What can isolated skeletal muscle experiments tell us about the effects of caffeine on exercise performance?

Tallis, J. , Duncan, M.J. and James, R.S.

Original citation & hyperlink:
Tallis, J. , Duncan, M.J. and James, R.S. (2015) What can isolated skeletal muscle experiments tell us about the effects of caffeine on exercise performance?. British Journal of Pharmacology, volume 172 (15): 3703-3713.
http://dx.doi.org/10.1111/bph.13187

What can isolated skeletal muscle experiments tell us about the effects of caffeine on exercise performance?

Jason Tallis (✉), Michael J. Duncan, and Rob S. James.

Centre for Research in Applied Biological and Exercise Sciences, Coventry University, Coventry, CV1 5FB

Email: tallisj2@uni.coventry.ac.uk

Abstract

Caffeine is an increasingly popular nutritional supplement due to the legal, significant improvements in sporting performance that it has been documented to elicit, with minimal side effects. Therefore, the effects of caffeine on human performance continues to be a popular area of research as we strive to improve our understanding of this drug and make more precise recommendations for its use in sport. Although variation in exercise intensity seems to affect its ergogenic benefits, it is largely considered that caffeine can promote significant improvements in endurance, power and strength based activities. There are a number of limitations to testing caffeine induced effects on human performance that can be better controlled when testing isolated muscle under *in vitro* conditions. The hydrophobic nature of caffeine results in a post digestion distribution to all tissues of the body making it difficult to accurately quantify the key mechanism of action. This review considers the contribution of evidence from isolated muscle studies to our understating of the direct effects of caffeine on muscle during human performance. The body of *in vitro* evidence presented suggests that caffeine can directly potentiate skeletal muscle force, work and power which may be important contributors to the performance enhancing effects seen in humans.

Introduction

Caffeine is the most commonly consumed drug in the world (Graham, 2001) and its ability to induce legal improvements in exercise performance has made it an increasingly popular ergogenic supplement. Mechanistically, the action of caffeine in the whole body is difficult to pinpoint due to the nature of its wide distribution to bodily tissues (Magkos & Kavouras, 2005). It is largely considered that caffeine will act as a central nervous system (CNS) stimulant, however glycogen sparing, increases in fatty acid mobilization, catecholamine release and direct muscle effects, are also reported but debatable mechanisms attributing the ergogenic effect (See reviews by Graham, 2001; Magkos & Kavouras, 2005; Davis & Green, 2009). The usage of *in vitro* experiments to apply caffeine to isolated muscle provides an important method for quantifying the direct effect of caffeine on muscle as a potential mechanism for improvement in sports performance. A number of more recent publications (James *et al.*, 2005; Tallis *et al.*, 2012; 2013b) have used advances in methodology to more accurately examine the direct effect of caffeine on skeletal muscle mechanical performance and as such have significantly contributed to our understanding of the caffeine response. The evidence presented indicates that physiological concentrations of caffeine can directly affect skeletal muscle to cause a significant enhancement in mechanical performance increasing the ability of the muscle to produce force, work and power. Such effects could be used in humans to increase training stimulus and to improve performance in competition.

Caffeine & Sport Performance

It is widely accepted that caffeine ingestion can promote performance enhancing effects on endurance (activity lasting greater than 30 minutes), power and strength activities, although there is debate regarding the magnitude of effect (Graham *et al.*, 2001). Evidence demonstrates that caffeine has greater potency when used as an acute supplement in endurance based activities, whilst results from studies using short term high intensity exercise protocols appear to be a more ambiguous (Graham *et al.*, 2001; Davis & Green, 2009; Goldstein *et al.*, 2009). The effect of caffeine ingestion on sport performance has been extensively explored in a number of reviews (Graham *et al.*, 2001; Burke, 2008; Davis & Green, 2009; Goldstein *et al.*, 2009; Astorino and Roberson, 2010).

Evidence from these articles suggest that mode and intensity of exercise, caffeine consumption habits, fitness level, treatment dose and individual differences in caffeine digestion, distribution and sensitivity could greatly influence the caffeine induced response in human performance (Fig 1). It is likely that the varied caffeine response and conflicting evidence demonstrated throughout the literature can largely be attributed to methodological differences between studies.

[Insert Figure 1 here]

In the most part, the previously cited literature reviews suggest that the performance enhancing effect of caffeine is greater in trained athletes compared to non-trained athletes (Graham, 2001; Astorino and Roberson, 2010). Although there is a distinct dearth of studies directly assessing this, Leblanc (1985) demonstrated that trained individuals had increased resting metabolic rate, adrenaline and free fatty acids

compared to an untrained population. Furthermore, Collomp *et al.,* (1992) reported faster swim speeds in trained athletes that were not paralleled in an untrained group following a 250mg caffeine dose. The mechanism responsible for these response differences is largely unknown, but it is considered that as many experimental procedures require participants to work maximally, trained individuals will have greater motivation to perform fatiguing exercise, will have better nutritional preparation, and the day-to-day performance variation will be reduced (Burke, 2008).

Furthermore evidence suggests a greater ergogenic benefit in non-habituated consumers (Bell & McClellan, 2002). Caffeine is rich in the western diet and recruiting participants that consume similar quantities is near impossible, and in many studies participants are considered as habitual users (Tarnoposlky & Cupido, 2000; Bridge & Jones, 2006; Duncan *et al.,* 2014). Although it appears that the effects of habituation on the magnitude of response needs further investigation (Graham *et al.,* 2001; Astorino *et al.,* 2010), this mechanism is in part attributed to the division of responders and non-responders to caffeine treatment that has been reported in studies examining responses on an individual level (Skinner *et al.,*2009).

Another methodological debate relates to the withdrawal of caffeine prior to completion of the experimental trial. It is common practice for researchers to restrict caffeine consumption 12-48 hours prior to completion of the exercise protocol (Bell & McLellan, 2002; Glaister *et al.,* 2008; Duncan *et al.,* 2014). Although evidence indicates that withdrawal has limited effects on exercise performance, there is a wealth of literature demonstrating negative effects on mood, stress, fatigue, alertness and short term memory (Smith, 2002). James (1994) suggested that caffeine has no behavioural effect, but consumption merely removes negative effects associated with withdrawal.

Although it is common to administer caffeine per unit body mass, a number of studies have used absolute doses (Collomp *et al.,* 1992; Kovacs *et al.,* 1998), thus potentially resulting in erroneous results due to vastly different relative doses between individuals. It is generally considered that $3mg.kg^{-1}$ is the lowest level to elicit ergogenic benefit on exercise performance (Graham *et al.,* 2001), and it is common practice to administer caffeine in doses of 5-6 $mg.kg^{-1}$ (Jackman *et al.,* 1996; Bridge & Jones, 2006; O'Rouke *et al.,* 2006; Carr *et al.,* 2008). Despite research assessing a variety of doses ranging between 0.5-13 $mg.kg^{-1}$ (Wiles *et al.,* 1992; Pasman *et al.,* 1995; Graham & Spriet, 1991; Bruce *et al.,* 2000; Cohen *et al.,* 2006), only a small number of studies have examined the dose response relationship on human performance (Perkins & Williams, 1975; Graham & Spriet, 1995; Cohen *et al.,* 1996; Kovacs *et al.,* 1998; Bruce *et al.,* 2000; O'Connor *et al.,* 2004). Few of these studies actually demonstrate an ergogenic benefit of caffeine (Graham & Spriet, 1995; Kovacs *et al.,* 1998; Bruce *et al.,* 2000; O'Connor *et al.,* 2004), and thus, conclusions regarding dose dependant effects are based on a limited number of studies. It is generally considered that an increased caffeine dose fails to elicit a further response; however contradictory evidence is also presented (Kovacs *et al.,* 1998). It is further considered that inter-individual side effects related to consumption of high caffeine concentrations may actually result in decreased performance (Graham & Spriet, 1995). Although there is some ambiguity in a caffeine dose response relationship, anecdotal evidence suggests caffeine induced dose related relationships in the reduction in pain perception and increased plasma epinephrine and free fatty acid

concentration (Graham & Spriet, 1995; Pasman *et al.,* 1995; O'Connor *et al.,* 2000), which may evoke performance enhancing benefits in other modes of exercise. The variety of methodological approaches and results demonstrated, make meaningful conclusions and recommendations to athletes difficult to fully quantify. Furthermore, there is difficulty isolating the direct effect of caffeine from systematic effects due to the number of potential mechanisms evoked from its wide distribution within the body. It is commonly reported that caffeine acts as a central nervous system stimulant due to its action as an adenosine receptor antagonist (Fredholm et al., 1999). Additionally, the increased effectiveness of caffeine on endurance based sports has led to a common misconception that caffeine may increase the utilisation of free fatty acids as an energy source thus permitting glycogen sparing. The evidence supporting this claim is inconclusive (Graham; 2001; Davis & Green, 2009). The action of caffeine to promote increased adrenaline release, evoke greater Ca^{2+} release from the sarcoplasmic reticulum, improve the function of the Na^{+}/K^{+} pump and reduce pain perception are further mechanisms believed to contribute to caffeine's performance enhancing effect (Graham, 2001; Magkos & Kavouras, 2005; Davis & Green, 2009). Although the effectiveness of caffeine as a performance enhancer is widely reported, the outlined discrepancies have confounded our ability to make an accurate judgement on the specific action of caffeine.

Benefits of Testing the Direct Effect of Caffeine on Isolated Muscle

Many of the aforementioned variables that limit our ability to fully review results from whole body, *in vivo*, testing of the effects of caffeine can be controlled in studies assessing the direct ergogenic effect of caffeine on isolated skeletal muscle. During such *in vitro* studies a target muscle(s) is isolated, usually from a rodent/amphibian, and placed in an organ bath circulated with oxygenated Krebs-Henseleit/Ringer solution, which is high in glucose and contains other salts to mimic blood plasma. Maximal muscle activity is induced by subjecting the muscle to an external electrical stimulation. A caffeine dose is added directly to the Krebs/Ringer solution, and the mechanical performance of the muscle is reexamined. Typical assessments include the measurement of maximal isometric twitch and tetanus force, and associated activation and relaxation times. During isometric studies the muscle is held at a constant length and subjected to a single stimulation (twitch) or multiple stimulations (tetanus) to determine peak force, muscle length is adjusted until maximal force is achieved (Luttgau & Oetliker, 1968; Allen & Westerblad, 1995; Germinario *et al.,* 2004). More recently, the work-loop technique has been implemented as a method of assessing the effects of caffeine on muscle power output during the types of dynamic muscle activity that are more common during *in vivo* muscle action (James *et al.,* 2004; 2005; Tallis *et al.,* 2012; 2013b; 2014b).

Evidence suggests that caffeine metabolism and consequently magnitude of the potential effect may be related to variations in genotype. It has been reported that a single substitution of a gene can cause individuals to be slow or fast caffeine metabolisers (Sokmen *et al.,* 2008). Additionally as caffeine is distributed evenly to all tissues of the body, those with a greater body fat will have a greater adipose tissue concentration, thus reducing the quantity acting at the tissues that can improve sports performance. A direct skeletal muscle caffeine treatment avoids the potential limitations associated with digestion and metabolism, and this method assures that that the same dose reaches each examined tissues.

In human studies it is difficult to isolate factors that result in a direct muscle performance improvement from a muscle performance improvement resulting from central mechanisms. An isolated muscle is externally stimulated and its metabolism controlled, thus it is possible to exclusively examine the skeletal muscle reaction to a caffeine dose. Furthermore, lab animals from which the muscle preparations are taken have a controlled low caffeine diet which reduces the potential issue of habituation and pre-activity withdrawal effects influencing the results. Implementation of such methods within this research area uniquely allow the examination of muscle fiber type specific effects of caffeine treatment, which have been proposed as a mechanistic rational for the increased potency of caffeine in relation to endurance based events. Isolated muscle also allows improved analysis of a dose-response relationship, without the adverse side effects of high caffeine consumption seen during *in vivo* work (Graham & Spriet, 1995). The effect of caffeine on exercise mode can be considered in greater detail *in vitro*, allowing the investigation of maximal and submaximal contraction, fatigue and recovery, using both isometric and dynamic work loop protocols. Such *in vitro* studies have been, and continue to be, vital to improving our understanding of the ergogenic effects of caffeine.

The Effect of mM Concentrations of Caffeine on Skeletal Muscle Contractility

Much of the evidence demonstrating the direct ergogenic properties of caffeine on skeletal muscle is derived from early *in vitro* studies such as Luttgau & Oetliker (1968) who tested millimolar (mM) caffeine concentrations (supraphysiological for humans) on isolated semitendinosus and iliofibularis muscle from *Rana temporaria*. The study concluded that significant increases in twitch force occurred following treatment with 6-10 mM caffeine, with an increased sensitivity to caffeine following a drop in temperature from 20^{o}C to 1-3^{o}C. At high concentrations caffeine has even been shown to produce contracture without stimulation (Huddart, 1968). A number of isolated muscle studies have demonstrated the potentiation of muscle force following a direct treatment with caffeine (Table 1). Furthermore, it is largely accepted that the ergogenic benefit is more pronounced in slow twitch muscle (Rossi *et al.* 2001; Wondmikun *et al,*. 2006; Tallis *et al.*, 2012), and that a reduction in temperature increases sensitivity to caffeine (Luttgau & Oetliker, 1968; Weber & Herz, 1968), particularly in slow twitch muscle (Wondmikun *et al.*, 2006).

[Insert Table 1 here]

Mechanistically caffeine will promote greater force output in skeletal muscle due to modification of excitation contraction coupling (Davis & Green, 2009). Weber & Herz (1968) was one of the earliest studies to investigate this theory by isolating sarcoplasmic reticulum (SR) from skeletal muscle of *Rana pipiens* and monitoring Ca^{2+} release to varying millimolar concentrations of caffeine. Caffeine treatment resulted in an immediate release of Ca^{2+} in 11 of 12 preparations, attributed to a shift in the voltage dependant Ca^{2+} release mechanism to a more negative membrane potential. This was later confirmed by Endo *et al.*, (1970) using skinned muscle preparations with SR left intact. More specifically, it is believed that caffeine operates directly as an adenosine receptor antagonist on A1 receptors on the skeletal muscle membrane and/or binds to Ryanodine receptors (RYR) of the SR as demonstrated *in vitro* with 10mM caffeine treatment and in RYR -/- mice (Damiani *et al.*, 1996; Bhat *et al,*. 1997; Fredholm *et al.*, 1999; Rossi *et al.*, 2001). Ultimately this has been shown

to result in a greater release of Ca^{2+} into the intramuscular space, increased myofibrillar Ca^{2+} sensitivity, slowing of the SR Ca^{2+} pump, and increased SR Ca^{2+} permeability, significantly modifying skeletal muscle performance (Allen *et al.,* 1989; Westerblad & Allen, 1991; Allen & Westerblad, 1995). The consequential decrease in rate of Ca^{2+} efflux from the intracellular space, due to the reduced action of the SR Ca^{2+} pump, is the mechanism underpinning the commonly reported caffeine induced increase in isometric relaxation time (Allen *et al.*, 1989; Westerblad & Allen, 1991).

These studies have proven important in enhancing our understanding of the direct effect of caffeine on isolated muscle performance; problems arise when attempting to link the outcomes of this research to human performance. The authors recognise that although this may not be the primary intention of all of these studies, the underlying mechanism of response to caffeine in human literature is commonly attributed to such research.

A significant limitation in many of these studies is the use of supraphysiological, millimolar, concentrations of caffeine (Luttgau & Oetliker, 1968; Endo *et al.*, 1970; Huddart, 1968; Weber & Herz, 1986; Allen & Westerblad, 1995; Rossi *et al.*, 2001; Germinario *et al.*, 2004) which would be toxic to humans (Fredholm *et al.*, 1999), and as such these studies have poor relevance to the effects of ingested caffeine on human performance. Fredholm et al., (1999) reported that blood plasma concentrations exceeding 1mM would be fatal for humans and common concentrations are usually between 20-50µM (Graham, 2001), with 70µM being the nontoxic limit (Fredholm et al., 1999).

Although it has been demonstrated that caffeine has increased potency at lower temperatures, most previous studies have used test temperatures that have little physiological relevance to humans (Ritchie, 1954; Luttgau & Oetliker, 1968; Weber & Herz, 1986; Fryer & Neering, 1989; Allen & Westerblad, 1995; Rossi *et al.*, 2001; Germinario *et al.*, 2004; Rosser *et al.*, 2009). Lower test temperatures are usually used as a method of reducing the metabolic rate of muscle preparations, subsequently maintaining its functional capacity for a longer duration. Mammals regulate core body temperature such that daily variation is less than $3^{o}C$ in order to maintain homeostatic conditions (Refinetti 1999; Wooden and Walsberg 2004). Although there is some variation in peripheral muscle temperature as a result of ambient conditions and exercise, the relationship between higher skeletal muscle temperature within a physiological range and improved mechanical performance has been well documented (James et al., 2013). It should further be considered that studies using amphibian or insect muscle (Ritchie, 1954; Luttgau & Oetliker 1968; Huddart, 1969; Rosser *et al.*, 2009) may evoke different caffeine response when compared to mammalian muscle.

Evidence in this area, bar the work of James and Tallis, has been gained via isometric testing methods, which although provide important information for assessing the effect of caffeine on maximal force, have poor relevance to *in vivo* power producing muscles (Josephson, 1985; James *et al.*, 1995; James *et al.*, 1996). It is rare for skeletal muscle to be acting completely isometrically with shortening required to perform work and to produce power (Rome, 2002). James *et al.*, (1996) concluded that isometric testing vastly underestimated the *in vivo* rate of force activation and relaxation and is limited by not considering the passive properties of muscle. A muscle cannot shorten indefinitely and will eventually have to re-lengthen. In

addition, locomotion is primarily determined by the ability of certain muscles to produce power (force x velocity), which cannot be estimated via isometric testing (James *et al.*, 1995; 1996).

Recent work by Tallis and James (James *et al.*, 2004; 2005; Tallis *et al.*, 2012; 2013b; 2014a) has addressed these limitations and provides a more accurate assessment of the direct ergogenic effect of caffeine on skeletal muscle that can be more closely related to human performance. In this body of work, caffeine induced changes in muscle power output were quantified using the work loop method as a more realistic estimation of *in vivo* muscle function during power production (Josephson, 1985, James *et al.*, 1995; 1996). As for *in vivo* power producing muscles, the work loop technique considers muscle force production over dynamic contractions accounting for the interaction of force production during shortening, resistance to muscle re-lengthening and changes in activation and relaxation time using length change waveforms and stimulation parameters that more closely replicate those used *in vivo* (Josephson, 1985; James *et al.*, 1995; 1996). More significantly, these studies examine the skeletal muscle response to 70 micromolar (µM) caffeine treatment that represents the likely normal *in vivo* human maximum (Graham, 2001) and is markedly lower than millimolar caffeine concentrations used in previous works. In addition, experiments are carried out on whole mammalian locomotory skeletal muscle at physiologically relevant test temperatures.

The Effect of µM Concentrations of Caffeine on Skeletal Muscle Contractility

James *et al.*, (2004) was the first to examine the direct effect of 70µM caffeine on the mechanical performance of skeletal muscle, reporting no effect on force, work, or power output in fatigued EDL or soleus muscles. In contrast 10mM caffeine treatment evoked greater recovery of fatigued EDL, but a reduction in power output in fatigued soleus, and as such it was considered that caffeine, including when used in human performance, may not significantly affect the contractile performance of fatigued skeletal muscle. The aetiology of skeletal muscle fatigue is complex and a number of interacting mechanisms including a reduction in: SR Ca^{2+} release; sensitivity of the contractile proteins to Ca^{2+}; and SR Ca^{2+} pump function (Allen et al., 2008). The results presented by James *et al*., (2004) infer that the potential effect of a physiologically relevant caffeine concentration to elicit modulation of calcium handling is not great enough to offset the changes brought about by fatiguing contractions.

Additional work by James *et al.*, (2005) was the first study to demonstrate a direct ergogenic effect of 70µM caffeine, reporting a small, but significant, 2-3% increase in the power output of non-fatigued mouse EDL muscle. This effect on EDL was later confirmed by Tallis *et al.*, (2012), who also demonstrated a larger, 6%, increase in mouse soleus power output, uniquely highlighting a fiber type specific effect at physiological doses. Although not directly measured, this increase in power output was attributed to a caffeine induced increase in Ca^{2+} release resulting in an increased ability of the muscle to produce work when electrically stimulated during shortening and a greater production of net work, as indicated via analysis of the work loop shape (Fig 2). The area encompassed by the work loop represents the net work done (see Fig 2) and this is calculated by subtracting the negative work (energy input required to

lengthen the muscle) from the positive (work output during shortening). Figure 2 demonstrates that when treated with caffeine the muscles produced greater force during shortening, than the control, leading to an increase in net work and power output. The demonstrated response outlined by Tallis *et al.,* (2012) may infer an amplified ergogenic effect of caffeine during prolonged submaximal activities that have a greater reliance on more oxidative fiber types.

[Insert Figure 2 here]

Tallis *et al.,* (2012) further demonstrated that the ergogenic benefit of caffeine was of similar magnitude at both maximal and submaximal activation intensities. This is particularly interesting as evidence using millimolar concentrations of caffeine suggests that the caffeine induced potentiation of twitch force is greater than that in tetani (Wondmikun *et al.,* 2006). Theoretically, during submaximal stimulation there is a larger pool of Ca^{2+} in the SR which could allow a greater release in the presence of caffeine resulting in greater force production. In light of these results it is considered that the mechanism by which caffeine acts directly at the muscle may be more complex than first thought and that the caffeine-induced release of Ca^{2+} is in some way limited. This warrants further investigation, using physiological doses, of the direct mechanism of the action of caffeine.

The findings by Tallis *et al.,* (2012) are the first to demonstrate no caffeine related dose-response relationship when physiologically relevant concentrations are used directly on the muscle, similar to previous findings in a large proportion of the *in vivo* human performance literature (Pasman *et al.,* 1995; Graham & Spriet, 1995; Bruce *et al.,* 2000; O'Connor *et al.,* 2004). In fact the findings of Tallis *et al.,* (2012) demonstrate an 'all or none' relationship, whereby treatment will either cause the potentiation of force or there is a lack of response. Consequently, it is considered that much higher concentrations of caffeine are needed to promote a dose response effect as reported by Fryer and Neering (1989), and as such there is little human relevance of such work. Interestingly, the results of Tallis *et al.,* (2012) indicate that the direct ergogenic benefit of caffeine can be achieved using only 50µM, making it increasing likely that direct caffeine induced improvements in the mechanical performance of skeletal muscle contribute to the ergogenic benefit demonstrated *in vivo*.

An inter-individual variation in the magnitude of response and a division of responders and non-responders has been reported in the human literature (Skinner *et al.,* 2009; Astorino, 2011). Recent *in vitro* studies have also demonstrated contrasting responses to caffeine between muscles isolated from different individuals (James *et al*., 2005; Tallis *et al.,* 2012). This is particularly interesting as previously this varied response has been attributed habituation to the caffeine response due to regular exposure. As the rodents used in this study do not consume a high caffeine diet, this confirms further mechanisms are responsible for this effect.

James *et al.,* (2005) and Tallis *et al.,* (2013b) were also the first to measure the effect of physiologically relevant caffeine treatment on the ability of the muscle to sustain power output. 70µM caffeine had no effect on maximally fatigued EDL (James *et al.,* 2005), but time to fatigue was significantly increased in maximally fatigued (by 17.6 %) and prolonged in submaximally fatigued (by 19.2%) soleus muscle (Tallis *et al.,* 2013b). Indirectly these results confirm the action of physiologically relevant

concentrations of caffeine as a modulator of excitation contraction coupling which can be seen by examining the work loop shapes generated in these studies (Fig 3). Here work loops shapes 0.4s, 2.4s, 4.8s, and 7.2s from the start of the fatiguing protocol are plotted for control and caffeine treated conditions and a further comparison between maximal and submaximal stimulation is made. In all examples the area of the work loop becomes smaller over time as the ability of the muscle to produce work is reduced. Interestingly, in the maximally stimulated protocol, the caffeine treated muscle produced greater force during the re-lengthening phase post active shortening when compared to controls (as indicated in Fig 3a&b), which will greatly influence the net work achieved. The net work produced is the sum of the work generated during shortening minus the work required to lengthen the muscle. If the muscle is active to a greater degree while it is being elongated, the energy required to stretch the muscle is increased, thus reducing the net work. The outlined decrease in time to fatigue was attributed to a caffeine induced increase in basal intramuscular Ca^{2+} concentration and reduced activity of the SR Ca^{2+} pump (Allen *et al.*, 1989; Westerblad & Allen, 1991; Allen & Westerblad, 1995) causing a more exaggerated slowing of relaxation throughout the fatiguing protocol. In support of this it was further reported that the ability of the caffeine treated muscles to recover was significantly reduced indicating damage from the fatigue run, attributed to a caffeine evoked increase in high intensity eccentric activity.

[Insert Figure 3 here]

It is important that the effects of caffeine on acute power and on the fatigue response, as reported by James *et al.,* (2005) and Tallis *et al.,* (2012), are not viewed in isolation. In these studies the muscle is treated with caffeine and then the decline in peak muscle power output as a percentage of this maximal (100%) is plotted over time, thus masking any acute effect of the treatment. More simply, if EDL muscle is able to produce 3% more power but fatigues at the same rate as controls (James *et al.*, 2005), a positive caffeine induced fatigue response is realised. A review of this work has presented a number of novel findings which may highlight the significance of the skeletal muscle response in caffeine induced improvements during human sports performance.

Applications to Human Performance

The evidence presented infers that physiological concentrations of caffeine can directly affect skeletal muscle to cause a significant enhancement in mechanical performance increasing the ability of the muscle to produce force, work and power. Although the 3% and 6 % improvements in power output for fast and slow twitch muscle respectively (Tallis *et al.,* 2012) may seem small, these gains could prove meaningful in competitive performance, that at elite level is decided by narrow margins, or as an effective training aid promoting an amplified training stimulus. The demonstrated fiber type specific effect (Fryer & Neering, 1989; Germinario *et al.*, 2004; Tallis *et al.,* 2012) indicates an amplified ergogenic benefit during prolonged submaximal activities that have a greater reliance on oxidative fibers, providing further evidence supporting the increased potency of caffeine in endurance based activities.

Interpretation of the possible benefit of caffeine during fatiguing exercise is complex, but if muscle is able to produce a greater maximal power *in vivo*, the desired muscle power output may be achieved with a smaller number of recruited fibres, thus delaying the recruitment of further fibres and potentially the fatigue response. Alternatively, during human performance it may be possible to produce a greater maximal power output, but a similar fatigue response following caffeine treatment (James *et al.*, 2005), enabling a faster performance time.

The work loop method is a valuable tool for assessing the mechanical performance of skeletal muscle, however it should be noted that the length change wave forms and stimulation patterns used *in vitro* are simplified approximations of what may occur *in vivo*. *In vivo* the patterns of fiber stimulation and length change waveforms are likely to be manipulated throughout movement in order to maximise muscle economy and prevent the onset of fatigue (Wakeling, 2005). This may be particularly true when it comes to fatiguing stimulation, as it is likely that activation and length change patterns will be modified to prevent the muscle damage seen in some of the *in vitro* caffeine treated muscle (Tallis *et al.*, 2013b). With consideration of these limitations, it may be that the magnitude of the direct effect of caffeine on isolated skeletal muscle during fatiguing activities is greater than that portrayed in this review.

Although the current review presents substantial evidence demonstrating the ability of caffeine to cause significant improvements in muscle contractility, this may be one of only a number of mechanisms that works synergistically to promote the performance enhancing effect seen in humans. Most noteworthy is the action of caffeine as a central adenosine receptor antagonist, particularly on A1 and A2a receptors, promoting an elevated release of neurotransmitters due to withdrawal of the adenosine effect (Garrett & Griffiths, 1997; Fredholm, 1999; Ribeiro & Sebastião, 2010). A primary central mechanism of caffeine is to prevent the adenosine induced suppression of dopamine release (Okada *et al.*, 1997; Davis *et al.*, 2003), contributing to the commonly reported increase alertness and arousal (Nehlig, 2010). Evidence further suggests that caffeine may modify CNS function by inhibiting phosphodiesterase activity resulting in elevated cAMP, blocking $GABA_A$ receptors and mobilising intracellular calcium, although it is considered that the dose required to promote such effects is greater than that necessary to block adenosine receptors (Garrett & Griffiths, 1997; Davis *et al.*, 2003). Due to the interaction of these mechanisms it is likely that the effect of caffeine in whole body human performance may is greater than that portrayed in this review alone.

Furthermore the interaction of caffeine with adenosine receptors has been shown to stimulate lipolysis (Garrett & Griffiths, 1997), however the literature is rife with evidence demonstrating performance enhancing effects of caffeine in the absence of increased plasma FFA's, changes in RER, and the popularised glycogen sparing mechanism (see review by Graham, 2001). Moreover this mechanism would not contribute to the performance enhancing effect of caffeine demonstrated in short term anaerobic events.

The freely available and socially acceptable nature of caffeine consumption within society, and the issues with accurately measuring consumption form the primary rationale for its removal for the World Anti-Doping Agency (WADA) prohibited list. With the demonstrated magnitude of its effects, and the seemingly unpredictable

division of responders and non-responders to the drug, it is conceivable that individuals could elicit a significant legal enhancement in performance that may not be comparable in all competitors.

The majority of research evaluating the ergogenic effects of caffeine has been conducted on subjects within the range of physiological maturity. With the associated age related changes in muscle fiber type composition and reduced efficiency of the excitation-contraction coupling process (Deschenes, 2004; Tallis *et al.,* 2014b), it is conceivable that the ergogenic benefit of caffeine may differ in children and older populations. Work by our research group has indicated that direct 70μM caffeine still adequately produces significant increases in muscle power across a wide age range of mice, however the effectiveness of the treatment is reduced with increasing age (Tallis, 2013a). Although a comparably under researched area, support for the ergogenic effect of caffeine in older adults has been demonstrated in human performance literature (Norager *et al.,* 2005; Duncan *et al.,* 2014).

Conclusion

This review considers the contribution of evidence from isolated muscle studies to our understating of the direct effects of caffeine on muscle during human performance. The body of *in vitro* evidence presented suggests that caffeine can directly potentiate skeletal muscle force, work and power which may well contribute to the overall performance enhancing effects seen in humans. The established fibre type specific effect adds clarity to the demonstrated increased potency of caffeine when used to promote enhancements in endurance activities. Interestingly, the evidence from *in vitro* studies demonstrates a division between responders and non-responders to caffeine treatment that cannot be attributed to habituation or inter-individual differences in digestion and distribution. Importantly it is considered that future *in vitro* experimental design and interpretation is improved to more accurately replicate physiological conditions in humans, if it is the intention of such studies to relate their results to potential changes in human performance.

References

Allen D G, Lamb G D, Westerblad H (2008). Skeletal muscle fatigue: Cellular mechanisms. Physiol Rev 88: 287-332.

Allen D G, Lee JA, Westerblad H (1989). Intracellular calcium and tension during fatigue in isolated muscle fibers from Xenopus laevis. J Physiol 415: 433-458.

Allen D G, Westerblad H (1995). The effects of caffeine on intracellular calcium, force and the rate of relaxation of mouse skeletal muscle. J Physiol 487: 331-342.

Astorino TA, Martin BJ, Schachtsiek L, Wong K, Ng K (2011). Minimal effect of acute caffeine ingestion on intense resistance training performance. J Strength Cond Res 25: 1752-1758.

Astorino T A, Roberson D W (2010). Efficacy of acute caffeine ingestion for short-term high-intensity exercise performance: a systematic review. J Strength Cond Res 24: 257-265.

Astorino T A, Rohmann R L, Firth, K (2008). 'Effect of caffeine ingestion on one repetition maximum muscular strength. Eur J Appl Physiol 102: 127-132.

Bell D G, Mclellan T M (2002). Exercise endurance 1, 3, and 6 hours after caffeine ingestion in caffeine users and non-users. J Appl Physiol 93: 1227-1234.

Bhat M B, Zhao J, Zang W, Balke C W, Takeshima H, Wier W G, *et al.* (1997). Caffeine-induced release of intracellular Ca2+ from Chinese hamster ovary cells expressing skeletal muscle ryanodine receptor. Effects on full-length and carboxyl-terminal portion of Ca2+ release channels. J Gen Physiol 110: 749-762.

Bridge C A, Jones M A (2006). The effect of caffeine ingestion on 8 km run performance in a field setting. J Sports Sci 24: 433-439.

Bruce C R, Anderson M E, Fraser, S F, Stepto N K, Klein R, Hopkins W G, *et al.* (2000). Enhancement of 2000-m rowing performance after caffeine ingestion. Med Sci Sport Exerc 32: 1958-1963.

Burke L M (2008). Caffeine and Sports Performance. Appl Physiol Nutr Metab33: 1319-1334.

Carr A, Dawson B, Schneiker K, Goodman C, Lay B (2008). Effect of caffeine supplementation on repeated sprint running performance. J Sports Med Phys Fitness 48: 472-479.

Cohen B S, Nelson A G, Prevost M C, Thompson G D, Marx B D, Moris G S (1996). Effects of caffeine ingestion on endurance racing in heat and humidity. Eur J Appl Physiol 73: 358-363.

598 Collomp K, Ahmaidi S, Chatard J C, Audran M, Prefaut C (1992). Benefits of caffeine
599 ingestion on sprint performance in trained and untrained swimmers. Eur J Appl
600 Physiol 64: 377-380.
601
602 Conway K J, Orr R, Stannard S R (2003). Effect of a divided caffeine dose on
603 endurance cycling performance, postexercise urinary caffeine concentration and
604 plasma paraxanthine. J Appl Physiol 94: 1557-1562.
605
606 Davis J K, Green J M (2009). Caffeine and anaerobic performance: Ergogenic value
607 and mechanisms of action. J Sports Med 39: 813-832.
608
609 Davis J M, Zhao Z, Stock H S, Mehl K A, Buggy J, Hand G A (2003). Central nervous
610 system effects of caffeine and adenosine on fatigue. Am J Physiol Regul Integr
611 Comp Physiol 287: 399-404.
612
613 Damiani E, Larsson L, Margreth A (1996). Age-related abnormalities in regulation of
614 the ryanodine receptor in rat fast-twitch muscle. Cell Calcium 19: 15-27.
615
616 Deschenes M (2004). Effects of aging on muscle fiber type and size. J Sports Med 34:
617 809-824.
618
619 Duncan, M.J, Clarke, N., Tallis, J., Leddington Wright, S. (2014) The Effect of acute
620 caffeine ingestion on functional performance in older adults. J Nutr Health
621 Aging [in press]
622
623 Endo M, Tanaka M, Ogawa Y (1970). Calcium induced release of calcium from the
624 sarcoplasmic reticulum of skinned skeletal muscle fibers. Nature 228: 34-36.
625
626 Fredholm B B, Battif K, Holmen J, Nehlig A N, Zvartau E E (1999). Actions of
627 caffeine in the brain with special reference to factors that contribute to its
628 widespread use. Pharmacol Rev 51: 83-133.
629
630 Fryer M W, Neering I R (1989). Actions of caffeine on fast- and slow-twitch muscle of
631 the rat. J Physiol 416: 435-454.
632
633 Garett B E, Griffiths R R (1997). The role of dopamine in the behavioural effects of
634 caffeine in animals and humans. Pharmocol Biochem Be 57: 533-541.
635
636 Germinario E, Esposito A, Megighian A, Midrio M, Betto R, Danieli-Betto D. (2004).
637 Effects of modulators of sarcoplasmic Ca^{2+} release on the development of
638 skeletal muscle fatigue. J Appl Physiol 96: 645-649.
639
640 Glaister M, Howatson G, Abraham C S, Lockey R A, Goodwin J E, Foley P, *et al.*
641 (2008). Caffeine supplementation and multiple sprint running performance.
642 Med Sci Sport Exer 40: 1835-1840.
643
644 Goffart M, Ritchie J M (1951). The effect of adrenaline on the contraction of
645 mammalian skeletal muscle. J Physiol. 116 : 357-371.
646

Goldstein E R, Ziegenfuss T, Kalman D, Kreider R, Campbell B, Wilborn C, *et al.* (2010). International society of sports nutrition position stand; caffeine and performance. J Inter Soc Sports Nutr 7: 1-15.

Graham T E (2001). Caffeine and exercise. Metabolism, endurance and performance. J Sports Med 31: 785-807.

Graham T E, Hibbert E, Sathasivam P (1998). Metabolic and exercise endurance effects of coffee and caffeine ingestion. J Appl Physiol 85: 883-889.

Graham T E, Spriet L L (1991). Performance and metabolic responses to a high caffeine dose during prolonged exercise. J Appl Physiol 71: 2292-2298.

Graham T E, Spriet L L (1995). Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. J Appl Physiol 78: 867-874.

Huddart H (1968). 'Caffeine-induced activation in stick insect skeletal muscle' Comp Biochem Physiol 28: 1185-1192.

Jackman M P, Wendling P, Friars D, Graham T E (1996). Metabolic, Catecholamine, and endurance response to caffeine during intense exercise. J Appl Physiol 81: 1658-1663.

James J E (1994). Does caffeine enhance or merely restore degraded psychomotor performance? Neuropsychobiology 30: 124-125.

James R J (2013). A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. J Comp Physiol B

James R S, Altringham J D, Goldspink D F (1995). The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. J Exp Biol 198: 491-502.

James R S, Kohlsdorf T, Cox V M, Navas C A (2005). 70μM caffeine treatments enhances in vitro force and power output during cyclic activities in mouse extensor digitorum longus muscle. Eur J Appl Physiol 95: 74-82.

James R S, Wilson R S, Askew G N (2004). Effects of caffeine on mouse skeletal muscle power output during recovery from fatigue. J Appl Physiol 96: 545-552.

James R S, Young I S, Cox V M, Goldspink D F, Altringham J D (1996). Isometric and isotonic muscle properties as determinants of work loop power output. Eur J Physiol 432; 767-774.

Josephson R K (1985). Mechanical power output from striated muscle during cyclical contraction. J Exp Biol 114: 493-512.

Kovacs E M R, Stegen J H C H, Brouns F (1998). Effect of caffeineated drinks on substrate metabolism, caffeine excretion and performance. J Appl Physiol 85: 709-715.

LeBlanc J, Jobin M, Cote J, Samson P, Labrie A (1985). Enhanced metabolic response to caffeine in exercise-trained human subjects. J Appl Physiol. 59: 832-837.

Luttgau H C, Oetliker H (1967). The action of caffeine on the activation of the contractile mechanism in striated muscle fibers. J Physiol 194: 51-74.

Magkos F, Kavouras S A (2005). Caffeine use in sports, pharmacokinetics in man, and cellular mechanisms of action. Crit Rev in Food Sci Nutr 45 535-562.

Nehlig A (2010). Is caffeine a cognitive enhancer? J Alzheimer Dis 20: 85-94.

Norager C B, Jensen M B, Madsen M R, Laurberg S (2005). Caffeine improves endurance in 75-yr-old citizens; a randomized, double-blind, placebo-controlled, crossover study. J Appl Physiol 99: 2302-2306.

O'Connor P J, Motl R W, Broglio S P C Ely M R (2004). Dose-dependent effect of caffeine on reducing leg muscle pain during cycling exercise is unrelated to systolic blood pressure. Pain 109: 291-298.

Okada M, Kiryu K, Kawata Y, Mizuno K, Wada K, Tasaki H, Kaneko S (1997). Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis. Eur J Pharmacol 26: 181-188

O'Rourke M P, O'Brien B J, Knez, W L, Paton C D (2006). Caffeine has a small effect on 5-km running performance of well-trained and recreational runners.' J Sci Med Sport 11: 231-233.

Pasman W J, van Baak M A, Jeukendruo A E, de Haan A (1995). The effect of different dosages of caffeine on endurance performance time Int J Sports Med 16: 225-230.

Perkins R, Williams M H (1975). Effect of caffeine upon maximal muscular endurance of females. Med Sci Sports 7: 221-224.

Reffinetti R (1999). Amplitude of the daily rhythm of body temperature in eleven mammalian species. J Therm Biol 24: 477–481.

Ribeiro J A, Sebastião A M (2010). Caffeine and Adenosine. J Alzheimers Dis 20: 3-15.

Ritchie J M (1954). The effect of nitrate on the active state of muscle. J Physiol 126: 155-168.

Rosser J I, Brandon W, Hogan M C (2009). Effect of physiological levels of caffeine on Ca^{2+} handling and fatigue development in Xenopus isolated singe myofibers. American J Physiol Regul Integr Comp Physiol 296: 1512-1517.

Rossi R, Bottinelli R, Sorrentino V, Reggiani C. (2001). Response to caffeine and ryanodine receptor isoforms in mouse skeletal muscles. Am J Cell Physiol 281: 585-594.

Skinner T L, Jenkins D G, Coombes J S, Taaffe S R, Leveritt M D (2009). Dose response of caffeine on 2000-m rowing performance. Med Sci Sport Exerc 42: 571-576.

Smith A (2002). Effects of caffeine on human behaviour. Food Chem Toxicol 40: 1243-1255

Sokmen B, Armstrong L E, Kraemer W J, Casa D J, Dias J C, Judelson D A, Maresh C M (2008). Caffeine use in sports: Considerations for the athlete. J Strength Cond Res 22: 978-986.

Spriet L L (2014) Exercise performance with low doses of caffeine. Sports Med 44: 175-184.

Tallis J (2013a). Effects of physiological caffeine concentration on isolated skeletal muscle force, power and fatigue resistance. PhD thesis. Coventry: Coventry University

Tallis J, James R S, Cox V M, Duncan M J (2012). The effect of physiological concentrations of caffeine on the power output of maximally and sub maximally stimulated mouse EDL (fast) and soleus (slow) muscle. J Appl Physiol 112: 64-71.

Tallis J, James R S, Cox V M, Duncan M J (2013b). The effect of a physiological concentration of caffeine on the endurance of maximally and submaximally stimulated mouse soleus muscle. J Physiol Sci 63: 125-132.

Tallis J, James R S, Cox, V M, Duncan, M (2014a). The effect of physiological concentrations of taurine alone and taurine and caffeine on acute muscle power output, fatigue and recovery of isolated skeletal muscle. Can J Physiol Pharmacol 92 42-49.

Tallis J, James R S, Little A G, Cox, V M, Duncan M J, Seebacher F (2014b). The Early Effects of Ageing on the Mechanical Performance of Isolated Locomotory (EDL) and Respiratory (diaphragm) Skeletal Muscle Using the Work Loop Technique. Am J Physiol. Regul Integr Comp Physiol 15: 307.

Tarnopolsky M, Cupido C (2000) Caffeine potentiates low frequency skeletal muscle force in habitual and non habitual caffeine consumers. J Appl Physiol 89: 1719-1724.

Wakeling J M (2005). Motor unit recruitment during vertebrate locomotion. J Anim Biol 55: 41-58.

Weber A, Herz R. (1968). The relationship between caffeine contracture in intact muscle and the effect of caffeine on reticulum. J Gen Physiol 52: 750.

Westerblad H, Allen D G. (1991). Changes of myoplasmic calcium concentration in single mouse muscle fibers. J Gen Physiol 3: 615-635.

Wiles J D, Bird S R, Hopkins J (1992). Effects of caffeinated coffee on running speed, respiratory factors, blood lactate and perceived rate of exertion during 1500-m treadmill running. Br J Sports Med 26: 116-120.

Wondmikun Y, Soukup T, Asmussen G. (2006). Effects of caffeine at different temperatures on contractile properties of slow-twitch and fast-twitch rat muscles. Physiol Res 55: 641-652.

Wooden K M, Walsberg G E (2004). Body temperature and locomotor capacity in a heterothermic rodent. J Exp Biol 207:41–46.

Conflict of Interests

None

Tables

Table 1 – Sample of the literature examining the direct effect of caffeine on contractile performance of isolated skeletal muscle

Figures

Figure 1 – Variables that Limit our Ability to Compare Between Research Studies Examining the Ergogenic Effects of Caffeine in vitro

Figure 2 - Typical effects of caffeine treatment on work loop shapes in mouse EDL (left) and soleus (right) stimulated maximally at 5-Hz cycle frequency. Solid loops, control; dashed loops, caffeine treated (Tallis et al., 2012). Each work loop cycle started at length 0 (optimal length for producing isometric force). Each muscle was lengthened by 5% of its resting length and electrically stimulated to produce force. Each muscle was stimulated to produce force during shortening. Near to the end of shortening, the electrical stimulation ceased and the muscle was lengthened back to the initial length, 0). The area inside the loop represents the net work done (active work – passive work).

Figure 3- Typical effects of fatigue on work loop shape for maximally and submaximally stimulated mouse soleus muscle (A 140Hz stimulation frequency & C 40Hz stimulation frequency respectively) compared with those treated with 70µM caffeine (B 140Hz, caffeine and D 40Hz, caffeine) [Arrows indicate where stimulation typically started, towards the end of lengthening, and finished, during shortening; 0.4s, 2.4s, 4.8s, & 7.2s represent time since the start of the fatigue protocol for each of the work loops shown] (Tallis et al., 2013).