

**A comparison of the effects of three different
forms of caffeine supplementation
on 5000-metre running performance**

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submitted in partial fulfilment
of the requirements for the Degree of
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Abstract

Background: Caffeine is a well established ergogenic aid to enhance sporting performance, and there is a variety of commercially available caffeine supplements. However, there is limited research investigating caffeine effects on running performance and comparing different forms of caffeine supplementation.

Aim: The purpose of this research was to compare how a moderate dose of caffeine (3-4.5mg.kg⁻¹ of body mass), administered acutely and in three different supplementation forms (caffeine gum, tablets, and strips), affected 5000-metre track running performance.

Method: Using a randomised, placebo-controlled, crossover design, 10 males (mean \pm SD, age 40 \pm 9 years, body mass 73.4 \pm 10.1kg, height 180.6 \pm 10.2cm), and 4 females (age 40.7 \pm 8.8 years, height 166.7 \pm 6.8cm, body mass 58.5 \pm 2kg), completed five x 5000-metre time trials. Trials were conducted at a self-paced maximal effort and took place on an outdoor 400-metre athletics track. Trials were spread over a 9-week period, separated by no less than 3 days and a maximum of 28 days. After conducting a familiarisation, participants ingested a single dose of caffeine (200mg for body mass <65kg or 300mg for body mass >65kg), 10-15 minutes before each trial, in the form of either caffeine chewing gum (CG), caffeine strips (CS), caffeine tablets (CT), or placebo (P). Performance and physiological measurements collected were total time, 800-metre lap pace, heart rate (HR) and rate of perceived exertion (RPE). On completion of each time trial, participants also provided a urine sample for analysis of caffeine and paraxanthine metabolites.

Results: In comparison with P, caffeine supplementation improved running performance for CT = 2.0% \pm 1.1%, CG= 0.9 \pm 1.4% and CS= 1.2 \pm 1.0 % (95% CL), with only CT significant (p=0.02). Overall pace trended faster in all caffeine trials compared with P and was significant in CT at 2600m-3400m (p=0.013) and 3400m-4200m (p=0.015). Acute caffeine supplementation did not affect HR or RPE. There was no relationship between the urinary concentration content of caffeine, paraxanthine, and associated metabolic ratio and running performance.

Conclusion: This study concludes that 3-4.5mg kg⁻¹ body mass of caffeine, consumed 10-15 minutes before exercise, can produce small yet significant improvements in 5000-metre running performance when taken in tablet form. However, why this only occurred in the tablet supplement is unclear and requires additional research.

Keywords: caffeine, ergogenic aid, supplements, caffeine gum, caffeine strips, caffeine tablets, running performance, pacing, RPE, caffeine urinary metabolites

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Abbreviations

Bpm: Beats per Minute

CG: Caffeine Gum

CL: Confidence Limit

CNS: Central Nervous System

CS: Caffeine Strips

CT: Caffeine Tablets

CV: Coefficient Variation

HR: Heart Rate

International Society of Sports Nutrition: (ISSN)

MR: Metabolic Ratio

P: Placebo

RPE: Rate of Perceived Exertion

SD: Standard Deviation

Std: Standard

UHPLC: Ultra High-Performance Liquid Chromatography

VO₂ max: Maximal oxygen uptake

WADA: World Anti-Doping Agency

Units of measure

%: percent

µg: micrograms

µL: microliters

µm: micrometre

h: hours

kg: kilograms

kJ: kilojoules

km: kilometres

L: litre

m: meters

mg: milligrams

min: minutes

mL: millilitres

mm: millimetres

°C: degrees Celsius

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Chapter One: Introduction

1.1 Background

Athletes at all levels are often looking for ways in which they can boost performance on race day, including through the use of external products and instruments (Silver, 2001). Products include nutritional supplements such as caffeine, which is a widely used socially acceptable legal drug most commonly found in coffee (Ferré, 2013). The numerous proposed physiological effects associated with acute caffeine consumption include increased central nervous system (CNS) activity, enhanced muscle activation, and increasing pain threshold (Burke, 2012). These mechanisms form the basis for caffeine use in sport, and there is a significant amount of literature determining how caffeine is best used to enhance performance (Ganio, Klau, Casa, Armstrong, & Maresh, 2009). The majority of research investigating the effects of caffeine on repetitive sprints, maximal strength, and time trial performance, have highlighted small yet substantial gains in performance (Burke, Desbrow, & Spriet, 2013). Sports science and nutrition has progressed from consuming quantities of strong coffee before the competition. Nowadays, many products have been developed to contain a highly concentrated dose of caffeine such as beverages, gum, strips, tablets, and carbohydrate-based gels and chews (Spriet, 2014).

1.2 Thesis rationale and significance

Caffeine supplements that are commercially available and accessible to consumers are aimed to be a convenient way for athletes to consume caffeine, through increased metabolism, absorption and bioavailability (Wickham & Spriet, 2018). However, current research has predominantly tested the effects of acute caffeine consumption in a laboratory (Ganio et al., 2009), limiting applicability to a real-world setting. Additionally, much of the research investigating caffeine supplementation is an isolated comparison with a placebo, rather than a cross-comparison of different commercially-available caffeine products (Wickham & Spriet, 2018). There is also a lack of research investigating how caffeine impacts an athlete's pacing strategy, with most research only analysing time to complete the task or time to exhaustion. With an array of products available for consumer purchase, further research is needed to understand which products are most beneficial for athletic performance, and how different forms affect the mechanism and timing of metabolism.

1.3 Research aims

On the basis that acute caffeine consumption can positively increase exercise performance, this research aims to:

- 1) Compare the effects of different forms of commercially available caffeine supplements on 5000-metre running performance, in a field setting.

Chapter Two: Literature Review

2.1 Preface

Caffeine (1,3,7-trimethylxanthine) is globally the most widely consumed of all psychoactive drugs (Ferré, 2013). Caffeine is an ergogenic aid which is any technique or substance, including nutritional, pharmacologic, physiologic or psychologic, used for performance enhancement (Thein, Thein, & Landry, 1995). Ergogenic aids can be legal or illegal in competition, and caffeine was removed from the World Anti-Doping Agency (WADA) list of prohibited substances in 2004. The change in legislation has enabled athletes to consume this substance freely to enhance performance, without the risk of prosecution or disqualification (World Anti-Doping Agency, 2004). As caffeine is now legal, Chester and Wojek (2008) highlighted the widespread use of caffeine in the sport. Their survey of approximately 500 elite and sub-elite British cyclists and track and field athletes indicated that 60% of cyclists and 32% of those competing in track and field consumed caffeine for ergogenic benefits. Also, Desbrow and Leveritt (2007) found 89% of the 124 athletes surveyed at the 2005 Ironman Triathlon World Championships regularly used caffeine during training and racing. The authors found athletes used caffeine in a variety of products, including tablets (CT), carbohydrate-based gels and beverages, to alter perceptions of speed and concentration. These studies highlight caffeine is an accessible tool for athletes, raising questions of how caffeine is best used and the most effective delivery mode to maximise performance.

2.2 Physiological effects of caffeine

Caffeine has many proposed physiological effects in the body. The mechanisms impacting exercise performance are likely to be a combination of stimulating the central nervous system (CNS), increased adrenaline release which enhances pain tolerance, and direct effects on muscle recruitment and contractility (Burke, 2012). These effects are the result of caffeine blocking the receptors for adenosine that serve as a neuromodulator function to calm the brain and spinal cord neurons (Davis et al., 2003). The result is a positive effect on neuromuscular activity, by reducing the threshold for motor unit recruitment, altering excitation-contraction coupling, facilitation of nerve transmission, and increased calcium release in the muscle (Burke et al., 2013). These concepts are supported by Graham (2001) who also suggests that caffeine may create a more favourable intracellular ionic environment in active muscles, which facilitates production in each motor unit. McArdle, Katch, and Katch

(2010) also suggest that caffeine metabolites readily cross the blood-brain barrier to produce analgesic effects on the central nervous system, reducing perceived effort during exercise.

Caffeine is 99% completely absorbed within 45 minutes of consumption. When consumed in beverages such as coffee and tea or soft drinks, it is absorbed through the gastrointestinal tract of the mouth, stomach, small intestine, and liver and distributed throughout body water (Institute of Medicine (US) Committee on Military Nutrition Research, 2001). Further research has indicated that after oral ingestion of caffeine, peak plasma caffeine concentrations occur within in 30-60 minutes (Naderi, de Oliveira, Ziegenfuss, & Willems, 2016); (Sökmen et al., 2008). The wide variation in times may be due to the individual differences in gastric emptying time and the presence of other dietary constituents such as fibre (Sökmen et al., 2008). The by-products of caffeine breakdown appear within minutes of consumption and into primary metabolites of paraxanthine (85% of caffeine), theobromine (10%), and theophylline (5%) (Burke et al., 2013). Paraxanthine is the preferential pathway for caffeine metabolism and exhibits similar pharmacological and ergogenic properties, including stimulation of the CNS, increased plasma epinephrine, and increased blood pressure (National Center for Biotechnology Information, 2018). Additionally, paraxanthine is responsible for the enhanced transport of potassium ions in skeletal muscle. Research has shown that consumption of caffeine before and during exercise results in an increase in paraxanthine concentration levels post exercise in urine (Del Coso et al., 2012) and plasma (Conway, Orr, & Stannard, 2003). Sökmen et al. (2008) details the half-life of caffeine, which is the time required for caffeine to reduce half its initial value, is around 4-5 hours and may increase when the dose exceeds 300mg or vary between acute and chronic users. This understanding of the physiological effects of caffeine consumption highlights the use of caffeine as an ergogenic aid to enhance exercise performance. Furthermore, research investigating how to produce and maximise these ergogenic effects has been conducted by exploring modes of caffeine delivery, dose size and timing of consumption.

2.3 Caffeine use for exercise performance

A large quantity of research has investigated how caffeine can be used to enhance exercise performance, the required dose, and optimal timing of consumption. The majority of findings are that acute caffeine consumption can result in small yet significant enhancements in performance. Ganio et al. (2009) conducted a meta-analysis and systematic review

investigating acute caffeine ingestion in swimming, cycling, running, and rowing time trial performance > 5-minutes. This analysis of 21 studies determined that in comparison with placebo (P), consumption of caffeine can result in a 3.2-4.3% performance improvement. However, a wide variation between studies (-0.3% to 17.3%) was noted. The authors suggested this variance is due to differences in ingestion timing, ingestion mode, and subject habituation. Also, 12 of the studies had exercise protocols \geq 60 minutes, which raises the need for research focused on shorter duration activities (\leq 30 minutes), where exercise intensity is higher. The review conducted by Ganio et al. (2009) stipulated that the optimal amount of caffeine to consume was 3-6mg.kg⁻¹ of body mass, and higher doses do not elicit more significant results. The findings of the study by Ganio et al. (2009) are consistent with the International Society of Sports Nutrition's (ISSN) position on caffeine (Goldstein et al., 2010). The ISSN acknowledges consistent research findings support the use of caffeine as an ergogenic aid in submaximal exercise, time trial performance, and high-intensity intermittent team sports (Goldstein et al., 2010). Additionally, Goldstein et al. (2010) highlight the ergogenicity of caffeine is more significant when consumed in an anhydrous state as opposed to consumption in coffee. Most importantly, Goldstein et al. (2010) confirm that caffeine consumption does not result in induced diuresis during exercise or any harmful change in fluid balance that may negatively affect performance. Most studies conducted on humans have indicated that moderate levels of caffeine consumption, below 400mg/day, pose no significant health risks (Heckman, Weil, & De Mejia Elvira, 2010). Furthermore, Food Standards Australia and New Zealand suggest a limit of 500mg of caffeine per day, as higher intakes may increase anxiety, and impair sleep and fine motor control (Smith, Smith, Miners, McNeil, & Proudfoot, 2000).

Caffeine has been shown to be an effective ergogenic aid in high-intensity aerobic activities less than 30 minutes, where athletes are working at near maximal aerobic capacity for the entire exercise duration. Astorino, Cottrell, Talhami Lozano, Aburto-Pratt, and Duhon (2012) investigated the effects of caffeine on trained versus active but not endurance-trained subjects in short duration (<20minutes) high-intensity cycling performance. Sixteen subjects in total (8 in each group) conducted three separate 10km time trials, 1 P and 2 trials where 5mg.kg⁻¹ body mass caffeine ingested in a drink form 60 minutes before completing each trial. The authors found that in comparison to a P treatment, trained men improved performance by 1.6% and 2%, in both caffeine time trials, while active men improved their performance by 0.3% and 1%. In comparison, a study by Jenkins, Trilk, Singhal, O'Connor, and Cureton (2008) involved administering 1, 2, and 3mg.kg⁻¹ body mass of caffeine, or P to 13 participants, 60 minutes

before exercise. The test protocol consisted of 15 minutes of cycling at 80% of their maximal aerobic capacity ($\text{VO}_2 \text{ max}$), then after 4 minutes active recovery, 15 minutes at VO_2 peak performance. Work output (kJ/kg) was used to measure performance. Compared with P, the authors found performance increased by 4% (95% CI: 1.0-6.8%, $p=0.02$) and 3% (95% CI: -0.4% to 6.8%, $p=0.077$), in caffeine doses of $2\text{mg}\cdot\text{kg}^{-1}$ and $3\text{mg}\cdot\text{kg}^{-1}$ body mass, respectively, and no increase in $1\text{mg}\cdot\text{kg}^{-1}$ dose. These studies by Astorino et al. (2012) and Jenkins et al. (2008) highlight that caffeine is an effective method for increasing performance in short duration high-intensity exercise, yet the optimal dose size is not clear. In addition to these findings, both Astorino et al. (2012) and Jenkins et al. (2008) noted large inter-subject variability, which is a complex issue requiring additional explanation.

2.3.1 Caffeine and running performance

Caffeine has been proven effective in a laboratory setting and on cycling performance, yet there is limited research as to how it impacts on running performance (Schubert & Astorino, 2013). Schubert and Astorino (2013) also highlighted there are considerable physiological differences between running and cycling, indicating a need for more running specific trials to take place in future research. Of the limited research available, Birnbaum and Herbst (2004) found caffeine improved respiratory efficiency during submaximal running. In this study, participants consumed $7\text{mg}\cdot\text{kg}^{-1}$ of caffeine before completing a 30-minute run on a treadmill at 70% $\text{VO}_2 \text{ max}$, where. The authors found that there was a significant difference ($p<0.05$) between caffeine and P in tidal volume, alveolar ventilation and rate of perceived exertion. In a field setting investigating effects on running performance, O'Rourke, O'Brien, Knez, and Paton (2008) highlighted acute caffeine consumption can result in small yet substantial improvements. In this study, subjects consumed of $5\text{mg}\cdot\text{kg}^{-1}$ body mass of caffeine in the form of CT before completing a 5km time trial. Compared with P, caffeine trials resulted in a mean decrease in a finish time of 11 seconds, and relative performance gain of 1.1% (CI 0.4-1.6) for well-trained runners, and a reduction by 12 seconds, equivalent to an improvement of 1% (CI 0.2-2%) in recreationally trained runners. This magnitude of performance gain is similar to that found by Bridge and Jones (2006) where 8 male subjects ingested $3\text{mg}\cdot\text{kg}^{-1}$ body mass of caffeine 60 minutes before completing an 8km running time trial in a field setting. Compared with a P and control trial, ingestion of caffeine resulted in a significant ($p<0.5$) mean improvement in performance of 1.2% (95% CI=0.7-1.8%), by decreasing finish time by a mean

of 23.8s (95% CI = 13.1 to 34.5 s). These studies emphasise the ergogenic benefits of caffeine for running performance, and more research will enhance these findings.

An element yet to be explored is how caffeine impacts running pace. Although laboratory conditions enable enhanced control of variables, including atmospheric conditions, and easier monitoring of participants, there are disadvantages. Stevens et al. (2015) indicated that the difficulty of using a motorised treadmill to analyse running performance is that speed is set and maintained until consciously adjusted. A set speed eliminates subconscious changes in pace and disables analysis of pacing strategy. Furthermore, the treadmill speed must be manually adjusted for athletes to demonstrate a fast finish, which is common in a time trial and is a fundamental part of an optimally paced performance. Absolute performance may be hindered if the participant selects the wrong speed. However, field testing provides an accurate analysis of running pace as small changes can occur without conscious knowledge due to fatigue. By researching a field setting, this will be enhanced understanding of the implications of caffeine use in a real-world race environment.

2.3.2 Pain, pacing and caffeine

During exercise, pain and discomfort arising from repeated muscular contractions are used by an athlete to gauge their current exercise intensity. Achieving optimal performance requires an athlete to find an equilibrium of tolerable discomfort and work rate, for the exercise duration (Mauger, 2014). The process is defined as pacing strategy and is the regulation of total energy expenditure during exercise on a moment to moment basis to complete the exercise bout in the minimum time (Baron, Moullan, Deruelle, & Noakes, 2009). By increasing pain tolerance, this may increase work rate and pace able to be achieved during intense exercise. Tarnopolsky (2008) suggests that caffeine is beneficial in intense exercise due to its analgesic properties from increased nerve stimulation and muscle activity. Beedie (2010) highlighted that as pain is highly placebo-responsive, ergogenic aids with analgesic properties, including caffeine, may be favoured by athletes in sports where a prominent level of pain and little relief is experienced, such as prolonged endurance or short and intense activity. Studies by Lynn, Rodgers, and Ranchordas (2016), Birnbaum and Herbst (2004), and Motl, O'Connor, and Dishman (2003) have found caffeine ingestion can result in a reduction in RPE and isolated muscle pain post-exercise. These concepts and findings support the use of caffeine as a means

of increasing pain threshold during exercise and in turn, enhancing exercise performance by enabling a faster pace and exercise intensity to be maintained.

There is limited research as to how caffeine affects pacing strategy. In a 4000m cycling time trial, Santos et al. (2013) noted power output was significantly higher in caffeine trials compared with P ($p < 0.05$) at 1200m, 1400m, 2200m, 2400m and 2600m, increasing overall performance. However, the trials conducted by Santos et al. (2013) were short (<440 seconds), indicating further research is needed to determine how caffeine affects pace during continuous endurance activity. Furthermore, Nummela et al. (2006) analysed the neuromuscular factors in 5km time trial running performance and found a significant correlation between the average velocity and average muscle activity. This study indicated that average muscle activity typically decreased at the 3km mark, resulted in a slowing of pace. Nummela et al. (2006) suggested that neural input is a significant factor in distance running performance and that runners who can keep their level of muscle recruitment at a high level during this critical phase performed better than those whose level of muscle recruitment decreases. As caffeine consumption has consistently been associated with increases in neural stimulation and muscle activity (Burke et al., 2013), (Graham, 2001), (Schneiker, Bishop, Dawson, & Hackett, 2006), these mechanisms in conjunction with an increased pain tolerance may offer an explanation as to how caffeine supplementation can enhance pace during short duration high intensity running.

2.4 Different forms of caffeine supplementation

Caffeine supplements are more advantageous than traditional consumption methods, including coffee or soft drink, as they have a high dose quantity and an enhance absorption metabolism rate (Goldstein et al., 2010). Supplements are portable and easily accessible, making them a practical tool for athletes to use during competition, such as mid-race or between periods of play in team sports. Wickham and Spriet (2018) conducted a comprehensive review of the various forms of caffeine administration and highlighted historical research used caffeine tablets (CT), capsules, and coffee. The authors indicated there is emerging yet limited research on caffeine consumed in bars, gels, chewing gum (CG), energy drinks, mouth rinses and nasal sprays. Limited research into other caffeine forms was also evident in the review by Ganio et al. (2009) as all studies in this review administered caffeine either in beverages or CT, due to the quantity and quality of research, which indicates high

reliability. Studies on caffeine forms such as CG weren't included due to insufficient research, which the authors acknowledge may be advantageous.

Consuming CT still requires digestion through the gut to be metabolised which may have a delay, which has led to the creation of new products, including CG and caffeine strips (CS), which aim to solve this issue. Research by Kamimori et al. (2002) indicated CG has a faster absorption and higher bioavailability than capsules, as absorption occurs via the oral mucosa in the mouth, rather than the liver for CT. Kamimori et al. (2002) compared the metabolism of caffeine via blood plasma and found in 200mg doses, the time to reach caffeine peak was lower in CG when compared with caffeine capsules (1.34 ± 1.6 hours and $2.0 \text{ hours} \pm 1.0$, respectively). However, this research also found that the relative caffeine peak was lower in CG than capsules ($3.70 \text{mg.l}^{-1} \pm 1.49$ and $4.13 \text{mg.l}^{-1} \pm 1.92$, respectively). The absorption rate may be as fast as five minutes to reach the bloodstream in some individuals (Kamimori et al., 2002). In a military setting, CG has been shown to enhance vigilance and marksmanship motor skill control (McLellan et al., 2005), and increase concentration and alertness in sleep deprivation scenarios (Syed, Kamimori, Kelly, & Eddington, 2005). These findings form the basis for the use of CG in athletic performance.

Research on the benefits of CG in exercise is emerging. Paton, Costa, and Guglielmo (2015) found that in comparison to P, $\sim 3\text{-}4 \text{mg.kg}^{-1}$ CG has been shown to improve performance during a simulated 30km cycling race. Subjects conducted a maximal 0.2km effort every 10km. The results of this study were in comparison to P, CG resulted in an increase in mean power over the final 10km ($3.8\% \pm 2.3\%$), and sprint power at the 30km mark ($4.0\% \pm 3.6\%$). Also, the authors noted that the ergogenic properties were evident within 20 minutes of consumption. In a separate study, Paton, Lowe, and Irvine (2010) measured the effectiveness of CG in repeated sprint cycling performance. Participants conducted 4 sets of 5 x 30 seconds sprints on a cycling ergometer and ingested 240mg of caffeine via CG (equivalent to approximately 3mg.kg^{-1} body mass) after the second set of sprints. The findings were that in comparison to a P, caffeine was effective in delaying fatigue and enabled mean power output to be maintained. In addition, CG also has been shown to have a positive effect on running performance. In a study conducted by Lynn et al. (2016) participants consumed 300mg of caffeine via 3 pieces of CG, before completing a 5km time trial. In comparison to a P, CG resulted in a mean reduction in time of 17.28 seconds, and a decreased rate of perceived exertion (RPE) by 1.21. The studies

by Paton et al. (2015), Paton et al. (2010), and Lynn et al. (2016) highlight CG is useful method of caffeine delivery for enhancing performance. However, all these studies have been conducted in isolation against a P, and there was no cross-comparison to other caffeine products. Therefore it is difficult to determine if CG is more effective than a CT form. Furthermore, these studies observed finish time and power output only and did not look at the effect caffeine had on pacing strategy among athletes, and whether the rapid absorption of caffeine influenced the pacing strategy, particularly during the early phases of exercise, due to the proposed increased feelings of alertness.

CS is an emerging product that is designed to dissolve on the tongue for fast absorption mechanism through the same oral mucosa, like CG. For example, caffeine strips marketed under the brand name Revvies Energy Strips (Revvies Energy Strips, Caringbah, NSW, Australia). Launched in Australia and New Zealand in 2014, each strip contains 40mg of caffeine (Revvies, 2018). Revvies Energy Strips meet Informed-Sport program guidelines, which conforms to WADA standards and is therefore safe and legal for athletes to consume in competition (Informed-Sport, 2018). However, to the extent of our knowledge, there is as yet no research conducted on this product.

Other caffeine supplementation methods have been trialled with little apparent effect. These have included a caffeine mouth rinse, where researchers repeatedly exposed participants to caffeine through the buccal cavity for 10 seconds. However, this did not improve cycling performance or have an impact on RPE, HR, oxygen consumption or blood lactate concentration (Doering, Fell, Leveritt, Desbrow, & Shing, 2014). Another product that has been tested is highly concentrated caffeine “energy shots”. A study by Schubert, Astorino, and Azevedo (2013) found no conclusive evidence that these “energy shots” improved 5km running performance in comparison with P. The authors acknowledged the caffeine dose contained in these commercially available energy shots was low at 80mg and 140mg respectively, which is below the dose recommended by Ganio et al. (2009) and Goldstein et al. (2010) required to elicit ergogenic effects. The authors followed the manufacturer's consumption recommendations but suggest this may account for the lack of result.

Wickham and Spriet (2018) highlighted that although there are many products now available on the market, there is a lack of research comparing the effects of different caffeine forms on the same group of participants receiving alternative treatment. In conjunction, Goldstein et al. (2010) highlighted caffeine in various anhydrous forms is effective when ingested 15-30min before exercise. However, these authors noted most research had utilised a protocol where caffeine is ingested 60 minutes before commencing the protocol. A comparison of different products, notably those that are rapidly absorbed such as CG, will determine which supplementation form is most effective in a short time frame. Additionally, a short timeframe between consumption and commencement of exercise will determine how fast the ergogenic properties of different supplements are elicited. The independent effects of caffeine can be verified in CG, CS and CT as they have few other ingredients. In contrast, caffeinated energy gels have a high carbohydrate content which restricts analysis of the isolated effect of caffeine.

Chapter Three: Method

3.1 Design

The Eastern Institute of Technology Research Ethics & Approvals Committee approved this research (Appendix A). The study was a randomised, placebo-controlled, crossover design. All participants completed a series of five 5000-metre running time trials. The first trial was a familiarisation, and in the subsequent four intervention trials, participants were administered a different form of caffeine supplementation or placebo before the time trial commenced. Trials took place at the same time of day (7:30-9:00 am) for all participants and were spread over a 9-week period, separated by a minimum of 3 days and a maximum of 28 days. All trials took place on an outdoor 400-meter running track. The facility used met the international Tier 1 standard for athletics (Sports Park Hawke's Bay, 2018), and has grandstand seating and a raised stop bank around the perimeter to provide shelter from the wind.

3.2 Participants

16 healthy participants' (males $n = 11$), (females $n = 5$) volunteered to participate in this study. Participants were informed of study requirements (Appendix B), provided their written informed consent (Appendix C) and completed a Pre-Exercise Screening form (Appendix D), to ensure they were injury free before commencing trials. Any participants who had notable medical conditions were asked to seek their doctor's permission before participating in the research. Two participants (1 male and 1 female) withdrew from the study and were not included in data analyses. The participants ($n = 14$) who completed all 5 trials mean (\pm SD) physical characteristics of age, body mass and weight were: males ($n = 10$) 40 ± 9 years, 73.4 ± 10.1 kg, 180.6 ± 10.2 cm, and females ($n = 4$) 40.7 ± 8.8 years, 166.7 ± 6.8 cm, 58.5 ± 2 kg, respectively.

Before trials commenced, all participants indicated their current training history and regular caffeine use (Appendix E). Participants were trained runners, with a self-estimated weekly training volume ranging from 2 to > 7 runs per week, and the mean being 3-4 runs per week. Additionally, many participants also engaged in cycling, kayaking, strength training and swimming. Total aerobic training time was estimated to be between 3 to >10 hours per week, with the mean being 5-6 hours per week. 10 participants identified themselves as habitual

caffeine users (consuming coffee, tea, or soft drinks such as Coca-Cola daily), while the other 4 identified as non-habitual caffeine users. All participants were non-smokers. Participants were asked to refrain from strenuous exercise and caffeine consumption for 48 hours before each trial, including the familiarisation, which is recommended to obtain the highest possible ergogenic effect from caffeine (Addicott & Laurienti, 2009). Specific products to avoid included coffee, tea, soft drinks, energy drinks, chocolate, and any medications that contained caffeine, including allergy medications and some paracetamol tablets. In prolonged periods where trials are unable to be conducted due to unavailability, participants were asked to maintain their regular training volume.

3.3 Supplementation forms

Supplement administration was randomised using a 4 x 4 Latin Square model (Giesbrecht & Gumpertz, 2004) (for supplementation administration order see Appendix F). The supplementation forms each participant consumed throughout this study were:

- a) Military Energy caffeine chewing gum (CG), 100mg of caffeine per piece (Military Energy Gum, Spearmint Flavour, Marketright Inc. USA)
- b) Revvies Energy Caffeine strips (CS), 40mg of caffeine per strip (Revvies Energy Strips, Arctic Charge Flavour, Caringbah, NSW, Australia)
- c) NoDoz tablets (CT), 100mg of caffeine per tablet (NoDoz, Cedar Rapids, IA, USA)
- d) Placebo (P), ~300mg glucose powder in a gelatin capsule

Caffeine was administered as an absolute dose of 200mg (equating to 2 x CG, 2 x CT, or 5 x CS) for participants weighing under 65kg body mass (females n = 4, males n = 2), and 300mg (equating to 3 x CG, 3 x CT, or 7.5 x CS) for participants over 65kg body mass (males n = 8). The caffeine dose equated to 3-4.5mg.kg⁻¹ body mass for all participants. The presence of P was not disclosed before the study and was administered as a single capsule to all participants.

3.4 Test protocol

Upon assembly, participants conducted a prescribed standardised warm-up for approximately 20-25 minutes. The warm-up consisted of low to moderate intensity running, dynamic stretching, speed mechanics (including heel flicks and skipping), and gradually accelerated sprints. Excluding the familiarisation, supplement administration occurred during the warm-

up, a period of 10-15 minutes before the commencement of each time trial. CT and P were swallowed, CS sucked until dissolved, and CG was chewed for approximately 5 minutes then discarded into a container. In all trials, participants were started individually at 1-minute intervals and completed the 5000m running time trial (consisting of 12.5 laps of the track), at a self-paced maximal effort. Participants ran in lane 1 for the entirety of the time trial unless they were required to overtake anyone.

3.5 Data Collection

3.5.1 Finish time and pace recording

Timing was conducted using Webscorer timing software (Webscorer Inc, Woodinville, WA, USA), loaded onto a portable tablet device. Webscorer software provided manual timing to the nearest second, lap recording and reporting of results. The software also enabled preprogramming of participant start lists and individual countdown times. Lap times taken were the first 200 meters then every 400 meters afterwards, with 13 laps in total recorded.

3.5.2 Heart Rate and RPE

Participants wore a Garmin 920 XT heart rate monitor and chest strap (Garmin International, Olathe, KS, USA), for the duration of each trial. Within 5 minutes of completing each trial, participants were asked to estimate their rate of perception of exertion (RPE), with respect to the entire time trial, using the Borg 6-20 RPE scale (Borg, 1982).

3.5.3 Weather recording

Weather details, including temperature and humidity, was recorded at the start of each trial using a TESA Pro WS1151 wireless weather station (Fine Offset Electronics, Shenzhen, China). The recording was obtained from the same point at each trial, approximately 1 meter from the finish line on the infield of the track.

3.5.4 Urine samples and analysis of urinary caffeine and metabolites

All participants provided a urine sample within 30 minutes of completing each trial. Samples were collected in an individually pre-assigned LabServ 70mL polypropylene airtight container

(ThermoFisher Scientific, Auckland, New Zealand). These samples were transported in a 23L temperature-controlled container (Rubbermaid, Atlanta, GA, USA) and stored in a fridge at 4°C (± 2 °C). Urine samples were prepared within 72 hours of collection using the protocol outlined by Furge and Fletke (2007). Briefly, a 2.5mL aliquot was adjusted to pH 3.1-3.3, by addition of HiPerSolv Chromoanorm acetic acid 100% (VMR Chemicals, Radnor, PA, USA, Batch 12D030505). Once achieved, each sample was filtered with a Membrane Solutions cellulose acetate 0.45 μ m syringe filter (Membrane Solutions, Kent, WA, USA), and 1.5mL was transferred into an Interlab V923 clear screw cap glass vial (Interlab, Wellington, New Zealand). Samples were then stored in a Thermo Scientific Revco Value Plus freezer set to -80°C (Cole-Parmer, Vernon Hills, IL, USA), and stored until completion of all trials before analysis in one batch.

Prepared samples were analysed using a Shimadzu Nexera X2 LC-30AD Ultra-High-Performance Liquid Chromatography (UHPLC) system, and chromatograms were visualised using Shimadzu LabSolutions Software (Shimadzu Europa GmbH, Duisburg, Germany). Reversed-phase separation was performed, using a GraceSmart RP18 250mm x 4.6mm 5 μ m column (Fisher Scientific, Loughborough, England, Lot 60/121). The mobile phase consisted of a 90:10 ammonium acetate/acetonitrile solution and had a flow rate of 1.0mL.min⁻¹. The temperatures of the UHPLC autosampler chamber and column oven were set to 4°C and 40°C, respectively, for the duration of the runtime. Sample and standard injections were 20 μ L. The average retention time for paraxanthine and caffeine was determined to be 2.5 min and 4.1 min, respectively.

External standards of ReagentPlus C0750 caffeine (Sigma-Aldrich, Auckland, New Zealand, Lot BCBS9512V), and D5385 Sigma 1,7-dimethylxanthine paraxanthine (Sigma-Aldrich, Auckland, New Zealand, Lot 06740) were analysed to determine retention times and concentrations. A stock standard solution of each metabolite was made as follows. For caffeine, 100 mg of the standard was weighed and dissolved in the mobile phase in a 100 mL volumetric flask to achieve a final concentration of 1 mg.mL⁻¹. This stock standard was diluted 1:10 with the mobile phase and then serially diluted to create 5 caffeine standards between 0.1000 and 0.0063 mg.mL⁻¹. For paraxanthine, 50 mg of the standard was weighed and dissolved in the mobile phase in a 100 mL volumetric flask to achieve a final concentration of 0.5 mg.mL⁻¹. This stock standard was diluted 1:10 with the mobile phase and then serially diluted to create 5 paraxanthine standards between 0.0250 and 0.0016 mg.mL⁻¹. For standard concentration, analysis and figures, see Appendix H.

3.6 Statistical analysis methods

Simple group statistics are shown as means \pm between supplement-standard deviations. The magnitude of the effect of caffeine on performance and physiological measures were determined using a made for purpose Excel spreadsheet (Hopkins (2017)), in accordance with recommendations by Batterham and Hopkins (2006). The mean effect of caffeine and its confidence limits (CL) of 95% was estimated using the unequal-variance, *t*-statistic computed for change scores between placebo and caffeine supplements across all participants. To control for difference in ability between participants, scores were expressed as a percent of placebo score via analysis of log-transformed values, to reduce any bias arising from non-uniformity of error. The magnitude of standardised effect was interpreted and reported using Cohen effect size thresholds (D) of 0, 0.2, 0.5, and 0.8 for trivial, small, medium and large respectively, with recommendations and guidelines of Cohen (1988). The error of measurement, calculated as the difference between familiarisation and placebo trial, is expressed as a coefficient variation (CV).

Additionally, using GraphPad Prism version 4.00 (GraphPad, San Deigo, CA, USA), a one-way repeated measure analysis of variance (ANOVA), followed by Tukey's post-hoc analysis was conducted to compare differences between all caffeine and placebo supplements on performance and physiological measures. Differences were considered significant at alpha $p < 0.05$.

Running pace analysed by grouping the time trial into six equal segments of 800m after the first 200m. The paraxanthine and caffeine standard curves were calculated with linear regression (see Appendix H). Peak height was used to assess concentration [Height x slope] correct for y-intercept. The metabolic ratio comparing the concentration of paraxanthine to caffeine in individual samples was calculated using the relationship $MR = \frac{[\text{paraxanthine}]}{[\text{caffeine}]}$ described by Furge and Fletke (2007). Furthermore, all figures were created using GraphPad Prism version 4.00 (GraphPad, San Deigo, CA, USA).

Chapter Four: Results

CG, CS and CT all decreased mean finish time in comparison with P, with CT eliciting the fastest mean finish time of 1219s \pm 172s (see *Table 1*). There was no significant difference in HR or RPE between supplements. (See Appendix G for raw data).

Table 1: Overall group mean time, HR, and RPE, for each 5000m time trial

	Time (s)	HR (bpm)	RPE
Familiarisation (F)	1246 \pm 192	163 \pm 13	16.6 \pm 0.8
Placebo (P)	1245 \pm 192	164 \pm 11	16.0 \pm 0.8
Gum (CG)	1231 \pm 162	165 \pm 12	16.3 \pm 1
Strips (CS)	1229 \pm 179	166 \pm 11	15.8 \pm 2
Tablets (CT)	1219 \pm 172	165 \pm 12	16.1 \pm 1

Mean finish time compared with P decreased by $-0.9\% \pm 1.4\%$, $-1.2\% \pm 1.0\%$, and $-2.0\% \pm 1.1\%$ for CG, CS and CT, respectively (see *Table 2*). A significant improvement ($p=0.02$) was only between the pairing CT and P. All effect sizes were deemed trivial ($D<0.02$)

Table 2: Differences in mean finish time between supplements

Pairwise supplement comparison	Mean % (\pm 95% CL) difference between supplements	p value	Cohen effect size (D)
CG- P	-0.9 ± 1.4	>0.05	0.06
CS – P	-1.2 ± 1.0	>0.05	0.08
CT – P	-2.0 ± 1.1	0.02*	0.13
CS – CG	-0.3 ± 1.2	>0.05	0.02
CG - CT	1.1 ± 1.2	>0.05	0.07
CS - CT	0.8 ± 1.6	>0.05	0.05

*significant at $p<0.05$

Caffeine supplementation in at least one form had a positive effect for all participants in comparison with P (see *Figure 1*). In comparison with P, taking into consideration intra- and inter-participant variability, overall mean finish time was fastest in CT.

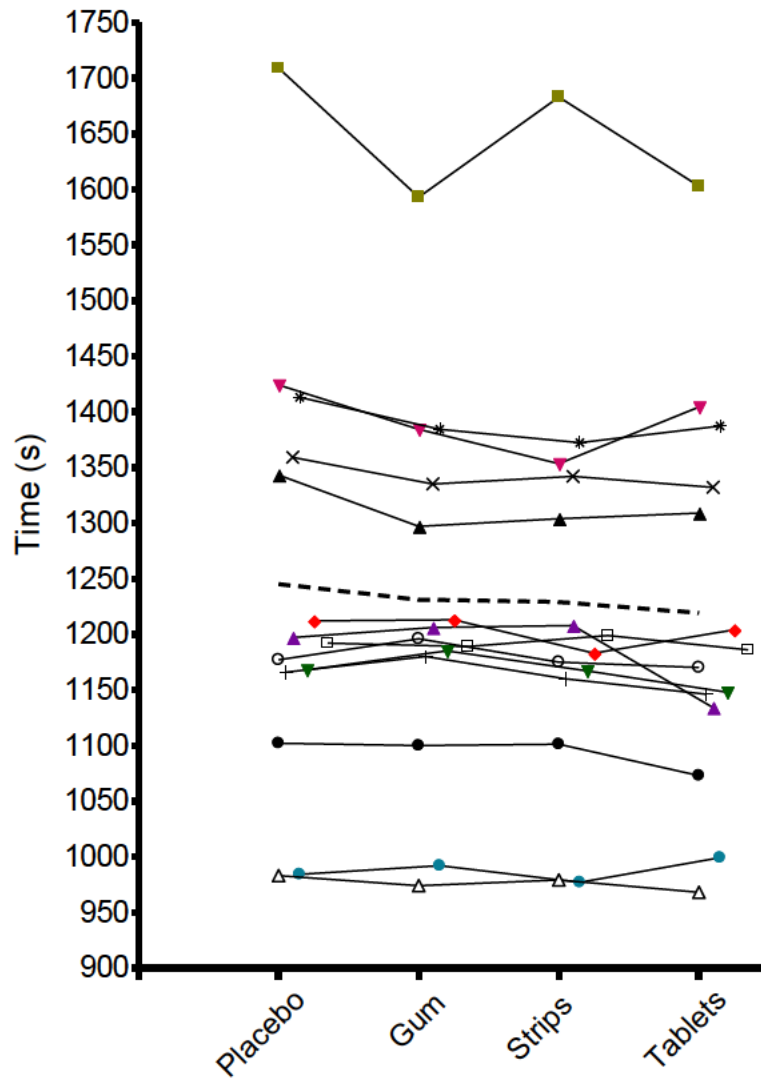
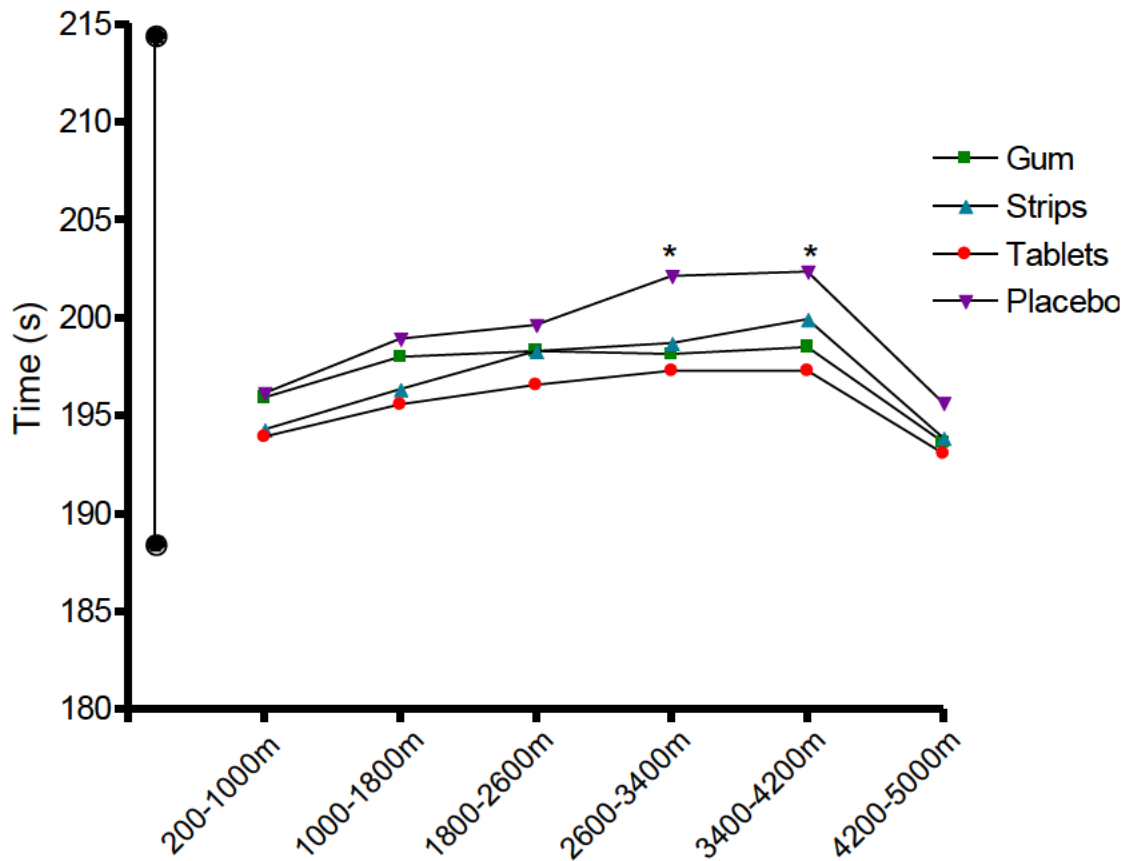


Figure 1: Individual participant (n = 14) finish time for three different forms of caffeine supplementation and placebo. Note: The black dashed line represents the overall group mean time for each supplement.

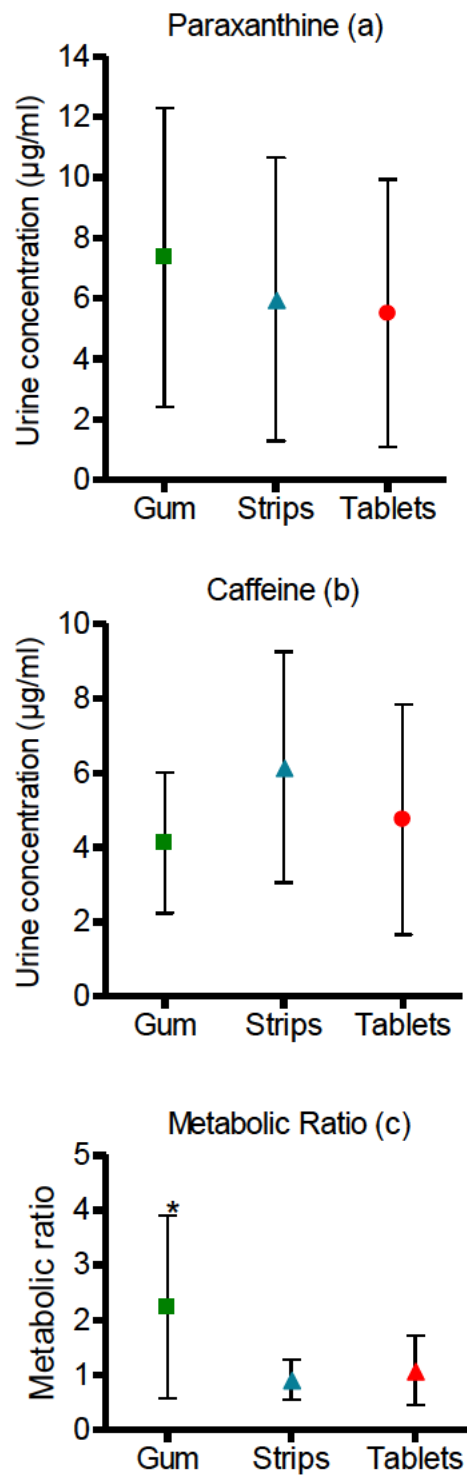
Compared with P, overall pace trended faster in all caffeine trials (see *Figure 2*). Significant differences at 2600m-3400m ($p=0.013$) and 3400m-4200m ($p=0.015$) were observed in CT compared with P, with mean % increase in pace equivalent to $2.3\% \pm 1.3\%$ and $2.4\% \pm 1.4\%$, respectively. There was no difference in pace between trials over the first 200m.



*significant at $p < 0.05$

Figure 2: Comparison of group mean pace for each caffeine supplement. Note: The black vertical line aligned with the vertical axis represents the mean between-subject standard deviation (± 27) on all laps observed.

Urine concentration of paraxanthine (a), caffeine (b) and the metabolic ratio (c) are indicated in Figure 3. There was no level of significance or relationship observed in urine paraxanthine or caffeine concentration. The differences in metabolic ratio were significant in CG compared with both CS and P ($p=0.004$).



*significant at $p < 0.05$

Figure 3: Comparison of group mean \pm urinary paraxanthine and caffeine concentration and associated metabolic ratio for each caffeine supplement

Chapter Five: Discussion

This research aimed to compare the effects of caffeine gum (CG), caffeine strips (CS), and caffeine tablets (CT) on 5000m running performance. The major finding of this study is that compared with placebo (P), only CT resulted in a significant improvement in performance ($p=0.02$). Additionally, running pace determined by the mean time of measured 800m segments was significantly faster in CT compared with P at 2600m-3400m ($p=0.013$) and 3400m-4200m ($p=0.015$). Caffeine supplementation had no significant effect on RPE or HR, and there was no discernible relationship between urinary caffeine and metabolite concentration and running performance.

5.1 Effects of caffeine supplementation on running performance

Compared with P, only CT elicited a significant improvement ($p=0.02$), with a $2.0\% \pm 1.1\%$ decrease in performance time. Improvements in performance following CG and CS were similar and somewhat smaller at $0.9\% \pm 1.4\%$ and $1.2\% \pm 1\%$ respectively ($p>0.5$). The effect size for all forms of caffeine supplementation was however deemed trivial ($D<0.2$). However, the magnitude of improvement for all caffeine products is consistent with research by Hopkins and Hewson (2001), who suggested that the smallest worthwhile change in performance is approximately 1% in events of this distance. Furthermore, this percentage increase in performance is similar to other studies investigating the effects of acute caffeine consumption on running performance. For example, O'Rourke et al. (2008) observed a mean increase in performance of $\sim 1-1.1\%$ over 5km, and Bridge and Jones (2006) found a 1.2% decrease in mean finish time over 8km. Therefore, our findings suggest that although CG, CS, and CT compared with P improved 5km running performance, CT is likely to produce the greatest beneficial improvements.

Due to the wide diversity of participants in our study, there was a notable inter-individual variation in response to caffeine supplementation. The results of our study indicate that performance improvements were higher also in the slower participants, with the bottom 7 participants observing an increase in performance of $2.8\% \pm 2\%$, $2.3\% \pm 2.4\%$ and $2.1\% \pm 1.7\%$ (95% CL), for CT, CG, and CS, respectively. Additionally, only CT compared with P resulted in a small size effect ($D=0.23$). How training status or level of ability affects caffeine ergogenicity is not known. O'Rourke et al. (2008) noted no significant interaction between the effectiveness

of caffeine and training status. In contrast, Astorino et al. (2012) investigated caffeine supplementation in cycling over a similar exercise duration to our study (~1100s). In contrast to our findings these authors found mean performance improvement was greater in endurance-trained men (~1.8%) compared with non-trained but active men (~0.6%). These previous studies coupled with our results highlight that responses to caffeine may be dependent on more than training status and requires further research for clarification.

Pacing is the manipulation of power output over an exercise bout, by balancing energy expenditure and speed in a way that will allow completion of the activity to the best of the individual's capacity (Mauger, Jones, & Williams, 2011). In our study pace trended faster in all caffeine trials compared with P. However, only CT was significantly different at 2600m-3400m ($p=0.013$) and 3400m-4200m ($p=0.015$), with an equivalent improvement in mean time of $2.3\% \pm 1.3\%$ and $2.4\% \pm 1.4\%$, respectively. Overall, all caffeine and P trials observed a parabolic pacing strategy, which is a progressive reduction in speed during the middle of the event, before an increase of speed during the later portion (Abbiss & Laursen, 2008). Caffeine supplementation did not appear to alter participants overall pacing strategy in our study, which is consistent with the findings of Saunders, Farias de Oliveira, Pires da Silva, Painelli, and Gualano (2016). These authors noted caffeine enhanced 16km cycling time trial performance by $3.0\% \pm 5.8\%$ through an increased mean power output while maintaining the same pacing profile. Generally, there are a lack of studies investigating the effects of caffeine supplementation and prolonged adopted pacing strategy. Further research is needed to determine how different supplementation forms can be best employed by athletes to influence pace during critical stages of an event.

The physiological mechanisms by which caffeine enhanced performance are likely via a combination of positive neural and skeletal muscle effects. Researchers including O'Rourke et al. (2008), Schneiker et al. (2006), Kalmar and Cafarelli (2004), and Conway et al. (2003) have suggested that acute caffeine consumption stimulates the central nervous system (CNS), which positively increases muscle activity. The enhanced activity increases the speed of muscle contraction, which can lead to improved performance. O'Rourke et al. (2008) and Lindinger, Graham, and Spriet (1993) have speculated that mechanism in which caffeine effects skeletal muscle is by increasing sodium/potassium (Na^+/K^+) pump activity, enabling more forceful muscle contractions and enhances speed performance, as observed in this study through a

faster finishing time. Additionally, Bridge and Jones (2006) proposed that caffeine increases calcium production and mobilisation in the active skeletal muscle. Calcium activity usually diminishes during prolonged intense exercise due to repeated muscular contractions, resulting in fatigue (Jensen et al., 2007). This coincides with findings by Nummela et al. (2006) that pace typically decreases during a 5000m running time trial due to fatigue, causing muscle activity to decline. Consequently, this enhanced calcium activity may have increased the fatigue threshold and enabled a higher exercise intensity to be maintained (Burke et al., 2013). Furthermore, Paton et al. (2010) suggested that caffeine inhibits adenosine activity in the brain, increasing motor unit recruitment and reducing the perception of effort. A combination of these mechanisms is likely to explain our results. Caffeine supplementation appeared to raise the fatigue threshold and enabled participants to maintain a higher intensity running pace which resulted in a faster finish time. Suggestions by McArdle et al. (2010) and Costill, Dalsky, and Fink (1978) that caffeine increases fatty acid oxidation resulting in superior performance are unlikely, as glycogen stores are not a limiting factor in short duration high-intensity exercise (O'Rourke et al., 2008). As to why only CT was significant, Kamimori et al. (2002) highlighted that although slower to metabolise, capsules had higher relative caffeine peak plasma than CG. A higher plasma value may have also been present in our study, and therefore resulted in greater performance. However, additional research is needed to clarify this.

5.2 Physiological measures

Exercise-induced pain is a strong determinant of endurance performance, as it facilitates awareness of the physiological state of muscle and consequently helps to regulate pace (Mauger, 2014). Our study found no significant difference in RPE from caffeine supplementation. Astorino et al. (2012) and Santos et al. (2013) have similarly not found caffeine to have a significant impact on RPE during maximal exertion. Conversely, a systematic review conducted by Doherty and Smith (2005) exploring the relationship between RPE and caffeine found caffeine reduced RPE during constant a load ($-5.6\% \pm -5.3\%$). However, the authors noted that caffeine did not significantly affect the RPE obtained at the end of strenuous and exhaustive exercise protocols ($0.01\% \pm 4.2\%$). These authors suggested that relative effort and fatigue experienced by an individual during intense exercise is unchanged.

In the present study caffeine increased mean heart rate by a non-significant ~ 1 -3bpm in comparison with placebo. In comparison to other research, low doses of caffeine (1.5 & 3mg.kg⁻¹ body mass), have been found to significantly ($p < 0.05$) reduce heart rate during submaximal exercise by 4-7 bpm in comparison with placebo, but not at near maximal and maximal exercise (McClaran & Wetter, 2007). In conjunction with the perception of effort, Doherty and Smith (2005) suggest limitations of performance during maximal exercise are due to maximal oxygen consumption and maximal heart rate. These factors are not influenced by caffeine and may explain why they were affected.

In our study, there was no obvious relationship between urinary caffeine and paraxanthine concentration, the associated metabolic ratio, and exercise performance. Conway et al. (2003) also determined that in urine samples, the relative contributions of caffeine and paraxanthine to performance enhancement were unclear. Also, research by Del Coso et al. (2012) found that consumption of a caffeinated energy drink before ~ 2 hours of intense exercise increased urinary caffeine and paraxanthine concentration, through analysis of pre and post exercise samples. The authors also noted post-exercise urinary paraxanthine concentration was lower than caffeine concentration. They suggested that because the half-life for caffeine ranges from 2.5 to 10 hours, their exercise protocol of ~ 2 hours was not long enough to produce a transformation of caffeine into paraxanthine. In contrast, a limitation of our study is pre-exercise urine samples were not obtained. Therefore, our analysis of caffeine metabolites relied upon a comparison between supplements and trials. Measuring changes in metabolites pre and post-exercise may have provided a clearer picture as to which supplement had the highest uptake. Also, the time from caffeine consumption until sample collection was within 60 minutes, which may explain as to why there were no significant observations in urinary paraxanthine concentration content.

Researchers including Furge and Fletke (2007) and Weimann, Sabroe, and Poulsen (2005) have deemed urine analysis via UHPLC is a fast, robust, and cost-effective method to analyse caffeine and its metabolites. However, to examine total intake and metabolism, Burke et al. (2013) highlighted urine might not be an appropriate measure. The caffeine in urine has not been metabolised and has been excreted unchanged. Therefore, there may be individual differences in the amount of caffeine that escapes during metabolism. A wide variability of caffeine concentration between participant samples may emerge, as observed in our study. An

alternative and potentially more thorough way of determining caffeine uptake is an analysis of blood plasma, as conducted by Kamimori et al. (2002). However, this was not feasible in this study due to cost.

5.3 Factors influencing performance

The order effect of trials was calculated using the Hopkins (2017) spreadsheet. The change in mean finish time between trials 1-4 (excluding familiarisation) across all participants increased by 0.1%-0.4%. These figures highlight that there was no learning or training effect that may have influenced performance and outweighed the caffeine supplementation effects.

Our experimental protocol was deemed ecologically valid, as 5km time trials have a low intra-subject variability in trained runners (Fisher, Clark, Newman-Judd, Arnold, & Steele, 2017). Therefore, it is suitable for assessing nutritional strategies and ergogenic aids. A test-retest error of measurement of ~1.8% indicates the reliability between trials in our study. However, 2 participants recorded notable differences in times between familiarisation and P of -72 sec and +55 seconds respectively, inflating this margin. In comparison to other running studies, Bridge and Jones (2006) had a test-retest error of 0.6% using a small group of 8 highly trained runners. Using highly trained participants is advantageous as they have lower variability due to more experience with pacing (Stevens & Dascombe, 2015). Also, the study conducted by O'Rourke et al. (2008) had a separate group of 15 recreationally trained participants who had a test-retest error of 1.4%. The reliability may be attributed to grouping a larger number of participants with similar ability together, reducing variation in times. The range of ability and diversity of participants in our study was a limitation, and a higher inter-participant variation was evident. Furthermore, most research is conducted in isolation to placebo. In contrast, our study required multiple trials which enhances complexity and is likely to result in greater intra-participant variation.

There may be several contributing factors that influence the effectiveness of caffeine supplementation among individuals. In our study, caffeine was administered as a set dose of 200mg or 300mg, as opposed to a set relative level, resulting in the dose varying between 3-4.5mg.kg⁻¹ body mass for participants. A set dose is more practical to measure CG, CT and CS as whole or half pieces than to accurately cut and weigh them. Improvements in performance

were higher in those who received a lower dose. In comparative studies, Bridge and Jones (2006), O'Rourke et al. (2008), and Lynn et al. (2016) observed similar improvements, yet administered different caffeine doses of $3\text{mg}\cdot\text{kg}^{-1}$ body mass; $5\text{mg}\cdot\text{kg}^{-1}$ body mass; and 300mg total, respectively. In conjunction, the participants in our study who recorded the most considerable improvements also had the slowest overall mean time, lowest body mass and all 4 were females. The sample size of females is too small to determine if gender influenced responses to caffeine and the diversity of subjects was too broad to determine if training status affected the outcome. Additionally, habitual caffeine consumption did not impact response to caffeine supplementation, which is supported by Gonçalves et al. (2017). Therefore, determining the ergogenicity of caffeine may be a combination of factors such as training status, dose size, habitual caffeine use and gender. Conversely, Pickering and Kiely (2018) suggest the variation of effectiveness among individuals may be due to genetic factors relating to caffeine, which influence caffeine metabolism speed and nervous excitability. These authors proposed the current caffeine consumption guidelines of $3\text{-}6\text{mg}\cdot\text{kg}^{-1}$ body mass (Goldstein et al., 2010), (Ganio et al., 2009), is not optimal for everyone but it is unclear what is. Consequently, future research into individual responses and determinants of ergogenicity is necessary.

In the present study, caffeine was ingested a period of 10-15min before commencing exercise, based on research by Kamimori et al. (2002) that CG is metabolised faster than CT due to rapid absorption through the oral mucosa. In our study, the mean difference in finish time between CS and CG equated to only $-0.3\% \pm 1.2\%$ difference in performance, which highlights their similarities of effectiveness and potentially metabolism mechanisms. However, as CT was more effective than CG and CS, employing the proposed rapid absorption mechanisms requires refining. Paton et al. (2015) found CG appeared to elicit its ergogenic properties within the first 20 minutes of consumption, yet the most substantial improvements in performance were during the later stages of the exercise bout, which was $\sim 30\text{-}40$ mins after caffeine ingestion. These findings suggest that in our study the duration between caffeine consumption and commencing exercise may not have been optimal for CG and CS due to a short test protocol. Further research investigating the ideal timing of ingestion before and during exercise for these forms of caffeine supplementation is warranted.

Why CT was the most effective supplement in our study may be due to several factors. In conjunction with potential differences in peak plasma values and timing of consumption, product familiarity may have influenced participants performance. Research suggested the pharmacological effects of caffeine and expectation are related, and anticipation of consumption can improve performance (Elliman, Ash, & Green, 2010). CT is more common than CG and CS, highlighted by the pre-participation survey (Appendix E) which found 7 participants commonly consumed a variety of caffeine products, including CT, yet none had used or were familiar with CS or CG. In turn, the expectation of CT may be higher due to the familiarity of the product, resulting in an inadvertent additional placebo effect.

Chapter Six: Conclusions

The purpose of this study was to compare three different forms of caffeine supplementation on 5000m running performance. The main results of this investigation were that in comparison with a placebo, only caffeine tablets resulted in a significant improvement in performance. Caffeine supplementation did not affect heart rate, the perception of effort, and there was no observable relationship between urinary caffeine and metabolite concentration and performance. With caffeine gum and strips designed for rapid absorption, it is unclear why tablets were most effective and the mechanism that caused these results.

6.1 Future research

While there is consistent evidence for the use of caffeine tablets and emerging research on caffeinated chewing gum, there is little research investigating caffeine strips or other forms of delivery. Further study comparing different forms of caffeine supplementation on short exercise duration (≤ 30 minutes), the optimal timing window, and how pacing strategy is affected will provide greater insight as to how to best use caffeine supplements during exercise performance.

To investigate the metabolism of different forms of caffeine supplementation, analysis of blood plasma to determine caffeine peak time and peak max, as demonstrated by Kamimori et al. (2002) is justified. In conjunction, additional research exploring how the link between caffeine response and individual biological factors will enhance understanding of caffeine uptake.

6.2 Limitations

A limitation of this study was a diversity of participants and sample size. There was a broad performance variation between all participants, due to the inability to recruit elite runners. As it was evident performance gain was larger slower participants, a larger sample size of participants with similar ability would enhance the applicability of findings for 'elite' and 'recreational' athletes.

A further limitation of this study is that it was conducted in a field setting where atmospheric conditions are uncontrollable. Across all trials, mean temperature and humidity were $7.3^{\circ}\text{C} \pm 3.7$; $65\% \pm 10.1$, respectively, and there several days in which there was frost on the track or light precipitation. Although variation was small, aspects such as track surface moisture may have had some impact on performance outcome. Also, as participants were started at 1-minute intervals and ran multiple laps, they were able to see other participants on the track, which may have provided some positive external motivation.

Consumption of caffeine was a set absolute dose, as opposed to a set relative level in proportion to individual body mass was a limitation. Although more practical, this variation in dose size may have resulted in differences in performance. Furthermore, apart from asking participants to abstain from caffeine products, for 48 hours before each trial, no dietary control was conducted, such as following food diary or consumption of a standardised meal before each trial. Changes in diet may have had an impact on individual performance.

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Appendices

Appendix A: Research Ethics Approval



Our Ref: PG18/05

23 May 2018

Pete Whalley
Master of Health Science
EIT

Dear Pete

Thank you for your application for your research project *"A comparison of different forms of caffeine supplements on running performance in well trained athletes"*.

The Reviewers commend you for your well written RAD form, in particular good coverage in sections 2.4 and 2.5.

You are requested to amend the Research Consent Form, to also consent to photos; ie. **I consent to my interview/activity being videotaped/audiotaped/photographed.**

I am pleased to inform you that your research application was approved on 21 May 2018.

You are reminded that should the proposal change in any significant way, you must inform the Committee. Please quote the above reference number on all correspondence to the Committee. Please send all correspondence to REACapprovals@eit.ac.nz.

The Committee wishes you well for the project.

Yours sincerely

Jeanette Fifield
Secretary - Research Ethics & Approvals Committee

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Regional Learning Centres: Central Hawke's Bay, Hastings, Maraenui, Ruatoria, Tokomaru Bay, Wairoa

www.eit.ac.nz

Appendix B: Participation information



Information for Research Participants

Date:	16/05/2018
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Project Title:	A comparison of different forms of caffeine supplements on running performance
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To:	Research subjects
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Researcher(s):	Pete Whalley, Dr Carl Paton & Dr Chey Dearing (supervisors)
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Affiliation:	Health and Sport Science, Eastern Institute of Technology
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Description of the research:

The aim of this research is to investigate the effect of different forms of commercially available caffeine supplements on short duration (~25 minutes) high intensity (>80% max heart rate) running performance.

What will participating in the research involve?

<p>Subjects will be required to complete a series of five 5-kilometre running time trials in a 12-week period. Tests will be separated by a minimum of 6 days, and a maximum of 28 days</p> <p>The five tests will consist of: 1 x familiarization to collect a baseline 5km time, then 4 x subsequent tests in which you will consume a different form of supplement prior to completing the time trial.</p> <p>This research will take place at the Hastings Sports Park on the athletics track, on Saturday or Sunday morning. You will be asked to arrive at 7.30am, to allow time for set up and conduct a standardised warm up.</p> <p>10-15 minutes before each test, (excluding the initial familiarization trial), subjects will be randomly assigned a supplement to consume. A different form of supplement will be assigned and consumed before each test.</p> <p>At 8am, the test will commence, with participants starting at 1-minute intervals. You will complete a 5km time trial at maximal effort.</p>

For the duration of the time trial, you will be required to wear a Garmin 920XT wrist-based GPS unit and heart rate chest strap to record data.

Data that will be measured and analysed includes: finish time, pace (by 400-meter splits), heart rate, and rate of perceived exertion (RPE), to determine if caffeine positively or negatively impacts these measurements.

On completion of each test, a urine sample will be collected. This will undergo laboratory analysis, to determine the caffeine content and the metabolites present in your body.

The total time you will be required for each trial will be approximately 90minutes.

Prior to participation in this study, you will be required to complete a survey detailing current training load and racing history. This will also ask you about your caffeine use, both on a daily basis, and during training and racing.

You will be asked to abstain from caffeine intake 48 hours prior to each trial, and to maintain a light-moderate training load in this time also. This is to elicit the greatest effect from caffeine supplementation, and to ensure that residual fatigue does not inhibit your performance.

Photographs of you are likely to be taken of the running trial for presentation purposes only.

What are the benefits and possible risks to you in participating in this research?

Healthy participants are at no greater risk than they would be participating in normal running training or competitions. The risk of injury from a high intensity activity, however this will be mitigated through completing a thorough warm up prior to completing each trial.

The caffeine dosage administered for this research will be 3-4mg/kg of body weight per trial. This is classed as a low to moderate dosage and is deemed safe, in accordance with the NZ Food Standards Authority recommendations.

Benefits of participation in research will provide you with information as to the potential use of caffeine supplementation in your own training and racing to achieve personal best race times,

Your rights:

- You do not have to participate in this research if you do not wish to.
- If you are a student at EIT and decide to take part, you can withdraw from the research at any time and this will not affect treatment or assessment in any courses at EIT.
- Once you have completed the research you have 2 months after all data is collected within which you can withdraw any information collected from you.
- You are welcome to have a support person present (this may be a member of your family/whanau or other person of your choice)
- You may request a summary of the completed research

Confidentiality:

All personal information gained in this study will be considered confidential. Identifiable information about you will not be made available to any other people without written consent. Anonymous group information may be used for publication purpose by researchers, through completion of a Master of Health Science thesis and in peer reviewed journals.

Electronic data will be held only by principal researchers in (password protected files. Hard copies of data will be stored in a locked filing cabinet accessible by the named researchers only. Data will be kept for a minimum of 5 years where data may be disposed of in an irretrievable manner.

Biological samples collected for this research will be stored in air tight, temperature-controlled containers, and stored in a locked and secured storage facility.

If you wish to participate in this research, or if you wish to know more about it, please contact

Contact Person:	Pete Whalley		
EIT School/Section:	Health and Sport Science		
Work phone #		Email address	
Mobile phone #			

Supervisor Name(s): (if applicable)	Carl Paton Chey Dearing		
Work phone #		Email address	cpaton@eit.ac.nz cdearing@eit.ac.nz

Head of School/Manager:	Kirsten Westwood		
Work phone #	06 9748000 ext 5240	Email address	kwestwood@eit.ac.nz

For any queries regarding ethical concerns, please contact:

Chair, Research Approvals Committee, EIT. Ph. 974 8000

This study has been approved by the EIT Research Ethics and Approvals Committee on 23 May 2018 Reference # PG18/05.

Appendix C: Informed consent



Research Consent Form

Project Title: A comparison of different forms of caffeine supplements on running performance in well trained athletes

Researcher(s): Pete Whalley, and supervisors Dr Carl Paton & Dr Chey Dearing

I have read and I understand the Information for Research Participants sheet dated 16/05/2018 for volunteers taking part in this study. I have had the opportunity to discuss this study and am satisfied with the answers I have been given.

I understand I am able to withdraw all of my information until 2 months after test completion.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the testing at any time and this will in no way affect my employment.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.

I have had time to consider whether to take part and know who to contact if I have any questions about the study.

I agree to take part in this research

	Yes	No
I consent to my interview/activity being videotaped/audiotaped/photographed		
I wish to receive a summary of the results		

Signed: _____ Name: _____

I/We as researcher(s) undertake to maintain the confidentiality of information gather during the course of this research.

Signed _____ Dated _____

This study has been approved by the EIT Research Ethics & Approvals Committee, on 23/05/18; Reference #PG18/05

Appendix D: Pre-Exercise Screening Questionnaire



PRE-EXERCISE SCREENING QUESTIONNAIRE

Name		Occupation		
Address		Day Phone		
		Evening Phone		
Contact Person (1)		Contact Person (2)		
Phone		Phone		
Today's Date		Doctor		
DOB	Height	Weight	BIA	
Gender	Age	H/R	B/P	
Please answer the following questions by placing a tick ✓ in the appropriate box.				
Health Status			Yes	No
1 Have you ever had a stroke or heart condition?				
2 Have you ever had high blood pressure?				
3 Have any family members had heart problems before age 60?				
4 Have you experienced chest pain when engaged in physical activity?				
5 Have you experienced chest pain when not engaged in physical activity?				
6 Have you ever had, or do you currently have, high blood cholesterol?				
7 Have you ever suffered from asthma or breathing difficulties?				
8 Have you ever smoked – cigarettes, pipes or cigars?				
9 Are you pregnant or have you been pregnant within the last three months?				
10 Have you been hospitalised within the last six months?				
11 Are you currently taking any medication(s)?				
12 Have you ever had, or do you currently have, diabetes, epilepsy, hernia, dizziness or loss of consciousness?				
13 Have you ever had any disease or injury of the back, joints, bones or muscles that may be aggravated by exercise?				
14 Are you aware of any other health-related issues that may affect your participation in physical exercise?				

Pre-exercise Screening Questionnaire (part two)

Name				
Details of "Yes" answers, medications, possible contraindications to exercise, etc.				
Please answer the following questions by placing a tick ✓ in the appropriate box.				
Exercise Participation	Yes	No		
1 Have you been participating in regular physical activity? If yes, what type?				
2 How would you describe your current physical condition? (Tick ✓ one or more boxes).				
unwell	overweight	unfit	healthy	fit
3 What are your exercise goals? (Tick ✓ one or more boxes).				
fat reduction	improve fitness	maintain fitness	health / wellness	stress reduction
muscle tone	increased mass	sport training	injury prevention	social contact
<ul style="list-style-type: none"> • I HAVE UNDERSTOOD ALL THE QUESTIONS AND HAVE ANSWERED THEM TO THE BEST OF MY KNOWLEDGE. • I CERTIFY THAT I HAVE DISCLOSED FULLY ANY CONDITIONS THAT MAY AFFECT MY PARTICIPATION IN PHYSICAL EXERCISE. 				
Date		Staff Name		
Client Signature		Staff Signature		

Appendix E: Pre-participation questionnaire-Training and caffeine use

Name

Age

Height

Weight

Gender:

Current training history: Number of runs per week (circle answer)

1-2 3-4 5-6 6-7 7 or more

Estimated total aerobic training time per week (hours)

<2 hours per week 3-4 hours 5-6 hours 7-8 hours 9-10 hours >10 hours

Other aerobic activity: detail type and total time per week (swimming, cycling etc)

Race history: 5km PB and approx date

Recent performance history: ie most recent half marathon or 10k and approx date

Do you consume caffeine products regularly?

Yes No

If yes, how many cups of coffee do you usually consume per day?

1-2 3-4 5-6 6<

How many cups of tea do you usually consume per day?

1-2 3-4 5-6 6<

Do you use other caffeine products during your regular day such as Red Bull, V, Coca-Cola etc and how much do you consume?

Do you use caffeine products during training and or races?

Yes No

If yes, what caffeine products do you use during training and races?

Gels Gum Tablets (ie NoDoz) Energy drinks (V, Redbull, Monster etc)

Soft drink (Coca-Cola, Mountain Dew, Pepsi etc) Other...

I consent to participate in this survey: Yes No

Appendix F: Supplement administration order

Participant	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
1		a	b	d	c
2		b	c	a	d
3		c	d	b	a
4		d	a	c	b
5		a	b	d	c
6		b	c	a	d
7		c	d	b	a
8		d	a	c	b
9		a	b	d	c
10		b	c	a	d
11		c	d	b	a
12		d	a	c	b
13		a	b	d	c
14		b	c	a	d

Key:

	Familiarisation
a	Gum
b	Strips
c	Tablet
d	Placebo

Appendix G: Individual participant raw data for all trials

Trial	Subject #	Finish Time	Ave HR	RPE	First 200m	Pace (seconds)					
						2 & 3 (200m-1000m)	4 & 5 (1000m-1800m)	6 & 7 (1800m-2600m)	8 & 9 (2600-3400m)	10 & 11 (3400-4200)	12 & 13 (4200-5000m)
Familiarisation	1	1016	163	15	34	160	167	166	166	164	160
Familiarisation	3	1496	146	17	62	249	246	241	234	231	231
Familiarisation	4	1675	165	15	65	256	257	257	258	272	257
Familiarisation	5	1330	180	16	49	211	213	215	215	216	212
Familiarisation	6	1177	163	16	43	182	189	190	191	191	186
Familiarisation	7	1166	150	17	41	182	189	189	188	186	185
Familiarisation	8	1218	139	17	44	192	194	194	195	197	196
Familiarisation	9	1162	149	17	42	181	189	189	189	186	186
Familiarisation	10	1429	163	17	48	215	232	230	233	235	234
Familiarisation	11	1047	172	17	41	163	164	167	170	174	167
Familiarisation	12	1191	185	18	46	185	188	192	195	198	188
Familiarisation	13	1350	173	17	44	207	217	223	221	223	217
Familiarisation	14	1195	164	16	44	191	195	192	192	192	190
Familiarisation	15	994	175	17	39	158	160	161	160	160	156
Gum	1	992	165	16	33	151	159	163	163	163	161
Gum	3	1384	157	16	54	223	218	218	216	210	202
Gum	4	1593	171	15	61	257	258	255	257	258	246
Gum	5	1335	180	16	51	214	213	216	217	215	212
Gum	6	1185	164	16	43	184	190	192	194	194	188
Gum	7	1206	146	15	43	195	200	195	192	192	188
Gum	8	1213	158	15	44	190	193	194	198	202	193
Gum	9	1180	141	16	43	186	190	191	191	190	189
Gum	10	1384	164	16	50	217	229	222	223	229	225
Gum	11	1100	162	16	48	183	178	179	176	174	164
Gum	12	1189	181	17	47	192	193	190	189	191	188
Gum	13	1297	178	16	46	202	205	211	209	210	213
Gum	14	1196	165	16	44	191	190	193	194	195	190
Gum	15	974	178	18	40	158	156	157	155	156	152
Strips	1	977	164	16	31	151	158	161	162	160	156
Strips	3	1353	149	15	57	224	219	220	216	220	199
Strips	4	1683	175	17	63	253	255	270	275	280	269
Strips	5	1342	178	17	50	208	214	215	218	221	217
Strips	6	1167	166	14	44	181	186	190	192	190	186
Strips	7	1208	157	16	44	202	203	197	191	187	186
Strips	8	1183	163	17	45	186	187	190	189	198	188
Strips	9	1160	145	16	43	184	188	189	186	187	183
Strips	10	1372	167	16	48	210	220	221	222	224	226
Strips	11	1101	164	16	47	180	177	176	177	176	169
Strips	12	1199	180	17	49	192	192	192	193	194	185
Strips	13	1304	178	17	47	204	207	210	211	215	207
Strips	14	1175	167	16	44	187	187	189	192	190	188
Strips	15	979	178	18	39	158	156	156	158	157	155
Tablets	1	999	170	17	31	150	161	166	164	163	163
Tablets	3	1404	144	13	62	238	231	226	226	214	207
Tablets	4	1603	165	13	61	254	256	255	261	262	254
Tablets	5	1332	177	15	48	210	213	216	217	216	211
Tablets	6	1148	167	16	43	175	183	184	188	189	186
Tablets	7	1134	154	17	43	191	196	186	182	181	176
Tablets	8	1204	160	16	50	193	189	192	194	195	191
Tablets	9	1146	141	19	40	181	185	187	185	185	183
Tablets	10	1387	165	18	48	214	220	223	225	230	226
Tablets	11	1073	172	17	41	168	168	173	174	177	172
Tablets	12	1186	182	14	48	192	191	193	191	190	180
Tablets	13	1309	178	16	46	201	201	208	215	220	218
Tablets	14	1170	163	12	46	191	188	190	187	185	183
Tablets	15	968	179	18	41	157	156	153	153	155	153
Placebo Cap	1	984	167	16	32	151	160	161	161	162	157
Placebo Cap	3	1424	146	15	61	239	237	235	223	222	208
Placebo Cap	4	1709	167	18	63	257	257	258	276	274	274
Placebo Cap	5	1359	179	17	49	205	213	218	228	226	222
Placebo Cap	6	1168	168	16	43	180	184	191	194	195	183
Placebo Cap	7	1197	145	14	44	191	196	194	193	192	187
Placebo Cap	8	1212	156	16	46	192	192	193	198	202	191
Placebo Cap	9	1166	144	16	42	182	188	189	189	190	184
Placebo Cap	10	1413	165	17	49	224	226	225	232	231	227
Placebo Cap	11	1102	167	17	45	174	175	177	177	180	173
Placebo Cap	12	1192	173	13	48	200	194	193	192	190	176
Placebo Cap	13	1343	178	17	49	205	215	215	219	223	217
Placebo Cap	14	1177	161	15	45	186	192	189	190	188	184
Placebo Cap	15	983	173	19	40	160	156	157	158	158	156

Appendix H: Urinary caffeine and metabolites analysis

Table 3: Caffeine and paraxanthine standards analysis and linear regression

Standard	Concentration (mg/ml)	Height	Best-fit values	Caffeine std linear regression	Paraxanthine std linear regression
Caffeine Std 2	0.1	962534	Slope	0.0000001040 ± 0.00000000521	0.00000005597 ± 0.00000000171
Caffeine Std 3	0.05	474274	Y-intercept when X=0.0	0.0001718 ± 0.0002579	0.000006885 ± 0.000007849
Caffeine Std 4	0.025	237851	X-intercept when Y=0.0	-1653	-123
Caffeine Std 5	0.0125	121498	1/slope	9619000	17870000
Caffeine Std 6	0.00625	59242	95% Confidence Intervals		
Paraxanthine Std 2	0.005	89302	Slope	0.0000001023 to 0.0000001056	0.00000005543 to 0.00000005651
Paraxanthine Std 3	0.0025	44406	Y-intercept when X=0.0	-0.0006490 to 0.0009926	-0.00001809 to 0.00003186
Paraxanthine Std 4	0.00125	22106	X-intercept when Y=0.0	-9664 to 6169	-573.4 to 320.9
Paraxanthine Std 5	0.000625	10919	r ²	0.9999	1
Paraxanthine Std 6	0.0003125	5735	P value	< 0.0001	< 0.0001

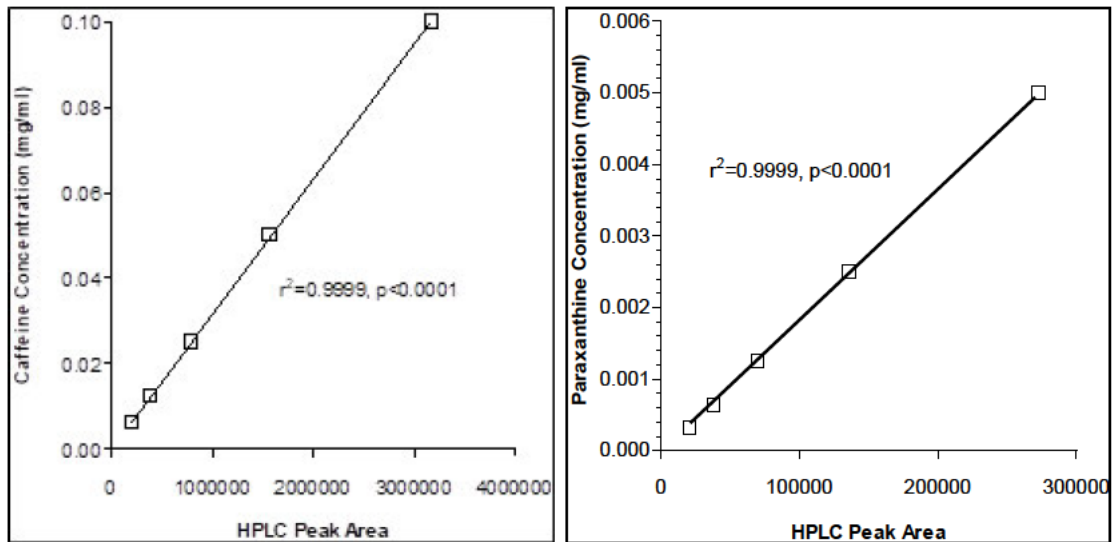


Figure 4: Caffeine (a) and Paraxanthine (b) standard linear regression