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The possible actions of methylxanthines on various tissues

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1. INTRODUCTION

After a series of three critical studies [1-3] on the effects of caffeine ingestion on metabolism and exercise endurance, Costill and coworkers [2] proposed that caffeine caused an increase in circulating catecholamines which in turn mobilized free fatty acids (FFA) from adipocytes. This subsequently resulted in an increased delivery of FFA to active muscle, and since FFA were thought to be taken up passively, there would be increased fat available for metabolism. The increased beta oxidation would cause an inhibition of carbohydrate catabolism via the "Randle effect", resulting in decreased glycogenolysis. This theory would account for their findings that caffeine increased endurance during prolonged exercise and was associated with elevated plasma FFA and a sparing of muscle glycogen.

This hypothesis, proposed in 1980, was based on studies conducted *in vitro*, with animal models, and with resting humans, and is still commonly used to explain findings in today's research. While we [4], and others [2,5] have demonstrated that caffeine ingestion can be associated with muscle glycogen sparing and is frequently associated with increases in plasma adrenalin and FFA [4-8], there can be little doubt that this hypothesis is far from complete.

There are several reasons for questioning this hypothesis. First, the only direct evidence that fat metabolism is enhanced during exercise following caffeine ingestion is that of Essig *et al.* [2] who reported greater intramuscular triglyceride use with caffeine ingestion (but the amount of decrease in triglyceride was so large that if it was entirely oxidized it would require more than 100% of the O_2 consumed during the exercise). It is commonly found that plasma FFA levels are elevated following caffeine ingestion, but muscle does not necessarily take up FFA proportional to the plasma concentration [9]. Numerous reports [6-8] have failed to show a decline in respiratory exchange ratio (RER) during such exercise.

Second, we noted [4] that the decreased net glycogenolysis rate was transient, only occurring within the first 15 min of an exercise period lasting 60-90 min while the plasma adrenalin was elevated throughout the exercise. In addition, there was no caffeine-induced increase in the active muscle in either of the putative mediators (citrate or acetyl CoA) of the Randle effect, which is proposed to inhibit carbohydrate oxidation. In contrast, MacLean and Winder [10] found that infusing caffeine into the isolated rat hindlimb at rest resulted in no change in citrate but a lowering of malonyl-CoA, a potent inhibitor of FFA oxidation in rat muscle. (This occurred with no increase in cAMP.) More recently, we [11] found that a low

dose of caffeine (3 mg/kg) resulted in increased endurance without a measurable increase in plasma adrenalin, FFA or RER. Furthermore, when adrenalin was infused to mimic the response of caffeine [12] there was no change in active muscle carbohydrate metabolism. Chesley *et al.* [12] found that such an increase in adrenalin increased the mole fraction of phosphorylase a in the active muscle but did not change the net glycogenolysis rate or the muscle or blood lactate concentrations or the blood FFA and glycerol concentrations during 15 min of exercise at 80% VO_2max. Recently, Greer *et al.* (unpublished data) found that caffeine ingestion can increase endurance in prolonged exercise (85% VO_2max) without glycogen sparing.

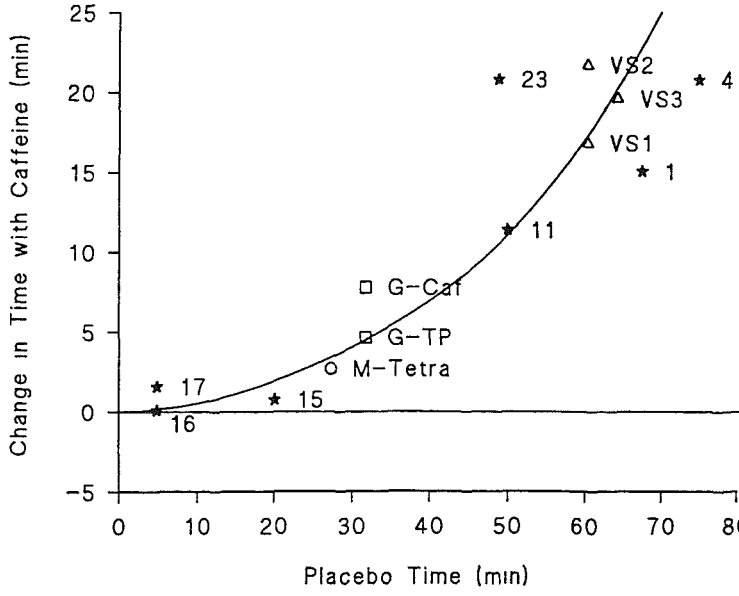

Figure 1. A summary of the effect of methylxanthine ingestion on endurance. The mean increase in endurance following caffeine ingestion is compared to placebo; these are represented by asterisks and the number beside the asterisk corresponds to the reference. In addition unpublished data from three studies are presented: the open circle is work by Mohr (M) *et al.* with tetraplegic patients (Tetra), the open squares are from Greer (G) *et al.* for subjects ingesting either Caffeine (Caf) or theophylline (TP), and the open triangles are from a study by van Soeren (VS) *et al.* for caffeine ingestion following 0, 2, or 4 days of withdrawal (VS1, VS2 and VS3).

In addition to these data, several investigators [13-16] have shown that caffeine can increase endurance and/or power output in exercise situations (fatigue in < 4.5 min) when neither muscle carbohydrate supply nor fat metabolism would be limiting factors. We have recently [17] examined the impact of caffeine on exercising at a power output equivalent to VO_2max; the subjects were able to exercise significantly longer, but the rate of muscle glycogenolysis was not affected and at exhaustion (5-8 min) the muscle glycogen was only decreased by approximately 50%. Figure 1 summarizes the literature addressing human endurance/performance with caffeine and it is evident that at durations when muscle glycogen

would not be limiting, caffeine is having a profound effect on the human body. Performance enhancement has been reported for activity over a wide range of durations when different factors are probably limiting performance. It is not clear if there is a minimal duration of activity in which caffeine would cease to be effective; it is likely that our ability to make precise measures of endurance limits our measurements of activity lasting a few seconds.

The theory proposed in 1980 [2] is not compatible with various results such as the inability to demonstrate clearly and consistently an increased fat metabolism, the increased endurance when carbohydrate supply is not limiting and the inability to relate any of the metabolic responses with the changes in plasma adrenalin. This is not to say that these responses do not occur in some circumstances, but rather that the theory can not explain the ergogenic effects of caffeine in many circumstances. Exercise physiologists have consistently viewed caffeine as a simple metabolic agent that alters fuel supply to active muscle. It must be recognized as a potent drug that directly and/or indirectly can alter the function of virtually every tissue of the body and that some of the effects are not primarily metabolic.

2. THE CAFFEINE "SIGNAL(S)"

Caffeine is completely absorbed within an hour of ingestion and the liver begins to metabolize it via the P450 system [18]. The initial enzyme (1A2) in this pathway becomes saturated when the dose of caffeine is in the 5-6 mg/kg range [11,19,20]. The demethylation of caffeine (a trimethylxanthine) produces three dimethylxanthines, paraxanthine, theophylline and theobromine. In adult humans the major (approximately 80%) product is paraxanthine; the dimethylxanthines all have the potential to have similar effects to those of caffeine. In fact, we have recently demonstrated that theophylline is an ergogenic drug (Greer *et al.*, unpublished results) (Figure 1). The peak plasma caffeine concentration occurs in 60-90 min post-ingestion and the half life is 2.5-4.5 h [18]. By 8-10 h post ingestion the plasma concentration of paraxanthine exceeds that of caffeine [18]; Lelo *et al.* [21] reported that the half life for caffeine was 4.1 h and that for paraxanthine was 3.1 h. In contrast, the data for theophylline and theobromine were 6.2 and 7.1 h. All of these compounds can stimulate the central nervous system (CNS) and such an altered level of arousal within the CNS could account for some of the caffeine effects. In addition, caffeine results in an increased secretion of adrenalin; thus the various affects that are attributed to caffeine could also be the result of direct actions on peripheral tissues of not only the CNS but also dimethylxanthines and/or adrenalin.

With many possible signals, understanding the events can be very complicated even for a single tissue or process (Figure 2). Using adipose tissue and FFA mobilization as the example, caffeine and/or the resulting dimethylxanthines could be directly stimulating the mobilization of FFA (i.e. it could be a "primary" target) via adenosine antagonism (see below). Alternatively, the sympathetic stimulation of the adipocytes either by the CNS or by the adrenal medulla (via rise in plasma adrenalin) could result in adipose tissue being a secondary response. Subsequently, the mobilization of FFA could then be a metabolic stimulus to muscle or liver, and in this way these tissues would be secondary (or even tertiary) targets. Furthermore, it becomes even further complicated as these tissues have their own triglyceride stores which could be affected directly by methylxanthines, or by sympathetic stimuli. There are often redundancies in regulatory systems and several of these mechanisms could act in concert. Figure 2 summarizes these complexities for a single response , that of FFA

mobilization. The goal of this review is to address which of these are critical to the various responses to caffeine.

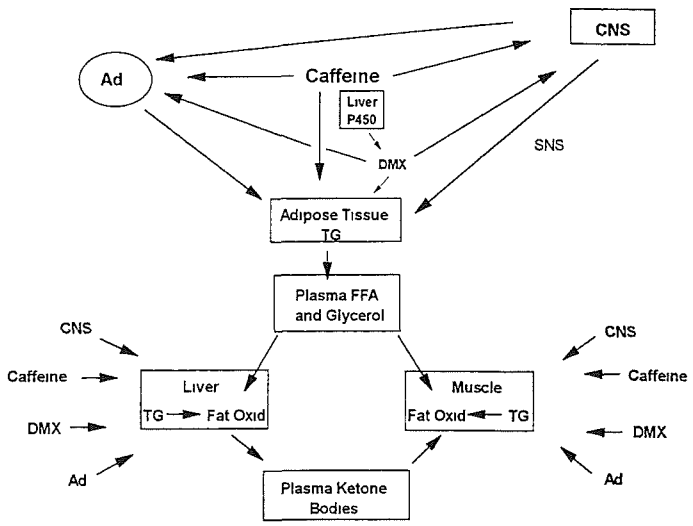

Figure 2. This is a scheme that summarizes the various "signals" and "routes" that could result in FFA mobilization and metabolism. Ad is plasma adrenalin, P450 is the cytochrome enzyme system for drug metabolism, DMX are the three dimethylxanthines, SNS is the sympathetic nervous system (noradrenalin), TG is triglyceride, and oxid is oxidation. Each arrow is a potential positive signal.

3. MECHANISMS FOR DIRECT EFFECTS

As described in numerous reviews [22-24] the three common mechanisms that are considered to account for the actions of caffeine are increases in the sensitivity of intracellular Ca translocation mechanisms, inhibition of phosphodiesterase resulting in an increase in cAMP, and inhibition of adenosine receptors on cell membranes. The latter process is commonly accepted as the main (or exclusive) mechanism because it has been shown to occur with physiological (μM) concentrations of caffeine while the other mechanisms require pharmacological (mM) concentrations. If the latter is the sole mechanism then any tissue being directly stimulated by methylxanthines must have adenosine receptors.

The adenosine receptors are a subclass of purinergic receptors that bind adenosine and adenine nucleotides. The adenosine receptors are P1 receptors which are identified by their high affinity for adenosine compared to adenosine nucleotides and by their antagonism by methylxanthines. Within this subgroup there are A1, 2 and 3 receptors; the former are the most sensitive to physiological concentrations of caffeine [25]. They are coupled through Gi proteins to adenylate cyclase and mediate inhibitory signals, which are blocked by caffeine. The involvement of A1 receptors in caffeine responses are clearly demonstrated in many tissues, especially in the brain, heart, kidney, and adipocytes. The less sensitive A2 receptors are found in the brain, platelets, liver and smooth muscle. The affinity of the different

methylxanthines for the adenosine receptors varies but generally theobromine is the weakest antagonist and theophylline and paraxanthine the strongest.

Comparison of the pharmacological effects of methylxanthines and adenosine analogues on various tissues supports the impression that the methylxanthines are acting as adenosine antagonists. They dilate most arteries (but constrict the cerebral arteries), are inotrophic and chronotrophic to the heart, cause bronchodilation, diuresis, promote lipolysis, platelet aggregation and stimulate the CNS. Adenosine and its analogues generally result in the opposite effects.

There has been a great deal of investigation of adenosine receptors in the CNS and since caffeine is an adenosine antagonist, it and various analogues have been commonly employed in this work. Caffeine is a relatively weak adenosine antagonist (Ki 40-50 μM); paraxanthine is more potent (Ki 33 μM), but caffeine crosses the blood brain barrier more easily. It is well known that the methylxanthines are psychoactive, causing increased alertness, well-being, motivation, a sense of "energy", and mental concentration but in high doses they can be anxiogenic. Similarly there is a great deal of knowledge about the distribution of the various adenosine receptor types in the CNS and their sensitivity to methylxanthines. However, the receptors are found in a wide range of CNS pathways and affect a wide range of neurotransmitters. Furthermore most of the work has employed pharmacological doses of drugs, often with *in vitro* preparations such as brain slices. The physiological significance of these findings is not clear.

Work by Daly [25], Barraco [26], Fredholm [27] and others has revealed a great deal about the interactions of caffeine and adenosine receptors. The A1 receptors are present in most areas of the CNS, particularly the cortex, hippocampus, thalamus, cerebellum, raphe nucleus, locus coereleus and ventral tegmental area. These receptors are commonly upregulated with chronic caffeine exposure. There inhibitory actions are conducted via various mechanisms including lowering cAMP, stimulating K channels and perhaps Cl conductance as well. They are inhibitory to neurons which are involved in a wide range of neural pathways (noradrenalin, dopamine, serotonin, acetylcholine, GABA and glutamate). Despite this formidable list on neurotransmitters, in recent years more attention has been given to a subset of the A2 receptors (A2a). The A3 receptors are sparse in the CNS and A2b receptors are low affinity receptors that are widely distributed in the brain and are thought to have a more modulating role rather than a controlling role. In contrast, the A2b receptors are more specific in their location, being abundant in the dopamine-rich areas of the ventral striatum of the basal ganglia (caudate nucleus, the tuberculum olfactorium and nucleus accumbens. The latter is both richly innervated by dopaminergic terminals and has the highest density of adenosine A2a receptors.) Within these regions there are subsets of dopamine receptors (D1 and D2). The A2a receptors are sparse in those neurons that have D1 receptors and which express preprotachykinin A, but are rich in the neurons that have D2 receptors and that also express enkephalin.

It is these latter dopaminergic neurons that are thought to be stimulated by methylxanthines due to their antagonism of the A2a adenosine receptors. The ventral tegmentum and reticular formation contain such dopaminergic neurons that project to the frontal cortex and the limbic system. It appears that these may be critical sites for the behavioural properties of caffeine. Barraco [26] has demonstrated that infusion of an adenosine agonist for the A2a receptors into the nucleus accumbens reduces locomotor activity, while A1 agonists have no effect. In contrast, caffeine analogues blocked the effects of the A2a agonists. This suggests that caffeine may induce behavioural effects by modulating the A2a receptors of mesolimbic neurons of the ventral striatum. He proposed that the ventral striatum and especially the nucleus

accumbens is the key for integrating and co-ordinating the limbic and motor systems. He also suggested that the accumbens nucleus is heavily involved in the dopamine-mediated psychomotor function and brain reward processes and in specific aspects of spontaneous and drug-induced locomotion.

While these aspects have received a great deal of attention, it must be emphasized that the CNS is extremely complex and given the wide range of neural pathways that are sensitive to methylxanthines it is too simplistic to consider that only one aspect is critical. This area may be vital to behavioural responses but caffeine has many psychogenic components and also may influence aspects of the CNS that are fundamental to functions such the cardiovascular system, endocrine secretion and the degree of activity in the sympathetic nervous system.

While the CNS effects appear to be mediated via A1 and A2 receptor agonism, Verma [28] has demonstrated that Ca induced Ca release (CICR) pools in the brain and heart are sensitive to caffeine. These are localized with ryanodine binding sites rather than with inositol 1,4,5-triphosphate (IP3). The CICR pools are greatest in the CA-3 region of the hippocampus, medial septum and olfactory bulb in the brain and in the ventricular and atrial aspects of cardiac muscle. (They are also localized in skeletal muscle in terminal cisternae, but these pools have not been examined for sensitivity to physiological concentrations of caffeine *in vivo*.) This opens the possibility for additional mechanisms of action.

We commonly observe that subjects are more alert, outgoing and talkative following caffeine ingestion and they feel more "energetic" following caffeine ingestion, and subjectively feel that exercise is less taxing. It is also common to see a muscular tremor. All of these suggest CNS stimulation; in contrast when they fill out a questionnaire after each experiment regarding what treatment they believe that they received, the majority are incorrect in identifying whether or not they received caffeine. Thus despite the apparent CNS effects at the level of consciousness the subjects do not perceive the actions clearly.

In addition to these traditional sites of action, one should also consider the peripheral nervous system. Silinsky and Redman [29] have found that at the myoneural junction ATP is released concurrently with acetylcholine. The ATP is degraded producing adenosine which binds to the motor neuron's A1 receptors to inhibit further neural transmitter release. Caffeine could disinhibit this feedback mechanism and enhance the motor unit function. As discussed below, muscle has not been shown to have adenosine receptors, but there could be direct actions of the methylxanthines on the myoneural junction or even on the vascular smooth muscle. These would not be detected in studies of an entire muscle sample.

There is no question that binding of adenosine receptors is a major mechanism for caffeine's actions but there are findings that are difficult to explain exclusively through this mechanism. The results of caffeine ingestion by humans are well documented; however, it is far from resolved what the effective signals are and what mechanisms are involved in mediating the signals. The remaining sections of this review will address these issues.

4. WHAT IS THE CAUSE OF THE ERGOGENICITY- METABOLISM OR CONTRACTION?

As mentioned previously, several studies including one from this laboratory have demonstrated that caffeine ingestion prior to exercise can result in reduced net glycogenolysis. However, ergogenic effects have been demonstrated in intense, short-duration exercise when glycogen is not limiting and fat metabolism is not important. Furthermore, we recently [17]

found that there is no effect on net muscle glycogenolysis in such exercise. In addition, we have also failed to demonstrate that there is less net glycogenolysis in exhaustive exercise lasting 25-35 min (Greer *et al.*, unpublished data). This suggests that the traditional glycogen-sparing explanation may not be the critical mechanism.

It would be simple if the effects of caffeine were mediated exclusively via its stimulatory effects of the CNS and the resulting effects on perception and both sympathetic and motor control. One could imagine that this could result in increased heart rate, elevated blood pressure, increased adrenalin secretion, increased FFA mobilization, suppression of the CNS perception of fatigue, altered motor recruitment, and so on.

The CNS actions may be involved in some of the responses during exercise but recent work by van Soeren and colleagues [30] demonstrates that they are not essential for many of the metabolic actions. We studied the effects of caffeine on tetraplegic patients [30]. The patients had low circulating concentrations of catecholamines and a denervated adrenal medulla. After caffeine ingestion there was no change in adrenalin concentration, suggesting that the normal caffeine-induced increase in adrenalin is secondary to stimulation of the CNS and an increase in SNS stimulation of the medulla. In contrast, the patients had a large and prolonged increase in FFA, demonstrating that SNS and/or adrenalin stimulation of the adipocytes was not essential in order to have this caffeine response. There was also an increase in blood pressure. These results clearly demonstrate that caffeine's actions originate in some cases indirectly as a result of CNS activity and in other cases via direct actions on the peripheral tissue.

In a second study of tetraplegic patients (Mohr *et al.*, unpublished data) the subject's lower limb muscles were electrically stimulated in a co-ordinated manner so that fictive exercise could be performed. Following caffeine ingestion the patients had a significant increase in endurance (Figure 1). This obviously was independent of the CNS and also was not dependent on changes in adrenalin. While there was a rise in plasma FFA there was no change in RER suggesting that metabolism was not a factor. The data are consistent with the hypothesis that caffeine could have a direct effect on skeletal muscle.

One needs to bear in mind that normally excitation-contraction coupling drives metabolism, not vice versa. In other words, making more substrate available will not influence muscle function unless the metabolic need is created. Lopes *et al.* [31] studied the force-velocity relationship in the human adductor policies with electrical stimulation. Ingestion of 500 mg of caffeine potentiated muscle tension development at submaximal frequencies (i.e. the force-frequency curve was shifted to the left) both before and after fatigue. This clearly did not involve metabolism and was independent of the central nervous system. It must be the result of either greater force per motor unit or the recruitment of more motor units.

Similarly Tarnopolsky *et al.* [32] in a preliminary report, suggested that caffeine (6 mg/kg) ingested before electrical stimulation of the quadriceps muscle resulted in potentiation in torque at 20 Hz; this frequency is believed to cause fatigue due to impairment of excitation-contraction impairment and a reduced Ca release from the sarcoplasmic reticulum. These studies force us to reconsider a Ca mechanism, although it is commonly dismissed because it has only been demonstrated directly with pharmacological concentrations of caffeine. Since electrical stimulation of the muscle was employed, any caffeine effects via the CNS or myoneural junction are eliminated.

Both Challiss *et al.* [33] and Vergauwen *et al.* [34] have demonstrated that adenosine can influence the insulin-mediated glucose uptake in skeletal muscle and this action is inhibited by caffeine and methylxanthine analogues. There are two major concerns regarding these studies; they found completely opposite effects of adenosine on the insulin action (Challiss

et al. found that adenosine increased and methylxanthines decreased the insulin concentration required for stimulation, while Vergauwen *et al.* reported that glucose uptake was inhibited 30-50% by caffeine). Vergauwen *et al.* proposed that skeletal muscle must have A1 adenosine receptors but several studies have failed to demonstrate the presence of adenosine receptors in skeletal muscle. Challiss *et al.* [33] using 8-cyclopentyl-1,3-dipropylxanthine (CPX) and a rat muscle homogenate had negative results. Similarly Sajjadi and Firstein [35] found evidence of gene expression for the A3 receptor primarily in human lung, liver, kidney and heart, but not in brain or skeletal muscle. In addition, van Soeren *et al.* [36], using PIA and human skeletal muscle also found no evidence for adenosine receptors in human or rat skeletal muscle.

It has been suggested [24] that caffeine may have a direct effect on ryanodine receptors. This could account for the effects seen in muscle fatigue, but not those reported for insulin-glucose transport. Furthermore the research suggesting that caffeine stimulates Ca release indicate this only happens with pharmacological concentrations of caffeine. One common example of this is the use of caffeine-induced contractures of human muscle biopsies during clinical tests for malignant hyperthermia. Kalow *et al.* [37,38] reviewed clinical data from patients suspected of having malignant hyperthermia. They found that in those samples (approximately 1,200) which were not classified as malignant hyperthermia, there were several subsets of people showing different thresholds of sensitivity to caffeine. The range of threshold response among the subjects was approximately 30-fold. Furthermore biopsies from men were more sensitive to caffeine than were women. Thus it appears that, at least pharmacologically there are several populations of responsiveness of skeletal muscle to caffeine and gender differences may also exist.

The studies that have directly demonstrated that caffeine can have an effect on Ca mechanisms have been conducted *in vitro* with pharmacological concentrations of caffeine. It is not known if *in vivo* conditions would result in Ca responses to physiological levels of caffeine. It is possible that changes in the internal environment of the cell can facilitate its responses to a metabolic signal. Recently Lee [39] using sea urchin eggs, demonstrated that when cyclic ADP-ribose, a naturally occurring metabolite of NAD, was present in sea urchin eggs in trace amounts the effect of caffeine on CICR was potentiated; the concentration of caffeine required for CICR was reduced 10-20 fold. This is particularly interesting given the findings of Verma [28] concerning the CICR mechanisms as reviewed above. This type of facilitation could mean that, with metabolic disturbances due to the demands of exercise, active muscle could be more sensitive to low levels of caffeine.

As noted earlier MacLean and Winder [10] reported an effect of caffeine infusion in the resting rat hindlimb muscle. A high, physiological dose of caffeine (120 μM) decreased malonyl-CoA, and this occurred with no change in cAMP. Only a large pharmacological dose of caffeine (3 mM) stimulated cAMP in resting muscle. This both clearly demonstrates that direct effects of caffeine occur on skeletal muscle and also that these appear to occur without changing cAMP, the "traditional" methylxanthine signal and also without a change in adrenalin.

There is another area of research that demonstrates that caffeine has direct effects on skeletal muscle. When the muscle myofibre depolarizes, some intracellular K crosses the membrane into the interstitial space. Immediately the NA-K ATPase attempts to re-establish the ionic distribution across the membrane. During repeated depolarizations (e.g. exercise) the pump is not 100% successful and as the interstitial K increases some of it is "washed out" via the blood stream and there is a rise in plasma K. Immediately the body attempts to maintain

homeostasis and resting muscle increases its uptake of K by increasing the activity of its Na-K ATPase.

Humans who ingested caffeine prior to exercising to exhaustion had less rise in venous K concentration during the exercise [15,40]. The muscle ATPase is sensitive to both adrenalin and to methylxanthines. Both these signals were increased and thus either could be critical and both resting and the active muscles would be exposed to the compounds. However, it is likely that the ATPase in exercising muscle is normally fully activated [41], thus it is probable that the K effect is the result of increased stimulation of the Na-K ATPase in resting muscle. Lindinger *et al.* [40] have shown that rat hindlimb muscle has a greater K uptake when exposed to the dimethylxanthine, paraxanthine and van Soeren *et al.* [30] found that tetraplegics at rest showed a decline in plasma K concentration following caffeine ingestion even though there was no increase in adrenalin. These are additional examples of methylxanthines having a direct action on a peripheral tissue independent of the CNS and independent of adrenalin. The consequences of the direct stimulation the Na-K ATPase activity in resting muscle are unknown, but Sjogaard [42] has suggested that K loss from active muscle could precipitate fatigue.

There are many aspects of the effects of caffeine that are unknown; however, if one reconsiders Figure 2 and identify of the potential "signals" (arrows) as being nonessential (Figure 3), this exercise not only simplifies the phenomenon but may also allow us to focus

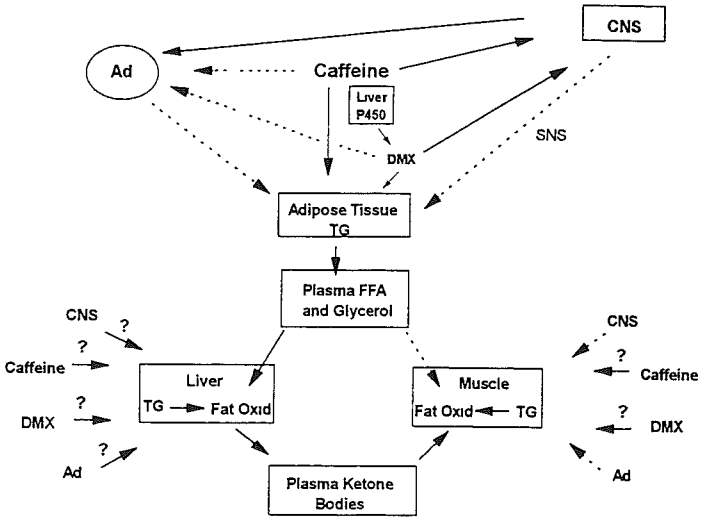

Figure 3. This is the same figure as Figure 2 for the mobilization and oxidation of FFA, however, now the "potential signals that have been shown to be not essential are represented by dashed lines. The signals with question marks are mechanisms that need to be explored in more depth.

on what are the key mechanisms. They may function in the normal human, but they do not appear to be essential. The traditional catecholamine-FFA mobilization-glycogen sparing explanation is very inadequate; there are various circumstances where catecholamines are not involved and muscle does not appear either to increase fat metabolism or spare glycogen. Furthermore, one needs to realize that there may be direct actions of the methylxanthines on peripheral tissues including muscle and that the etiology for the ergogenic effects may not be metabolic but rather may be related to aspects of excitation-contraction coupling.

5. ACKNOWLEDGEMENTS

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Discussion: The Possible Actions of Methylxanthines on Various Tissues

P.M. Clarkson:

I have had the opportunity to hear one of your colleagues, Lawrence Spriet, speak on caffeine, and in discussing the limitations of research, he suggested that there is very little research done on females. Most of the research has been done on males. Is there any theoretical basis to believe that females would respond differently than males?

T.E. Graham:

I do not think that we can generalize in terms of saying females. Caffeine is metabolized by the P-450 system and oestradiol is as well. One of our areas of research deals with differences in the metabolism of caffeine in women with a range of reproductive status, from amenorrheics to eumenorrheics to oral contraceptive users, and certainly there is evidence, at least in women at rest, that those with the highest oestrogen levels will have a different caffeine metabolism, so I am not sure that all women will respond the same way. There is limited evidence that eumenorrheic women may respond somewhat differently metabolically to exercise, probably in terms of being perhaps more fat-oriented and more carbohydrate-sparing oriented. But, we should keep in mind that metabolism is not always the cause of such an effect. The cause could be at the level of excitation/contraction. Finally, the most convincing evidence to support gender differences is from Kalow; with pharmacological *in vitro* tests he has shown gender differences in caffeine induced muscle contracture.

D.A. Cowan:

Is there a big influence of tolerance to caffeine and xanthines and is not it rather difficult to get people who are totally xanthine-free at the start of your experiments?

T.E. Graham:

The metabolism of caffeine itself does not appear to be inducible. Non-users and users metabolise it at a very similar rate and in a similar way. We have looked at non-users, and I have served some individuals the first cup of coffee they have ever had. The only real difference appears to be in terms of sensitivity. The non-user is more sensitive and more easily overdosed than is the user. In our experience -our experiments are double blind- it is only the non-user who can successfully identify what they have received. The users guess incorrectly as often as they guess correctly. In terms of getting them xanthine free, we traditionally use a two-day withdrawal. What you end up with is with people who have barely detectable or non-detectable caffeine levels but they usually do have residual paraxanthine levels. We do not feel that this is a serious problem, but certainly even two-day withdrawal is not enough. We did do a withdrawal study, looking at everything from 0 to 4 days, and at least in terms of exercise endurance response it really did not matter whether you were 0 day withdrawn or 4 day withdrawn. Certainly it was easier to overdose the 0 day withdrawn people, because they already had a residual amount of caffeine in their circulation.

Y. Hellsten:

Endothelium has adenosine receptors and I do not know if anybody has looked specifically at muscle endothelium and you may not be able to pick it up because the endothelium is such

a small portion of your muscle homogenates. Do you think it is too far-fetched to imagine that adenosine receptors in the endothelium or the capillary endothelium would somehow signal further to the muscle?

T.E. Graham:

I am sure that there are adenosine receptors in the endothelium, but we do not know how important that is in terms of a caffeine response. I am currently doing some work with Eric Richter and Berte Kiens looking at leg metabolism during exercise with caffeine and subjectively it does look as if there is a decrease in exercise leg blood flow with the caffeine. Whether it turns out to be statistically significant, I do not know, so I do think that there could be effects at least at the level of the endothelium.

D.P.M. MacLaren:

Are there any differences between elite and sub-elite performers in terms of their responses to caffeine?

T.E. Graham:

No one has tested that quantitatively, but I think if one took untrained individuals and tried to show a performance effect, it would be a waste of time, because day to day performance of untrained individuals is so variable. In our first study, we used what I would classify as elite runners. There were anything from Top Ten olympic marathon finishers to national level distance runners, and in that particular study, we showed the biggest effect we have ever seen. Subsequently, because we tend to get more invasive, we are not able to convince such people to be poked and probed, and we use anything from university level athletes to recreational athletes. We still see the effects, but they are not as exaggerated, and I do not know whether the elite individual is more sensitive to the signals or whether they can drive themselves so hard that you are going to see a bigger separation in the data.

A.J.M. Wagenmakers:

Is it known whether caffeine has an effect on lipase activity in the muscle? I have a second question. Do you think that the muscle effect of caffeine plays a role in the improvement of performance or is the ergogenic effect the consequence of central effects?

T.E. Graham:

No one has any idea what is regulating muscle hormone-sensitive lipase, so whether there is a specific effect of caffeine is totally unknown. If one draws an example from adipose tissue where the effects seem to be mediated through cyclic AMP, then that is probably going to require a very high pharmacological concentration. Concerning your second question, I have started to believe or theorize that there may well be an excitation contraction aspect to the caffeine, a very direct action which is independent of the central nervous system.

F. Brouns:

My question deals with motivation. If you look to the psychological measurements after taking caffeine, people experience less subjective fatigue. They experience more anxiety and drive to do the work. Could a part of the performance improvement be related to being more motivated to do the work?

T.E. Graham:

I think so. Our subjects feel more aroused, they are more alert and they are certainly a lot more talkative. You can almost see personality changes in some of them. But in studies with electrical stimulation of muscle groups or individual muscles, caffeine still has effects, and this makes me think that the central nervous component is not essential.