

# **Exercise-Induced Hyperammonemia: Skeletal Muscle Ammonia Metabolism and the Peripheral and Central Effects**

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## **1. Introduction**

The area of ammonia metabolism in skeletal muscle is a vast topic and one that involves both directly and indirectly carbohydrate, fat and protein. To gain an appreciation for hyperammonemia associated with exercise one must also understand the membrane-associated events as well as the clearance mechanisms for ammonia. These cannot be dealt with in detail in this paper and those who wish more information can refer to a variety of recent reviews by this author (1,2) and others (3,4,5,6). This manuscript will address the possible effects that exercise-induced ammonia hyperemia could have on the feelings of fatigue and also will address whether there are local effects within the active muscle. Attention will also be given to the clearance mechanisms that are active in association with exercise states both because they are critical to determining the circulating ammonia concentration and also since they represent aspects of how ammonia affects various tissues. However, before these issues can be addressed the basic aspects of ammonia metabolism in muscle will be reviewed.

There are many excellent studies using animal models and they form the bases for what we understand regarding ammonia metabolism in the human. However there are many qualitative and quantitative differences between humans and, for example, rodents in terms of both muscle and liver metabolism. Thus whenever possible I will base my statements on research with human subjects.

## **2. Overview of Skeletal Muscle Ammonia Metabolism**

At rest skeletal muscle is consistently an ammonia consumer; in my experience the human quadriceps has an uptake of approximately 1 umol/min. This would mean that there is a clearance of approximately 0.3 umol/kg wet wt/min by resting muscle. Assuming that the body is 40% muscle then there would be 8 umol/min taken up by resting muscle. This is not insignificant since Eriksson (7) reported that splanchnic ammonia uptake at rest was 12 umol/min.

During exercise muscle rapidly shifts within 30 seconds to releasing ammonia. Originally it was believed that exercise intensity needed to be severe i.e. at least 80%  $\text{VO}_2$  max before ammonia was produced. However we have found that at as little as 60%  $\text{VO}_2$

**Table 1.** Cumulative Data for Single Leg Extensor Exercise.

Exercise Conditions		NH <sub>3</sub> 'load'			Net Total flux (nmoles/kg)					
Intensity (% thigh max)	Duration (h)	Release (mmoles)	Δart NH <sub>3</sub> <sup>c</sup> (nmol)	NH <sub>3</sub>	Gln	Ala	Glu	La	Pyr <sup>a</sup>	
140	0.05	1.9	45	0.9	?	?	?	26.5	53 <sup>b</sup>	
80	1	4.4	38	1.5	1.2	0.9	0.6	33.6	166	
70	1	5.0	15	1.4	1.4	1.2	0.3	56.0	181	
65	1.5	8.4	25	3.0	2.2	1.7	0.6	64.0	132 <sup>b</sup>	
60	3	10.9	15	4.4	6.4	3.6	1.8	15.6	312	

<sup>a</sup> (glucose uptake plus decrease in Glycogen) x 2 = pyruvate flux.

<sup>b</sup> Glucose uptake not considered.

<sup>c</sup> Δart NH<sub>3</sub> is the increase above rest in the arterial ammonia concentration.

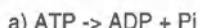
max(an intensity that can be maintained for several hours) there is a continuous ammonia production. The arterial concentration is virtually constant (table 1) as clearance matches release and so if one does not measure release of ammonia directly the production would go undetected. It has not been determined what the minimum intensity of activity is in which there is no net ammonia release.

## 2.1. Ammonia from AMP Deamination

It is commonly believed that the source of ammonia is from AMP deamination and there is no question that it is the main source (perhaps the only source) when the exercise is brief (a few minutes or less) and intense (90% VO<sub>2</sub> max or greater). Under these conditions it has been shown that the rise in the product of AMP deamination, IMP is stoichiometrically matched very closely with the net ammonia production (7,8,9). However, even this well accepted statement must be accepted cautiously as most investigators have only measured the net increase in muscle ammonia as a reflection of production. As described below one needs to include the ammonia that is released during the exercise as well as that which is removed as glutamine and possibly as alanine in order to get an accurate measure of production.

Despite these reservations there is no question that AMP deaminase (reaction (c) figure 1) is the key enzyme in ammonia production during intense exercise. The fundamentals of the regulation of this process are well documented (1-6) and generally it appears that shifts in the energy status of the tissue resulting in an increase in AMP as well as rises in ADP and

### Ammonia from AMP deamination



**Figure 1.** Ammonia from AMP deamination. A series of reactions take place during high metabolic stress to try to maintain a high ATP/ADP ratio. Myosin ATP'ase (and others) cause ATP to go to ADP (reaction (a)). As ADP accumulates adenylate kinase forms ATP and AMP (reaction (b)). To encourage ATP formation AMP is removed via AMP deaminase (reaction (c)) and ammonia is formed.

**Ammonia from aspartate deamination  
(The purine nucleotide cycle)**



**Figure 2.** Ammonia from aspartate deamination. The purine nucleotide cycle is a series of 3 reactions IMP and aspartate form adenylosuccinate (S-AMP) and requires GTP in the adenylosuccinate synthase step (reaction (a)). This is converted to fumarate and AMP by adenylosuccinate lyase (reaction (b)). Finally, the AMP is deaminated to ammonia and IMP (reaction(c)). Thus the cycle results in the deamination of aspartate, the formation of fumarate and ammonia and requires GTP.

H<sup>+</sup> and a decline in CP will increase the activity of the enzyme. Rundell and coworkers (10,11) have demonstrated that during intense exercise the AMP deaminase shifts to a bound form on myosin and this may be a critical factor in regulation. It is not clear what promotes the binding and recently we (12) have demonstrated that this may not be vital in human muscle. For example, while the percent of bound enzyme rose during moderate exercise (despite no change in the nucleotide pool) there was no further increase when the subjects increased their power output even though the ammonia production rapidly increased and fatigue occurred.

## 2.2. Ammonia from Aspartate Deamination

A second possible source of ammonia is the purine nucleotide cycle (PNC) (figure 2). AMP deaminase is one step of this process (reaction (c) figure 2) and is often referred to as the initial step. However, if the PNC is cycling it is difficult to identify a start and end. In the PNC AMP is deaminated to IMP and ammonia and the IMP is then processed by the "reaminating arm" of the cycle (reactions (a) and (b) of figure 2). The amino acid aspartate and IMP form adenylosuccinate and the step is energy (GTP) requiring (reaction (a) figure 2). The process is not fully understood, but it is inhibited by IMP, AMP, and Pi. Hence it is difficult to accept that this step would be active during exercise. The adenylosuccinate is then converted to fumarate, a TCA cycle intermediate and AMP (reaction (b) of figure 2). Thus when the PNC actually cycles the net effect would be aspartate being deaminated to fumarate with the production of ammonia and the consumption of GTP. The purpose of this cycle appears to be the anaplerotic effect for the TCA cycle (discussed below) and it is apparent that the process can not contribute to nucleotide management. In contrast when the deaminating step occurs with no further cycling then there should be a benefit to the nucleotide pool in that the removal of AMP would promote adenylate kinase activity in the ATP formation direction and so help to maintain a high ATP/ADP ratio (figure 1).

It is controversial whether cycling occurs during exercise; Terjung and coworkers argue that if it does then it is in fibers that have already fatigued and which are recovering even as the exercise continues (5,6). Broberg and coworkers (13) propose that cycling occurs in humans during moderate exercise. However, their evidence is very circumstantial and they



**Figure 3.** Ammonia from branched chain amino acid deamination. The three branched chain amino acids use alpha ketoglutarate to accept the amino group in a near equilibrium reaction (a) catalysed by branched chain amino transaminase. The keto acids are dehydrogenated by the key non-equilibrium enzyme branched chain keto acid dehydrogenase (reaction (b)), and depending on the original amino acid one of three acyl CoA derivatives is formed (alpha ketoisovalerate (KIV), alpha ketoisocaproate (KIC) and alpha keto 3-methylvalerate (KMV)). These are converted to TCA intermediates by a series of dehydrogenases and other reactions.

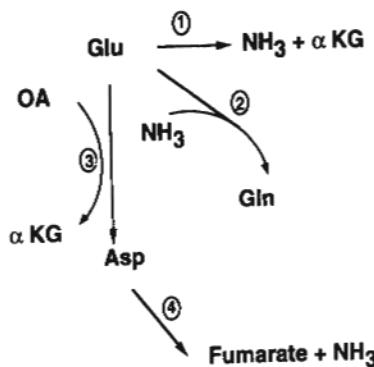
fail to consider factors such as branched chain amino acid deamination(see below). Recently Hood and Parent (14) have presented evidence that rat fast twitch red fibers do have cycling during prolonged activity. However, given that the metabolic characteristics of human muscle are not as distinctly differentiated between fiber types as that of the rat, one cannot generalize these findings to the human.

### 2.3. Ammonia from Branched Chain Amino acid Deamination

The third process by which skeletal muscle can produce ammonia is by the deamination of the branched chain amino acids (figure 3). Unlike the metabolism described above this takes place in the mitochondrial compartment. The three branched chain amino acids, leucine, isoleucine and valine can be deaminated to their respective keto acids with alpha ketoglutarate accepting the ammonia group and forming glutamate (reaction (a), figure 3). This is a near equilibrium reaction and the keto acids can either be released and transported to the liver for further oxidation or they can be catabolized to TCA intermediates in the muscle mitochondria and oxidized (reactions (b) and (c), figure 3). At rest the former appears to be dominant while during prolonged, steady state exercise the latter process is very active. In the latter case the key enzyme is branched chain keto acid dehydrogenase (BCKAD) (reaction (b) figure 3) and this exists in both an active and an inactive form. At rest the muscle form is very inactive but during prolonged exercise the activity increases considerably (4,12), presumably due to the increase in branched chain keto acids in the mitochondria. It has been suggested that a decline in mitochondrial ATP is also a factor (4), but we found that there is considerable BCKAD activation when there is no disturbance in the nucleotide pool (12).

### 2.4. Ammonia, Glutamine and Alanine

The glutamate produced by the branched chain amino acid deamination does not accumulate but in fact from the onset of exercise there is a marked drop in concentration to less than 50% of rest levels and it remains at this level throughout the exercise. Glutamate is the only amino acid to decline significantly in muscle during exercise and this occurs



**Figure 4.** The fate of Glutamate. This illustrates some of the metabolic processes involving glutamate (Glu). It can be deaminated by glutamate dehydrogenase (reaction (1)). It can form glutamine (reaction 2) via glutamine synthase. It can also be involved in transaminations. The one represented by reaction (3) is aspartate transaminase which uses oxaloacetate (OA) and forms aspartate (Asp). Asp can then be deaminated by the purine nucleotide cycle (4) to fumarate and ammonia. Note how frequently TCA cycle intermediates are involved.

despite the fact that the muscle is continuously taking up large quantities of glutamate. It is a very active amino acid involved in numerous reactions (figure 4). It can be deaminated by glutamate dehydrogenase to form ammonia and alpha ketoglutarate (reaction (1) of figure 4). It can also take on another ammonia group to form glutamine and it can take part in various transamination reactions. The two critical ones in muscle are alanine aminotransferase and aspartate aminotransferase. The former requires pyruvate and results in alanine leaving the muscle while the latter requires oxaloacetate and the aspartate can go to ammonia and fumarate via the PNC (reactions (3) and (4) of figure 4).

We do not know the importance of each of these processes but it appears that they are all active during exercise. This obviously complicates studies of ammonia metabolism. For example just to quantify the ammonia production one needs to evaluate the release and change in intramuscular concentration of not only ammonia but also glutamine and alanine. Very few scientists have done so and even when this is performed one cannot be certain whether the glutamine "counts" for one or two ammonia and whether alanine represents one or no ammonia. In order to establish this one has to know whether the glutamate that was used was synthesized from alpha ketoglutarate or whether it came from an existing glutamate pool. Nevertheless as reviewed below there is a large production and release of ammonia, glutamine and alanine in most forms of exercise.

There have been a number of measures of muscle ammonia during exercise. The values at the end of intense exercise are typically approximately 1 mmol/kg wet wt and at the end of prolonged exercise the concentration is usually about half of this value (2), while resting ammonia ranges from .15-.30 mmol/kg wet wt. The rise in plasma or blood concentration is also reasonably modest with typical values at exhaustion being 100-150 uM regardless of exercise intensity. There has been a report of values as high as 250 uM in one study of elite athletes (15) and as high as 3-400 uM in McArdle's patients (16). While changes in plasma concentration generally are a qualitative reflection of ammonia release and production there is no question that they cannot be used quantitatively (8,17). There have been very few measures of circulating ammonia concentrations in other species, but the horse appears to be able to generate greater levels of ammonia than that found in humans. During intense exercise

**Table 2.** Cumulative Data for Single Leg Extensor Exercise.

Exercise Conditions		Net total flux (umoles/kg·min)					
Intensity (% thigh max)	Duration (h)	NH <sub>3</sub>	Gln	Ala	Glu	La	Pyr <sup>a</sup>
140	0.05	300	?	7	?	8,800	17,700 <sup>b</sup>
80	1	25	20	15	10	560	2,800
70	1	23	23	20	5	970	3,000
65	1.5	33	24	19	7	710	1,470 <sup>b</sup>
60	3	24	36	20	10	87	1,730

<sup>a</sup> (glucose uptake plus decrease in Glycogen) x 2 = pyruvate flux.<sup>b</sup> Glucose uptake not considered.

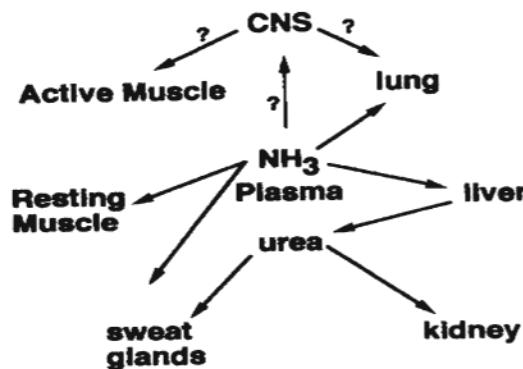
lasting 1–4 minutes horses achieve values of 300–1000 uM (18,19). Furthermore based on the increase in muscle IMP (20) the intramuscular ammonia may be 3–4 mmol/kg wet wt. It is not clear whether this is due to the enzyme compliment of the animals or whether it is due to their tremendous sprinting power.

Rarely have studies reported the responses of ammonia, glutamine and alanine during exercise. We have been fortunate to be able to do so in a series of studies that have all used the same experimental model. We have studied humans exercising the quadriceps of one leg (the single leg knee extensor model) in brief, exhaustive exercise as well as during various intensities of prolonged exercise. Table 1 and 2 summarize these studies for both the rate of metabolite production as well as the total quantity of metabolite formed during the entire exercise. It can be seen that while intense exercise naturally has a higher rate of production the total quantity of formation is greater during prolonged exercise. Thus the latter state will place a greater demand on the supply and removal mechanisms even though the former may generate greater changes in plasma metabolites as reflected in the change in arterial ammonia. Unfortunately we did not measure alanine or glutamine release in the study in which the subjects worked at 140% VO<sub>2</sub> max (8) but Katz et al (9) found that humans released at least as much alanine and glutamine as ammonia during whole body exercise at 100% VO<sub>2</sub> max. It was not established whether this was due to production or a washout of intramuscular stores. It is clear that multiple measures are necessary to quantify ammonia production.

## 2.5. Plasma Clearance

Associated with the release of ammonia and alanine and glutamine there is a release of essential amino acids (17). This occurs even though there is no change in the intramuscular free pool of essential amino acids and so this must represent a net protein catabolism. It is impressive that the efflux of the various amino acids and ammonia are great enough to potentially produce huge changes in the circulating concentrations yet very little change is observed (17). This must mean that clearance mechanisms are very sensitive to plasma changes and they have a large capacity. It has been shown that the splanchnic clearance of amino acids is quite active during exercise and most are probably used for gluconeogenesis (21,22,23) and Carraro et al (24) have reported that the liver can also synthesize small, short-lived plasma proteins during exercise. This latter response would result in the conservation of amino acids and reduce the need to produce urea.

The clearance mechanisms for ammonia have not been clearly quantified or even identified (figure 5). As mentioned above resting muscle can clear ammonia but its role during exercise has not been quantified. The liver takes up ammonia but Eriksson and coworkers (7) could not show an increase in extraction during exercise. Sweat contains not only urea (25), but also ammonia (26). The magnitude of these processes have not been determined. In addition we demonstrated that the clearance of ammonia decreased very



### **Actions of Plasma Ammonia**

**Figure 5.** An illustration of how plasma ammonia may affect various tissues. It may be cleared by the lung, resting muscle, sweat glands and the liver. The latter forms urea which can be excreted in urine and sweat. In addition, plasma ammonia may affect the brain and in turn stimulate ventilation and/or cause fatigue. Because these are more speculative question marks are beside these arrows.

rapidly in the first few seconds of recovery and speculated that the lung could be an important clearance organ (8).

Thus it is evident that the ammonia production of exercise is associated with many metabolic processes and exits the muscle in several forms. As described below these events have been used to construct several theories of central (i.e. CNS) fatigue and peripheral fatigue. In addition the changes in plasma ammonia and assorted amino acids cause immediate changes in a variety of tissues that are involved in the clearance of these substances.

## **3. Central Effects of Ammonia and Associated Amino acids**

In all considerations of ammonia and the central nervous system one must keep in mind that the exercise hyperammonemia rarely exceeds 150 uM and that these changes are very transient, lasting only minutes or perhaps even seconds. Furthermore Cooper and Plum (27) point out that while ammonia is metabolised in many compartments in the brain it is largely metabolized in the astrocytes, which act as a metabolic trap for ammonia. Ammonia can result in numerous changes in neural membranes, including depolarizing resting membrane potentials, inhibition of axonal action potentials, and depression of excitatory postsynaptic potentials (27). However, the ammonia concentrations and duration of exposure appear to be considerably greater than those that a person would encounter during exercise.

### **3.1. Ammonia and Ventilation**

As discussed previously ventilation during exercise may serve as a means of reducing the ammonia load. In addition it has been suggested that the increase in plasma ammonia may act to stimulate the ventilatory response during exercise (figure 5). Certainly the temporal patterns in plasma ammonia during an exercise in which the power output rises rapidly (eg. a standard, progressive  $\text{VO}_2$  max test) follow the curvilinear rise in ventilation. Similarly during prolonged, steady state exercise the plasma ammonia concentration tends to drift

upwards as does ventilation. However, these correlations cannot be considered proof of a causal relationship.

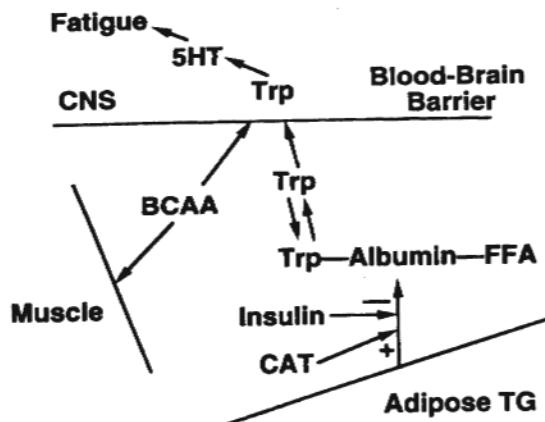
In some pathological conditions associated with chronically high plasma ammonia as well as when plasma ammonia is massively elevated by infusion, ventilation may be stimulated. For example, Wichser and Kazemi (28) infused ammonia into anaesthetized dogs to a blood ammonia concentration of 400  $\mu\text{M}$  and observed a marked increase in ventilation. Similarly direct infusion of the ventriculocisternal system with a high glutamate concentration also stimulated ventilation (29). Obviously it is a large extrapolation from such studies to conscious human who experience transient elevation in plasma ammonia during exercise.

### 3.2. Ammonia and Central Fatigue

Some investigators have demonstrated a correlation between plasma ammonia concentration and the perception of exertion (exertion is rated on a numerical scale) and from this observation have suggested that the ammonia is influencing the perception within the CNS (16,30) (figure 5). Similarly Banister and Cameron (31) speculate that plasma ammonia may cause ammonia toxicity which leads to a lack of coordinated movement, ataxia and stupor. There is no evidence to support any of these claims and given the transient nature of the moderate increases in plasma ammonia together with the protective role of the astrocytes these theories seem to be unlikely.

The second theory involving central fatigue that pertains to this discussion focuses not on ammonia but on the plasma amino acid profile (figure 6). This theory is included in this review since the branched chain amino acids are major factors in this theory. The hypothesis that has been put forward by Newsholme (32) is based on several well established facts. The neural transmitter serotonin or 5-hydroxytryptamine is known to cause impaired mental performance and to induce sleep. It is also clear that the synthesis of this monoamine is

#### BCAA and Central Fatigue



**Figure 6.** A scheme for how branched chain amino acids may cause fatigue (adapted from Newsholme (32)). Tryptophan (Trp) is both free and bound to albumin in the plasma. During exercise catecholamines (CAT) rise and insulin falls causing a rise in free fatty acids (FFA). These displace the tryptophan from albumin. The rise in free amino acid allows it to enter the brain and stimulate the synthesis of serotonin (5HT).

dependent on the availability of its amino acid precursor, tryptophan. This amino acid is transported across the blood brain barrier by the same transport mechanism that carries the branched chain amino acids. Thus there is a competition for entry into the central nervous system and any stress that alters the ratio of these amino acids could also be reflected in a change in serotonin.

Tryptophan is not abundant in proteins so it is difficult to increase its plasma concentration with diet changes. However, one can increase the intake of branched chain amino acids and lower the tryptophan/branched chain amino acid ratio (TRP/BCAA). In addition tryptophan is unique among amino acids in that it is bound to plasma albumin, the same protein that carries free fatty acids. As a result there is a bound and free fraction of tryptophan in the plasma. Furthermore albumin prefers free fatty acids and the fraction of bound tryptophan varies inversely with the plasma fatty acid level and it is the free fraction that competes with the branched chain amino acids for entry into the brain. Newsholme (32) points out that when the plasma free fatty acid concentration exceeds approximately 1 mM there is a rise in free tryptophan. Importance is placed on factors that would either change the concentration of the branched chain amino acids or the free fatty acids. During intense exercise there would be little change in either factor but in prolonged exercise several aspects can change. It is well known that insulin levels decline and also that catecholamines increase. These can elevate free fatty acid levels and hence increase the free fraction of tryptophan. In addition the active muscle can extract plasma branched chain amino acids and so both actions could increase the TRP/BCAA ratio. Newsholme (32) proposes that the resulting shift increases the brain serotonin and promotes central perceptions of fatigue.

There is very limited experimental knowledge in this area. Blomstrand and coworkers (33) examined rats following sustained running to complete exhaustion. They found that the plasma TRP/BCAA ratio was elevated (due mainly to an increase in the free/bound tryptophan) and the serotonin concentration was increased 15% in the brain stem and hypothalamus. Of course it is speculative as to what is the functional significance of these changes. These workers subsequently studied humans running in a marathon. They administered branched chain amino acids to half of the runners prior to the race but they did not control the intake of fluids either as water or as carbohydrate/electrolyte solutions during the race. Thus the study is questionable. Furthermore performance was not improved unless only the slower runners(3:05–3:30 h) were examined separately. Even then there was only a 3% improvement. The administration of branched chain amino acids is probably not the best way in which to alter the TRP/BCAA ratio; while it certainly lowers the ratio it also results in a large increase in the plasma ammonia concentration (4,34) and we have recently observed that this is due to a large increase in the activation of BCKAD in the active muscle.

Davis et al (35) had cyclists ride to exhaustion and they ingested either a placebo or a 6 or a 12% carbohydrate solution both prior to and during the exercise. Carbohydrates resulted in an increase in endurance and in plasma insulin. There was also a lower concentration of plasma free fatty acids and together with this, less free tryptophan and a lower TRP/BCAA ratio. Of course it is unknown whether the endurance benefit came from the carbohydrate or from the TRP/BCAA shift. In contrast Madsen et al (unpublished observations) have recently completed a similar study. Their cyclists ingested either placebo or carbohydrate or carbohydrate plus branched chain amino acids. There was no difference in performance and the carbohydrate-branched chain amino acid trial had greater plasma ammonia levels.

This theory remains as a theory and since it involves the central nervous system it will be difficult to examine in any detail. Perhaps it will require the use of pharmacological treatments to influence the synthesis and degradation of serotonin to resolve the hypothesis in humans.

## **4. Peripheral Effects of Ammonia and Associated Amino acids**

### **4.1. Possible Roles of Ammonia in Skeletal Muscle**

There have traditionally been four possible roles attributed to ammonia formation in active skeletal muscle. In actual fact several of these should be proposed for the PNC or at least AMP deamination rather than to ammonia itself.

It has been suggested that ammonia will act as a buffer against metabolic acidosis. With a pKa of 9.3 it will be predominantly in the form of ammonium ion in physiological solutions. However, there is a large disparity in the capacity for ammonia and lactate production (table 1 and 2); for example in several minutes of strenuous exercise we estimated that the net ammonia formation was 2 mmoles while that of lactate was 127 mmoles (8) and corresponding values for an hour of moderately strenuous exercise were 4.4 and 92.6 mmoles (17). Thus the buffering capacity of ammonia must be very minor.

It has also been suggested that ammonia is a modulator of the key glycolytic enzyme, phosphofructokinase. However, it is not generally viewed as an important regulator and there are many examples where the rates of ammonia and lactate formation vary independent of each other. For example, we observed that ammonia formation continues to rise during prolonged exercise while lactate formation increases early in the activity and then decreases (17,36) and during the breathing of hyperoxic gas lactate formation is depressed while ammonia formation is not altered (36).

The third putative role is for the prevention of adenosine nucleotide degradation, i.e. by forming ammonia (and IMP) the accumulation of AMP is less. If AMP accumulates presumably some would be degraded to adenosine. However, this apparent advantage of conserving the adenosine nucleotide pool is more accurately attributed to the AMP deaminase process than to ammonia per se. Furthermore it would only be associated with ammonia if the ammonia is formed from AMP deamination (as opposed to amino acid degradation) and if there is no PNC cycling.

The fourth possible role is that of maintaining a high ATP/ADP ratio. As mentioned earlier the assumed purpose of AMP deamination is to attenuate the rise in AMP which in turn creates an environment in which ATP formation via adenylate kinase reaction would proceed. Again this is a property of the process of AMP deamination rather than something that can be directly attributed to ammonia. Furthermore it would not be associated with ammonia formed by amino acid deamination.

In addition to these commonly listed possible roles ammonia formation due to amino acid deamination is associated with anaplerotic actions for the TCA cycle both when aspartate goes to fumarate in the PNC (figure 2) or when branched chain amino acids are catabolized to succinyl CoA and/or acetyl CoA (figure 3). If these actions are important it is most likely to be during prolonged exercise as discussed below.

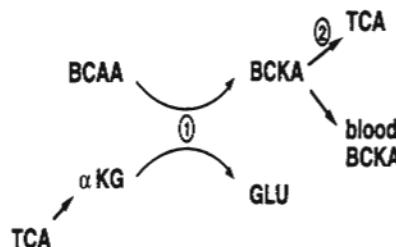
Besides these possible actions ammonia has been associated with fatigue processes within the muscle, i.e. local or peripheral fatigue. Brouns et al (15) examined endurance cyclists during extremely long rides and speculated that muscle cramps and fatigue were caused by ammonia influencing the muscle membrane potential. This is rather similar to the possibility that ammonia is influencing the central nervous system in that the muscle ammonia levels do not get very high compared to those of in vitro studies and the high levels only occur transiently. There is also the possibility that the muscle ammonia acts as a stimulant to the peripheral sensory nervous system. In addition to the Ia, Ib and II afferents which originate from muscle spindles, Golgi tendon organs and Pacinian corpuscles, skeletal muscle has group III and IV afferents. Their receptors are "free" nerve terminals or unencapsulated nerve endings. These receptors are responsible for reflex cardiovascular responses that occur with static contractions (37) and some of the receptors are termed nociceptors. These are activated by noxious chemical stimuli and are responsible for the sensation of muscle pain. Rotto and Kaufman (38) demonstrated that lactate, adenosine and inorganic phosphate were

ineffective stimuli of these group III and IV afferents, while lactic acid and cyclo-oxygenase products (prostaglandins and thromboxanes) were potent stimuli. Lewis and Haller (39) speculated that ammonia would also be a stimulus since McArdle's patients have an exaggerated ammonia production and pressor responses to dynamic exercise. This is still open to speculation, but in a preliminary report they found that ammonia had little or no effect on these afferents (40).

In 1990 two investigators (4,41) proposed that a decrease in the TCA intermediates during prolonged exercise may be the main fatigue factor. This is a very interesting proposition and it is fascinating that while both scientists put forward the same theory and both link it to increased ammonia production and decreased carbohydrate supply, the fundamental nature of their proposed mechanisms is completely different!. Perhaps this is the best reflection of the controversy and complexity of this field when two excellent scientists propose similar explanations for fatigue but have very different mechanisms for the same factors. It is well documented that during prolonged exercise there is a continuous and progressive release of ammonia from active muscle (13,17). It has also been accepted for decades that carbohydrate supply, mainly as muscle glycogen is a major limiting factor in prolonged exercise, i.e. when the glycogen stores become low the person must stop or at least decrease the power output. Traditionally it has been stated that while there are abundant fat stores available the maximal rate of ATP supply from fats is limiting.

However, both of the present theories suggest that as fatigue approaches there is a progressive decline in TCA intermediates either because they are involved in other reactions or because there is a slowing of some of the anaplerotic reactions. This in turn might result in a decreased capacity to maintain a high rate of ATP production. Sahlin and coworkers (41) made a detailed study of the TCA intermediates during 75 minutes of exhaustive exercise. They found that there was an initial 10-fold rise in TCA intermediates and that this slowly decreased over the course of the exercise to only 68% of the initial rise. However, it was still 6-fold greater than the rest level. They propose that as the carbohydrate supply decreases, the rate of glycolysis declines. This results in less pyruvate and phosphoenolpyruvate being available for the synthesis of TCA cycle intermediates. The reduced flux through the TCA cycle and thus decreased aerobic ATP supply causes an increase in cytosolic ADP, AMP, Pi and creatine. These would both lead to a greater AMP deamination and ammonia production as well as stimulation of the regulatory enzymes of the TCA cycle by ADP and Pi. To a degree this argument seems circuitous and it hinges on the ammonia production being related to only AMP deamination. It is clear that the ammonia production during most of the duration of prolonged exercise is not associated with IMP accumulation and thus AMP deamination is not the source. Furthermore, it is clear that branched chain amino acids are oxidized in muscle during prolonged exercise (2,6) and this would provide TCA intermediates as would PNC cycling. Furthermore, one might expect to observe a decrease in  $\text{VO}_2$  and an increase in NADH if mitochondrial function is impaired and these were not observed in the study by Sahlin. In addition we (42) found that at the point of exhaustion in prolonged exercise the muscle acetyl CoA concentration was 1.3-fold greater than rest. Thus it is difficult to accept that the TCA cycle is limiting.

Wagenmakers and coworkers (4,43) have made a proposal quite similar to that of Sahlin. They suggest that there is a depletion of TCA intermediates at exhaustion and that it is associated with ammonia metabolism. However, in their theory the ammonia is derived from the deamination of the branched chain amino acids. They have demonstrated that the activity of the BCKAD increases as the glycogen stores are depleted (43) and if carbohydrate is given during the exercise the enzyme's activity declines (4) as does plasma ammonia. We (44) have shown that giving a branched chain amino acid supplement results in increased plasma ammonia and recently (unpublished data) have found that there is an increase in ammonia production and a marked increase in BCKAD activity. This demonstrates that the rate of this pathway is sensitive to substrate supply. Since the branched chain aminotransferase requires alpha ketoglutarate Waganmakers suggests that this acts as a drain



**Figure 7.** A scheme to illustrate how branched chain amino acid deamination could lower TCA intermediates (adapted from Wagenamakers (4,43)). When branched chain amino acids are transaminated they take alpha ketoglutarate from the TCA. The keto acids may leave the muscle or form TCA intermediates (reaction (2)). Depending on this and the fate of the glutamate (Glu) (see figure 4) there could be an impact on the TCA.

on the TCA cycle (figure 7). The fate of the products of the transamination are critical to this theory; if the branched chain keto acids leave the muscle and if the glutamate proceeds on to glutamine and is released (figure 4) then the theory would be supported. Our understanding of the fate of the keto acids is incomplete, but it appears that oxidation within the muscle is likely (45). This is supported by the increased activation of the BCKAD and the fact that it is greater when there is more branched chain amino acids available. If this is the case then the oxidation would provide succinyl CoA and acetyl CoA. The fate of the glutamate is more difficult to resolve. Certainly there is glutamine formation, but glutamate is also involved in alanine and aspartate synthesis. Both would regenerate the alpha ketoglutarate and the latter would also provide fumarate for the TCA cycle (figure 4). One could argue that the alanine formation would be a drain on pyruvate and thus indirectly a loss for the TCA cycle, but in prolonged exercise we estimated that at most 1% of the pyruvate goes to alanine (17) (table 1 and 2). In our experience active muscle releases similar amounts of glutamine, ammonia and alanine throughout prolonged exercise. Both of these theories are interesting and hopefully will stimulate more research. However, at this time it seems unlikely that either of them is correct. There have been two studies that have examined ammonia and fatigue in a more general manner. Miller-Graber and coworkers (46) administered ammonium acetate to horses and exercised them to exhaustion with an exercise intensity that lead to fatigue in 15–20 minutes. They observed no difference in exhaustion time even though the plasma ammonia was 200uM at exhaustion after the ammonium acetate rather than 110 uM as in the control; in fact there was a tendency for a longer endurance time with the ammonium load and the ventilation also tended to be lower not higher! Similarly Beaunoyer et al (47) infused monosodium glutamate into horses and ran them to exhaustion with intense exercise. The infusion resulted in almost 50% less increase in plasma ammonia and yet there was a nonsignificant decrease in exercise time (3:55 vs 3:30 minutes for control and treatment respectively). Neither of these studies suggest that ammonia has any relationship with any aspect of fatigue.

## 5. Concluding Remarks

The metabolism of ammonia in active skeletal muscle clearly elevates both the intramuscular and the plasma concentrations of ammonia. The roles of the various metabolic pathways in the production of ammonia are not clear. It is likely that AMP deamination is dominant in intense exercise lasting only a few minutes and that branched chain amino acid

oxidation is a major factor in prolonged exercise. The rise in plasma ammonia is associated with rapid responses by tissues involved in clearance. However, even the relative importance of each of these tissues remains to be established. There have been suggestions about the possible roles of ammonia accumulation in muscle, but it is likely that it is a "necessary evil" and any functional roles are attributed to associated changes in metabolic modulators such as AMP and IMP and to increased production of TCA intermediates. Ammonia and some amino acids have been speculated to alter brain function, but there is little evidence for this. Similarly ammonia production has been suggested to result in fatigue in the active muscle during prolonged exercise, but again the evidence is lacking.

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