Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling

Mohr, T., M. Van Soeren, T. E. Graham, and M. Kjaer. Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling. J. Appl. Physiol. 85(3): 979–985, 1998.—Normally, caffeine ingestion results in a wide spectrum of neural and hormonal responses, making it difficult to evaluate which are critical regulatory factors. We examined the responses to caffeine (6 mg/kg) ingestion in a group of spinal cord-injured subjects (7 tetraplegic (C5–7) and 2 paraplegic (T4) subjects) at rest and during functional electrical stimulation of their paralyzed limbs to the point of fatigue. Plasma insulin did not change, caffeine had no effect on plasma epinephrine, and there was a slight increase (P < 0.05) in norepinephrine after 15 min of exercise. Nevertheless, serum free fatty acids were increased (P < 0.05) after caffeine ingestion after 60 min of rest and throughout the first 15 min of exercise, but the respiratory exchange ratio was not affected. The exercise time was increased (P < 0.05) by 6% or 1.26 ± 0.57 min. These data suggest that caffeine had direct effects on both the adipose tissue and the active muscle. It is proposed that the ergogenic action of caffeine is occurring, at least in part, by a direct action of the drug on muscle.

Lactate; metabolic regulation; insulin; free fatty acids; glucose; norepinephrine; epinephrine; functional electrical stimulation; metabolic regulation; insulin; free fatty acids; glucose; lactate

INGESTION OF CAFFEINE, a trimethylxanthine, has been repeatedly reported to increase endurance time during prolonged exercise (5, 10–12, 30, 32). The traditional explanation for this is that caffeine increases plasma catecholamines that enhance lipolysis, thereby allowing for greater fat metabolism in contracting muscle. This, in turn, could spare glycogen usage and increase exercise capacity (7, 30).

This hypothesis is difficult to test by using human subjects because caffeine ingestion results in a variety of responses that may induce their own effects. For example, there is an increase in circulating epinephrine (11–13) both at rest and during exercise as well as in plasma concentrations of caffeine and paraxanthine (a dimethylxanthine derivative of caffeine) (12), which may result in stimulation of both the peripheral and central nervous system. Thus the possible cause-effect actions resulting from caffeine ingestion could include epinephrine stimulation of α- and β-adrenergic receptors as well as methylxanthines' antagonizing of adenosine receptors in both peripheral tissues and the central nervous system. The latter involvement in these effects could also include recruitment of motor units and/or central perception of fatigue (3, 14). With these various possibilities it is apparent why it is very difficult to establish which, if any, of these factors is vital to the ergogenic effect after caffeine ingestion.

There have been reports of delay of fatigue by caffeine in activity only lasting seconds (2), a few minutes (38), or ∼20 min (24). In the first case, it is unlikely that fat metabolism would be important and in none of them would it be likely that muscle glycogen was limiting. Recently, our laboratory (13) reported that during exercise requiring maximal O2 uptake (VO2), endurance was extended by caffeine ingestion whereas net muscle glycogenolysis was unaffected. None of these results can be explained by the traditional glycogen-sparing hypothesis. However, it is possible that caffeine resulted in a decrease in the perception of fatigue via central nervous system-mediated adenosine receptor antagonism.

It is also possible that the methylxanthines have direct actions on the active muscle, independent of the brain and catecholamines. These could have metabolic or excitation-contraction coupling effects. In support of the latter possibility, Lopes et al. (23) reported that caffeine ingestion caused the force-velocity curve of electrically stimulated human pollicis muscle to shift to the left. Similarly, Tarnopolsky et al. (33) reported that caffeine resulted in delayed fatigue in human muscle during low-frequency stimulation. In addition, there have been reports that Na+–K+–ATPase (22) and glucose transport (35, 36) in isolated rodent muscle respond directly to physiological concentrations of methylxanthines.

We (34) have recently demonstrated that studying spinal cord-injured (SCI) subjects allows one to differentiate among some of the possible effects of caffeine ingestion. With tetraplegia, the entire sympathetic and motor outflow is separated from cerebral control and the spinal cord functions independently of the brain (25). Tetraplegic subjects, on ingestion of caffeine, had no increase in circulating catecholamines, and yet they demonstrated a progressive rise in plasma free fatty acids (FFA). This and other caffeine-associated responses in the periphery must have been due to direct action of the plasma methylxanthines on the tissues, independent of both the brain and circulating epinephrine. Therefore, SCI patients present a physiological model in which the mechanisms associated with the endurance-enhancing effects of caffeine can be evaluated. These subjects can perform involuntary or fictive exercise induced by functional electrical stimulation (FES) of their paralyzed limbs (16, 18, 27), thereby excluding any role for the brain. In the present investigation, we studied a group of SCI subjects under these circumstances to test the hypothesis that caffeine ingestion would increase exercise endurance when there was no involvement of the brain in either motor control or
involuntary leg exercise with a mean maximal V\textsubscript{O2} of 1.2 l/min. They were all in a steady-state training status, able to perform all subjects were enrolled in a long-term training program, with the type of exercise described below, and they were all in a steady-state training status, able to perform involuntary leg exercise with a maximal V\textsubscript{O2} of 1.2 l/min.

Procedures. Subjects were studied on two occasions with a double-blind procedure. All subjects were asked to avoid intake of caffeine-containing substances 48 h before experiments, and compliance was confirmed through analysis of plasma methylxanthines. Trials were held in the morning, and subjects were asked to consume a normal diet before the trials and identical diets before each of the two trials. None of the subjects smoked or consumed medication (analogesics and preventative antibiotics) on the days of the experiments. None of the subjects took antispasmodic medication.

On arrival at the testing area, the subject was placed in a hospital bed after emptying his bladder. The subject ingested capsules containing 6 mg caffeine/kg body weight or placebo capsules (dextrose). After 60 min of rest, the subject was placed on a modified ergometer bicycle connected to an FES system. The system (ERGYS 1) consists of an ergometer attached to a computer and a six-channel electrical stimulator. The quadriceps, hamstrings, and gluteal muscles were stimulated sequentially via skin electrodes. The pattern of the stimulation was determined from pedal position and was controlled by the computer. The subjects were thereby stimulated to perform involuntary exercise with their paralyzed legs, and the exercise intensity was adjusted by adding load to the flywheel of the ergometer (for a detailed description, see Refs. 18, 19, and 27). The ergometer resistance was constant during a given experiment, and as a result V\textsubscript{O2} was quite constant. On the basis of pretrial experiments, the resistance of the cycle ergometer was individually selected to result in fatigue within 20–25 min. Fatigue was defined as the inability to maintain 35 revolutions/min in the face of maximal voluntary effort. The stimulator was automatically switched off when this point was reached. During FES the use of the upper extremities was limited (but not excluded). The subjects did not appear to use their arms; all subjects had a complete blockade and therefore did not transform force from the upper to the lower body by rocking back and forth. Furthermore, they were blind to the procedure and could not have purposely biased their endurance in one particular trial. After the exercise, subjects were asked to identify which treatment they believed they had just received. The result appeared random because four of the nine guessed incorrectly.

In seven of the subjects (6 tetraplegic subjects and 1 paraplegic subject) a cannula with a saline lock was placed into a hand vein before the hour of rest and was kept patent by being filled with 1 ml sodium citrate after each blood sampling. A blood sample was taken before capsule ingestion, at the end of the hour of rest, at 5 and 15 min during exercise, and at exhaustion. Analysis. All blood sampled was analyzed for catecholamines, FFA, glycerol, lactate, potassium, insulin, and methylxanthines as described below. Heart rate was measured by light absorption in a digital puls (Propaq), and expired air was obtained for analysis of \textsubscript{O2} and \textsubscript{CO2} fractions (\textsubscript{O2} analyzer and \textsubscript{CO2} detector), and 

\begin{align*}
\text{V\textsubscript{O2}} &= \text{maximal electrical stimulation (135 mA). The stimulator was automatically switched off when this point was reached.} \\
\text{max tertile} &= \text{maximal electrical stimulation (135 mA). The stimulator was automatically switched off when this point was reached.} \\
\text{max tertile} &= \text{maximal electrical stimulation (135 mA). The stimulator was automatically switched off when this point was reached.} \\
\end{align*}

Blood samples were separated into aliquots with relevant preservatives and centrifuged at 4°C. Blood glucose and lactate concentrations were determined with a rapid automatic glucose analyzer (YSI 2300, Yellow Springs Instruments) within 5 min after sampling. Plasma potassium was analyzed by using a Radiometer analyzer (Copenhagen, Denmark). Heparinized blood was treated with EGTA and reduced glutathione, and the plasma was stored at –80°C for catecholine (HPLC as described by Weiker et al. (37)), methylxanthine (HPLC (34)), and insulin (\textsubscript{125I} radiolmmunoassay, Diagnostic Products) analysis. Serum was stored at –30°C for FFA (Wako Instrument kit) and glycine (34) analysis.

Statistics. Because of the unique and sensitive nature of this population and because the primary question was whether human skeletal muscle that lacked cerebral control would respond to a physiological concentration of caffeine by increasing endurance, every effort was made to include all of the subjects, even though two were paraplegic (and thus would have some rise in plasma catecholamines) (25) and two (a tetraplegic and a paraplegic subject) did not have blood-based data. Statistical analysis was performed by using two-way analysis of variance for repeated measures for the seven subjects for which blood-based data were available. Because one of the seven subjects had a lower level of injury, i.e., he was a paraplegic individual, analysis was repeated with his data excluded. As noted in RESULTS, if this analysis produced different findings, a protected least significant difference post hoc test was performed to resolve interactions. Time to exhaustion was compared by using a paired t-test for all nine subjects. Significance was accepted at P < 0.05. Data are presented as means ± SE. In the text, differences are only described as an increase or decrease if the difference was significant at P < 0.05.

RESULTS

Average V\textsubscript{O2} was similar during exercise in both trials (1.03 ± 0.12 vs. 1.03 ± 0.15 l/min for caffeine and placebo, respectively). This represented ~80–85% of the maximum response that this technique can elicit in these same subjects. There were no differences between trials for the respiratory exchange ratio (RER), but it declined modestly and significantly during exercise. RER data in the placebo trial at 5, 15 min, and at exhaustion were 1.09 ± 0.03, 1.00 ± 0.03, and 0.98 ± 0.04, respectively, and corresponding data for the caffeine tests were 1.06 ± 0.02, 0.99 ± 0.03, and 1.00 ± 0.05, respectively.

Time to fatigue was significantly longer in trials with caffeine ingestion (27.24 ± 2 min) compared with placebo (25.48 ± 1.48 min), an increase of 6%. Seven of the nine subjects exercised longer after caffeine ingestion, and the average increase was 1.26 ± 0.57 min (Fig. 1). There were no systematic differences in the responses of the two paraplegic subjects compared with the tetraplegic subjects. Plasma methylxanthine analy-
sis confirmed that the subjects had adhered to the withdrawal instructions; only one had a detectable concentration of caffeine (~1 µM). After caffeine ingestion, the plasma concentration of caffeine increased to an average of 57.3 ± 7.4 µM after 1 h, and it remained close to this level during exercise.

Resting plasma catecholamine concentrations were extremely low in subjects before caffeine ingestion (Figs. 2 and 3). Caffeine ingestion had no effect on plasma epinephrine; in addition, the six tetraplegic subjects did not have a significant increase in epinephrine during exercise, but when the paraplegic subject was included there was a significant but small increase in epinephrine at exhaustion (Fig. 2). Plasma norepinephrine increased modestly but significantly during exercise to a level approximating a resting concentration in healthy, able-bodied subjects (Fig. 3). The entire group (n = 7) of subjects had a greater norepinephrine concentration at exhaustion during the caffeine test, whereas the six tetraplegic subjects had a significantly higher concentration at 15 min of exercise during the caffeine trial compared with control.

Serum FFA concentration (Fig. 4) increased 1 h after caffeine ingestion; in the six tetraplegic subjects, the difference remained significant at 5 and 15 min of exercise as well. Plasma glycerol concentration rose during exercise, but there was no effect of caffeine (Table 1).

Plasma insulin concentration showed no changes during either trial, although blood glucose decreased during the course of the experiment. There was no
difference between the two tests (Table 1). Blood lactate increased during exercise, and there were no differences between caffeine and placebo for the group of seven subjects. In the tetraplegic subjects, after caffeine ingestion, blood lactate was lower at 5 min and greater at exhaustion (Table 1). In the placebo trial, during the hour before exercise there was no significant change in lactate, insulin, glucose, glycerol, or FFA. Plasma potassium concentration rose during exercise, but there were no differences between trials (Table 1).

**DISCUSSION**

We studied a group of SCI subjects during involuntary FES of their paralyzed limbs. The hypothesis tested was that caffeine ingestion would increase exercise endurance when there was no involvement of the brain in either motor control or the perception of fatigue. There was no or minimal disturbance of the circulating catecholamines, and the exercise conditions were such that a metabolic substrate and/or glycogen limitation for performance was unlikely. Nevertheless, caffeine ingestion resulted in a significant increase in muscular endurance. These results support the hypothesis that caffeine in a physiological concentration can have a direct ergogenic effect on human skeletal muscle.

The increase in endurance was 6% or 1.26 min, and it could be argued that such an increase could occur by random chance or that the improvement would be greater in healthy individuals. The former would appear to be extremely unlikely because the study was double blind and, if there were such random variation, there would be no statistical difference between treatments. In many previous studies (9, 32) the endurance time has been increased to a greater extent. However, frequently the placebo time has also been longer than the placebo time has been increased to a greater extent. Nevertheless, the magnitude of the improvement with caffeine ingestion appears to be similar to what one would predict (9) on the basis of the literature and given the endurance time in the placebo trial. The finding is important in understanding the mechanisms that result in the increased endurance.

Most discussions of caffeine and exercise have favored a metabolic substrate explanation for caffeine's ergogenic effects. However, caffeine ingestion is ergogenic when there is no evidence of a metabolic (muscle glycogen) limitation (2, 13, 24, 38). Furthermore, the majority of the studies (9, 10, 32) that have measured plasma FFA concentrations and/or RER data have not supported the concept that caffeine ingestion increases fat metabolism during exercise. The one exception is the early study by Essig et al. (7), in which they report a greater decrease in muscle triglycerides after caffeine ingestion during nonexhaustive exercise. Although the metabolic data in the present study are limited, there was no caffeine effect on RER and it was very high (0.97) throughout both trials. This high RER may, in part, be due to the elevated blood lactate and associated acidosis. In addition, it may reflect a high degree of carbohydrate metabolism. In normal subjects, one would not expect 25–30 min of exercise to lead to a glycogen-limited state. We did not measure glycogen, but previously (19) similar activity in similar subjects was found to only decrease glycogen stores by ~30%. Thus, although it would appear that a large proportion of the energy came from carbohydrate oxidation, this was not a limiting factor in the placebo condition.

Furthermore, although the FFA concentration was elevated when caffeine was ingested, the RER and blood lactate and glucose concentrations were not altered. These data suggest that, although caffeine directly stimulated lipolysis in the adipose tissue during the hour of rest before exercise [as we reported previously (34) in resting SCI subjects], the total fat oxida-

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**Table 1. Blood metabolites, potassium, and insulin**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>5</th>
<th>Exh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4.72 ± 0.18</td>
<td>4.42 ± 0.17</td>
<td>4.38 ± 0.12</td>
</tr>
<tr>
<td>Caffeine</td>
<td>4.78 ± 0.27</td>
<td>4.35 ± 0.13</td>
<td>4.22 ± 0.28</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.80 ± 0.13</td>
<td>0.58 ± 0.05</td>
<td>3.56 ± 0.64</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.57 ± 0.06</td>
<td>0.58 ± 0.03</td>
<td>2.74 ± 0.48</td>
</tr>
<tr>
<td>Caffeine</td>
<td>45.83 ± 5.32</td>
<td>68.69 ± 11.44</td>
<td>75.35 ± 6.24</td>
</tr>
<tr>
<td>Glycerol, µM</td>
<td>46.70 ± 6.02</td>
<td>71.05 ± 11.16</td>
<td>82.15 ± 7.97</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.02 ± 0.05</td>
<td>4.03 ± 0.05</td>
<td>4.45 ± 0.11</td>
</tr>
<tr>
<td>Caffeine</td>
<td>4.10 ± 0.11</td>
<td>4.08 ± 0.07</td>
<td>4.55 ± 0.06</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>34.42 ± 5.44</td>
<td>25.86 ± 2.02</td>
<td>28.72 ± 3.19</td>
</tr>
<tr>
<td>Placebo</td>
<td>30.51 ± 4.29</td>
<td>24.90 ± 2.06</td>
<td>32.50 ± 2.52</td>
</tr>
<tr>
<td>Caffeine</td>
<td>30.51 ± 4.29</td>
<td>24.90 ± 2.06</td>
<td>32.50 ± 2.52</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 tetraplegic and 2 paraplegic subjects. Exh, exhaustion.
tion was not affected significantly. However, one must be cautious in interpreting such changes in plasma concentrations because they can be altered by either release or clearance. Recently, Kjaer et al. (Refs. 16, 18, and 19; personal communication) have found that FES results in only a modest fat mobilization, and SCI individuals have less uptake of FFA by active muscle than one would predict for a given FFA delivery (16). The present data suggest that, although caffeine increased FFA mobilization (at least at rest), there was no evidence of an increase in FFA uptake and oxidation by the active muscle.

It is surprising that an exercise demanding only \( \sim 1 \) l/min \( \dot{V}O_2 \) should have blood lactate levels of 6–7.4 mM. However, this exaggerated lactate response has been reported previously (17, 19) in healthy men who received epidural anesthesia at L3-4 followed by FES. Thus it appears that the exaggerated lactate response is a reflection of FES and not a selective phenomenon in SCI patients. The elevated lactate may represent a more rapid net glycogenolysis than that of voluntary exercise at the same \( O_2 \) cost. However, as noted above, it is likely that the glycogen stores were far from depleted at exhaustion. Furthermore, in the present study, the blood lactate response was similar in both the placebo and caffeine trials.

Vergauwen et al. (35, 36) have reported that electrically stimulated, perfused rat muscle responds to caffeine in the presence of insulin to decrease glucose uptake (35) and increase glycogenolysis (36). These results were attributed to the antagonism of both \( A_1 \) and \( A_2 \) receptors. Although the subjects in the present study had muscles stimulated electrically and certainly had circulating insulin, there was no indication of increased fat oxidation (on the basis of RER) or decreased glucose uptake (on the basis of no differences between trials for blood glucose) and, as noted above, it is unlikely that muscle glycogen or blood glucose was a limiting factor in the control experiment. The mechanism of caffeine action remains to be identified. For example, the presence of adenosine receptors in mammalian muscle has been demonstrated functionally; however, attempts to identify them by labeled ligands have been unsuccessful despite several attempts (4, 28). It should be noted that altering availability of the various metabolic substrates will not necessarily change metabolism or endurance unless one or more of them is limiting. It is unlikely that this is the case in the present study, and it is more likely that there are direct actions on the motor units of the active muscles.

Previously, Lopes et al. (23) reported that caffeine ingestion in humans before electrical stimulation of the adductor pollicis potentiated tension development at submaximal frequencies. This clearly did not involve alterations in metabolism and was independent of the brain. It must be the result of either more force per motor unit or more units depolarizing in response to the stimulus. Similarly, Tarnopolsky et al. (33) in a preliminary report suggested that caffeine ingestion potentiated the torque generated by the quadriceps in response to electrical stimulation at 20 Hz. This frequency is believed to cause fatigue due to excitation-contraction impairment. These investigations would support the concept that caffeine can enhance muscle function via a calcium mechanism and could account for the increased endurance in the present study.

Calcium mechanisms are usually dismissed as pharmacological events in considerations of possible in vivo mechanisms for the actions of caffeine. However, several in vitro studies suggest that metabolic changes such as previous stimulation of the ryanodine receptors (31), an increase in palmitoyl-CoA (8), or an increase in cADP-ribose (a metabolite of NAD) (20) can dramatically increase the sensitivity of calcium mechanisms to caffeine. Although this is speculative, the sensitivity of calcium to caffeine could be much higher under such situations, and this could result in greater force development per motor unit.

It has been demonstrated (6) in healthy subjects that caffeine can influence the threshold magnitude for spinal monosynaptic reflexes. Although the SCI subjects had a complete lesion, these spinal reflexes are still present and are free of cerebral control. Their H-reflexes have been shown (26) to be less depressed than those in healthy subjects after muscle stretch. The effect of caffeine on such reflexes in tetraplegic subjects is unknown, but it could alter the lower motor neuron excitability to the electrical stimulation in the present study and contribute to the improved endurance.

Previously, we (34) found that caffeine ingestion by resting tetraplegic subjects did not cause the rise in plasma epinephrine that is generally observed in normal subjects (11–13). In the present study the plasma catecholamine concentrations were subnormal, in agreement with previous reports (1, 18, 25, 34). Caffeine ingestion had no impact on plasma epinephrine, confirming our previous observation; however, it was associated with a modest increase in norepinephrine when the exercise was performed. In addition, exercise itself caused a small increase in norepinephrine and, in the paraplegic subject, there was a modest rise in epinephrine as well. The cause of this minimal catecholamine response is due to impaired sympathetic activity to the adrenal medulla, in association with the high spinal cord lesion disrupting the sympathetic outflow from the brain. Interestingly, in the one paraplegic subject with a lower lesion, there was a modest catecholamine response. However, there were no obvious differences in the metabolic (FFA and RER) responses of this subject. Insulin normally declines during exercise due to sympathetic stimulation of the beta cells (15). The minimal sympathetic response in the SCI subjects is in agreement with the lack of decrease in the insulin concentration.

We feel that the very low levels of plasma catecholamines and the general stability of their concentrations are evidence that the responses to caffeine were not mediated via the catecholamines but rather were caused by direct actions of caffeine on various tissues. For example, there was a large increase in serum FFA whereas the catecholamines were unchanged, suggesting that caffeine was directly stimulating the adipo-
cytes, probably via adenosine antagonism. It is possible that the subjects had developed a hypersensitivity to catecholamines and that we are underestimating the effect of the low concentration on various tissues (29). Mathias and Frankel (25) point out that stimuli such as mental arithmetic, loud noise, and cutaneous stimulation in areas above the lesion are dependent on sympathetic activation that either originates in or is modulated by the brain. These stimuli do not induce a response in tetraplegic subjects. However, stimulation such as pain or cold applied below the lesion results in an exaggerated response, likely via reflex sympathetic activation. Mathias and Frankel also note that tetraplegic patients do not demonstrate the classic denervation sensitivity with impaired norepinephrine clearance or exaggerated plasma concentration. In the present study the catecholamine concentrations were very similar in both conditions throughout the two trials, and this was particularly true during the period when the FFA concentration was elevated by caffeine. Furthermore, the subjects who had higher norepinephrine and epinephrine concentrations did not have greater FFA responses or vice versa.

There have been reports that exercise in association with caffeine ingestion results in less increase in plasma potassium in human subjects (21, 24). Furthermore, resting rodent muscle increases its potassium uptake in the presence of methylxanthines (22), suggesting that there is a direct stimulation of Na\(^{+}\)-K\(^{-}\)-ATPase. We recently reported (34) a lowering of plasma potassium in resting tetraplegic subjects after caffeine ingestion. However, the present data did not confirm this observation. There are two possible reasons for this. First, the rise in potassium from the active muscles may have overwhelmed the clearance process in the resting muscles, and thus the stimulatory effect of the caffeine could not be detected. Second, with the previous observations in tetraplegic subjects, the potassium concentration did not show a caffeine effect until 140 min post-caffeine ingestion. The present protocol only lasted ~90 min and thus may have ended before such an effect would be evident.

Recently, Kjær et al. (16, 18) demonstrated that patients who lack neural input from the motor centers and from working muscles had impaired lipolytic activity and glucose appearance in the blood during FES. Our present data for the placebo condition are in agreement with these findings and, even when lipolytic activity is further stimulated by caffeine, there is no apparent increase in fat oxidation. It appears that bloodborne mechanisms alone cannot provide normal substrate regulation during exercise. Caffeine ingestion failed to stimulate a rise in plasma catecholamines but was able to increase the blood FFA concentration and to increase muscle endurance. These two events do not appear to represent cause and effect, and the data support the theory that methylxanthines are stimulating adipose and muscle tissues directly.

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