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REVIEW

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

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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 continue 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 variations in exercise intensity seems to affect its ergogenic benefits, it is largely thought that caffeine can induce 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 investigating its effects on isolated muscles 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 its 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.

Abbreviations

–/–, knockout; A_1 receptor, adenosine receptor 1; Ca^{2+}, calcium ions; RyR, ryanodine receptor; SR, sarcoplasmic reticulum

Tables of Links

TARGETS	
GPCRs[a]	**Ion channels**[b]
A_1 receptor	$GABA_A$ receptor
A_{2A} receptor	RyR

LIGANDS
Adrenaline
Caffeine
cAMP

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b]Alexander *et al.*, 2013a,b).

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 and Kavouras, 2005). It is largely considered that caffeine acts as a CNS stimulant; however, its glycogen-sparing effects, ability to increase fatty acid mobilization and induce catecholamine release as well as direct effects on muscle have all been reported as mechanisms that contribute to its the ergogenic effect (see reviews by Graham, 2001; Magkos and Kavouras, 2005; Davis and Green, 2009). The use of *in vitro* experiments, in which caffeine is applied directly to isolated muscle has been shown to be an important method for quantifying the direct effect of caffeine on muscle and to assess its potential mechanism for improving sports performance. A number of more recent publications (James *et al.*, 2005; Tallis *et al.*, 2012; 2013) have used advances in methodology to more accurately examine the direct effect of caffeine on the mechanical performance of skeletal muscle and as such have significantly contributed to our understanding of the caffeine response. The findings presented indicate that physiological concentrations of caffeine can directly affect skeletal muscle to cause a significant enhancement in mechanical performance, so increasing the ability of the muscle to produce force, work and power. Such effects could be used in humans to facilitate training and improve performance at competitions.

Caffeine and sports performance

It is widely accepted that caffeine ingestion can promote performance-enhancing effects on endurance (activity lasting greater than 30 min), power and strength activities, although the magnitude of this effect is debatable (Graham, 2001). There is also evidence that caffeine is more potent when it is used as an acute supplement in endurance-based activities, while results from studies showing its effects on short-term high-intensity exercise protocols appear to be more ambiguous (Graham, 2001; Davis and Green, 2009; Goldstein *et al.*, 2010). The effect of caffeine ingestion on sports performance has been extensively explored in a number of reviews (Graham, 2001; Burke, 2008; Davis and Green, 2009; Astorino and Roberson, 2010; Goldstein *et al.*, 2010).

Evidence from these articles suggests that mode and intensity of exercise, caffeine consumption habits, fitness level, treatment dose and individual differences in caffeine digestion, distribution and sensitivity greatly influence the effects of caffeine on human performance (Figure 1). It is likely that the different responses to caffeine and conflicting evidence found in the literature can largely be attributed to methodological differences between studies.

In the most part, the previously cited reviews suggest that the performance-enhancing effect of caffeine is greater in trained athletes compared with non-trained athletes

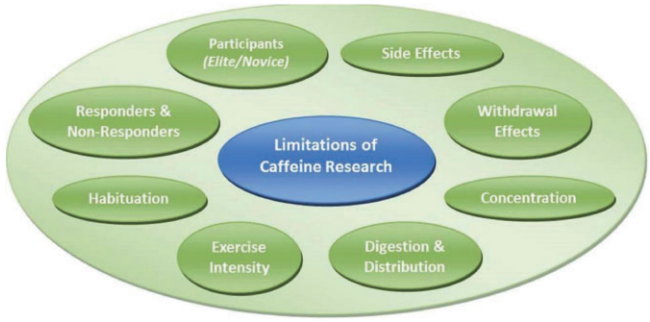

Figure 1
Variables that limit our ability to compare results between research studies examining the ergogenic effects of caffeine *in vitro*.

(Graham, 2001; Astorino and Roberson, 2010). Although there is a distinct lack of studies directly assessing this, LeBlanc *et al.* (1985) demonstrated that trained individuals have an increased resting metabolic rate, adrenaline levels and free fatty acids compared with an untrained population. Furthermore, Collomp *et al.* (1992) reported faster swim speeds in trained athletes following a 250 mg caffeine dose that were not replicated in an untrained group. The mechanism responsible for these different responses is largely unknown, but it is thought that as many experimental procedures require participants to work maximally, trained individuals will have greater motivation to perform taxing exercise and will have better nutritional preparation, and their day-to-day performance variation will be reduced (Burke, 2008).

Furthermore, it has been suggested that caffeine has a greater ergogenic benefit in non-habituated consumers (Bell and McLellan, 2002). Caffeine is rich in the Western diet and it is almost impossible to recruit participants who consume similar quantities of caffeine, and in many studies participants are considered to be habitual users (Tarnopolsky and Cupido, 2000; Bridge and Jones, 2006; Duncan *et al.*, 2014). Although the effects of habituation to caffeine on the magnitude of the response to a particular dose of caffeine need to be investigated further (Graham, 2001; Astorino and Roberson, 2010), this could be why there are responders and non-responders to caffeine treatment, as reported in studies examining responses at an individual level (Skinner *et al.*, 2009).

Another methodological debate relates to the withdrawal of caffeine before completion of the experimental trial. It is common practice for researchers to restrict caffeine consumption 12–48 h before completion of the exercise protocol (Bell and McLellan, 2002; Glaister *et al.*, 2008; Duncan *et al.*, 2014). Although there is evidence indicating that withdrawal has limited effects on exercise performance, there is a wealth of literature demonstrating its negative effects on mood, stress, fatigue, alertness and short-term memory (Smith, 2002). James (1994) suggested that caffeine has no behavioural effect, but its 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 3 mg·kg^{-1} is the lowest dose needed to elicit ergogenic benefit on exercise performance (Graham, 2001), and it is common practice to administer caffeine in doses of 5–6 mg·kg^{-1} (Jackman *et al.*, 1996; Bridge and Jones, 2006; O'Rourke *et al.*, 2006; Carr *et al.*, 2008). Despite research assessing a variety of doses ranging from 0.5 to 13 mg·kg^{-1} (Graham and Spriet, 1991; Wiles *et al.*, 1992; Pasman *et al.*, 1995; Cohen *et al.*, 1996; Bruce *et al.*, 2000), only a small number of studies have examined the dose–response relationship on human performance (Perkins and Williams, 1975; Graham and 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 and 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 thought that an increased dose of caffeine fails to elicit a further response; however, contradictory evidence is also presented (Kovacs *et al.*, 1998). Furthermore, it is also thought that inter-individual side effects associated with the consumption of high-caffeine concentrations may actually result in decreased performance (Graham and Spriet, 1995). Although there is some ambiguity as regards a caffeine dose–response relationship, there is anecdotal evidence suggesting that the caffeine-induced reduction in pain perception and increased plasma adrenaline and free fatty acid concentration (Graham and Spriet, 1995; Pasman *et al.*, 1995; O'Connor *et al.*, 2004), which may evoke performance-enhancing benefits in other modes of exercise, are all dose-dependent effects. The variety of methodological approaches and results obtained make meaningful conclusions and recommendations to athletes difficult to calculate. Furthermore, it is hard to isolate the direct effects 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 CNS 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 utilization of free fatty acids as an energy source, thus permitting glycogen-sparing. The evidence supporting this claim is inconclusive (Graham, 2001; Davis and Green, 2009). The ability of caffeine to promote increased adrenaline release, evoke greater Ca^{2+} release from the sarcoplasmic reticulum (SR), 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 and Kavouras, 2005; Davis and Green, 2009). Although the effectiveness of caffeine as a performance enhancer is widely reported, the discrepancies summarized have meant that we are unable 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 stimulus. A caffeine dose is added directly to the Krebs/Ringer solution, and the mechanical performance of the muscle is re-examined. 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 and Oetliker, 1968; Allen and 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; 2013; 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 metabolizers (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 the same dose reaches each tissue examined.

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 is 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. The implementation of such methods within this research area uniquely allows the examination of muscle fibre-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 enables an improved analysis of a dose–response relationship, without the adverse side effects of high-caffeine consumption seen during *in vivo* work (Graham and Spriet, 1995). The effect of caffeine on exercise mode can be considered in greater detail *in vitro*, allowing the investigation of maximal and submaximal contractions,

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 those by Luttgau and Oetliker (1968) who tested mM concentrations of caffeine (supraphysiological for humans) on isolated semitendinosus and iliofibularis muscles 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°C to 1–3°C. At high concentrations, caffeine has even been shown to produce a contraction without stimulation (Huddart, 1969). 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 and Oetliker, 1968; Weber and Herz, 1968), particularly in slow twitch muscle (Wondmikun *et al.*, 2006).

Mechanistically, caffeine will promote greater force output in skeletal muscle due to modification of excitation contraction coupling (Davis and Green, 2009). Weber and Herz (1968) were one of the earliest studies to investigate this theory by isolating SR from skeletal muscle of *Rana pipiens* and monitoring Ca^{2+} release induced by various mM concentrations of caffeine. Caffeine treatment resulted in an immediate release of Ca^{2+} in 11 of 12 preparations; this was attributed to a shift from 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 antagonist of A_1 adenosine receptors on the skeletal muscle membrane and/or binds to ryanodine receptors (RyRs) on the SR, as demonstrated *in vitro* with 10 mM 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 and Allen, 1991; Allen and 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 and 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 recognize that although this may not be the primary intention of all of these studies, the underlying mechanism of the response to caffeine in humans is commonly attributed to these effects observed *in vitro*.

A significant limitation in many of these studies is the use of supraphysiological, mM concentrations of caffeine (Luttgau and Oetliker, 1968; Weber and Herz, 1968; Huddart, 1969; Endo *et al.*, 1970; Allen and 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 1 mM would be fatal for humans and concentrations are usually between 20 and 50 μM (Graham, 2001), with 70 μM 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 and Oetliker, 1968; Weber and Herz, 1968; Fryer and Neering, 1989; Allen and 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°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, 2013). It should further be noted that studies using amphibian or insect muscle (Ritchie, 1954; Luttgau and Oetliker, 1968; Huddart, 1969; Rosser *et al.*, 2009) may evoke different caffeine response when compared with mammalian muscle.

Evidence in this area, bar the work of James and Tallis, has been gained by use of isometric testing methods, which although providing 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; 1996). It is rare for skeletal muscle to act completely isometrically with a shortening required to perform work and to produce power (Rome, 2002). James *et al.* (1996) concluded that isometric testing vastly underestimates the *in vivo* rate of force activation and relaxation and the results obtained are limited, as the passive properties of muscle are not taken into account. 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 × velocity), which cannot be estimated by isometric testing (James *et al.*, 1995; 1996).

Recent work by Tallis and James (James *et al.*, 2004; 2005; Tallis *et al.*, 2012; 2013; 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

Table 1

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

Reference	Muscle preparation	Test temperature (°C)	Caffeine concentration	Results
Goffart and Ritchie (1951)	Rat diaphragm	37	1×10^{-4}, w v^{-1}	Increased maximal twitch force accompanied by prolongation of contraction time
Ritchie (1954)	Frog sartorius muscle	0	6×10^{-5}, w v^{-1}	Augmented twitch force
Luttgau and Oetliker (1968)	Single fibres of semitendinosus and iliofibularis of Swiss mountain frogs	20 to 1–3	6–10 mM	Increased twitch force. Increased caffeine sensitivity at lower temperature.
Weber and Herz (1968)	SR isolated from leg and back muscles of frogs and rabbits	5 and 24	1–10 mM	High concentration resulted in immediate release of Ca^{2+}. Drop in temperature increased the caffeine-induced Ca release.
Huddart (1969)	Stick insect skeletal muscle	Not cited	1–10 mM	Potentiation of twitch and tetanus tension. Caffeine-induced contraction.
Fryer and Neering (1989)	Fibre bundles from mouse soleus and EDL	25	0.2–20 mM	Potentiated twitch force of soleus and EDL in a dose-dependent manner. Increased caffeine sensitivity in soleus muscle. Potentiation of tetanus force and increased Ca^{2+} release
Allen and Westerblad (1995)	Mouse flexor brevis	22	5 mM	Increased tetanic tension and slowed the rate of relaxation. Increased Ca^{2+} concentration and sensitivity
Rossi *et al.* (2001)	Mouse EDL, soleus and diaphragm	22	5–30 mM	Ergogenic effect related to RYR composition. Caffeine treatment potentiated isometric force. In adult mice, caffeine-induced responses were greater in diaphragm, lower in EDL and intermediate in soleus.
Germinario *et al.* (2004)	Mouse EDL and soleus	30	2 or 5 mM for soleus and EDL muscles	Increased tetanic force of EDL and soleus. Faster time to fatigue in both muscles.
James *et al.* (2004)	Mouse EDL and soleus	35	10 mM and 70 μM	10 mM caused greater recovery of power output of fatigued EDL, reduced power output in fatigued soleus. No effect of 70 μM on recovery of power output in fatigued muscle.
James *et al.* (2005)	Mouse EDL	35	70 μM	Increased work loop power output. No effect on time to fatigue
Wondmikun *et al.* (2006)	EDL and soleus	20 and 35	0.1–60 mM	0.3–10 mM caused potentiation of both twitch and tetanus force for EDL and soleus. Soleus demonstrated effect at lower caffeine concentrations and the magnitude was significantly increased at lower temperatures.
Rosser *et al.* (2009)	Single fibres of the lumbrical muscles of *Xenopus laevis*	20	70 μM	No difference in peak tetanic force generation, time to fatigue, cytosolic Ca^{2+} levels or relaxation times between the non-caffeinated and caffeinated trials
Tallis *et al.* (2012)	Mouse EDL and soleus	36	35, 50, 70 and 140 μM	Significant fibre-type specific potentiation of work loop power. 35 μM treatment had no effect, all other treatments elicited effects of the same magnitude. No effect of concentration. No difference between maximal and submaximal stimulation.
Tallis *et al.* (2013)	Mouse soleus	36	70 μM	Significantly faster fatigue when maximally stimulated compared with when submaximally stimulated.
Tallis *et al.* (2014a)	Mouse soleus	36	70 μM+ taurine	Ergogenic benefit of caffeine was not enhanced in the presence of taurine

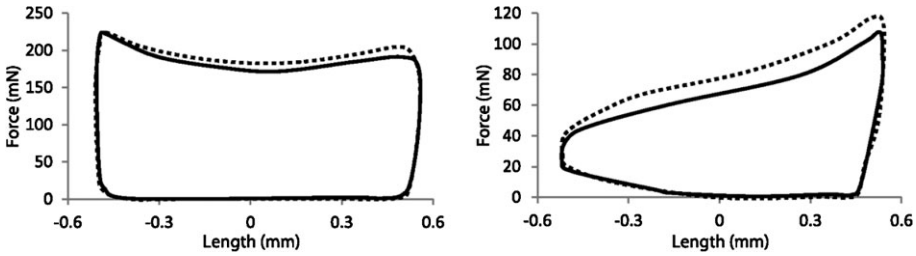

Figure 2

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 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).

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 μM caffeine treatment that represents the likely normal *in vivo* human maximum (Graham, 2001) and is markedly lower than the mM caffeine concentrations used in previous studies. In addition, the experiments were 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) were the first to examine the direct effect of 70 μM caffeine on the mechanical performance of skeletal muscle, and reported no effect on force, work, or power output in fatigued extensor digitorum longus (EDL) or soleus muscles. In contrast, 10 mM caffeine treatment evoked a greater recovery of fatigued EDL, but a reduction in power output in fatigued soleus, and as such it was concluded that caffeine, including when it is used to increase 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; decreased sensitivity of the contractile proteins to Ca^{2+} and reduced SR Ca^{2+} pump function are involved (Allen *et al.*, 2008). The results presented by James *et al.* (2004) infer that the potential effect of a physiologically relevant caffeine concentration to affect 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 to demonstrate a direct ergogenic effect of 70 μM 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 fibre-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 by analysis of the work loop shape (Figure 2). The area encompassed by the work loop represents the net work done (see Figure 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 response obtained by Tallis *et al.* (2012) indicates an amplified ergogenic effect of caffeine during prolonged submaximal activities that have a greater reliance on more oxidative fibre types.

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 results obtained using mM concentrations of caffeine suggest 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 the production of a greater force. In the light of these results, it is proposed 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 investigations of the mechanism of the direct effects of caffeine at physiological doses.

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

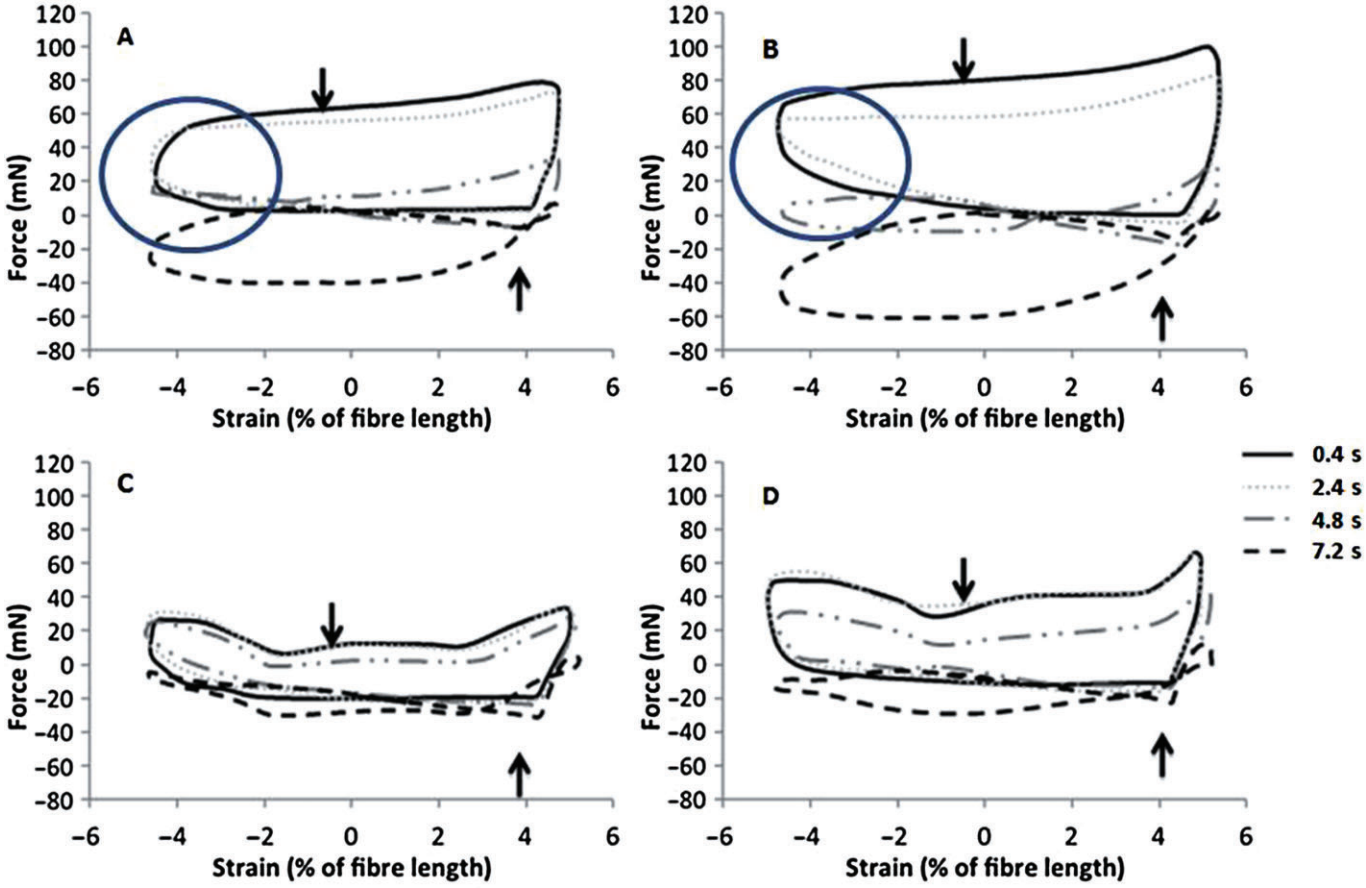

Figure 3

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

of the *in vivo* human performance literature (Graham and Spriet, 1995; Pasman *et al.*, 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 no response. Consequently, it is thought 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 50 μM, making it increasingly 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 the response to caffeine in humans and the finding that the population can be divided into responders and non-responders have been reported in the literature (Skinner *et al.*, 2009; Astorino *et al.*, 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 was attributed to 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.* (2013) were also the first to measure the effect of physiologically relevant caffeine concentrations on the ability of the muscle to sustain power output. Up to 70 μM 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.*, 2013). Indirectly, these results confirm that physiologically relevant concentrations of caffeine act as a modulator of excitation contraction coupling, which can be seen by examining the work loop shapes generated in these studies (Figure 3). Here, work loop shapes 0.4, 2.4, 4.8 and 7.2 s 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 proto-

col, the caffeine-treated muscle produced greater force during the re-lengthening phase post active shortening when compared with controls (as indicated in Figure 3A and 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 and Allen, 1991; Allen and 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; this was attributed to a caffeine-evoked increase in high-intensity unusual activity.

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 realized. 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 of 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, so 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 muscles, 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 fibre-type specific effect of caffeine demonstrated (Fryer and 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 fibres, providing further evidence for the increased potency of caffeine in endurance-based activities.

Clarifying the possible benefit of caffeine during strenuous 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 waveforms and stimulation patterns used *in vitro* are simplified approximations of what may occur *in vivo*. *In vivo*, the patterns of fibre stimulation and length change waveforms are likely to be manipulated throughout movement in order to maximize 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 muscles (Tallis *et al.*, 2013). If these limitations are taken into account, 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 work 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 A_1 and A_{2A} receptors, promoting an elevated release of neurotransmitters due to withdrawal of the adenosine effect (Garrett and Griffiths, 1997; Fredholm *et al.*, 1999; Ribeiro and 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); this contributes to the commonly reported increase alertness and arousal (Nehlig, 2010). There is also evidence suggesting that caffeine modifies CNS function by inhibiting phosphodiesterase activity resulting in an elevated level of cAMP, blocking $GABA_A$ receptors and mobilizing intracellular calcium, although it is thought that the dose required to promote such effects is greater than that needed to block adenosine receptors (Garrett and Griffiths, 1997; Davis *et al.*, 2003). Due to the interaction of these mechanisms, it is likely that the effect of caffeine on whole body human performance may be greater than that portrayed in this review alone.

Furthermore, the interaction of caffeine with adenosine receptors has been shown to stimulate lipolysis (Garrett and Griffiths, 1997); however, the literature is rife with evidence demonstrating performance-enhancing effects of caffeine in the absence of increased plasma free fatty acids, changes in respiratory exchange ratio and the popularized 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 problems associated with accurately measuring consumption form the primary rationale for its removal from the World Anti-Doping Agency 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 fibre-type composition and reduced efficiency of the excitation–contraction coupling process (Deschenes, 2004; Tallis *et al.*, 2014b), it is conceivable that the ergogenic benefits of caffeine may differ in children and older populations. Work by our research group has indicated that direct application of 70 μM caffeine effectively 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, 2013). 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 that preparations can be divided into responders and non-responders to caffeine treatment that cannot be attributed to habituation or inter-individual differences in digestion and distribution. Importantly, future *in vitro* experimental design and interpretation should be changed 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.

Conflict of interest

None.

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