steady-state settings to indirectly evaluate the relative contribution of carbohydrates and lipids to total energy expenditure (Pendergast et al., 2000; Simonson & DeFronzo, 1990), which is key for our study.

Based on primary research that examined the effects of caffeine on exercise performance (Graham et al., 1994; Cruz et al., 2015; Domaszewski et al., 2021; Hopker et al., 2010), it is hypothesized that the participants will have higher gross energy efficiency as well as a higher fat oxidation rate (thus a lower RQ or RER value) during the low-intensity cycling exercise after ingesting the caffeinated coffee drink. The results of the proposed study will provide insight into the physiological effects of caffeine during low-intensity exercise and can be further used to determine whether or not caffeine can be used as an effective ergogenic performance aid for endurance athletes.

2. Materials & Methods

2.1: Participants

Ten participants (5 male and 5 female) participated in testing for the present study. All participants were students who attend Thompson Rivers University (TRU) and were between the ages of 18-22. Participants were screened prior to testing to make sure each individual met the requirements for the study. All participants were considered to be moderately active to very active individuals, meaning they participate in either competitive sports, or recreational sports and activities such as going to the gym, walking, or jogging on a weekly basis. Out of these 10 participants, 7 participated on a competitive sports team, and 3 participated in physical activity at a recreational or moderate level. Additionally, all participants self-reported they were “regular

caffeine drinkers" to ensure they were familiar with the effects of caffeine. Based on our requirements, this meant they ingested caffeine at least 3-4 a week in the form of coffee, energy drinks, or other caffeine supplements.

2.2: Pre-experimental

The beverages for each condition (caffeinated coffee and decaffeinated coffee) were prepared prior to the participant's arrival. The amount of caffeine, in milligrams (mg) per kilogram (kg) of body weight, was measured for each participant and was dependent on their body weight, following the work done by Hodgson et al. (2013), Engels & Haymes (1992), and Jenkins et al. (2008). Nescafe Original instant coffee was used for the caffeine condition. Hodgson et al. (2013) stated that Nescafe Original instant coffee contains 3.4 grams (g) of caffeine per 100g of instant coffee, which translates to 0.15g coffee/kg of body weight, the equivalent of 5 mg caffeine/kg of body weight. Hodgson et al. (2013) confirmed this measurement by completing a caffeine content analysis. Nescafe Original Decaffeinated coffee was used for the control condition. The caffeine content of this product was also analyzed by Hodgson et al. (2013) and was found to have minimal amounts of caffeine. The instant coffee and instant decaffeinated coffee was prepared in the same way for each participant. Jenkins et al. (2008) found that caffeine at a lower dose (2-3 mg/kg) was capable of enhancing performance, thus 2.5mg of caffeine/kg of body weight was used for the present study, which translates to 0.075g of coffee/kg of body weight. Hot water (100mL) was added to the pre-weighed coffee grounds in a mug, and an artificial sweetener was added for taste, as done by Engels & Haymes (1992). All the contents were then stirred until dissolved. Artificial sweeteners are not

metabolized by the body, so there is no energy intake from their consumption thus the addition of an artificial sweetener will not affect the results of the experiment (Sharma et al., 2016).

The COSMED Quark Cardiopulmonary Exercise Test (CPET) was used to measure the breath-by-breath analysis of pulmonary gas exchange, recording the participant's physiological response and measuring expired gases continuously ("COSMED", 2022). Prior to the participant's arrival, the gas analyzer and flow turbine of the machine was calibrated with the use of precision gases and the 3L calibration syringe, respectively. Preparation of the informed consent form and both the pre-exercise and post-exercise questionnaires were also prepared before the participant's arrival.

2.3: Experimental trials

Once the participant arrived at the laboratory, they were informed of the protocol and any potential risks of the study (which was considered minimal risk). After being debriefed and clarifying any questions or concerns, they were asked to sign the informed consent form. The participants made two separate visits to the lab. Each visit followed the same protocol, differing only in the drink they consumed. The drink condition that the participant received first (coffee or decaffeinated coffee) was randomly chosen by the student researchers. The participant's weight, height, and gender was recorded; their weight (in kg) was used to accurately measure the dosage of coffee or decaffeinated coffee needed.

Caffeine has a half-life of 4-6 hours and thus can remain in one's system for 8-12 hours ("Spilling the Beans", 2018). Since the testing session had scheduled start times between 9:30 am and 11 am, the participants were asked to refrain from caffeine starting at 7 pm the night before testing, so a minimum of 12 hours had passed before the testing session. Previous studies

by Engels & Haymes (1992), Ruiz-Moreno et al. (2022), and Hodgson et al. (2013), also restricted caffeine consumption before testing to avoid drastically varied caffeine levels between participants. Additionally, the participants were asked to describe their meals on the day of the experiment prior to the time of testing; they were not instructed to record a food log nor were they asked to fast before testing, however, their response was recorded to compare their food intake before visit 1 and visit 2. Ideally, the amount of food ingested before testing would be similar for both sessions in terms of calories and macronutrients (carbohydrates, fats, and proteins). To ensure this, visits 1 and 2 were scheduled at the same start time for each participant, which would make it easier to eat the same meal on the day of testing for both visits.

The participants were blindly given their assigned condition (either caffeinated coffee or decaffeinated coffee), meaning they were unaware of which condition they had been given for the entire duration of the experiment. Once handed the condition, they were given 10 minutes to consume the beverage. Caffeine takes 35-45 minutes to reach peak absorbance in the body (Liguori et al., 1997); to ensure the caffeine had been absorbed, the participants would wait 30 minutes after ingesting the drink to start the cycling exercise. During this 30-minute break, the participant was asked to select their desired seat post position as well as the seat height on the stationary bike. The selected values were recorded in the researcher's notebook to ensure the participant would have the same seat post and seat height position for the second visit. The power level for each participant was kept constant for each testing session at 70 Watts. A value of 60-70 RPM is necessary to reach a steady state at our prescribed resistance, so participants were instructed to maintain an RPM level within this range for the entirety of the exercise bout. A Bluetooth heart rate monitor belt was attached to the participant's lower chest area and was connected to the COSMED Quark CPET machine. Right before exercise, the participant was

instructed to fill out a 5-point Likert questionnaire to self-report their current state of fatigue, focus, alertness/energy, and awakeness. The participant then seated themselves on the stationary bike and a face mask was fitted to their face. The mask was tested to ensure there was a proper seal and no air was escaping between the mask and the participant's face. The participants were asked to refrain from speaking if not necessary when the recording began and were encouraged to give non-verbal hand signals when researchers periodically checked in during exercise.

Once set up, a metabolic test for pulmonary exercise, measured breath-by-breath, was started. The TacX iPhone app was simultaneously used and was placed in front of the participant so they were able to monitor their RPM range. The TacX app was also used to set the Watts on the bike, which represented the resistance of pedaling. The participant was asked to remain still for 2 minutes while the CPET took baseline measurements, and for another 2 minutes after the recording was started as this data is not stored. After these 4 minutes, the participant started the exercise bout and cycled continuously for 30 minutes. It typically takes 5 minutes to reach steady state (Borges et al., 2016), so 30 minutes was the selected time to allow for data collection after reaching a steady state. Data was continuously recorded throughout the exercise, recording the volume of oxygen consumed (VO_2), the volume of carbon dioxide produced (VCO_2), the respiratory exchange ratio (RER), as well as heart rate (HR) (Lutsch et al., 2020; Hodgson et al., 2013). After the 30-minute exercise bout, the mask was removed from the participant, and the participant was instructed to fill out the same 5-point Likert questionnaire as previously used (based on how they felt after exercise). The data was then saved to the participant's file and stored in the researcher's USB drive. The equipment was properly cleaned after the participant left with the Peroxigard solution.

2.4 Data Analysis

Energy expenditure was calculated using a modified version of the Weir-equation. Gross efficiency (GE) was calculated as a percentage through a ratio of work rate (Watts) divided by energy expenditure (J/s). $\dot{V}O_2$ and $\dot{V}CO_2$ are expressed in units of L/min. Both formulas are included below (Moseley & Jeukendrup, 2001).

$$Energy\ Expenditure\ (J \cdot s^{-1}) = [(3.869\ x\ VO_2) + (1.195\ x\ VCO_2)]\ x\ (\frac{4.186}{60})\ x\ 1000$$

$$Gross\ Efficiency\ (\%) = \frac{(\text{Work Rate (W)})}{\text{Energy expended } (J \cdot s^{-1})}\ \text{x}\ 100\%$$

The abbreviated Weir-equation includes a conversion of kcal to joules (1 kcal = 4.186 J), minutes to seconds (1 minute = 60 seconds), and liters to milliliters (1 liter = 1000 milliliters). Work rate is represented by Watts (which was held at 70 throughout the exercise). Gross efficiency is represented as a percentage, where 100% would confer perfect efficiency and 1% would confer low efficiency.

Pre-exercise and post-exercise questionnaires were analyzed for each participant based on their subjective ratings. “Perceived energy” or PE scores were calculated by adding the self-reported scores for awakeness, alertness/energy, and focus (all energy-promoting qualities) and subtracting self-reported scores for fatigue (an energy-reducing quality). The questionnaire was created using a standard, 5-point Likert scale; after the participant read the statement (for example, “I am fatigued”), they proceeded to check the box which reflected how they felt at that exact moment in time. A score of 1 would mean they “strongly disagree” with the statement; 2 would mean they “moderately disagree” with the statement; 3 would mean they “neither agree/disagree” with the statement; 4 would mean they “moderately agree” with the statement; 5 would mean they “strongly agree” with the statement.

2.5: Statistical Analysis

All statistical analyses were conducted using RStudio software, Version 2022.12.0+353 by Posit Software, PBC. Paired two-sample t-tests were conducted to determine any difference between the caffeinated and non-caffeinated conditions. This included t-tests of $\dot{V}O_2$, $\dot{V}CO_2$, RER, EE, and GE. To ensure that only steady-state $\dot{V}O_2$ and $\dot{V}CO_2$ values were used to calculate energy expenditure, we removed any subject's data for which the RER (respiratory exchange ratio) exceeded 1.0.

Additionally, all $\dot{V}O_2$ and $\dot{V}CO_2$ data were measured per kilogram of body weight for each participant. This allows us to account for gas consumption differences based on body weight, which varied from 52 kg to 88.5 kg in our 10 participants. Using participant data, we can visually analyze differences between conditions. When calculations of energy expenditure and energy efficiency were conducted, respiratory data was in units of liters per minute as required in the energy expenditure equation.

3. Results

Ten individuals participated in the present study to compare energy efficiency percentages between caffeine and non-caffeine conditions. $\dot{V}O_2$, $\dot{V}CO_2$, and RER were tracked using CPET, and mean values for each condition were found by averaging the last 290 seconds (4.83 minutes) of submaximal stationary bike exercise to include only steady-state measurements in data analyses.

Out of all the variables of interest, none of the variables saw a significant change between caffeinated and non-caffeinated conditions. As presented in Table 1 of the appendix, $\dot{V}O_2$ per kg (mL/min/kg) saw no change (, p-value = 0.5024), and $\dot{V}CO_2$ per kg (mL/min/kg) saw no change

(p-value = 0.4057). Similarly, the calculated variable of energy expenditure (J/s) saw no change (p-value = 0.4761). RER also was not different across condition (p-value = 0.8347). Table 1 of the appendix demonstrates means of each variable of interest between each condition (caffeinated and non-caffeinated). Mean differences between each variable are also displayed and the p-values of those differences were found using a paired two-sample t-test. The mean differences were all insignificant, with $p > 0.3625$ across all variables. The respiratory exchange ratio (RER) did not exceed 1.0 in any condition, meaning steady-state values represented the only data that was analyzed.

Additionally, our main variable of interest, gross energy efficiency (%), saw no change and was therefore not significantly affected by caffeine consumption as well (p-value = 0.3625). See Figure 1 below for a graphical visualization of gross energy efficiency (%) between caffeine and decaffeinated conditions. Mean data for the variables of interest measured and used for gross energy efficiency calculations are included in Figure 2 and Table 1, located in the appendix.

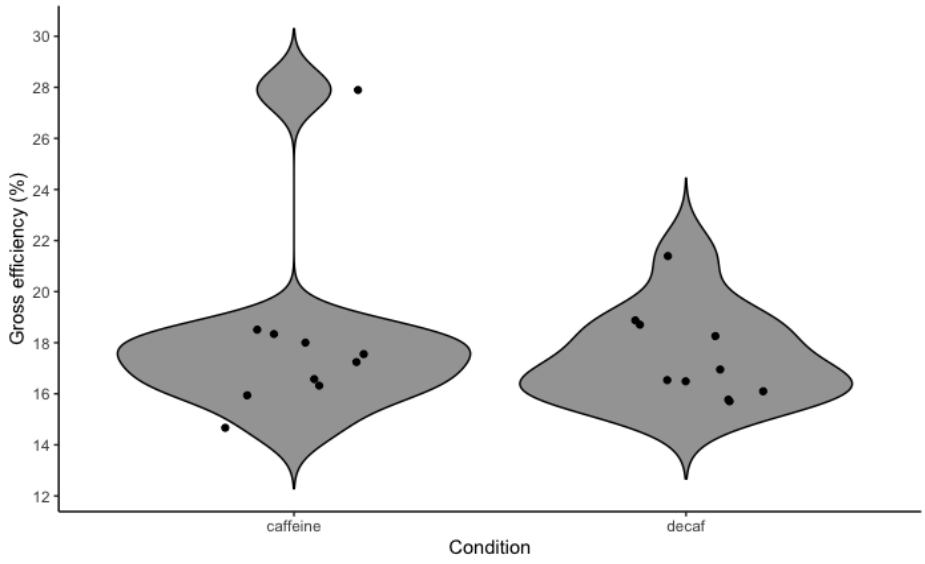

Figure 1. Violin plot of gross efficiency (%) data between caffeine and decaffeinated conditions (n=10). Gross efficiency was calculated by finding the ratio of work rate (Watts) to energy expended (J/s). Energy expended was calculated through the modified Weir-equation.

Table 2 in the appendix displays the "perceived energy" or PE scores derived from the pre-exercise and post-exercise questionnaires that were filled out by each participant. The group average PE scores were highest in the pre-exercise caffeine group with a value of 8.1, which then decreased to 7.8 in the post-exercise caffeine group. For the pre-exercise non-caffeine group, the group average PE score was 6.2, which then increased to 7.4 in the post-exercise non-caffeine group.

4. Discussion

The main findings of the present study suggest that there is not enough evidence to support the initial prediction that the caffeinated coffee (CC) condition increased energy efficiency in participants during low-intensity, steady-state cycling compared to the decaffeinated coffee (DC) or control condition. The results found that there was no change in energy efficiency in the participants ($p = 0.3625$). These results were contradictory to our expectations, given that previous studies have found that caffeine enhanced energy performance (Graham et al., 1994; Hopker et al., 2010). However, Lutsch et al., (2020) found that caffeine consumption increased energy expenditure, which would decrease gross energy efficiency using our calculation, contrary to our original prediction. Ruiz-Monero et al., (2022) also found similar results and concluded that acute caffeine ingestion before an exercise session induced a higher total work output and therefore higher overall energy expenditure. Both of these studies contradict our prediction that the performance enhancements of caffeine is related to energy efficiency and that we would see an increased energy efficiency after caffeine consumption. In these studies, the exercise related improvements were explained in part by the alterations in metabolic rate and gas exchange increasing energy expenditure, which would have an opposite

effect on gross energy efficiency. Since all participants were kept at a constant power output (70 Watts) and speed (60-70 RPMs) for the same amount of time (30 minutes of exercise), it was predicted that energy expenditure would decrease and would indirectly increase energy efficiency, since energy efficiency is calculated by work rate (Watts) divided by energy expenditure (J/s). The results of our study do not align with this prediction. However, the results are also not fully in alignment with Lutsch et al., (2020) or Ruiz-Monero et al., (2022) as they found increases in energy expenditure with caffeine use, whereas our results found no change between conditions. Nevertheless, many factors can indirectly affect energy efficiency in participants, such as individual cardiovascular fitness or anatomical gender differences (Hunter et al., 2015; Westerterp, 2007). Future work is needed to further determine if caffeine is effective in enhancing endurance exercise performance, or if it acts as more of a placebo in individuals.

The results of the present study also found that the CC condition had no effect on RQ (or RER) compared to the DC condition ($p = 0.8347$). This means that there was no statistical difference in RQ values between CC and DC conditions. This does not support the initial prediction that participants would have a higher fat oxidation rate on the CC condition, thus a lower RQ value. Some studies found no difference in RQ values between caffeine and control or placebo conditions during exercise (Hodgson et al., 2013), which is more reflective of our results. Other studies, however, found increased lipid oxidation during exercise, which enabled glycogen sparing for later use during prolonged exercise. Glycogen sparing would provide subsequent energy for skeletal muscle contractions and therefore enhanced endurance exercise performance in participants (Ryu et al., 2001; Spriet et al., 1992). The reported RQ values for both conditions in the present study were roughly 0.9. This means participants in our study were predominantly utilizing carbohydrates as their primary energy source, with some fat metabolism

also occurring. These RQ values are normal as the average person relies more on carbohydrate oxidation during low to moderate exercise (McArdle et al., 2006). This process is not as effective as increased fat oxidation during exercise (thus an RQ closer to 0.7) because the limited carbohydrate (glycogen) stores could be spared for later on in the exercise rather than being oxidized sooner, which would aid in maintaining a steady power output for longer. It is important to note that RQ values and exercise performance do not have a direct correlation, and other factors such as anatomical gender differences, cardiovascular fitness of the individual, or caloric consumption before exercise also play a role in exercise performance. While these factors were generally controlled in the present study, there are inevitable physiological differences between participants that could not be controlled.

The present study also surveyed each participant before and after exercise on both the CC and DC conditions to examine perceived levels of fatigue, awakeness, alertness/energy, and focus. During analysis, the PE scores pre-exercise and post-exercise were calculated for each participant for each of the two conditions. The results of the analysis found that, on the CC condition, 4 participants had higher PE scores post-exercise, while 6 participants had higher PE scores post-exercise on the DC condition. On average, participants' PE scores decreased post-exercise on the CC condition and increased post-exercise on the DC condition. This means that, on average, participants had higher perceived energy levels after exercise when on the non-caffeine condition. These results contradict our initial prediction; if caffeine increased energy efficiency in participants during exercise, it is assumed that the participants' PE scores would be higher on the CC condition post-exercise compared to the DC condition as they would have been more efficient at conserving their energy, therefore would be less fatigued and have higher energy levels by the end of the exercise bout.

The present study had several limitations. First, caffeine sensitivity could be variable in some of the participants. Before testing, participants were screened to make sure they were familiar with drinking caffeine, therefore considered "regular coffee drinkers". While most of the participants ingested caffeine on a daily basis in some form, some participants only reported drinking caffeine 2-3 times a week. Additionally, some participants reported drinking coffee every day, while others reported that the majority of their caffeine consumption came from energy drinks or other caffeine supplements, meaning they rarely drank coffee. When a person consumes caffeine regularly, there are different physiological adaptations that occur within the brain. It is widely known that the stimulatory effects of caffeine are caused by caffiene's ability to act as a competitive antagonist to adenosine receptors in the brain (Fiani et al., 2021; Davis et al., 2003; Elmenhorst et al., 2012). Adenosine acts presynaptically to inhibit the release of neurotransmitters such as glutamate, norepinephrine, and GABA, among others (Prince & Stevens, 1992; Harvey & Lacey, 1997; Rongen et al., 1996; Saransaari & Oja, 2005). Caffeine promotes release of these neurotransmitters by competitively inhibiting adenosine onto its receptors (i.e., A1, A2A, A2B, and A3) (Lazarus et al., 2011). An influx in these neurotransmitters in the central nervous system (CNS) causes increased alertness and decreased perceived exertion during exercise (Daly et al., 1983; McLellan et al., 2016). The aforementioned CNS effects of caffeine (and therefore their performance enhancing effects) can be reduced, however, as tolerance to caffeine increases with frequent consumption (Fredholm, 1982). Specifically, tolerance can develop from the upregulation of adenosine receptors in the brain on which caffeine acts as an antagonist. This may have reduced our participants' sensitivity to caffeine as most of them reported to drink coffee daily; this ultimately may have resulted in a lack of improved performance between caffeinated and decaffeinated conditions and a

significant variability between participants based on different caffeine tolerances. Furthermore, those that drank coffee may have had a stronger belief that they became more alert or awake after consumption, which could have caused a potential placebo effect (even though the participants were unaware of whether they were ingesting the CC or DC condition at the time of testing). This variability could have impacted how the participants responded to the CC condition. Each participant was administered 0.075g of both CC and DC coffee conditions per kg of body weight, so every participant was given an equivalent amount of caffeine after adjustment. Although this variable was kept consistent, the potential variability of caffeine sensitivity among participants could have affected our results.

Another possible reason for the lack of change in RER could have been due to the amount of caffeine used in the present study not being high enough to cause peripheral changes in the body. Researchers have found that coffee increases sympathetic nervous system (SNS) activity, which has been found to play a key role in increased lipolysis (Corti et al., 2002; Acheson et al., 2004). SNS regulation of lipolysis occurs through a mechanism of stimulatory β-adrenoceptors (G-protein coupled transmembrane proteins) and inhibitory α2-adrenoceptors on adenylyl cyclase. Caffeine's SNS stimulation activates β-adrenoceptors, leading to increases in lipolysis through adenylyl cyclase. Adenylyl cyclase can also be inhibited by adenosine receptor binding (Levitzki, 1988). As we've discussed, coffee has antagonistic qualities for adenosine, meaning that caffeine stimulates lipolysis through both its stimulation of the SNS and inhibition of adenosine (Hetzler et al., 1990; Vannucci et al., 1989). Stimulated adenylyl cyclase produces higher levels of cAMP, which activate cAMP-dependent Protein Kinase A (PKA). In turn, PKA activates other substrates such as hormone-sensitive lipase and perilipin (both responsible for lipolysis) (Fricke et al., 2004). Caffeine is also known to inhibit phosphodiesterase, which breaks down cAMP. This would further increase cAMP levels in cells and enhance lipolysis (Fiani et al.,

2021). This enhancement of lipolysis would be reflected in a lower RQ in the caffeine condition compared to decaf condition (Schutz, 1995). By increasing lipid oxidation during exercise through these pathways, it's been hypothesized that this can increase exercise performance by allowing the body to spare endogenous carbohydrate stores and deploy them later, therefore prolonging time to exhaustion. Findings from Cruz et al. (2015) supported this theory as they observed a 22.7% increase in time to exhaustion accompanied by a significant reduction in RQ. McNaughton et al. (2008) also supported this theory finding that performance was improved from a greater reliance on fat metabolism, as indicated by increased FFA and a lower respiratory exchange ratio (RER). Importantly, these effects would not be observed if the acute caffeine load was insufficient. A meta-analysis by Callado-Mateo et al. (2020) found that doses equal to or below 3.0 mg/kg did not effectively enhance fat oxidation during exercise, and doses in the range of 3.1 and 7.0 mg/kg are necessary to obtain a change in lipid oxidation. This could mean that we simply did not give our participants enough caffeine to produce a significant change in lipid substrate use and glycogen storage, as our participants only received 2.5mg of caffeine/kg of body weight. If the caffeine dose was insufficient, this would have caused the desired effect of enhanced lipid oxidation and glycogen sparing to not occur, therefore causing the calculated RQ values to remain unchanged between caffeine and decaffeinated conditions.

Differences in cardiovascular fitness between participants could have also caused potential confounds in our results. The sample size of the present study consisted of 5 males and 5 females. Before testing, the participants were asked how regularly they participated in physical exercise; if the participant did not engage in any regular exercise and was considered sedentary, they did not meet the requirements of the study. Although all 10 participants were considered to be at least at a moderate physical activity level, there was variability within the sample. In

particular, all 5 of the male participants are currently competing at a high level (in either soccer or hockey), meaning their cardiovascular fitness is most likely higher than the average person who exercises at a moderate or recreational level. Of the 5 female participants, 2 of these participants are currently competing at a competitive level in soccer, while the other 3 female participants reported that they engage in recreational sports or activities such as going to the gym, walking, or jogging. This variability in cardiovascular fitness may have affected the results of our study. The effect of physical fitness on performance enhancement from caffeine is widely debated, with different studies having contradictory findings. Brooks and Wyld (2015) and Boyett et al. (2016) both found that untrained participants performed better in fitness tests than trained individuals when ingesting caffeine; on the contrary, studies conducted by Collomp et al. (1992) and Astorino et al. (2012) had opposite findings, where trained individuals saw greater performance increases through caffeine ingestion compared to untrained individuals. Our findings reflect that the physical fitness of our participants may have suppressed any ergogenic effects of caffeine, similar to Brooks and Wyld (2015) and Boyett et al. (2016). The question of whether physical fitness influences the improvement of caffeine performance remains unclear, therefore future work should aim to highly regulate this variable when studying the effects of caffeine on exercise performance.

Anatomical gender differences between males and females play a role in exercise. Performance between genders can be impacted by hormonal differences or body composition, as well as aerobic and anaerobic capacities (Mielgo-Ayuso et al., 2019). It has been found that males have larger lungs and bronchi airways which could provide a slight advantage over females during exercise (Dominelli & Molgat-Seon, 2022). A study by Bara et al. (2019) found that men had a higher VO_2 max than females due to a lower body fat percentage, higher

hemoglobin levels, and greater heart size (on average). These anatomical differences allowed men to have higher performance levels than females. A study done by Temple and Ziegler (2011) found gender differences in both the cardiovascular and subjective response to caffeine, which are thought to be caused by changes in circulating steroid hormones. These anatomical differences in gender could have affected response to caffeine, RQ, energy expenditure, and energy efficiency in our study. It is therefore important to note that gender differences need to be accounted for when examining both male and female participants as this could have affected the results of our study.

In terms of pre-exercise and post-exercise questionnaires, there is always the possibility of bias or dishonesty while conducting self-report measures for psychological variables. The participant's perceived feelings of energy or fatigue could highly depend on the day and other situational, life, relationship, or other external factors. While all participants were tested within the same time frame (9:30 am to 11 am), this could yield different results in participants who do not wake up as early as others, or who did not sleep for as many hours the night before.

Regarding future research, a sample size larger than 10 participants should be used for increased precision and better representativeness. Additionally, if all participants were selected at the same or similar cardiovascular fitness level, then this may eliminate potential confounding variables when calculating energy efficiency. This could mean selecting a sample of competitive athletes and a sample of sedentary individuals and analyzing the results within these groups and between them. Similarly, selecting participants with the same or similar caffeine tolerance may eliminate potential confounding variables in caffeine sensitivity. Additionally, if another form of caffeine were used during testing (for example, anhydrous caffeine powder), this may eliminate any biases or placebo effects that may result from an individual's personal beliefs about coffee.

Individual factors such as the amount of calories consumed before testing could be more highly regulated in future research. Although our participants were asked to consume the same meal before testing on both visits, their caloric consumption was not monitored in the days leading up to testing. If there was variability in whether the participant was in a caloric deficit or caloric surplus, or even the amount of carbohydrates/fats being consumed by participants well before testing began, this could have affected our RQ values. For example, a caloric deficit is often found to cause a reduction in energy expenditure and may cause an individual to have higher levels of fat oxidation if an insufficient amount of carbohydrates are being consumed and therefore cannot be used for energy (Most & Redman, 2020). A reduction in energy expenditure would increase energy efficiency based on the modified Weir-equation, however, it is important to note that the body compensates during a caloric deficit by trying to conserve energy, therefore fatigue or lethargy levels may increase, which would cause the individual to have lower energy levels during exercise. This is one example of how caloric consumption may impact energy efficiency, which is why it is an important variable to regulate.

Sleep was also a factor that was uncontrolled in our experimental design. Jung et al. (2010) found sleep to be a significant factor in energy expenditure, which was key to finding energy efficiency in our study. Specifically, Jung et al. (2010) found sleep deprivation to significantly increase energy expenditure, which would lower energy efficiency. Although the exact mechanism through which sleep controls energy expenditure and metabolism is unclear, epidemiological studies have shown a strong association of short sleep duration with lower leptin and higher ghrelin levels (Taheri et al., 2004). Pandit et al. (2017) found that leptin increased energy expenditure (possibly through increased thermogenesis), and grehlin promoted energy conservation. Leptin's proposed effects on thermogenesis would result in more energy being

dissipated through the production of heat, which would increase energy expenditure as the body responds to this energy loss. Thus, due to sleep deprivation, the effects of altered leptin and grehlin levels could have altered energy expenditure between each testing period and between each participant. In the context of our study, it was possible that participants may have gotten more or less sleep before either testing day, which would either result in an increase or decrease of energy efficiency, respectively.

Finally, the difference in energy efficiency between CC and DC conditions may have been more significant if the participants were to exercise for longer. For example, Lutsch et al. (2020) tested participants on 3 separate occasions, each time including a 60-minute treadmill exercise when comparing energy expenditure levels between caffeine and pre-workout conditions. Higher levels of fatigue, resulting from prolonged, low-intensity exercise bouts, may affect energy efficiency by depleting glycogen stores in the muscle. This depletion may cause an increase in oxygen demands to the fatigued muscles because muscle cells can no longer produce enough ATP to maintain the same exercise intensity; this would therefore decrease energy efficiency (Murray & Rosenbloom, 2018). If participants were tested for longer than 30 minutes and reached higher fatigue or exhaustion levels, this may allow for a more accurate comparison of which condition was more effective during exercise in terms of energy efficiency.

5. Conclusions

Our statistical analysis demonstrated that there is not enough evidence to support the initial prediction that caffeine would increase energy efficiency and fat oxidation in participants during low-intensity, steady-state cycling. The results of the analysis concluded there was no statistical difference in energy efficiency between the CC and DC conditions ($n=10$, $p = 0.3625$); similarly, there was no statistical difference in RQ values between the CC and DC conditions ($n=10$, $p=0.8347$). Additionally, self-report questionnaires were used to measure perceived levels of alertness/energy, awakeness, focus, and fatigue, which were used to calculate perceived energy (PE) scores. Participants on the DC condition had higher PE scores, on average, compared to the CC condition. While the results of the study did not support the prediction that caffeine enhances endurance energy performance through increased energy efficiency and increased fat oxidation, it is important to take into consideration other confounding factors that may have affected the results of our study.

6. Appendix

Table 1. *Mean respiratory and energy variable values between Caffeinated and Non-caffeinated participants (n = 10).*

Variable	Condition		*Mean difference*	*P-value*
	Caffeinated	Non-caffeinated		
VO_2 per kg (mL/min/kg)	16.7638 +/- 2.860	17.0450 +/- 2.038	-0.281 [CI: -1.19– 0.629]	0.5024
VCO_2 per kg (mL/min/kg)	15.49566 +/- 2.990	15.49566 +/- 2.309	-0.287 [CI: -1.03– 0.457]	0.4057
Respiratory exchange ratio	0.9045 +/- 0.048	0.9070 +/- 0.038	-0.003 [CI: -0.0288–0.0238]	0.8347
Energy expenditure (J/s)	404.0690 +/- 70.680	411.0213 +/- 51.340	-6.952 [CI: -28.1 –14.2]	0.4761
Gross energy efficiency (%)	17.8827 +/- 3.632	17.2705 +/- 2.147	0.612 [CI: -0.831–2.06]	0.3625

Table 2. *Perceived energy or PE scores of participants based on self-report questionnaires pre-exercise and post-exercise on caffeine (coffee) and non-caffeine (decaf coffee) conditions (n=10).*

	Caffeine Pre-exercise	Caffeine Post-exercise	Non-caffeine Pre-exercise	Non-caffeine Post-exercise
	10	6	6	5
	10	9	7	9
	9	12	7	10
	9	10	7	10
	12	13	3	-1
	3	4	4	6
	9	9	7	7
	5	1	5	10
	8	8	9	9
Participant 10	6	6	7	9
Group average	**8.1**	**7.8**	**6.2**	**7.4**

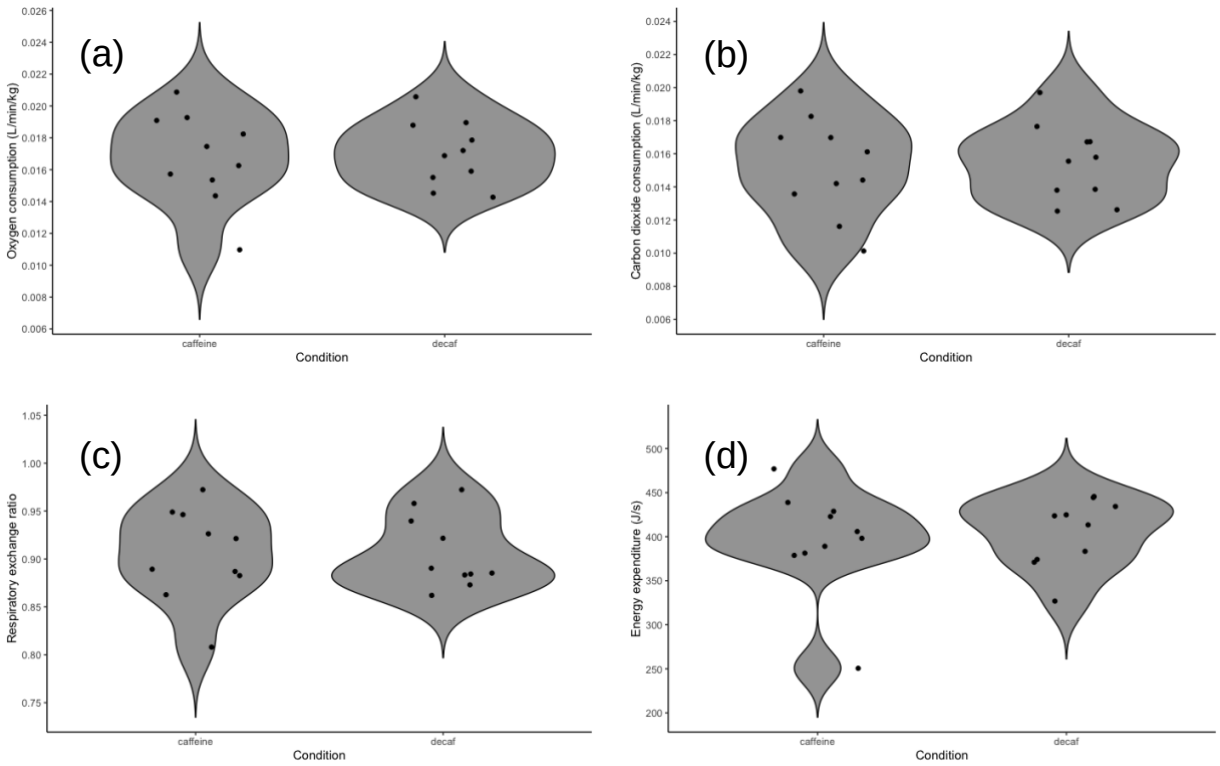

Figure 2. Violin plots of measured and calculated variables compared between caffeinated and non-caffeinated conditions.

(a) $\dot{V}O_2$ (L/min/kg) data for caffeine and decaffeinated conditions, (b) $\dot{V}CO_2$ (L/min/kg) data, (c) Respiratory exchange ratio data, (d) calculated energy expenditure (J/s) using modified Weir-equation.

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