Does a Physiological Concentration of Taurine Increase Acute Muscle Power Output, Time to Fatigue, and Recovery in Isolated Mouse Soleus (Slow) Muscle With or Without the Presence of Caffeine?

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Abstract

High concentrations of caffeine and taurine are key constitutes of many ergogenic supplements ingested acutely to provide legal enhancements in athlete performance. Despite this, there is little evidence supporting the performance enhancing effects of acute taurine supplementation. *In vitro* models have demonstrated that a caffeine induced muscle contracture can be further potentiated when combined with a high concentration of taurine. However, the high concentration of caffeine used in previous research would be toxic for human consumption. Therefore the present study aims to investigate the direct effect of a physiological dose of caffeine and taurine to potentiate skeletal muscle performance. Isolated mouse soleus muscle was used to examine the effects of physiological taurine (TAU), caffeine (CAF), and taurine-caffeine combined (TC) on: 1) acute muscle power output; 2) time to fatigue; and 3) fatigue recovery, compared to untreated controls (CON). Treatment with TAU failed to elicit any significant difference in the measured parameters. Treatment with TC resulted in a significant increase in acute muscle power output and faster time to fatigue. The ergogenic benefit posed by TC was not different from the effects of caffeine alone, suggesting no acute ergogenic benefit of taurine.

Key Words: Ergogenic Aid, Force, Skeletal Muscle, Work Loop

Introduction

The ergogenic effect of energy drinks to promote legal enhancements in sporting performance is largely attributed to high concentrations of caffeine due to the corresponding wealth of evidence demonstrating positive effects across endurance, power and strength activities (Alford et al. 2001; Davis & Green, 2009; Duncan & Oxford, 2011; Graham et al. 2001,). In addition to high concentrations of caffeine and various sugars, the amino acid taurine (Amino Ethane Sulfonic Acid) is a constituent of many energy drinks and is believed to contribute to the performance enhancing effect (Geib et al. 1994). Despite this there is little evidence to demonstrate an acute ergogenic effect of taurine.

Found in high concentrations in mammalian tissue, taurine is readily synthesized by the body and although the exact mechanisms of action are unclear, evidence suggests relationships to the functions of osmoregulation, regulation of oxidative stress, and cell signalling (Cuisinier et al. 2002; Matsuzaki et al. 2002; Oja & Saransaari, 2007; Schaffer et al. 2010). It is further suggested that taurine plays a role in maintaining mammalian myocardial and skeletal muscle contractile function (Schaffer et al. 2010). Warskulat et al. (2004) used taurine transporter knockout mice to demonstrate that the almost complete depletion of skeletal muscle taurine reduced exercise capacity by over 80%. Reducing the intracellular concentration of taurine has commonly been shown to result in a reduction in skeletal muscle function, primarily resulting in a decrease in muscle force production (Hamilton et al. 2006). Evidence is also presented to suggest that depletion of muscular taurine occurs during prolonged muscle activation (Kim et al. 1986; Matsuzaki et al. 2002), therefore suggesting a link between fatigue and muscular taurine concentration.

In vivo and *in vitro* evidence suggests that chronic taurine supplementation can positively enhance skeletal muscle performance (Imagawa et al. 2009). Chronic taurine supplementation in rats has been shown to lead to large increases in muscle taurine concentration and a consequential

75 enhancement of muscle force. This in turn has been shown to correspond to an increase in SR Ca^{2+}
76 storage protein, calsequestrin, which potentially relates to a greater intramuscular Ca^{2+}
77 concentration following muscle activation (Goodman et al. 2009).

78 Geib et al. (1994) concluded that 'Red Bull' energy drink had a greater effect at increasing cycling
79 endurance time in endurance trained male athletes if the taurine content was present, but generally
80 the evidence discussing the effect of acute taurine supplementation on physiological performance is
81 sparse. *In vitro*, high concentrations of taurine have been shown to increase isometric force in fast
82 skeletal muscles of rat and slow abdominal extensor muscle of crayfish (Bakker & Berg 2002; Galler
83 & Hutzler, 1990). The mechanism of action is believed to be modification of ion channel function and
84 an increase in Ca^{2+} sensitivity of the myofilaments (Camerino et al. 2004; Bakker & Berg 2002;
85 Cuisiner et al. 2000; Galler & Hutzler, 1990). Furthermore, a limited number of diverse studies
86 examining skinned rat heart to whole body human performance have demonstrated that taurine,
87 when combined with caffeine, can result in further potentiation of the effects of caffeine alone (Geib
88 et al. 1994; Steele et al. 1990). However, this effect has never been established using physiologically
89 relevant concentrations of taurine and caffeine to examine their direct effects on skeletal muscle.

90 The ergogenic properties of physiologically relevant concentrations (50-70µM) of caffeine on muscle
91 power output and fatigue have previously been evaluated (James et al. 2005; Tallis et al. 2012,
92 2013), and in agreement with the potential effect of taurine, modification in performance is
93 attributed to changes in intramuscular Ca^{2+} handling (Bhat, 1997; Daminai et al. 1996; Davis & Green,
94 2009; Fredholm et al. 1997; Magkos & Kavouras, 2005; Rossi et al. 2001).

95 The present study aims to uniquely assess if an acute physiological dose of taurine is sufficient to
96 improve muscular power and offset fatigue, and is the first to determine whether the ergogenic
97 effect of caffeine is augmented when combined with taurine when both are at a physiological
98 concentration. This research uses the work look technique as a closer representation of *in vivo*

muscle performance to compare the direct effects of physiologically relevant concentrations of taurine alone (2.64mM), caffeine alone (70μM), and taurine and caffeine combined on: 1) acute maximal muscle power output; 2) time to fatigue; 3) recovery following fatigue on isolated mouse soleus muscle.

Materials and Methods

Animals

The use of animals in this study was approved by the ethics committee of Coventry University. Female white mice (strain CD1 mice, Charles River, UK) were bred and kept at Coventry University. 8 -10 week old mice (body mass = 31.1±0.24 g, mean ± SE, *n* =72) were weighed and then killed by cervical dislocation in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1.

Soleus muscle was isolated from the right hind limb and pinned out at approximately its resting length at room temperature (19-21 ^{o}C). During dissection the muscle preparation was maintained in cooled (refrigerated) and frequently changed oxygenated (95% O_2; 5% CO_2) Krebs-Henseleit solution of composition (mM) NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54; pH 7.55 (at room temperature prior to oxygenation). For each preparation, the tendon and a small piece of bone was left attached at the proximal and distal ends. Aluminium foil T-clips were wrapped around each tendon leaving the bone at the back of the clip to help minimise tendon slippage when the muscle was producing force (James et al. 2005).

Isometric Studies

Isometric studies were used to determine the twitch and tetanus kinetics of isolated mouse soleus muscle. The foil clips at either end of the muscle preparation were clamped to a force transducer (UF1, Pioden Controls Ltd, UK) or a motor arm (DFG5.0, Solartron Metrology, UK) via crocodile clips. A Linear Variable Displacement Transformer was attached to the motor arm to measure the length changes the muscle was subjected to. The whole of the muscle was maintained in a flow through chamber containing circulated oxygenated Krebs-Henseleit solution at a constant temperature of 36

± 0.02°C. 10 minutes prior to the commencement of testing the muscle was allowed to equilibrate to the new environment. The preparation was then held at a constant length and stimulated via parallel platinum electrodes to generate a series of isometric twitch responses. The electrodes were not in contact with the nerve branch or the fibre itself but stimulated the muscle via the surrounding fluid. Muscle length and stimulation amplitude (12-16V) were altered to maximise isometric twitch force. In order to elicit a tetanus response the muscle was subjected to a 320ms burst of electrical stimuli and stimulation frequency was optimised (usually 140Hz) to achieve maximal isometric tetanus force. To ensure sufficient recovery a 5 minute rest period was imposed between each tetanus. An eyepiece graticule fitted to a microscope was used to measure muscle length (defined as L_0); mean muscle fibre length was then calculated as 85% of L_0 (James et al. 1995).

Work Loop Analysis of Muscle Power Output

The work loop technique was used in order to determine muscle power output during cyclical length changes (Josephson, 1985; James et al. 1996). Muscle preparations were subjected to four sinusoidal length change cycles at a strain of 0.10 around the previously determined L_0 that generated maximal isometric twitch and tetanus force. Therefore muscle lengthened by 5% from L_0 followed by a shortening to 5% shorter than L_0 before returning back to L_0. This occurred at a 5Hz cycle frequency that had previously been determined to elicit maximal mouse soleus muscle power output and is attainable *in vivo* (James et al. 1995). The strain employed also comes from previous estimates of maximal power output for mouse soleus muscle and again is attainable during *in vivo* locomotion (James et al. 1995). The stimulation frequency and amplitude found to yield maximal isometric force were employed to stimulate the muscle during the shortening phase of the work loop. Optimising the timing of muscle stimulation during the shortening phase maximises power output. In the present study a stimulus burst duration of 65ms was used, concurrent with the methods of James et

al. (1995) and Vassilakos et al. (2009). The stimulation phase shift was fixed at -10 ms, therefore muscle stimulation started 10 ms prior to the muscle reaching its maximal length and, with a burst duration of 65 ms, continued until 45 ms prior to the muscle reaching its shortest length. Muscle stimulation and length changes were controlled using custom written software (Testpoint, CEC, Massachusetts, USA) via a D/A board (KPCI3108, Keithley Instruments, Ohio, USA). Data were sampled at a rate of 10 kHz and then a work loop was formed, by plotting force against length, the area of which represents the net work done by the muscle during a single length change cycle (Josephson, 1985).

Evidence outlining the maximal acute consumption of taurine in humans is scarce and thus research defining maximal blood plasma taurine concentration is also limited. The physiologically relevant dosage of taurine used in the present study is therefore derived from maximal doses reported in humans without toxic effect. Taurine ingestion in doses up to 6g per day and further intravenous delivery of 5g of taurine has been reported in the literature (Milei et al. 1992; Mizushima et al. 1996). Based on results reported by Galloway et al. (2008) ingestion of 1.66g of taurine yields a peak blood plasma concentration of 750µM, assuming the rates of uptake and degradation scale proportionately, the present study uses a taurine concentration of 2.64mM equating to 5.8g, similar to maximal doses as previously outlined (5-6mg).

In order to test whether 2.64 mM of taurine alone (TAU) or 2.64 mM taurine and 70µM caffeine combined (TC) affected acute muscle power output, time to fatigue, or recovery from fatigue, each soleus muscle preparation was subjected to one of the following protocols. i.e. each muscle preparation was used to determine the effects of treatment on acute power output or time to fatigue or recovery from fatigue. 70µM is recognised as the human physiological maximum. The caffeine only treatment data at this concentration has been published previously (Tallis et al. 2012; 2013).

Acute Power Output

In order to test the acute effect of a given treatment the muscle was subjected to 4 work loop cycles at 10 minute intervals over 120 minutes. During the initial 3 measurements the muscle was maintained in standard Krebs-Henseleit solution. The fluid was then changed to Krebs-Henseleit solution containing either 2.64 mM taurine (TAU) or 2.64 mM taurine and 70µM caffeine combined (TC). This experimental treatment was maintained for 60 minutes. Following this, the solution was then changed to standard Krebs-Henseleit solution, for a wash out period of 40 minutes, to establish if the effect of treatment was reversed, returning the muscle to its pre treatment state. The second work loop of 4 was always used as the indicative measure of muscle power output at each time point.

Time to Fatigue

In order to test the effect of a given treatment on time to fatigue the muscle was again subjected to sets of 4 work loops at 10 minute intervals over 130 minute duration. During the initial 3 measurements (3 sets of 4 work loops) the muscle was maintained in standard Krebs-Henseleit solution. For the subsequent 40 minutes the fluid was changed to TAU or TC. The fourth measurement in the treatment solution was a fatigue run consisting of 100 consecutive work loop cycles; data was collected for every second loop. Forty minutes of incubation in the treatment solution was allowed prior to the fatigue run to give sufficient time to allow each preparation to respond to the treatment (Tallis et al. 2012; 2013). Directly after the fatigue run the circulating fluid was changed back to standard Krebs-Henseleit solution and recovery was monitored for 60 minutes. The control protocol followed the same process however the muscle was maintained in standard Krebs-Henseleit solution throughout.

Recovery from Fatigue

In order to measure the effects of taurine and/or caffeine on recovery from fatigue the same fatigue protocol as above was used, however, in the time up to and inclusive of the fatigue run the muscle was incubated in standard Krebs-Henseleit solution. Immediately after the fatigue run the solution was changed to TAU, TC or Caffeine (CAF) and recovery was monitored for the next hour.

Muscle Mass Measurements and Dimension Calculations

At the end of the experiment the muscle was disconnected from the apparatus and the tendons and bones removed leaving the muscle intact. Following this, the muscle was blotted on tissue paper to remove excess fluid. The muscle was then placed on an electronic balance (Mettler Toledo B204-S, Zurich, Switzerland) to determine the wet muscle mass. Mean muscle cross-sectional area was calculated from mean fibre length, muscle mass and an assumed muscle density of 1060 kg m^{-3} (Méndez and Keys 1960). Isometric stress was calculated as force divided by mean muscle cross-sectional area. Muscle power output was normalised to muscle mass to express power as Watts.kg^{-1}.

Statistical Analysis of Data

In order to compare the quality of muscle between treatment groups a single factor ANOVA was conducted on maximal untreated isometric stress and work loop power output data.

Due to the gradual development of an anoxic core in control conditions muscle power output will slowly decrease over time (Barclay, 2005). For the muscles tested in the present study power output decreased to an average 96 ± 1% of initial power over the 120 minute duration of the work loop protocol. In order to avoid the deterioration in muscle performance masking the effects of

235 treatment, a 1st order regression equation was calculated using the pre treatment control data and
236 post treatment washout control data in order to identify the linear relationship between control
237 muscle power output and time. This regression equation was then used to determine theoretical
238 control muscle power output for each time point during caffeine treatment (James et al. 2005; Tallis
239 et al. 2012).

240 Initially, for the acute power output data, pre treatment controls were compared against post
241 treatment washout controls for each experimental group. There was no significant difference
242 between these control data (Fig 1; paired t-test, $t<0.5$, $P>0.6$ in all cases) therefore, it was assumed
243 that any subsequent change in muscle power when incubated in TC, TAU or CAF was an effect of the
244 given treatment. In order to assess the effects of treatment for each acute response the control data
245 were compared directly against treatment data via paired t-tests. A single factor ANOVA was
246 conducted in order to identify whether the given increase in power output was different between
247 treatments (TC vs.TAU vs. CAF). Tukey post hoc tests were used to establish where these differences
248 occurred.

249 A two factor ANOVA (time and treatment) was used to determine whether muscle power output
250 changed over time. A second two factor ANOVA (time and treatment) was used to determine
251 whether the time to fatigue differed between treatment groups. In each case Tukey post hoc tests
252 were performed where applicable. However, single factor ANOVA were used, post hoc, at each time
253 point in order to assess whether power output was significantly different between treatments at
254 each time point of during the fatigue run.

255 A two factor ANOVA (time and treatment) was implemented to identify whether power output
256 increased significantly over recovery time and whether recovery differed between treatment groups,
257 Tukey post hoc tests were used for treatment. Single factor ANOVA were conducted at each time
258 point in order to identify if muscle power output differed between treatments at each time point.

259 Results were interpreted as significant when $p < 0.05$. Values are displayed as mean ± standard
260 error.

261 The original data regarding the effects of caffeine on maximal acute power output was taken from
262 that used in our previous publication, Tallis et al. (2012). Similarly, the original data assessing the
263 effects of caffeine on time to fatigue was taken from Tallis et al. (2013).

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278 **Results**

279 Comparisons between stress and power were made using only the original data presented in this
280 study (i.e. n=76 out of the n=92 total). The mean maximal isometric twitch and tetanus stress for
281 soleus muscle (n=76) was 38.6 ± 1.5 kN m^{-2} and 219 ± 7 kN m^{-2} respectively. Mean untreated maximal
282 muscle power output was 32.8 ± 1 W kg^{-1} (n=76). Untreated mean maximal isometric tetanus stress
283 and work loop power output were not significantly different between experimental groups (single
284 factor ANOVA main effect *F*= 1.13 *P*=0.35 and *F*=0.33, *P*=0.95 respectively). Therefore, it is assumed
285 that the muscles were of a similar quality between the experimental groups and that any
286 subsequent differences in response occurred due to the effects of treatment.

287 Mean maximal tetanus stress and power output of the original muscle samples used in the present
288 work are comparable to those reported in previous studies using similar methods (Askew et al. 1997;
289 James et al. 2004; Vassilakos et al. 2009). More significantly the present results for maximal stress
290 and power output were directly comparable to the 189 and 202 kN m^{-2} and 31.7 and 33.1 W kg^{-1}
291 previously reported by Tallis et al. (2012: 2013) respectively. This signifies that all of the soleus
292 muscle preparations used in the research by Tallis et al. were of similar quality pre-treatment, thus it
293 was viable to use the caffeine only treatment results from Tallis et al. (2012; 2013) for comparison to
294 the results reported in the present study, especially as it is the percentage response to treatment
295 that is of interest.

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297 **The Effect of Physiological Concentrations of Taurine and Caffeine on Muscle Power Output**

298 Treatment of soleus muscle with caffeine alone and taurine-caffeine combined resulted in a
299 significant increase in acute maximal muscle power output of 6.4% and 4.1% respectively (Fig 1;
300 paired t-test, *t*<-2.29, *P*<0.03 in both cases) compared to controls. Treatment with taurine alone
301 failed to elicit any significant increase in muscle power output (Fig 1; paired t-test, *t*=1.586, *P*=0.118).

The performance enhancing benefit was significantly dependant on treatment (Fig 1; single factor ANOVA main effect F=3.59, P=0.03). There was no significant difference between the benefit imposed by caffeine alone when compared with taurine and caffeine combined (Fig 1; Tukey P=0.628). The treatment effect of caffeine was significantly higher than that of taurine alone (Fig 1; Tukey P=0.027), however there was no significant difference between taurine and caffeine combined and taurine alone (Fig 1; Tukey P=0.178).

The Effect of Physiological Concentrations of Taurine and Caffeine on Time to Fatigue

The fatigue protocol was effective in inducing soleus muscle fatigue as power output reduced significantly over time (Fig 2; two factor ANOVA main effect of time F=110, P<0.001). There was a significant effect of treatment on time to net negative work (Fig 2; two factor ANOVA main effect F=15.68, P<0.001). Treatment of soleus muscle with caffeine alone or with taurine and caffeine combined resulted in a significantly decreased time to fatigue (time to net negative work decreased by 30% and 15% respectively) compared to controls (Fig 2; Tukey P<0.001 in both cases). Soleus muscle treated with caffeine fatigued significantly faster than muscle treated with taurine and caffeine combined (Fig 2; Tukey P<0.001). There was no significant difference in time to fatigue between soleus muscles treated with taurine alone compared to controls (Fig 2; Tukey P<0.001).

Soleus muscle treated with caffeine produced significantly less power when compared with: muscle treated with taurine from 3.2s onwards; controls from 3.6s onwards; taurine and caffeine combined from 4.8s (Fig 2; Tukey P<0.04 in all cases). There was no significant difference in the mean power output at any time point between any other treatments (Fig 2; Tukey P>0.05 in all cases).

The Effect of Physiological Concentrations of Taurine and Caffeine on Recovery from Fatigue

Power output significantly increased over time during recovery from fatigue (Fig 3; two factor ANOVA main effect F=8.29, P<0.001). The level of soleus muscle recovery was not significantly dependent on treatment (Fig 3; two factor ANOVA main effect F=2.59, P=0.054). Mean muscle recovery for the entire group was 87% of the pre fatigue maximum and occurred between 80 and 90 minutes following the start of the work loop protocol (Fig 3). Although during the latter stages of the measured recovery period, muscles treated with taurine and caffeine combined appeared to have reduced recovery, power output was not significantly different between treatments at any given time point during the recovery period (Fig 3; single factor ANOVA main effect p>0.156 in all cases).

Discussion

The Effect of Physiological Concentrations of Taurine and Caffeine on Muscle Power Output

Treatment of mouse soleus muscle with 2.64mM of taurine failed to elicit any significant increases in mean maximal muscle power output compared to controls. When taurine was combined with 70µM caffeine, mean maximal power output was significantly increased by up to 4.1%. This acute increase in maximal power output was not significantly different from the up to 6.4% increase induced with caffeine treatment alone. Therefore, the direct treatment of soleus muscle with an acute taurine supplement failed to potentiate short term maximal power output and failed to further potentiate the effects of caffeine when compared to caffeine treatment alone.

The results of the present study contradict the findings of Galler and Hutzler (1990) who demonstrated that physiological intramuscular concentrations of taurine (5mM) increased the submaximal isometric force of pig heart muscle and slow abdominal extensor muscle of crayfish. Such differences between studies may be related to the different muscle preparations used as cardiac muscle and crustacean skeletal muscle have previously been found to have different mechanical properties to vertebrate skeletal muscle. 20mM taurine treatment has previously been shown to potentiate force production of skinned rat EDL (Bakker & Berg 2002). Previous evidence suggests that the mechanism for this given increase in muscle performance following taurine treatment is largely attributed to short term modification of ion channel function, Ca^{2+} homeostasis and, increased myofilament Ca^{2+} sensitivity (Bakker and Berg 2002; Camerino et al. 2004; Cuisinier et al. 2000). Additionally, Cuisinier et al. (2000) demonstrated that taurine did not modify Ca^{2+} sarcoplasmic reticulum uptake. However, it has been suggested that the antioxidant properties of taurine may reduce oxidative stress on the sarcoplasmic reticulum Ca^{2+} pump (Schaffer et al. 2010). As no increase in acute muscle power was reported in the present study, it is suggested that taurine treatment failed to elicit improved skeletal muscle Ca^{2+} handling. The current research uses a

physiologically relevant taurine concentration (2.64mM) which is much lower than that of previous work (5-20mM) and uniquely examines treatment effects over dynamic muscle contraction, rather than constant muscle length studies, which may also contribute to the differences in results.

Steele et al. (1990) reported that taurine concentrations greater than 1mM contributed to larger caffeine induced (10mM) contracture of skinned rat heart tissue. This effect of taurine-caffeine combined was not transferable to skeletal muscle in the present study when used at a physiological level (70µM human maximal) of caffeine concentration and the combined effect was not greater than that of caffeine alone in the present study. Besides the differences in caffeine concentration, 10mM caffeine would be highly toxic for human consumption (Fredholm et al. 1999), it is considered that the effects of taurine are more profound in heart tissue which may further contribute to the differences in results (Schaffer et al. 2010).

The caffeine induced increase in muscle power has been attributed to a greater release of Ca^{2+} into the intracellular space, an increase in myofibrillar Ca^{2+} sensitivity, a decrease in the sensitivity of the SR Ca^{2+} pump, and an increased SR Ca^{2+} permeability (Allen et al. 1989, 1995; Tallis et al. 2012, 2013). The present results indicate that a physiological dose of taurine is unable to further modify the soleus muscle Ca^{2+} response to electrical stimulation.

The Effect of Physiological Concentrations of Taurine and Caffeine on Time to Fatigue

Treatment with 2.64mM of taurine failed to significantly affect the time to fatigue in maximally stimulated mouse soleus muscle compared to controls. When taurine was combined with caffeine time to fatigue was significantly decreased, although treatment of soleus muscle with caffeine alone resulted in significantly faster time to fatigue than all other treatments. These results suggest that in this instance, adding taurine to caffeine may in part block the effects of caffeine alone as the profile of fatigue is shifted to the right with respect to the caffeine only treatment data (Fig 2).

A reduction in skeletal muscle taurine content following prolonged muscle stimulation has previously been established *in vitro* (Kim et al. 1986; Matsuzaki et al. 2002). Chronic supplementation has been shown to significantly increase muscle taurine concentration resulting in increased muscle force production and partially counteracting the effect of intramuscular taurine depletion that occurs during fatigue (Goodman et al. 2009). Yatable et al. (2003) used a whole animal model to assess the effects of 7 days of 0.5g/kg taurine supplementation on rat skeletal muscle taurine content. Skeletal muscles of the leg displayed a significant increase in taurine concentration post treatment which was followed by an increased time to fatigue of subsequent treadmill running until exhaustion. Post exercise the supplemented rats demonstrated less of an exercise related reduction in skeletal muscle taurine concentration compared to controls. The effect demonstrated from prolonged dietary supplementation of taurine did not prevail in the present acute study suggesting that any increase in soleus muscle taurine concentration from the given treatment was not sufficient enough to offset fatigue.

Tallis et al. (2013) discussed that the decreased time to fatigue in caffeine treated soleus muscle was due to an increase in eccentric work during the lengthening phase of the work loop. In fatigue under control conditions muscle relaxation time is prolonged due to an increase in intracellular Ca^{2+} concentration (Askew, 1997). It is believed that this effect is amplified in the presence of caffeine resulting in the muscle being active to a greater extent at the end of shortening, hence greater work is required to stretch the muscle back to its resting length (Tallis et al. 2013). This provides a partial explanation as to why TC fatigues faster than controls. Silva et al. (2011) demonstrated that chronic supplementation of taurine in rats (1-ml 300 mg kg^{-1} per body weight) had a cytoprotective role in exercise induced muscle injury. Although this mechanism is not ruled out by the authors it is unlikely that the taurine concentration used in the present study will increase intramuscular taurine levels enough to generate this mechanism. Moreover, Tallis et al. (2012) highlighted the diverse individual acute response to 70μM caffeine and a clear division between responders and non-responders to

419 the treatment. The difference in time to fatigue between CAF and TC are most likely to be due to
420 individual differences in response to the caffeine treatment and the ratio of responders and non-
421 responders between the treatment groups. This is partly supported in the present study by the
422 increase in standard error for the TC trial compared to CAF (Fig 2).

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424 **The Effect of Physiological Concentrations of Taurine and Caffeine on Recovery from Fatigue**

425 Treatment of mouse soleus muscle with taurine (T), caffeine (CAF), or taurine and caffeine (TC)
426 combined failed to affect recovery compared to controls. Similarly, James et al. (2004) reported that
427 70μM caffeine treatment failed to affect the recovery of both mouse soleus and EDL following
428 fatigue. The established ergogenic benefit of caffeine on direct muscle power output of non-fatigued
429 muscle is not great enough to offset the fatigue induced reduction in calcium handling properties
430 (Allen et al. 2008; Tallis et al. 2012).

431 Previous work has shown that intramuscular taurine concentration will decrease during fatigue (Kim
432 et al. 1986; Matsuzaki et al. 2002). Consequently it is considered that the taurine concentration used
433 in the present work was not significant enough to increase total intramuscular taurine concentration
434 to a level to induce enhanced muscle recovery. With no significant effect of either taurine or caffeine
435 alone, it is unsurprising that combining these treatments also failed to elicit an enhanced fatigue
436 recovery.

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438 **Practical Implications of the Present Findings**

439 The present findings infer that taurine does not provide any acute ergogenic effect directly on
440 skeletal muscle. However, further research investigating the effect of taurine on other physiological
441 systems, particularly central effects, should also be considered. Furthermore, in rat skeletal muscle,

taurine concentration has been shown to be greater in relatively slow twitch solues muscle (33 µmol g wet weight^{-1}) compared to relatively fast twitch EDL (17 µmol g wet weight^{-1}; Iwata et al. 1986). Therefore an interesting area of future research may be to examine whether there is an acute effect of taurine on relatively fast twitch muscle where a given dose may represent a greater stimulus relative to normal muscle concentrations of taurine.

Although evidence for the prolonged supplementation of taurine has been established, achieving this through the regular consumption of caffeine containing products is not recommended. Regular consumption of caffeine has been shown to result in habituation and a dampening of the ergogenic effects, therefore increasing muscular taurine via this method may sacrifice the benefit of caffeine (Bell & Mcleellan, 2002). In addition to the high quantity of sugar in such drinks, the present findings and the previous work by Tallis et al. (2012; 2013) have demonstrated that caffeine is likely the key ingredient in energy drinks to provide an acute effect directly on skeletal muscle performance.

Acute treatment of taurine on isolated skeletal muscle failed to elicit any significant effect on mouse soleus muscle performance. One off maximal muscle power output, time to fatigue and recovery from fatigue were all unchanged. Taurine treatment also failed to further potentiate the effects of caffeine on the same parameters. In conclusion, a physiological level of taurine, to match the blood plasma that could occur from acute supplementation of taurine containing products such as energy drinks, did not provide any direct short term ergogenic benefit in isolated skeletal muscle.

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492 **References**

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

495 Allen, D.G., Lee, J.A., Westerblad,H. 1989. Intracellular calcium and tension during fatigue in isolated
496 muscle fibres from Xenopus laevis. J. Physiol. 415: 433-458.

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

499 Alford, C., Cox, H., Wescott, R. 2001. The effects of red bull energy drink on human performance and
500 mood. Amino Acids 21: 139-150.

501 Askew, G.N., Young, I.S., Altringham, J.D. 1997. Fatigue of mouse soleus muscle, using the workloop
502 technique. J. Exp. Biol. 200: 2907-2912.

503 Bakker, A.J., Berg, H.M. 2002. Effect of taurine on sarcoplasmic reticulum function and force in
504 skinned fast-twitch skeletal muscle fibres of the rat. J. Physiol. 538: 185-194.

505 Barclay, C.J. 2005. Modelling diffusive O(2) supply to isolated preparations of mammalian skeletal
506 and cardiac muscle. J. Muscle Res. Cell Motil. 26: 225-235.

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

509 Bhat, M.B., Zhao, J., Zang, W., Balke, C.W., Takeshima, H., Wier, W.G., et al. 1997. Caffeine-induced
510 Release of Intracellular Ca2+ from Chinese Hamster Ovary Cells Expressing Skeletal Muscle
511 Ryanodine Receptor. Effects on Full-Length and Carboxyl-Terminal Portion of Ca2+ Release Channels.
512 J. Gen. Physiol. 110: 749-762.

513 Camerino, D.C., Tricarico, D., Pierno, S., Desaphy, J.F., Liantonio, A., Pusch, M., et al. 2004. Taurine
514 and skeletal muscle disorders. Neurochem. Res. 29: 135-142.

515 Cuisinier, C., Gailly, P., Francaus, M., Lebacq, J. 2000. Effects of guandinoethane sulfonate on
516 contraction of skeletal muscle. Adv. Exp. Med. Biol. 483: 403-409.

517 Cuisinier, C., Michotte de Welle, J., Verbeeck, R.K., Poortmans, J.R., Ward, R., Sturbois, X., et al. 2002.
518 Role of taurine in osmoregulation during endurance exercise. Eur. J. Appl. Physiol. 87: 489-495.

519 Damiani, E., Larsson, L., Margreth, A. 1996. Age-related abnormalities in regulation of the ryanodine
520 receptor in rat fast-twitch muscle. Cell Calcium 19: 15-27.

521 Davis, J.K., Green, J.M. 2009. Caffeine and anaerobic performance: Ergogenic value and mechanisms
522 of action. J. Sports. Med. 39: 813-832.

523 Duncan, M.J., Oxford, S.W. 2011. The effect of caffeine on mood state and bench press performance
524 to failure. J. Strength Cond. Res. 25: 178-185.

525 Fredholm, B.B., Battif, K., Holmen, J., Nehlig, A.N., Zvartau, E.E. 1999. Actions of caffeine in the brain
526 with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51: 83-133.

527 Galloway, S.D.R., Talanian, J.L., Shoveller, A.K., Heigenhauser, G.J.F., Spriet, L.L. 2008. Seven days of
528 oral taurine supplementation does not increase muscle taurine content or alter substrate
529 metabolism during prolonged exercise in humans. J. Appl. Physiol. 105 643-651.

530 Galler, S., Hutzler, C. 1990. Effects of taurine on Ca2+ dependant force development of skinned
531 muscle fibre preparations. J. Exp. Biol. 152: 255-264.

532 Geib, K.R., Jester, I., Falke, W., Hamm, M., Waag, K.L. 1994. The effect of a taurine-containing drink
533 on performance in 10 endurance athletes. Amino Acids 7: 45-56.

534 Goodman, C.A., Horvath, D., Stathis, C., Mori, T., Croft, K., Murphy, R.M., et al. 2009. Taurine
535 supplementation increases skeletal muscle force production and protects muscle function during
536 and after high-frequency in vitro stimulation. J. Appl. Physiol. 107: 144-154.

537 Graham, T.E. 2001. Caffeine and exercise. Metabolism, endurance and performance. J. Sports Med.
538 31: 785-807.

539 Hamilton, E.J., Berg, H.M., Easton, C.J., Bakker, A.J. 2006. The effect of taurine depletion on the
540 contractile properties and fatigue in fast skeletal muscle of the mouse. Amino Acid 31: 273-278.

541 Imagawa, T.F., Hirano, I., Utsuki, K., Horie, M., Naka, A., Matsumoto, K., et al. 2009. Caffeine and
542 taurine enhance endurance performance. Int. J. Sports Med. 30: 485-488.

543 Iwata, H., Obara, T., Kim, B.K., Baba, A. 1986. Regulation of taurine transport in rat skeletal muscle. J.
544 Neurochem. 47: 158-163.

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

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

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

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

554 Josephson, R.K. 1985 Mechanical power output from striated muscle during cyclical contraction. J
555 Exper. Bio. 114: 493-512.

556 Kim, B.K., Baba, A., Iwata, H. 1986. Taurine transport in chronically stimulated fast- and slow-twitch
557 muscle of the rat. Jpn. J. Pharmacol. 42: 441-446.

558 Magkos, F., Kavouras, S.A. 2005. Caffeine use in sports, pharmokinetics in man, and cellular
559 mechanisms of action. Crit. Rev. Food Sci. Nutr. 45: 535-562.

560 Matsuzaki, Y., Miyazaki, T., Miyakawa, S., Bouscarel, B., Ikegami, T., Tanaka, N. 2002. Decreased
561 taurine concentration in skeletal muscles after exercise for various durations. Med. Sci. Sports Exerc.
562 34: 793-797.

563 Méndez, J., Keys, A. 1960. Density and composition of mammalian muscle. Metab. 9: 184-188.

564 Milei, J., Ferreira, R., Llesuy, S., Forcada, P., Covarrubias, J., Boveris, A. 1992. Reduction of
565 reperfusion injury with preoperative rapid intravenous infusions of taurine during myocardial
566 revascularization. Am. Heart. J. 123: 339-345.

567 Mizushima, S., Nara, Y., Sawamura, M., Yamori, Y. 1996. Effects of oral taurine supplementation on
568 lipids and sympathetic nerve tone. Adv. Exp. Med. Biol. 403: 615-622.

569 Oja, S.S., Saransaari, P. 2007. Pharmacology of Taurine. Proc. West Pharmacol. Soc. 50: 8-15.

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

572 Schaffer, S.W., Jong, C.J., Ramila, K.C., Azuma, J. 2010. Physiological roles of taurine in heart and
573 muscle. J. Biomed. Sci. 17.

574 Silva, L.A., Silveria, C.L., Ronsani, M.M., Souza, P.S., Scheffer, D., Vieira, L.C., et al. 2011. 'Taurine
575 supplementation decreases oxidative stress in skeletal muscle after eccentric exercise. Cell Biochem.
576 Func. 29: 43-49.

577 Steele, D.D., Smith, G.L., Miller, D.J. 1990. The effects of Taurine Ca^{2+} uptake by the sarcoplasmic
578 reticulum and Ca^{2+} sensitivity of chemically skinned rat heart. J. Physiol. 422: 499-511.

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

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

585 Vassilakos, G., James, R.S., Cox, V.M. 2009. Effect of stimulation frequency on force, net power
586 output and fatigue in mouse soleus muscle in vitro. Can. J. Physiol. Pharmacol. 87: 203-210.

587 Warskulat, U., Flogel, U., Jacoby, C., Hartwig, H.G., Thewissen, M., Merx, M.W., et al. 2004. Taurine
588 transport knockout depletes muscle taurine levels and results in severe skeletal muscle impairment
589 but leaves cardiac function uncompromised. FASEB. J. 18: 577-579.

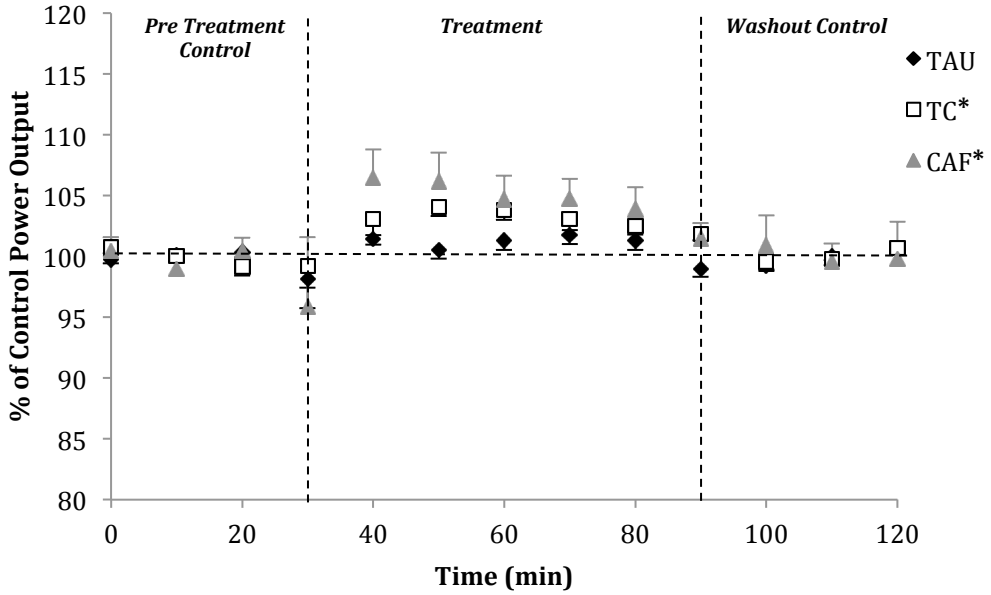
590 Yatabe, Y., Miyakawa, S., Miyazaki, Y., Ochiai, N. 2003. Effects of taurine administration in rat
591 skeletal muscles on exercise. J. Orth. Sci. 8: 415-419.

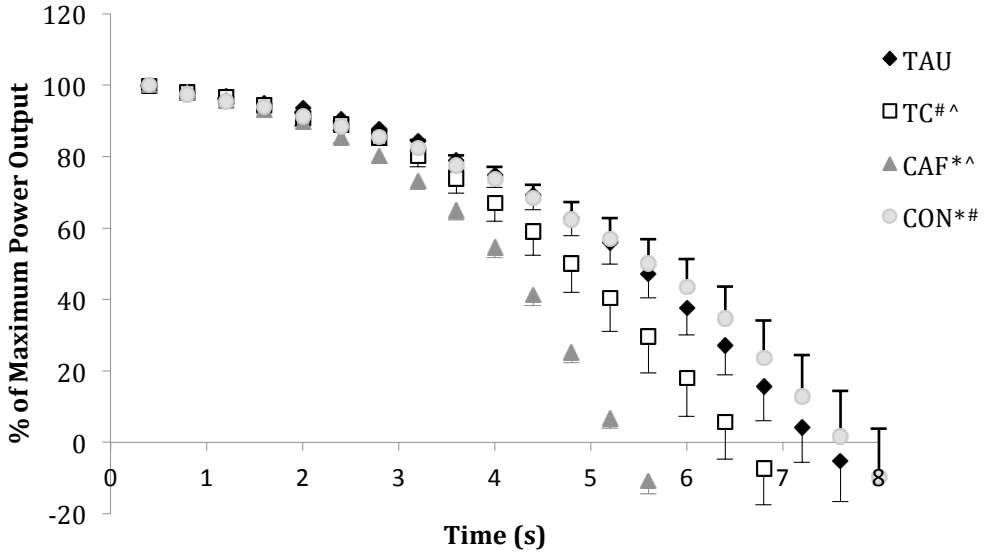
Figures

Figure 1. – The mean acute effect of physiological concentrations of taurine alone (TAU), caffeine alone (CAF), and taurine and caffeine combined (TC) on work loop power output in maximally stimulated mouse soleus muscle [Data represented as mean & SE; Original data for CAF published in Tallis et al. 2012; * represent significant (paired t-test $P<0.03$) differences in muscle power output, during the treatment phase, when compared to controls] n=10 for TAU and TC, n=8 for CAF.

Figure 2. – The effects of physiological concentrations of taurine alone (TAU), caffeine alone (CAF), and taurine and caffeine combined (TC) on time to fatigue in maximally stimulated mouse soleus muscle. Values are displayed as a percentage of maximal work loop power output. [Data represented as mean & SE; Original data from CAF published in Tallis et al. 2013; There was a significant effect of treatment on time to net negative work (ANOVA $P<0.001$), * # ^ represent significant (Tukey $P<0.001$) differences in time to fatigue between treatment groups, whereby any groups with the same symbol are significantly different from each other] n=8 in each case.

Figure 3 - Effect of physiological concentrations of taurine alone (TAU), caffeine alone (CAF), and taurine and caffeine combined (TC) on the recovery of mouse soleus muscle following fatigue at maximal stimulation frequency. Time 60 represents both 60 minutes after the initial treatment began and the time at which the fatigue run was performed. [Data represented as mean & SE] n = 8 in each case.


Pre Treatment Control
Treatment
Washout Control
TAU
TC*
CAF*
% of Control Power Output
Time (min)
120
115
110
105
100
95
90
85
80
0
20
40
60
80
100
120


120
100
80
60
40
20
0
-20
0
1
2
3
4
5
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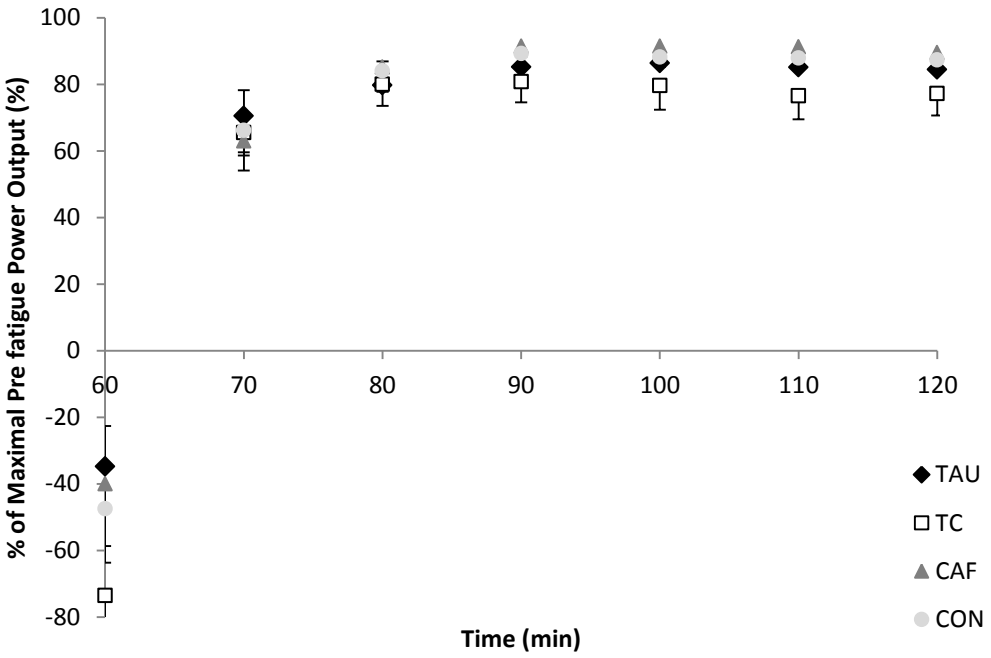
7
8
% of Maximum Power Output
Time (s)
TAU
TC#^
CAF*^
CON*#


100
80
60
40
20
0
-20
-40
-60
-80
% of Maximal Pre fatigue Power Output (%)
60
70
80
90
100
110
120
Time (min)
TAU
TC
CAF
CON