Caffeine enhances non-exercise activity thermogenesis in rats

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Abstract

Caffeine and its derivatives have been used, alone and in combination with other phytochemicals, as weight-loss supplements. Caffeine impacts several physiological and behavioral aspects of energy balance. Here, we investigate the potential for caffeine to enhance non-exercise activity thermogenesis (NEAT) even when activity level is held constant. To do this, muscle thermogenesis and energy expenditure (EE) were measured in rats during treadmill walking regimens, with and without caffeine (25 mg/kg, i.p.). Muscle heat dissipation was significantly increased by caffeine only at the end of the 25-min treadmill test. Activity-related EE, on the other hand, was significantly increased throughout the treadmill walking protocol. This study demonstrates that caffeine increases the calories used during physical activity even in the absence of altered physical activity, implicating decreased muscle work efficiency (fuel economy of activity).

Key words: Physical activity, energy expenditure, skeletal muscle, thermogenesis, NEAT

Introduction

As US obesity rates rise, new prevention and treatment options are considered. Intervention programs include not only medical options like weight-loss surgery or medications, but also public-health initiatives such as those designed to curtail consumption of sugar-sweetened beverages (Pereira, 2014). Sugar-sweetened beverages are a major contributor to caloric intake, particularly in children and adolescents (Reedy and Krebs-Smith, 2010). Ironically, components in some of these sugar-sweetened beverages may also hold answers and possibilities for treating this epidemic. Caffeine and caffeine derivatives have been used as dietary supplements, alone and in combination with other ingredients, often in attempts to control obesity or weight gain (Kovacs and Mela, 2006, Hursel and Westerterp-Plantenga, 2013). Caffeine-induced changes in thermogenesis and activity levels can promote negative energy balance (Kovacs and Mela, 2006), and caffeine may also affect metabolic efficiency.

Caffeine affects not only energy balance but also exercise endurance and performance (Bell and McLellan, 2002, Warren et al., 2010, Tallis et al., 2015). Caffeine can increase muscle performance, energy expenditure (EE), thermogenesis, and fuel utilization though several potential mechanisms (Magkos and Kavouras, 2005, Hursel and Westerterp-Plantenga, 2013). These include direct effects on behaviors including physical activity (Marin et al., 2011), effects on peripheral metabolism through alterations in sympathetic nervous system (SNS) activity (Dulloo et al., 1994), or direct effects on peripheral metabolic targets though inhibition of cAMP phosphodiesterase or adenosine receptors or activation of AMP-kinase (Dulloo et al., 1992, Egawa et al., 2009, Egawa et al., 2011a, Egawa et al., 2011c). Lastly, caffeine activates ryanodine receptors (RYR), including RYR 1 and 3 found in skeletal muscle (Magkos and

Kavouras, 2005). Caffeine also has direct effects in skeletal muscle physiology (Tallis et al., 2015); this could potentially occur though RYR-induced changes in the activity of sarco/endoplasmic reticulum Ca2+-ATPase (SERCA). Changes in SERCA activity, for example by phospholamban or sarcolipin, can induce thermogenesis (Rowland et al., 2014, Bal et al., 2012). The potential thermogenic effects of caffeine on skeletal muscle have not been explicitly tested, however.

Elevated non-exercise activity thermogenesis (NEAT) is associated with leanness in both laboratory models and humans (Novak and Levine, 2007, Kotz and Levine, 2005), suggesting that enhancing NEAT can be used to counter weight gain. We have also found that enhanced NEAT is associated with elevated skeletal muscle thermogenesis (Gavini et al., 2014), where decreased fuel economy of activity serves to increase activity EE, with caloric energy dissipated as heat from muscle. Here, we test the hypothesis that caffeine enhances EE partly through increasing skeletal muscle thermogenesis. To rule out the known effect of caffeine on physical activity levels (Marin et al., 2011), NEAT was assessed in rats while activity levels were controlled using treadmill walking protocols; this isolates the effect of muscle work efficiency on NEAT.

Methods

Animals. Two studies were conducted, both using adult male Sprague-Dawley rats, N=8 for muscle thermogenesis, N=6 for treadmill EE. Rats were housed in a temperature-controlled room (24°C ± 1°C) on a 12:12-h light-dark cycle with lights-on at 0700 Eastern Standard Time. Rats were given free access to both standard laboratory chow (5P00 MRH 3000, T.R. Last Co. Inc.) and water. All procedures were approved by Kent State University Institutional Animal Care and Use Committee.

Treatment. Dose and timing of caffeine treatment were chosen based on optimal effects on locomotor activity in rats (Marin et al., 2011). Caffeine solutions were prepared using pharmaceutical grade caffeine (Sigma- Aldrich 93784) and sterile saline; pre-made aliquots were frozen for a maximum 6 weeks. The rats were injected intraperitoneally (i.p.) with 25 mg/kg caffeine solution or an equal volume of vehicle (1ml/kg) before the treadmill test; each at was tested in one of two treadmills, with each rat undergoing all treatments in the same treadmill. Treatment order was counterbalanced and separated by at least 1 day, and treatments were given at the same time of day (within 2.5 hours) within rat to eliminate potential time-of-day effects. All treadmill tests were completed in an environmental temperature kept at a temperature thermoneutral for rats (25.0-25.9°C).

Muscle Thermogenesis. Temperature transponders (IPTT-300, BioMedic Data Systems) were surgically implanted in the hind legs bilaterally, against the gastrocnemius muscle group; the

sensor was used to measure temperature with 0.1°C resolution. Muscle temperature was first measured before injection of caffeine or vehicle. After injection, rats were placed on the stationary treadmill for 15 min, then muscle temperatures were measured again (0 min). The treadmill was then started, and temperatures were measured as follows: at 2, 5, and 10 min of 7 m/min at 0° incline; at 15 min at 9 m/min and 0° incline; at 20 min at 9 m/min and 10° incline; at 25 min at 11 m/min and 10° incline; and at 30 min at 11 m/min and 20° incline. Rats walked on the treadmill for 30 min or until they would no longer walk, whichever came first. Final temperature was measured immediately upon completion of treadmill walking regardless of test duration.

Treadmill Energy Expenditure. Immediately after injection, rats were placed into the enclosed treadmill apparatus, monitored by an Oxymax FAST small-animal calorimetry system (Columbus Instruments; Columbus OH) calibrated using primary gas standards. Fresh air was provided to the sealed treadmill at 3.5-3.6 LPM, and sampled at 0.5 LPM. At 20 min after injection, the treadmill was run at a constant low velocity (7 meters per minute) for up to 30 minutes while gas exchange was measured every 10 seconds.

Statistical Analyses. Caffeine's effect on activity-related muscle heat dissipation was analyzed using a repeated-measures ANOVA, with caffeine's effects on temperature over 25 min of treadmill running analyzed separately for the right and left legs. The effect of caffeine on final temperature was compared using a paired t-test (1-tailed). Calorimetric variables [EE; respiratory exchange ratio (RER = VCO_2/VO_2)] were averaged into 3 10-min blocks and analyzed using a 2X3 repeated-measures ANOVA; total EE and RER were compared using 1-tailed paired t-tests.

For the gas-exchange experiment, data from one rat were removed because the change in total activity-EE value fell above criterion for an outlier (Leys et al., 2013) due to an unusually low vehicle-induced EE measurement. Significance was determined as $p<0.05$.

Results

Muscle Thermogenesis. Body weight of rats did not significantly differ between the two conditions (caffeine, 599.8g±15.4g; vehicle, 596.2g±12.9g; p=0.306). The ANOVA showed a significant main effect of treadmill intensity on muscle temperature in both the right and left legs ($p<0.001$) with no main effect of caffeine on either the left or right leg muscle temperatures (p=0.212 for left leg, and 0.192 for right leg). As shown in Figure 1, there was a significant interaction between caffeine treatment and treadmill intensity in the left leg (p=0.006) but not in the right leg (p=0.221); this discordance may be due to minor differences in transponder placement. In the 5 rats which walked at least 25 min, temperatures in both the left and right gastrocnemius were significantly higher at 25 min compared to 0 min (immediately before treadmill running, 15 min after caffeine injection; right leg, p=0.016; right leg, p=0.010); there was no significant difference in either the left or right gastrocnemius temperatures at any other time point, including before injection or 15 min after injection, before treadmill walking commenced. As shown in Figure 1B, when final temperature was measured immediately after walking ceased, temperatures were significantly higher in both the left ($p<0.001$) and right (p=0.005) gastrocnemius. The time spent walking (in min) was not significantly longer after caffeine treatment, though there was a trend in this direction (p=0.079). 5 of the 8 rats walked longer after caffeine than after vehicle treatment; in all 3 rats that walked longer after vehicle treatment, the final temperature was still significantly higher in the caffeine condition in both the left (39.8 vs. 39.0°C, p=0.021) and right (40.1 vs. 39.3°C, p=0.039) gastrocnemius. Similarly, the change in temperature in both left and right gastrocnemius muscles between 0 min and after completing the walking protocol was

significantly higher after caffeine (Figure 1C). This effect remained significant when examining the 3 rats that walked longer with vehicle compared to caffeine (left gastrocnemius, 1.6°C vs. 2.3°C p=0.034; right gastrocnemius, 1.2°C vs. 1.8°C, p=0.038).

Treadmill Energy Expenditure. Body weight of rats did not significantly differ between the two conditions (caffeine, 473.9±9.3g; vehicle, 473.8±11.8; p=0.495). As illustrated in Figure 2A, caffeine significantly increase the kcal used to walk at a constant speed in rats. The ANOVA revealed a significant main effect of caffeine (p=0.003), and a significant main effect over the three 10-min measurement periods (p=0.001), but no significant interaction between treatment and time on treadmill (p=0.336). The increase in EE above vehicle levels ranged from 6.5% in the first 10 min to 10.0% in the final 10 min, with an average 8.3% increase for the 30-min duration of the walk. During the 5-min period before the treadmill was started, EE was also higher in the rats after caffeine treatment compared to vehicle treatment (vehicle, 2.65±0.22 kcal/hr; caffeine, 3.41±0.06; p=0.020), with the caffeine-induced increase from baseline EE ranging from < 1% to 70%.

The RER showed a significant interaction between treatment and time (p=0.003), and a significant main effect of time on treadmill (p<0.001), but no main effect of caffeine (p=0.301). Specifically, treadmill-walking RER started high and decreased over the 30-min walking test, but RER was significantly lower after caffeine treatment in the 1st 10-min period. When considering the 5-min period immediately before the rats walked on the treadmill, RER did not differ between treatments (vehicle, 0.83±0.02; caffeine, 0.82±0.01; p=0.408).

Discussion

Caffeine induces negative energy balance by altering multiple aspects of behavior and metabolism, including physical activity, thermogenesis, and EE (Dulloo et al., 1994, Dulloo et al., 1992, Bracale et al., 2014, Davoodi et al., 2014, Miles-Chan et al., 2015, Marin et al., 2011). Here, we demonstrate that caffeine enhances activity-related EE (i.e., NEAT) even when physical activity is not changed. Specifically, caffeine significantly increased EE by 8% during a treadmill walking protocol, implicating decreased skeletal muscle work efficiency. Skeletal muscle thermogenesis after completion of a graded treadmill walking protocol was also significantly increased by caffeine, suggesting that the additional caloric expenditure induced by caffeine was dissipated as heat energy. Altogether, these studies suggest that caloric expenditure during physical activity, at least moderate-level activity, is enhanced by caffeine.

The effect of caffeine on muscle work efficiency and thermogenesis seen here is consistent with the known ability of caffeine to enhance muscle power and exercise endurance (Bell and McLellan, 2002, Warren et al., 2010, Tallis et al., 2015, Magkos and Kavouras, 2005). The ability of caffeine to augment athletic performance is more pronounced when endurance is required, as opposed to short-term activity (Magkos and Kavouras, 2005); *in vitro*, slow-twitch type I fibers are more sensitive to caffeine compared to fast-twitch type II fibers (Magkos and Kavouras, 2005). The data presented here suggest that caffeine's enhancement of performance comes at a cost of decreased efficiency, resulting in additional calories burned for the same activity or workload.

Changes in skeletal muscle work efficiency have been implicated in adaptive thermogenesis during calorie restriction in humans (Rosenbaum et al., 2003) as well as

individual differences in EE and obesity resistance in rats (Gavini et al., 2014, Novak et al., 2010). In lean rats, elevated NEAT is also associated with higher muscle thermogenesis (Gavini et al., 2014). In the present study, whereas EE remained significantly elevated throughout treadmill walking (Figure 2), significantly elevated muscle heat dissipation was only observed at the final measurement (after walking 25 min) and at the completion of a graded treadmill walking test (Figure 1). Minor variance in duration of activity was not the determining factor in the elevated muscle heat dissipation in caffeine-treated rats: even the rats that walked for a longer duration after vehicle treatment compared to after caffeine treatment had significantly higher gastrocnemius temperatures and change in temperature after caffeine treatment. This diverges somewhat from our previous studies on muscle heat dissipation, where group differences or treatment-related changes in muscle thermogenesis typically occur during low level activity rather than at the end of the test (Gavini et al., 2014). It is likely that calorimetric measurement of gas exchange is a more sensitive measurement of the physiological processes underlying muscle work efficiency.

Activity-related fuel utilization was affected by caffeine as well. RER immediately before the treadmill test was not significantly affected by caffeine, despite the caffeine-induced difference in EE at this time. As shown in Figure 2C, RER increased during the first 10 min of activity, but RER did not increase to the same extent after caffeine treatment compared to vehicle, suggesting that fat oxidation was relatively enhanced by caffeine at this time. This is consistent with caffeine's lipolytic effects on white adipose tissue, attributed to its actions on adenosine receptors (Panchal et al., 2012), as well as its direct effect on fuel uptake and metabolism (Egawa et al., 2009).

The ability of caffeine to increase NEAT through alterations in muscle work efficiency may stem from one or more of its known actions on metabolism. First, caffeine may affect peripheral metabolism through changing autonomic outflow. For example, increased SNS outflow to skeletal muscle increases muscle glucose uptake (Minokoshi et al., 1994, Shiuchi et al., 2009). Caffeine's thermogenic actions on brown adipose tissue have been attributed to SNS outflow as they are prevented by chemical sympathetic denervation (Dulloo et al., 1994). Caffeine may also act directly on skeletal muscle through a multitude of possible routes (Magkos and Kavouras, 2005), including inhibiting phosphodiesterase activity (Dulloo et al., 1992), inhibition of adenosine receptors (Fredholm, 1995), activating AMPK (Egawa et al., 2011a, Egawa et al., 2011c, Egawa et al., 2009), or inhibition of glycogen phosphorylase (Magkos and Kavouras, 2005). Caffeine's thermogenic actions may also be a result of its potentiation of RYR-induced Ca^{2+} release from muscle sarcoplasmic reticulum (Magkos and Kavouras, 2005). Skeletal muscle is the primary expression site of RYR1, mutations of which are associated with malignant hyperthermia (Lanner, 2012). Enhanced sarcoplasmic Ca^{2+} release may also increase the sarco/endoplasmic reticulum Ca^{2+} ATP-ase (SERCA) activity. SERCA and its associated protein, sarcolipin, have been implicated in skeletal muscle thermogenesis, generating heat at the expense of ATP (Bal et al., 2012, Gamu et al., 2014, Sahoo et al., 2013). The potential role of these proteins in economy of physical activity is supported by the association between SERCA and enhanced muscle work efficiency seen during weight loss-induced adaptive thermogenesis in humans (Baldwin et al., 2011).

Conclusions

This study demonstrates that caffeine decreases fuel economy of physical activity, increasing the calories used for activity even when activity level or workload is held constant. This is accompanied by a delayed increase in muscle thermogenesis. This increase in calorie use may partly underlie the known ability of caffeine to induce weight loss or prevent weight gain in laboratory animals (Kim et al., 2015, Moy and McNay, 2013, Panchal et al., 2012) and promote negative energy balance in humans (Hursel and Westerterp-Plantenga, 2013, Davoodi et al., 2014, Miles-Chan et al., 2015). Similar to the ability of caffeine to enhance athletic performance (Magkos and Kavouras, 2005), the metabolic effects reported here may occur through multiple mechanisms, including elevated SRCA activity secondary to caffeine's increase of RYR-induced sarcoplasmic reticulum Ca^{2+} release. This study's demonstration of caffeine's amplification of activity-associated energy expenditure and increased skeletal muscle heat dissipation is consistent with these mechanisms and provides new quantification of physiological effects not previously demonstrated.

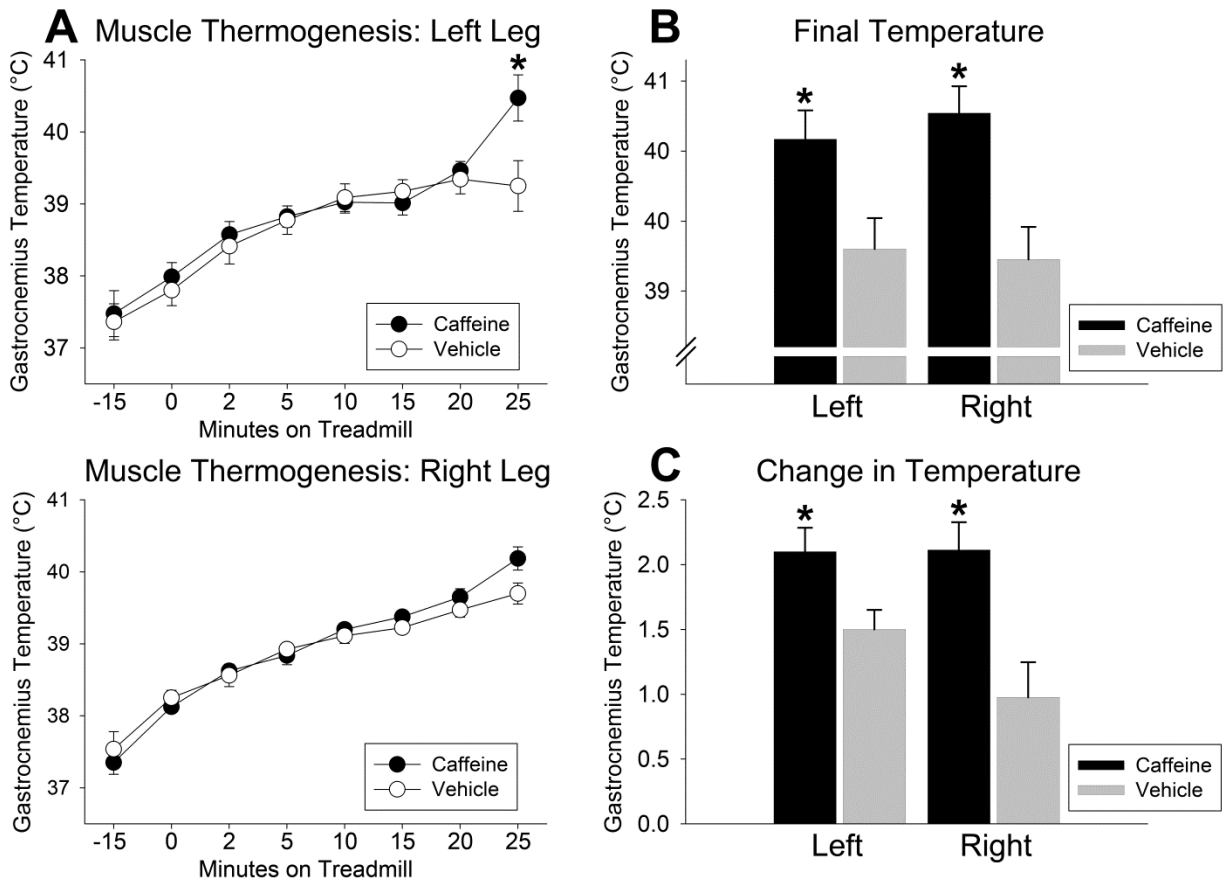

Figure 1. Caffeine increased skeletal muscle (gastrocnemius) activity thermogenesis. (A) Gastrocnemius temperature was significantly higher at 25 minutes of treadmill walking in the left leg. (B) The final temperature immediately upon completion of walking (B) and the change in temperature from 0 min of treadmill walking (C) were significantly enhanced by caffeine in both the left and right hindlimbs. *significantly greater than vehicle, $p<0.05$

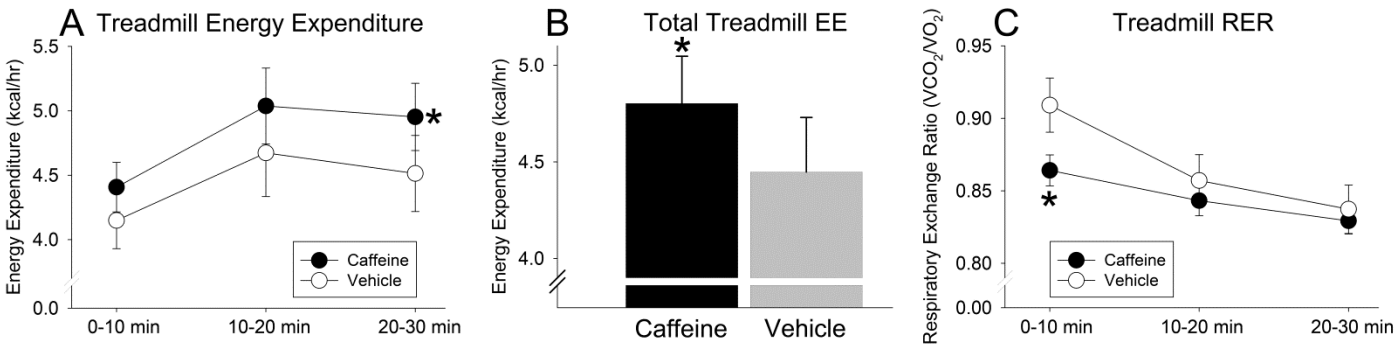

Figure 2. Caffeine amplified activity-associated energy expenditure. Compared to vehicle treatment, caffeine increased energy expenditure (EE) throughout the 30-min duration of treadmill walking (A) as well as the overall average EE (B). Caffeine also significantly decreased respiratory exchange ratio (RER; VCO_2/VO_2) in the first 10 min of treadmill walking only; RER during the 5 min preceding the initiation of activity was not significantly affected by caffeine. *significantly different from vehicle treatment, $p<0.05$

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Disclosures

The authors have no conflicts of interest to disclose.

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