American Journal of Sports Science and Medicine, 2015, Vol. 3, No. 5, 96-107
Available online at http://pubs.sciepub.com/ajssm/3/5/3
© Science and Education Publishing
DOI:10.12691/ajssm-3-5-3

The Proposed Effects of Nicotinamide Adenine Dinucleotide (NAD) Supplementation on Energy Metabolism

Benjamin David French*

Department of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, UK
*Corresponding author: Benfrench.r10@gmail.com

Abstract Energy metabolism is a process that is essential in the maintenance of life and has obvious roles with regards to sporting performance. Oxygen's role in aerobic respiration is to act as the final hydrogen/electron accepter to form water. If oxygen is not present the whole aerobic pathway cannot occur and so the body will rely on energy produced anaerobically. The question instantly raised is to whether oxygen is ever in short supply, does it become a limiting factor for energy metabolism? The body will adapt to training in a variety of manners so that only under extreme conditions is oxygen a limiting factor. All of these adaptations are beneficial from an exercise performance standpoint and increase the efficiency of the complex metabolic process. There are still however questions surrounding the idea that NAD+ plays a key role in this process and whether increasing the synthesis/concentration would be advantageous for the trained individual. Nicotinamide, nicotinic acid, tryptophan and nicotinamide riboside are all natural substances that are acquired in the diet. Due to the protein and other biosynthetic uses of tryptophan it may not be as efficient or indeed practical to use tryptophan as a supplement. Supplementation of nicotinamide and nicotinic acid appears to increase NAD+ biosynthesis and the intracellular NAD+ pool. Whether these effects can aid in sporting performance is currently unanswered with no research in this area.

Keywords: *Nicotinamide Adenine Dinucleotide (NAD), nicotinic acid, energy metabolism, biosynthesis, supplementation*

Cite This Article: Benjamin David French, "The Proposed Effects of Nicotinamide Adenine Dinucleotide (NAD) Supplementation on Energy Metabolism." *American Journal of Sports Science and Medicine*, vol. 3, no. 5 (2015): 96-107. doi: 10.12691/ajssm-3-5-3.

1. Introduction

Energy metabolism is a process that is essential in the maintenance of life and has obvious roles with regards to sporting/exercise performance. The body can produce energy both aerobically and anaerobically and the regulatory mechanisms underlying these pathways of energy modulation are complex [40]. Under aerobic conditions the Krebs cycle is crucial for energy production, the hydrogen's removed during the cycle are transferred to the electron transport chain and the energy released during electron transport is utilised in the formation of ATP [1]. Oxygen's role in aerobic respiration is to act as the final hydrogen/electron accepter to form water. If this is not present the whole aerobic pathway cannot occur and so the body will rely on energy produced anaerobically. The question instantly raised is to whether oxygen is ever in short supply, does it become a limiting factor for energy metabolism? Or are other factors limiting? Can increasing or maintaining NAD+ concentrations sustain the action of the Krebs cycle and bring about the continuation of oxidative phosphorylation and therefore reducing build up of lactate as a consequence? If this hypothesis were to be true then this could have advantageous implications in sporting performance (Figure 1).

Arterial oxygen content does not decrease at exercise intensities <75% of VO_2max [49]. VO2max is a measure of the ability of working muscles to oxidise metabolic substrates, with eventually a plateau in oxygen uptake occurring despite increases in work rate therefore achieving maximal oxygen uptake. This capacity is exceeded before circulating delivery of oxygen is limiting [28,55]. This is a significant, as it suggests that oxygen delivery is only limiting at VO_2max where beyond this point oxygen uptake and delivery will become limiting. With regards to the majority of sporting events, exercise is carried out sub maximally for the athlete and so oxygen supply will not be limiting. An early experiment concluded that it seems unwarranted at present to ascribe alterations in body lactate to oxygen deficiency [30]. This paper states that oxygen saturations exceeded 96% at every intensity set for the experiment (mild or severe). The phenomenon of the O_2 debt formation is a manifestation of the need for oxygen by the body tissues during exercise which is not met at the time. This occurs despite the rate of delivery of oxygen to the tissues being greater per minute than normal [30]. This is more supportive evidence that oxygen is not a limiting factor and is in fact transported efficiently to meet the demand,

at least at exercise intensities up to 85-90% VO_2max. Hence, why other metabolic factors appear to be limiting to such a degree that the cessation of aerobic respiration occurs. Blood flow redistribution is important to help compensate for the limits on O_2 delivery and uptake set by maximal cardiac output and O_2 extraction [55]. The oxyhaemoglobin dissociation curve demonstrates the extreme efficiency of haemoglobin at combing with O_2 in the lungs and unloading at tissues, this can be up to 90% of the O_2 carried by haemoglobin during intense exercise [49]. The myoglobin in the muscles functions as an oxygen store and transporter [35,43]. With regards to the respiratory system it has been identified that it only becomes limiting in untrained individuals with the endurance of respiratory muscles markedly improving in trained individuals [11].

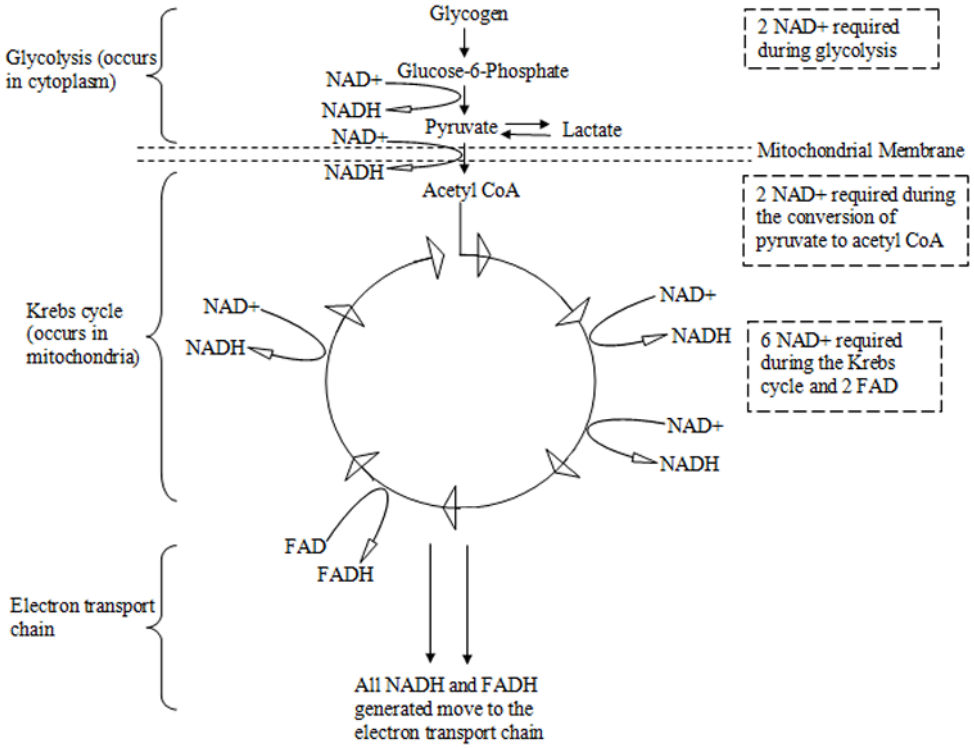

Figure 1. Flow diagram representing the stages in energy metabolism and how NAD affects each of these stages. NAD is required largely during the Krebs cycle but also is required during glycolysis and for the conversion of pyruvate at the end of glycolysis for its entry into the Krebs cycle

When the oxidative potential of a cell has diminished pyruvate can be converted to lactic acid by lactate dehydrogenase. This is important as energy can still be produced through the continuation of glycolysis. The rates of the oxidation for energy metabolism are not affected until NAD+ is affected [29]. If oxidative energy metabolism is so greatly impacted by NAD+, knowing that even during intense exercise oxygen is not in short supply nor are the delivery mechanisms efficiency, can increasing NAD+ concentration allow aerobic metabolism to continue? [Lactate] = [pyruvate] x k[DPNH2]/[DPN], the equation suggests, on a theoretical basis, that all instances of lactate production by tissues are influenced by the ratio of NAD+ and NADH (DPN or Diphosphopyridine nucleotide is another name for NAD) thus leading one to assume that lactate production can be manipulated by altering the ratio.

It has been stated that conclusions about tissue oxygen supply should not be drawn from determining lactate alone, suggesting the interaction between the anaerobic and aerobic energy systems are intricate. For example epinephrine has been found to increase lactate in muscle which is not due to diminished blood flow or blood arteriovenous oxygen difference [16]. Lactate produced during high intensity endurance activities appear to be occurring when the maximum rate of fat oxidation is inadequate to meet the demands of muscle contracting. This causes intracellular signalling events to occur which ultimately lead to the rate of pyruvate delivery to the mitochondria progressively exceeding the ability of the mitochondria to convert and transfer it into the Krebs cycle causing accelerated generation of lactic acid [34]. It has been argued that lactate formation will occur when NADH and pyruvate are available to lactate dehydrogenase regardless of how much O2 is present [26]. Lactate dehydrogenase can convert lactate back to pyruvate for further utilisation in the Krebs cycle, the reaction does make use of NAD+ [68]. The problem becomes one of fuel availability when exercise extends beyond approximately two hours but events lasting 15-30minutes (e. g. 5km and 10km running) the anaerobic contribution can be 10-20% of total ATP turnover. Total ATP turnover during endurance performance reflects the interplay of aerobic and anaerobic metabolism with lactate generation functioning to maintain the NAD+ needed for continuation of glycolysis. If more NAD+ could be supplied or synthesised could lactate be converted back to pyruvate for use in the Krebs cycle and could aerobic metabolism be sustained for longer with reduced lactate build up? Lactate is produced regardless of how much O2 is present as long as pyruvate is available but with increased NAD+ pyruvate would be converted to Acetyl CoA for its entry into the Krebs cycle. The exact mechanisms by which lactate plays a role in fatigue has remained elusive but it's clear it has some debilitating role as endurance trained individuals produce less [26]. Although only assumptions presently, the

implications that NAD+ could have on prolonging aerobic metabolism on exercise performance could be incredible, especially when considering the small margins between winning and losing in many sporting environments.

The human body will adapt in a variety of manners to physical training, these can effect both major systems/organs and more microscopic changes cellularly. These adaptations occur as a result of prolonged exposure to particular situations in an attempt to become a more efficient system. There is evidence that rats see an increase in mitochondria along with certain enzyme activities per gram/muscle (NADH dehydrogenase and NADH cytochrome c reductase), increasing approximately two fold in response to training. This results in a increased capacity of the electron transport chain which was associated with a concomitant rise in the capacity to generate ATP via oxidative phosphorylation [26]. A similar study conducted on rabbits using electrical stimulation of the muscle draws the same conclusion with an increased volume of mitochondria [50]. The exercise induced adaptation of increased mitochondria content appear to be essential for trained muscle to exhibit an increased O2 flux capacity, illustrating the significance of mitochondrial adaptations [53]. Trained endurance runners saw at least a 2. 5 times higher activity value in succinate dehydrogenase than untrained individuals, implying that enzyme activity of the Krebs cycle increases and adapts [26]. It is known that beta oxidation of fatty acids involves FAD and NAD, so it would seem feasible to suggest that increasing NAD concentration/synthesis could help increase or maintain utilisation of fat in doing so sparing glucose.

The Krebs cycle itself is an elaborate chain of intermediate compounds, enzymes and reactions. The cycle is responsible for approximately 67% of all generated reducing equivalents per molecule of glucose, highlighting the importance of Krebs cycle flux for oxidative phosphorylation. An increase in the total concentration of the Krebs cycle intermediates is also necessary to augment and maintain Krebs cycle flux during exercise [12]. NAD+ plays a central role throughout the cycling of reactions and so with the suggestion that Krebs cycle intermediates increase during exercise training, increasing NAD+ biosynthesis and therefore concentration/pool size could have beneficial effects on exercise performance. Research has been carried out on maximal one leg exercise and the results show that as maximal oxygen uptake increased to the muscle the maximal enzyme activity of citrate synthase, α-ketoglutarate dehydrogenase and succinate dehydrogenase increased to match demand [9]. α-ketoglutarate dehydrogenase average maximal activity is almost the same as the average flux through the Krebs cycle. This indicates that the enzyme activity is fully activated during maximal exercise (one leg exercise) and is one factor limiting the flux through the Krebs cycle. Enzyme activity within the Krebs cycle appears to increase in adaptation to exercise training but more research is needed to confirm if activity/concentration of all enzymes within the Krebs cycle adapt to training. It is interesting to note that α-ketoglutarate dehydrogenase is highlighted as one limiting factor in Krebs cycle rate/flux because this point coincides with an increased demand for NAD+ to oxidise isocitrate then α-ketoglutarate. Could NAD+ increased demand at this specific point potentially explain why the enzyme α-ketoglutarate dehydrogenase has been described as limiting? Would α-ketoglutarate dehydrogenase not be limiting if there was a sufficient input of NAD+? The evidence does possibly strengthen the theorised importance of NAD (Figure 2).

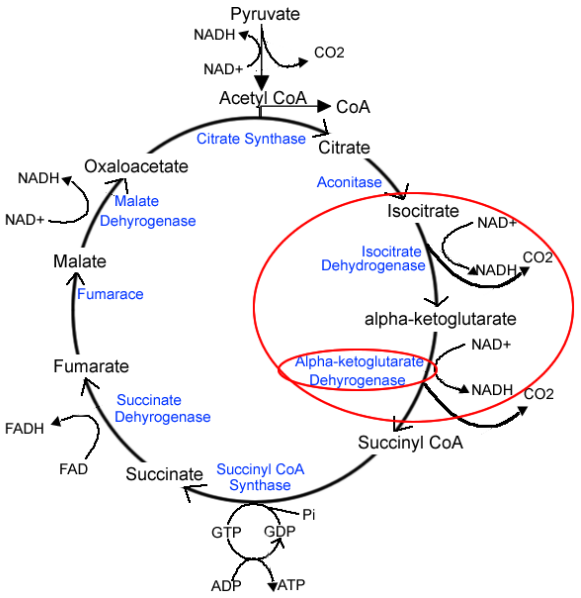

Figure 2. Diagram depicting the Krebs cycle in more depth. The red area highlights the limiting enzyme α-ketoglutarate and the increased demand for NAD at this point

Enzymes involved in aerobic metabolism become limiting only when the energy need of the cell requires a rate of substrate catabolism that exceeds the maximum catalytic ability of the rate limiting enzymes. However adaptations such as increased enzyme activity and increase in mitochondria in trained individuals results in these enzymes not being rate limiting [26].

Table 1. Table representing an experiment on untrained, medium trained and well trained individuals and their ability to generate ATP aerobically and anaerobically

Group	Gender	Anaerobic glycolysis	Oxidation via Krebs cycle
Untrained	Male	104	13
	Female	87	16
Medium Trained	Male	91	21
	Female	89	19
Well Trained	Male	72	26
	Female	61	29

Table 1 represents the results of an investigative experiment carried out on untrained, medium trained and well trained humans. The values of the maximum rate of O_2 uptake (VO_2max) were measured and the results show that values were 21% higher in medium trained and 49% higher in well trained compared to untrained individuals. α-ketoglutarate dehydrogenase activity was also analysed and the activities of the enzyme were 39% higher in medium trained and 90% higher in well trained compared with untrained individuals [8]. The results demonstrate that training increases the capacity to generate ATP aerobically and that α-ketoglutarate dehydrogenase activity also increases with training. Hexokinase (enzyme involved in phosphorylating glucose-6-phosphate) has been reported to change in parallel with the Krebs cycle enzymes as a result of exercise training and electrical stimulation of skeletal muscle [9]. The enzymes citrate synthase and NAD+ linked isocitrate dehydrogenase, both

involved in the Krebs cycle, are inhibited by ATP and low concentrations of calcium respectively and activated by ADP. Although these inhibitory and activating effects are removed when concentrations of isocitrate are high [1,61]. This evidence implies that as long as the Krebs cycle can continue the enzymes citrate synthase and NAD+ linked isocitrate dehydrogenase are not limiting factors in aerobic metabolism. In the same study it was found that in insect flight muscles the activities of both citrate synthase and NAD+ linked isocitrate dehydrogenase are high, indicating that the muscles involved in flight for insects depend on aerobic metabolism for energy production. The study concerns insect flight muscles but the premise is the same, in order for aerobic metabolism to continue at a high rate these enzymes and substrates involved (i. e. NAD) need to be high. It has been demonstrated that if the concentrations of NAD and isocitrate are sufficient the activities of the enzyme NAD+ linked isocitrate dehydrogenase can remain maximal, emphasising the key role NAD+ has in the continuation of the Krebs cycle and thus aerobic metabolism [1]. The slowest step of the Krebs cycle has been found to be between oxaloacetate and citrate and its at this point that pyruvate is converted to acetyl CoA with NAD+ being utilised in this reaction [67]. Citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase are major know sites for regulation and represent important branch points for the overall flux through the Krebs cycle [67,68]. All of these enzymes are effected by NAD+ concentrations. Citrate synthase and α-ketoglutarate dehydrogenase both have favourable conditions when NADH concentrations are low and NAD+ concentrations are high. Isocitrate dehydrogenase is stimulated by NAD+. So all three of the most important enzymes involved in the Krebs cycle need NAD+ concentrations to remain high.

The oxidative capacity of skeletal muscle is highly plastic in humans with adaptations occurring to the cardiovascular and respiratory systems. Changes are also seen in mitochondrial concentrations and in the activities of the various enzymes involved throughout the process of aerobic metabolism. All of these adaptation are beneficial from an exercise performance standpoint and increase the efficiency of the complex metabolic process. There are still however questions surrounding the idea that NAD+ plays a key role in this process and whether increasing the synthesis/concentration would be advantageous for the trained individual.

2. Discussion

The nicotinamide coenzymes are biological carriers of reducing equivalents. The most common function of NAD+ is to accept two electrons and a proton (H+ equivalent) from a substrate undergoing metabolic oxidation to produce NADH, the reduced form of the coenzyme. The chemistry of the NAD+ molecule allows it to readily accept electrons to transfer to the electron transport chain where donation of the electrons results in the concomitant generation of ATP, a molecule universally required for most energy consuming cellular processes [54,68]. Energy metabolism is largely mediated by the electron transport chain found within the mitochondrion and NADH plays a vital role in furnishing reducing equivalents to fuel oxidative phosphorylation [56]. The molecule NAD+ is formed from simple compound precursors such as nicotinic acid, nicotinamide, nicotinamide riboside and tryptophan. All of which can be taken up in the diet. Cells can also take up extracellular NAD+ from the surroundings. There are several pathways for NAD+ formation: 1. In the liver and other animal tissues, tryptophan degradation forms, among other products, quinolinic acid which is converted to nicotinate mononucleotide (or deamidonicotinamide mononucleotide/deamido-NMN) by quinolinate phosphoribosyltransferase, 2. In the cytosol of cells in many mammalian tissues there is the enzyme nicotinate phosphoribosyltransferase that forms deamido-NMN from nicotinate, 3. A very similar phosphoribosyltransferase present in the cytosol of all animal tissues acts on nicotinamide (Figure 3).

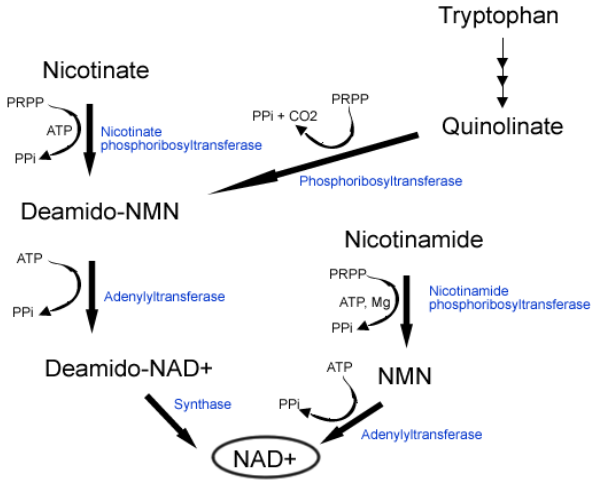

Figure 3. Diagram showing the different pathways of NAD formation

These transferases are responsible for the utilisation of nicotinate and nicotinamide in the diet with the role of ATP in the reaction unclear. Flavin adenine dinucleotide (FAD) also plays a similar role in energy metabolism as NAD+ but it does not appear as significant. FAD is formed from riboflavin which is an essential dietary constituent for mammals. The requirement for multiple synthesis/recycling pathways is a question with which a satisfactory explanation is needed. The most obvious rationalisation would be that the continued generation and preservation of NAD+ levels is so essential for metabolic processes that evolutionary selective processes resulted in the development of several pathways [21]. Therefore emphasising the critical role NAD+ has to play in the metabolic energy systems, again strengthening the claim that increasing NAD+ levels would bring about beneficial consequences.

Before elaborating on the biosynthesis of NAD the malate-aspartate shuttle and the glycerol-3-phosphate shuttle will first be looked at. It is known that one rate limiting step for aerobic metabolism is the shuttling of both NADH and pyruvate from the cytoplasm into the mitochondria. In this regard it is interesting to note that well conditioned and trained athletes actually have a higher number of mitochondria in their muscle cells, a possible adaptation to overcome the rate limiting factor of shuttling NADH and pyruvate into the mitochondria [14].

The malate aspartate shuttle is a beautifully complex array of reactions that occur at the inner membrane of the

mitochondria. NADH that is generated in the Krebs cycle takes place in the mitochondrial matrix and so has direct access to the electron transport chain. NADH generated during glycolysis on the other hand cannot reach the electron transport chain directly as there is no direct mechanism for the transfer of NADH across the mitochondrial membrane. The malate aspartate shuttle has evolved so that the energy of the reduced NADH can move across the membrane in the form of other reduced molecules. In the intermembrane space NADH donates its hydrogen and electrons to oxaloacetate to form malate, which can then move into the matrix of the mitochondria through the malate α-ketoglutarate transporter. The electrons and hydrogen present in malate are removed by NAD+ to generate NADH and oxaloacetate, with malate dehydrogenase catalysing the reaction. Therefore the electrons from glycolysis have entered the matrix and now inside can enter the electron transport chain (Figure 4). Approximately 2. 5 molecules of ATP are formed from each NADH that is oxidised in the mitochondria, the shuttling system does not produce as much ATP as can be obtained from mitochondrial NADH [68]. This is the same with the glycerol-3-phosphate shuttle.

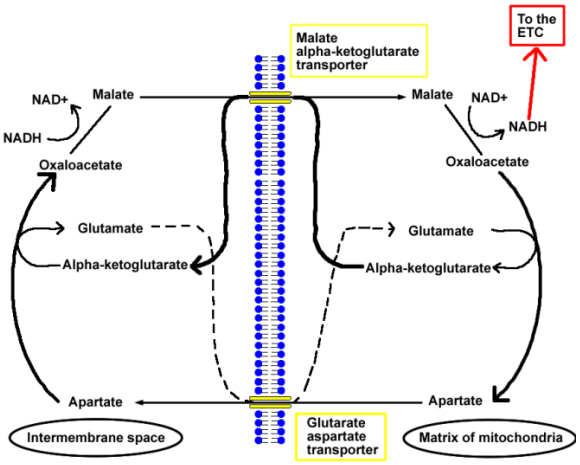

Figure 4. Diagram of the malate aspartate shuttle

The malate aspartate shuttle operates at a rate that seems to be faster than that of the Krebs cycle but the overall rate is slower than the aspartate aminotransferase reaction. This means NADH is quickly shuttled into the mitochondria for its entry into the electron transport chain. The significance of the rate of aspartate transferase being faster than the whole rate of the malate aspartate shuttle indicates that the whole process is not limited by the rate of this enzyme reaction. Because of the high activity of aspartate aminotransferase and the malate aspartate shuttle, it would seem feasible to speculate that this reaction serves as a buffer to maintain a sufficient balance of the intermediates in the Krebs cycle. This would imply that the Krebs cycle intermediates are not limiting due to the rate of the malate aspartate shuttle [67]. Inhibition of aspartate aminotransferase and hence the malate aspartate shuttle brings about decreased glucose oxidation, decreased acetylcholine synthesis and an increase in the cytosolic c redox state as measured by the lactate/pyruvate ratio [15]. Essentially, with the malate aspartate shuttle ceasing to perform, the rate of oxidative metabolism decreases. Experiments which came to these conclusions have been conducted on rat brain synaptosomes which certainly give promising results. There have also been similar experiments on pigs where the malate aspartate shuttle was inhibited through the enzyme glutamate-oxaloacetate transaminase in isolated carotid arteries. This had the following effects: inhibited O2 consumption by 21%, inhibited Krebs cycle, elevation in the NADH/NAD ratio, the rates of glycolysis and lactate production increased and glucose oxidation inhibited. From these effects one can deduce that: the malate aspartate shuttle is a primary clearance method of NADH reducing equivalents from the cytoplasm in vascular smooth muscle, and glucose oxidation and lactate production are influenced by the activity of the malate aspartate shuttle. An increased cytoplasmic NADH redox potential impairs mitochondrial energy metabolism so the malate aspartate shuttle plays a key role in preventing this by clearing NADH in doing so continuing oxidative metabolism. Smooth muscle is involuntary and primarily aerobically based so the results are easier to translate and apply to humans [3].

The glycerol-3-phosphate shuttle is another method of shuttling the potential energy from glycolysis into the mitochondria indirectly for use in the electron transport chain. NADH through the cyclic reaction is eventually converted to FADH in the inner mitochondrial space which is used in the electron transport chain. NADH at the beginning of the process is converted back to NAD+ and so NAD+ is recycled for use in glycolysis (Figure 5). FADH joins the electron transport chain at a later point than NADH, missing complex I, therefore produces less ATP.

A balance in the pyridine nucleotides NAD+ and NADH is a prerequisite for the continuation of metabolic reactions. A cellular imbalance favouring the reductant (NADH) has the potential to result in the production of reactive oxygen species [44]. The capacity of cells to modulate and control the NADH/NAD+ ratio impacts not only the redox state of metabolism but also the management of oxidative stress. A deficiency in the enzyme glycerol-3-phosphate dehydrogenase, a major enzyme in the glycerol-3-phosphate shuttle, results in an elevated NADH/NAD+ ratio [57]. This highlights the significance of the NADH/NAD+ ratio and the glycerol-3-phosphate shuttles role in helping to maintain the balance. Under normal conditions the glycerol-3-phosphate shuttle does not appear limiting especially with well conditioned athletes where as noted earlier an increase in mitochondria concentration and size would overcome any shuttling issues that could arise. There are two forms of the enzyme glycerol-3-phosphate dehydrogenase involved in the glycerol-3-phosphate shuttle: cytosolic and mitochondrial. In insects the activity of the cytosolic enzyme is 3-6 fold greater than that of the mitochondrial, so it is likely that the rate limiting step for the glycerol-3-phosphate shuttle cycle is mitochondrial glycerol-3-phosohate dehydrogenase. The maximum activity of mitochondrial glycerol-3-phosphate dehydrogenase in vertebrate muscle is very low so the overall capacity of the glycerol-3-phosphate shuttle must also be low. The activity is however higher in white than red muscle fibres. In vertebrates the glycerol-3-phosphate shuttle rate suggests that it only accounts for a small portion of the reoxidation of the glycolytically produced NADH and so there is a much greater emphasis on the malate aspartate shuttle [17].

On the whole, these shuttling systems for energy transfer from glycolysis into the mitochondria would not appear limiting due to afore mentioned adaptations to enzyme activity and mitochondria increases.

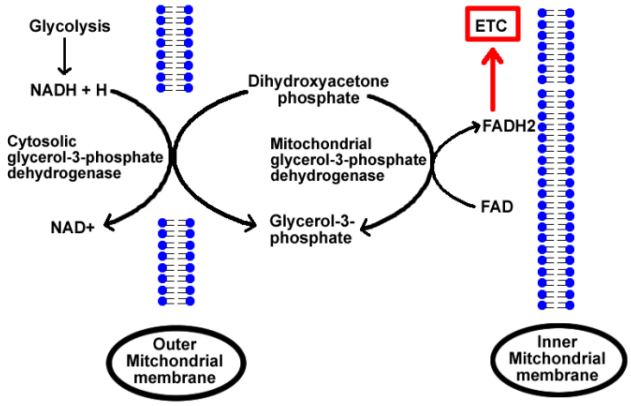

Figure 5. Diagram of the glycerol-3-phosphate shuttle

An in depth analysis of NAD+ biosynthesis from a large collection of research will now be presented. One biosynthetic pathway of NAD+ involves the aerobic degradation of tryptophan leading to the formation of quinolinic acid. Subsequent conversion of quinolinic acid to NAD occurs via a pathway common to all organisms. The role of tryptophan as a precursor in NAD+ biosynthesis first became evident when it was found that humans with pellagra (vitamin deficiency disease), recovered from the illness after the addition of tryptophan or niacin to their diets [21,22]. The term niacin is used for describing both nicotinamide and nicotinic acid, yet niacin on multivitamin and dietary supplements almost always mean nicotinamide [52]. The pathways of tryptophan catabolism are described as aerobic due to the strict oxygen requirements of some of the enzymes involved in the degradation process. Studies on bacteria have found that the enzyme tryptophan pyrrolase is feedback inhibited by NADH but not NAD+, suggesting that if NAD+ concentration could be increased the tryptophan pathway of NAD+ biosynthesis would not be inhibited [13]. Although a study conducted on a different species of bacteria found that the enzyme tryptophan oxygenase is likely to be inhibited by NAD+ [38]. In Escherichia coli (E. coli) exogenous NAD is not utilised directly by the cell but must be degraded and recycled through the pyridine nucleotide cycle [39]. The results reveal that an exogenous supply of NAD+ is not directly used by E. coli, but would the results be replicated in humans? A model was presented by Lundquist and Olivera [39] representing the balance of NAD+/NADH production on E. coli, the model proposed below modifies it slightly for application to humans [Figure 6]. The hypothetical model demonstrates that if R_B is increased, the biosynthesis or ingestion, NAD+ production will increase. One could conclude that the NADH/NAD+ ratio is important to the cell as well as the maintenance of a specific quantifiable level of NAD, the question is what is this level? With adaptations to exercise training occurring in the body, for example increases in mitochondria, does the requirement and this quantifiable level for NAD increase?

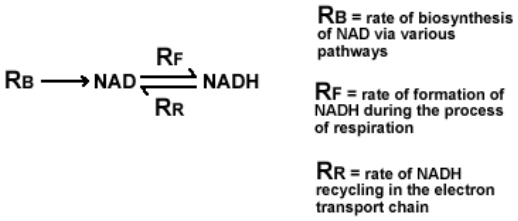

Figure 6. Lundquist and Olivera model of the NAD/NADP balance in E. coli modified to represent the NAD/NADH ratio in humans

A number of nutritional studies in the past have demonstrated that suitable levels of tryptophan can replace the requirement for niacin under normal conditions in several mammals and birds [46]. This may highlight the importance of tryptophan and its utilisation in the synthesis of NAD+ even without niacin. But the studies were done under normal conditions, can diet manipulation for training purposes effect NAD+ production in the body?

More recent research has found that mammals predominately use nicotinamide rather than nicotinic acid or tryptophan as a precursor for NAD+ biosynthesis. Nicotinamide phosphoribosyltransferase (Nampt) is the rate limiting enzyme that converts nicotinamide to nicotinamide mononucleotide in the NAD+ biosynthesis pathway in mammals [51]. The same protein Nampt has also been identified as a cytokine (pre-β-cell colony enhancing factor/PBEF) or as an insulin mimetic hormone (visfatin). Nampt plays a role both as an intra and extracellular NAD+ biosynthetic enzyme [52]. It is interesting that nicotinamide which is acquired from the diet is highlighted as the predominant precursor for NAD+ biosynthesis in mammals. Prior dated research had focused on tryptophan. So with the greater need for nicotinamide could dietary factors play a vital role in manipulating NAD+ biosynthesis? Also considering the enzyme Nampt has a role both intra and extracellularly, this could have great implications in being able to convert dietary precursors into NAD+ to shuttle into the cell for use. Further research lead to the same conclusion with nicotinamide being the predominant precursor for NAD+ biosynthesis. Instead of deamination to nicotinic acid, nicotinamide is directly converted to nicotinamide mononucleotide (NMN) by Nampt. NMN is then converted to NAD+ by nicotinamide/nicotinic acid mononucleotide adenylyltransferase (Nmnat). Therefore, there are only two steps to synthesise NAD+ from nicotinamide in mammals. In the case of restricted niacin availability mammals are able to synthesise NAD+ in the liver through the kynurenine pathway whereby tryptophan is metabolised to quinolinate and subsequently NAD+ to partially meet the NAD+ requirement [41,52]. One can infer from this information that nicotinamide is predominately used due to the fewer steps involved in the generation of NAD+ suggesting it is a quicker method. Nicotinic acid and nicotinamide require only three and two steps respectively [10]. Whereas formation of NAD+ from tryptophan has eight steps and so is not the fastest and most efficient way for generating NAD+, but may play more of a role if nicotinamide is low. Nicotinamide, a form of vitamin B_3, is absorbed from the diet and distributed to all organs/tissues through the blood circulation. In humans the nicotinamide concentration ranges from 0. 3-0. 5μm. Nampt can act as an extracellular

NAD+ biosynthetic enzyme, so a significant amount of NMN could be synthesised and transported into cells where Nmnat can then convert NMN to NAD+. There are two paths for dietary nicotinamide to take before it is eventually converted to NAD+. The first is where nicotinamide enters cells from the blood by diffusion or transport and is converted into NMN by intracellular Nampt and then to NAD+ by Nmnat. At the same time a significant fraction of nicotinamide may be converted to NMN by extracellular Nampt. NMN can then be distributed to tissues through the blood circulation and transported into cells. Once NMN is transported to the inside if cells, it would rapidly be converted to NAD+ by Nmnat, which is enzymologically more efficient than Nampt. The distribution of NMN through blood circulation may be of particular importance for organs/tissues that do not have sufficient levels of intracellular Nampt to synthesise NAD+ from nicotinamide. These tissues may be more susceptible to alterations in plasma or extracellular NMN levels (Figure 7).

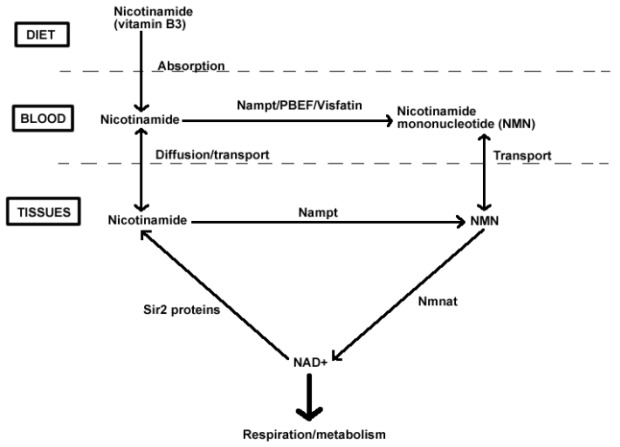

Figure 7. Diagram showing the two pathways dietary nicotinamide can take before being converted into NAD

By increasing dietary intake of nicotinamide could NMN levels in the blood increase for transport around the body to tissues in need of NAD+? This NAD+ would then be taken up and utilised in various steps of metabolism to continue energy production aerobically.

In terms of NAD+ being transported extracellularly there is circumstantial evidence that suggests that low NAD+ concentrations can be imported across the membrane to directly replenish the cellular NAD+ pools by bypassing biosynthetic pathways. Extracellular NAD+ could counteract the effects of intracellular NAD+ depletion. Additionally it has been shown that not only could NAD+ be transported across into the cell externally but that it can move up its concentration gradient, meaning that even when NAD+ concentrations are higher intercellular than extracellularly NAD+ can still transport across [7]. The evidence suggests that if NAD+ concentrations can be increased around the body extracellularly via potential supplementation, intracellular NAD+ pools can be replenished from these extracellular sources to be utilised during aerobic respiration. In certain cell types the transport of NAD+ is significantly reduced in the absence of extracellular Na+ therefore highlighting Na+ possible role in the transportation of NAD+ across cell membranes [7]. Up until this point nicotinamide, nicotinic acid and tryptophan have been identified as precursors for NAD+ synthesis; recently nicotinamide riboside has also been recognised as a precursor which has a two and three step pathway to form NAD+ [10]. It has been described that all of these precursors possess distinct and tissue specific biosynthetic activities [10]. Humans exhibit the complexity in NAD+ metabolism in which particular cells may utilise a specific precursor to produce an excess of NAD+ and export salvageable precursors to other cells. Not every cell is capable of converting each NAD+ precursor to NAD+ at all times because of the tissue/cell specific enzyme expression differences that exist. This means that the NAD+ precursors are utilised differently. The implications of this tissue specificity mean that NAD+ synthesis differs between the different substrates at a variety of places in the body. Also with NAD+ in particular areas being exported this implies that NAD+ transfer around the body occurs when necessary, possibly to be transported to meet the areas with the highest demand. To find which substrate precursor muscular tissue cells utilise would be of most interest with regards to the exercise physiological standpoint.

The question of whether supplementation can bring about increased NAD+ concentrations has been subtly hinted at but as of yet largely unexplored. This point will now be investigated.

It is known that most water soluble vitamins and vitamin E are involved in mitochondrial energy metabolism. Water soluble vitamins are B vitamins (including riboflavin and niacin) and vitamin C which play an integral part in mitochondrial energy metabolism and so could be beneficial if supplemented [59].

The recommended daily allowance (RDA) for niacin/vitamin B_3 is 16mg and 14mg per day for adult males and females respectively, and good sources of niacin include meat, fish, peanuts and fortified cereals [10]. Both nicotinic acid and nicotinamide are absorbed in the alimentary canal and can enter the blood stream for distribution to tissues, providing evidence that dietary intake is efficient and a very plausible method to deliberately increase the NAD+ pool. Nicotinamide riboside is incorporated into the cellular NAD+ pool via conversion to nicotinamide. It has been found that today the American population will consume more than adequate amounts of niacin [52]. These are guidelines generated by a government influenced organisation and are accurate within the realms of the majority of the general population. However it is known that an athletic/professional sporting population display different dietary requirements and so it could be argued that this will also apply to niacin specifically as well. For example there is a report completed in 2001 that states 2g. d^{-1} of creatine for a man weighing 70kg will suffice [47]. There is no RDA for creatine as it can be synthesised in the body based on variable mechanisms to fully satisfy the needs of a healthy human. Yet several research investigations conducted on the effects of creatine on exercise performance and physiological development have looked at amounts up to 80g. d^{-1}. On www. bodybuilding. com 20g. d^{-1} is stated as the amount necessary during a 'loading phase' and 5g. d^{-1} for a 'maintenance phase'. So RDA is adequate for the general population but the sporting demographic can benefit from manipulated inflated intakes. There is evidence to support the advantageous effects of creatine supplementation despite

RDA values so therefore can supplementing niacin/vitamin B_3 bring about beneficial implications for athletes.

It has been discovered that in Fischer-344 rats, 2 weeks of dietary nicotinic acid and nicotinamide supplementation resulted in elevated levels of NAD+ in the body [32]. Nicotinic acid supplementation (500mg and 1000mg/kg) caused increases in levels of NAD+ in the blood, liver, heart and kidney, whilst nicotinamide caused increases in NAD+ levels in the liver and the blood only compared to a control group fed a diet containing 30mg/kg of nicotinic acid. Both nicotinic acid and nicotinamide at 1000mg/kg caused elevations in liver NAD+ by 44% and 43% respectively.

With normal dietary intakes most of the dietary nicotinic acid is converted to NAD+ in the intestine or liver and cleared by NAD+ glycohydrolase to release nicotinamide into the circulation for use by extrahepatic tissues [18,25], whereas under the same conditions most nicotinamide is absorbed intact and used for NAD+ synthesis in the liver or extrahepatic tissues. With this information one would expect high doses of nicotinamide to be more effective than nicotinic acid in raising NAD+ levels in most tissues due to the more direct utilisation. However with large doses the physiological effects differ. Subsequently, it is difficult to raise nicotinamide levels in the peripheral blood beyond a certain point. Conversely large doses of nicotinic acid will overwhelm the clearance mechanism of the liver and intestine becoming present in the blood stream. Once present in the peripheral blood flow, all tissues are capable of utilising nicotinic acid to synthesise NAD+ with some using nicotinic acid preferentially to nicotinamide e. g. the kidney.

Supplementing niacin does appear to be effective in increasing nicotinamide concentrations in the blood. An increased niacin status, as assessed from blood nicotinamide concentrations and lymphocyte NAD+ concentrations has been observed when supplementing 50mg/day and 100mg/day [24]. The effect was most pronounced in individuals with lower initial NAD+ levels. Results indicate that supplementation effectively increases blood nicotinamide concentrations, but whether this would have a follow up affect on NAD+ biosynthesis, NAD+ concentrations and aerobic metabolism is still unanswered.

A separate study discovered that dietary niacin had no effect upon synthesis of liver pyridine nucleotides, even when fed at very high levels [62]. However, tryptophan added to non protein ration increased the pyridine nucleotide concentration significantly with the further addition of niacin having no effect. The study was conducted on rats and the results are of interest. This study suggests that tryptophan has a greater role in liver synthesis of NAD+ whilst niacin supplementation has a greater role in increasing blood concentrations of nicotinamide, NAD+ synthesis and concentration to the extrahepatic tissues [10,62]. This is supported and reflected when rats depleted of liver NAD+ were able to utilise dietary tryptophan to a greater extent than niacin. In the experiment niacin appeared to spare liver pyridine nucleotides in adult rats to some extent. If niacin does spare liver NAD+ then this would be of additional benefit because if NAD+ synthesis is increased in extrahepatic tissues the storage around the body is increased thus liver NAD+ becomes a reserve to be drawn on after the immediate tissue storage is depleted. In younger rats the results with niacin appeared conflicting and inconclusive but tryptophan brought about the same reaction as with the adult rats.

NAD+ uptake shows at least two phases with an initial rapid phase of transport followed by a prolonged phase of steady uptake of up to an hour. This point highlights the possible implications of how exercise/performance will be affected over time after ingestion. The notion that NAD+ is metabolised before transport can be excluded because there is evidence that shows the radioactivity remaining in the cellular medium assessed by high performance liquid chromatography (HPLC). The fate of transported NAD+ was determined by extracting nucleotides and analysing them by HPLC. The experiment found that 65. 4 ± 3% of the transported NAD+ ended up as NADH with most of the remaining being converted to NADP [7]. So approximately 2/3 of extracellular NAD+ ended up as NADH, suggesting it was used in either glycolysis, the conversion of pyruvate to CoA, Krebs cycle or even the shuttle mechanism. This evidence means that extracellular NAD+ can be used in metabolism therefore strengthening the original claim made that supplementing NAD+ could prolong exercise at an aerobic level subsequently improving performance.

When injected into the portal vein in small doses nicotinic acid appears to act very quickly (within 1-10mins) in the liver. When a large dose of nicotinamide is injected the effect on liver NAD+ occurs over a prolonged period of time as it appears to be fairly rapidly excreted from the liver and later reabsorbed by the liver and synthesised into NAD+. Experiments suggest that at a pH above 7. 4 nicotinamide is more permeable than nicotinic acid [31]. If taken orally through dietary supplementation would small doses of nicotinic acid be synthesised into NAD+ in the liver at the same rate as when injected? The time scale and pH aspect with regards to nicotinamide could impose important effects when considering supplementation for a sporting event. Large doses of nicotinamide produced more NAD+ in the liver but over a longer period of time, is this because when excreted by the liver it is being utilised by other tissues in the body? Also if formulating a nicotinamide supplement the pH would need to be taken into consideration due to the permeability increasing when above 7. 4. Overall the assumptions made from the study alluded to are inconclusive due to the injection methodology, but still cannot be completely disregarded.

A more recent study found that nicotinamide is rapidly ingested and circulated into the blood and is cleared to all tissues. The examined ranges were 25-50mg/kg^{-1}/day. These high doses administered orally or through injection are transiently metabolised in the liver to increase NAD+. Nicotinic acid also increases NAD+ content in the liver but is generally no more effective than nicotinamide [56]. 2 week treatment of rats with high doses of nicotinamide and nicotinic acid brought about responses in the blood and the liver with increases of NAD+ concentrations by 40-60%. The ability of nicotinamide to stimulate NAD+ synthesis in both the liver and the blood suggests that nicotinamide is converted to alternative forms of vitamin B_3; this increases nicotinamide bioavailability and potentially causes cellular adaptation leading to improved NAD+ biosynthesis.

Clearance rates of nicotinamide would have implications when considering length of time before competition to take a niacin supplement. The rates of clearance for nicotinamide have been found to be dose dependent: with a half life of 7-9hours for doses of 4-6g administered, ~4hours with 2g and ~1. 5hours with 1g dose. The time to reach peak plasma concentration ranged from 0. 7-3hours [58]. In this study nicotinamide had no detectable effect on blood pressure, pulse or body temperature.

Comparable studies have arrived at similar results. The effects of considerably higher doses than the RDA of 14-16mg have found that up to 80mg/kg/day of oral nicotinamide is feasible and clinically tolerated, giving no or few side effects. The time taken to reach peak concentration ranged from between 0. 8-4hours, producing similar results as highlighted previously [27]. However it was stated in one study that there may be side effects associated with high doses of nicotinamide but nicotinamide riboside does not cause flushing [10].

The risks and negative side effects to health posed by supplementing nicotinamide do not appear to be great but doses in excess of 3g/day (188x the RDA) should still be treated with care [36,48].

The protein and other biosynthetic uses of tryptophan mean that 1mg of tryptophan doesn't equate to 1mg of niacin. 60mg in fact is considered the equivalent of 1mg of niacin [10,56]. Because of this it may not be as efficient or indeed practical to use tryptophan as a supplement to attempt to increase NAD+ synthesis due to the large quantities that would be required compared to niacin.

The topic of vitamin supplementation and supplementation in general is one shrouded in inconclusive evidence and doubt. Scientific evidence for the ergogenic benefit of vitamin supplementation in athletes with an adequate vitamin status and a well balanced diet is lacking, and there is no real indications that long term vitamin intake among athletes in insufficient [59]. Theoretically an increased requirement can be caused by the certain demands as well as biochemical adaptations to training. Still the argument is raised in relation to creatine in that intake is not insufficient according to RDA's in athletes yet supplementation is still beneficial. A lot of doubt surrounds supplementation with the area short in evidence or studies. Studies on vitamin intake in general tend to show supplementation as ineffective, however studies on specific vitamins have been more successful for example the preliminary findings for vitamin C (ascorbic acid) appear to show positive effects on performance by reducing skeletal muscle damage and enhancing some aspects of immune function (Current statement gathered from the American College of Sports Medicine website). A comment on the US National Library of Medicine website (www. nlm. nih. gov) stated that when looking at using niacin (vitamin B_3) as a supplement it would seem plausible because it can be dissolved in water and it is absorbed well when taken orally. Nicotinic acid, nicotinamide and nicotinamide riboside are natural products which are incorporated into the intracellular NAD+ pool and thus could be used as a possible supplement [10].

Supplementation of niacin would appear to be a viable plausible option with no side effects but specific studies on the exact effects of niacin supplementation on NAD+ concentration and possible benefits in a sporting environment are currently not available and therefore unknown. But cellular NAD+ concentrations are linked to an organisms nutritional and physiological states suggesting that NAD+ concentrations can be manipulated with training and dietary intake [23].

The overall question proposed is can supplementation of nicotinic acid, nicotinamide or nicotinamide riboside increase the NAD+ pool beneficially for use in energy metabolism, and possibly reduce lactate production? If the body can continue aerobic metabolism it does not need to rely on the inefficient anaerobic process which can rapidly lead to fatigue due to the accumulation of protons. NAD+ accepts protons and so could reduce the accumulation and potentially increase work capacity in this manner [45]. If the NAD+/NADH ratio fell the effects would eventually result in acetyl CoA being directed away from the Krebs cycle to ketone body formation [5,63]. NAD+ and the NAD+/NADH ratio need to be kept at the right level or the Krebs cycle will cease and so will aerobic respiration, but could increasing NAD+ concentration through supplementation continue aerobic energy production? It is unknown if NAD+ concentration pools within the body are higher in athletic populations. There do appear to be situations in which cells actively increase and/or reduce the concentration of NAD+ and NAD+ metabolites to promote vital and regulatory functions including cell death [10,12]. Whether exercise or prolonged training is a situation in which NAD+ synthesis and concentration can be increased is as of yet not studied in any detail. Alternative methods have been studied for raising NAD+ concentrations for example increasing Nampt, the main rate limiting enzyme in NAD+ biosynthesis, increased total cellular NAD+ levels in mouse fibroblasts [51,65]. The gene manipulation method is not a viable or ethical option but nonetheless interesting. A separate study did also find that the levels of Nampt are correlated to NAD+ concentrations in cells and by increasing NAD+ levels the expression of the primary regulatory enzyme Nampt is increased to compensate [56]. There is the suggestion that the mitochondria maintain relatively high concentrations of NAD+ and the total distribution of NAD+ in cells is predominately mitochondrial, this premise is derived mostly from data obtained in myocytes. From this it is also apparent that relative NAD+ content in cells are cell and tissue specific [56]. Under stable isolated conditions studies have demonstrated that NAD+ concentration in mitochondria remain relatively constant. NAD+ concentration in isolated mitochondria does however become rapidly depleted upon the presence of Ca^{2+} [19]. Therefore under condition of stress (ie exercise) NAD+ pools may be depleted quickly with the addition of Ca^{2+} which is vital for muscular contraction. It would be of interest to test NAD+ levels before and after exercise under whole body conditions as appose to isolated cellular experiments. It has been hypothesised and proved that calorie restriction can stimulate sirtuin activity and extend the lifespan of organisms by increasing the levels of intracellular NAD+, a theory known as the NAD+ fluctuation model. Intracellular levels of NAD+ also increased in proportion to glucose restriction [65]. The fact that calorie restriction increases intracellular NAD+ levels is interesting but with regards to a sporting context if this technique was applied to an athlete it would be

counterintuitive due to factors related to total energy restriction.

There have been studies conducted that have revealed the potential clinical implications and various other effects of NAD+. One such effect has already been referred to with the addition of niacin in the diets of pellagra sufferers curing the individual [22,64]. Recently a broader range of biological functions of NAD+ have been identified including: poly (ADP-ribosyl) action in DNA repair, mono-ADP-ribosylation in both the immune response and to protein coupling signalling, the synthesis of cyclic ADP-ribose and nicotinate adenine dinucleotide phosphate in intracellular calcium signalling, and an important role in transcriptional regulation. It has also been reported that high doses of nicotinamide protect and improve β cell functions (pancreatic cells involved in insulin responses) in patients with onset type I diabetes [52]. The maintenance of NAD+ levels is also functional to the synthesis of several signal molecules known to be Ca^{2+} mobilising agents from intracellular stores [41]. By aiding the manipulation of Ca^{2+}, NAD+ could indirectly have implications with Ca^{2+} utilisation during muscular contraction. Findings suggest that nicotinamide supplementation can produce metabolic improvements in type I diabetes, also having some effect on reducing free radical formation. These metabolic influences that nicotinamide has are related to its pharmacological properties which bring about effects most likely via improvement in hepatic cell function and consequently normalisation of protein and amino acid metabolism. Further studies on diabetic rats have provided more evidence of the beneficial effects that NAD+ could have. It has been found that NAD+ and ATP content are significantly reduced in the diabetic brain [37]. Nicotinamide is a safe naturally occurring substance which appears to be non-toxic to humans, also it has been shown to be neuroprotective, anti inflammatory and an immunomodulator [10,48,56]. This evidence suggests that nicotinamide is not harmful and can be taken safely with possible clinical benefits of supplementation. Nicotinic acid administered in large doses was found to lower serum lipid and cholesterol and reduce the progression of coronary heart disease. Again additional evidence has found that NAD+ indirectly plays a role in mechanisms related to channel opening and calcium release which is key for muscular contraction [56]. One very interesting clinical implication of NAD+ and increasing concentrations in the body is the effect this has on lifespan and longevity. NAD+ biosynthesis is linked to the Sir2 protein which is related to the regulation of ageing and longevity. It has been demonstrated that by increasing NAD+ biosynthesis and therefore Sir2 concentration and activity the lifespan in yeast, worms and flies is extended [52] (Figure 7). NAD+ concentration levels seem to be a function directly proportional to life span with increasing levels of NAD+ extending the lifespan of human fibrolised cells [41,65]. In some instances experiments with nicotinic acid and nicotinamide have been found to rescue cells from cell death [7].

If conducting research in an attempt to build an understanding and a potential answer to the original question posed several techniques and factors would need to be considered. Intracellular levels of NAD+ can be determined using an acid extraction method followed by enzymatic geling technique [66]. By using radioactive labelled NAD+ and HPLC the fate of NAD+ can be determined with regards to how it is metabolised [7]. If this process could be carried out with ingested nicotinic acid, nicotinamide or nicotinamide riboside how the supplement is utilised could be established. The ratio of free NADH/NAD+ can be estimated using the following equation: [pyruvate][NADH][(H^+/lactate)][NAD+]=K [60]. If supplementation was effective at increasing NAD+ concentrations and helped the continuation of aerobic metabolism there would be a reduction in lactate production. The best method for calculating lactate production as accurately as possible is to analyse the muscles that are active in the exercise. Furthermore if the size of the active muscle mass is known and the amount of lactate which has escaped the muscle during the exercise can be determined, total net lactate produced can be estimated [2,42]. The approach of the experiment would be to recreate exercise which would be similar to a 1500m athletic race. The first test runs would be the control for comparison. The next trial runs would be with the supplement under the same conditions in an attempt to eliminate any other factors influencing performance. If the results increased NAD+, reduced lactate, increased NAD+ utilisation for aerobic metabolism continuation and ultimately performance this would be considered successful and further research would be necessary in the future.

3. Conclusion

In summary, currently at present there is not an incredible amount of research in the area of NAD+ on its own but even less or next to nothing with regard to its potential to provide a beneficial effect in the sporting arena. It would appear plausible with the evidence provided that NAD+ could insert an influence on sporting performance but due to lack of direct specific research it is unclear. Although NAD+ and its precursors were the predominant focus of this article it is worth mentioning that FAD and its respective precursors (riboflavin being one) could also bring about the same desired effect, but FAD does play less of a role in metabolism than NAD. Specific tailored research is required in this area before any conclusive conclusions can be drawn.

References

[1] Alp. P. R, Newsholme. E. A and Zammit. V. A (1976). Activities of citrate synthase and NAD+ linked and NADP+ linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates.

[2] Bangsbo. J, Gollnick. P. D, Graham. T. E, Juel. C, Kiens. B, Mizuno. M and Saltin. B (1990). Anaerobic energy production and O_2 deficit-debt relationship during exhaustive exercise in humans.

[3] Barron. J. T, Gu. L and Parrillo. J. E (1998). Malate-aspartate shuttle, cytoplasmic NADH redox potential, and energetics in vascular smooth muscle.

[4] Barron. J. T, Gu. L and Parrillo. J. E (2000). NADH/NAD redox state of cytoplasmic glycolytic compartments in vascular smooth muscle.

[5] Barron. J. T, Gu. L and Parrillo. J. E (1999). Relation of NADH/NAD to contraction in vascular smooth muscle.

[6] Bender. D. A, Magboul. B. I and Wynick. D (1982). Probable mechanisms of regulation of the utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of nicotinamide nucleotides in the rat.

[7] Billington. R. A, Travelli. C, Ercolano. E, Galli. U, Roman. C. B, Grolla. A. A, Canonico. P. L, Condorelli. F and Genazzani. A. A (2008). Characterization of NAD uptake in mammalian cells.

[8] Blomstrand. E, Ekblom. B and Newsholme. E. A (1986). Maximum activities of key glycolytic and oxidative enzymes in human muscle from differently trained individuals.

[9] Blomstrand. E, Rådegran. G and Saltin. B (1997). Maximum rate of oxygen uptake by human skeletal muscle in relation to maximal activities of enzymes in the Krebs cycle.

[10] Bogan. K. L and Brenner. C (2008). Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition.

[11] Boutellier. U, Büchel. R, Kundert. A and Spengler. C (1992). The respiratory system as an exercise limiting factor in normal trained subjects.

[12] Bowtell. J. L, Marwood. S, Bruce. M, Constantin-Teodosiu. D and Greenhaff. P. L (2007). Tricarboxylic acid cycle intermediate pool size: functional importance for oxidative metabolism in exercising human skeletal muscle.

[13] Brown. A. T and Wagner. C (1970). Regulation of enzymes involved in the conversion of tryptophan to nicotinamide adenine dinucleotide in a colorless strain of Xanthomonas pruni.

[14] Campell. M. K and Farrell. S. O (2003) Biochemistry fourth edition.

[15] Cheeseman. A. J and Clark. J. B (1988). Influence of the malate-aspartate shuttle on oxidative metabolism in synaptosomes.

[16] Cori. C. F, Fisher. R. E and Cori. G. T (1935). The effect of epinephrine on arterial and venous plasma sugar and blood flow in dogs and cats.

[17] Crabtree. B and Newsholme. E. A (1972). The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol-3-phosphate dehydrogenase in muscles from vertebrates and invertebrates.

[18] Dietrich. L. S (1971). Regulation of nicotinamide metabolism.

[19] Di Lisa. F and Ziegler. M (2001). Pathophysiological relevence of mitochondria in NAD(+) metabolism.

[20] Farrell. P, Wilmore. J. H, Coyle. E. F, Billing. J. E and Costill. D. L (1979). Plasma lactate accumulation and distance running performance.

[21] Foster. J. W and Moat. A. G (1980). Nicotinamide adenine dinucleotide biosynthesis and pyridine nucleotide cycle metabolism in microbial systems.

[22] Garrett. R. H and Grisham. C. M (1999). Biochemistry second edition

[23] Guarente. L (2006). Sirtuins as potential targets for metabolic syndrome.

[24] Hageman. G. J, Stierum. R. H, van Herwijnen. M. H, van der Veer. M. S and Kleinjans. J. C (1998). Nicotinic acid supplementation: effects on niacin status, cytogenetic damage, and poly(ADP-ribosylation) in lymphocytes of smokers.

[25] Henderson. L. M (1983). Niacin.

[26] Holloszy. J. O and Coyle. E. F (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences.

[27] Hoskin. P. J, Stratford. M. R, Saunders. M. I, Hall. D. W, Dennis. M. F and Rojas. A (1995). Administration of nicotinamide during chart: pharmacokinetics, dose escalation, and clinical toxicity.

[28] Howley. E. T, Bassett. D. R and Welch. H. G (1995). Criteria for maximal oxygen uptake: review and commentary.

[29] Huckerbee. W. E (1958). Relationships of pyruvate and lactate during anaerobic metabolism. I: effects of infusion of pyruvate or glucose and of hyperventilation.

[30] Huckerbee. W. E (1958). Relationships of pyruvate and lactate during anaerobic metabolism. II: exercise and formation of O_2 debt.

[31] Ijichi. H, Ichiyama. A and Hayaishi. O (1966). Studies on the biosynthesis of nicotinamide adenine dinucleotide III comparative in vivo studies on nicotinic acid, nicotinamide, and quinolinic acid as precursors of nicotinamide adenine dinucleotide.

[32] Jackson. T. M, Rawling. J. M, Roebuck. B. D and Kirkland. J. B (1995). Large supplements of nicotinic acid and nicotinamide increase tissue NAD+ and poly(ADP-ribose) levels but do not affect diethylnitrosamine induced altered hepatic foci in Fischer-344 rats.

[33] Joyner. M. J (1991). Modelling: optimal marathon performance on the basis of physiological factors.

[34] Joyner. M. J and Coyle. E. F (2008). Endurance exercise performance: the physiology of champions.

[35] Jürgens. K. D, Papadopoulos. S, Peters. T and Gros. G (2000). Myoglobin: just an oxygen store or also an oxygen transporter?

[36] Knip. M, Douek. I. F, Moore. W. P, Gillmor. H. A, McLean. A. E, Bingley. P. J and Gale. E. A (2000). Safety of high-dose nicotinamide: a review.

[37] Kuchmerovska. T, Shymanskyy. I, Bondarenko. L and Klimenko. A (2008). Effects of nicotinamide supplementation on liver and serum contents of amino acids in diabetic rats.

[38] Lester. G (1971). End-product regulation of the tryptophan-nicotinic acid pathway in Neurospora crassa.

[39] Lundquist. R and Olivera. B. M (1973). Pyridine nucleotide metabolism in Escherichia col. i II niacin starvation

[40] Ma. W, Sung. H. J, Park. J. Y, Matoba. S and Hwang. P. M (2007). A pivotal role for p53: balancing aerobic respiration and glycoysis.

[41] Magni. G, Amici. A, Emanuelli. M, Orsomando. G, Raffaelli. N and Ruggieri. S (2004). Enzymology of NAD+ homeostasis in man.

[42] Margaria. R, Edwards. H. T and Dill. D. B (1933). The possible mechanisms of contracting and paying the oxygen debt and the role lactic acid in muscular contraction.

[43] Masuda. K, Yamada. T, Ishizawa. R and Takakura. H (2013). Role of myoglobin in regulating respiration during muscle contraction.

[44] Millar. H, Considine. M. J, Day. A. A and Whelan. J (2001). Unravelling the role of mitochondria during oxidative stress in plants.

[45] Newsholme. E. A and Leech. A. R (1983) Biochemistry for the medical sciences.

[46] Nishizuka. Y and Hayaishi. O (1963). Studies on the biosynthesis of nicotinamide adenine dinucleotide: I enzymatic sythesis of niacin ribonucloetides from 3-hydroxyanthranilic acid in mammalian tissues.

[47] Pérès. G (2001). An assessment of the risks of creatine on the consumer and of the veracity of the claims relating to sports performance and the increase of muscle mass.

[48] Pociot. F, Reimers. J. I and Anderson. H. U (1993). Nicotinamide-biological actions and therapeutic potential in diabetes prevention.

[49] Power. K. S and Howley. E. T (2009). Exercise physiology: theory and application to fitness and performance.

[50] Reichmann. H, Hoppeler. H, Mathieu-Costello. O, von Bergen. F and Pette. D (1985). Biochemical and ultrastructural changes of skeletal muscle mitochondria after chronic electrical stimulation in rabbits.

[51] Revollo. J. R, Grimm. A. A and Imai. S (2004). The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells.

[52] Revollo. J. R, Grimm. A. A and Imai. S (2007). The regulation of nicotinamide adenine dinucleotide biosynthesis by Nampt/PBEF/visfatin in mammals.

[53] Robinson. D. M, Ogilvie. R. W, Tullson. P. C and Terjung. R. L (1994). Increased peak oxygen consumption of trained muscle requires increased electron flux capacity.

[54] Rongvaux. A, Andris. F, Van Gool. F and Leo. O (2003). Reconstructing eukaryotic NAD metabolism.

[55] Rowell. L. B (1974). Human cardiovascular adjustments to exercise and thermal stress.

[56] Sauve. A. A (2008). NAD+ and vitamin B3: from metabolism to therapies.

[57] Shen. W, Wei. Y, Dauk. M, Tan. Y, Taylor. D. C, Selvaraj. G and Zou. J (2006). Involvement of a glycerol-3-phosphate dehydrogenase in modulating the NADH/NAD+ ratio provides evidence of a mitochondrial glycerol-3-phosphate shuttle in Arabidopsis.

[58] Stratford. M. R, Rojas. A, Hall. D. W, Dennis. M. F, Dische. S, Joiner. M. C and Hodgkiss. R. J (1992). Pharmacokinetics of nicotinamide and its effect on blood pressure, pulse and body temperature in normal human volunteers.

[59] van der Beek. E. J (1991). Vitamin supplementation and physical exercise performance.

[60] van Dogen. J. T, Schurr. U, Pfister. M and Geigenbeger. P (2003). Phloem metabolism and function have to cope with low internal oxygen.

[61] Vaughan. H and Newsholme. E. A (1969). The effects of Ca^{2+} and ADP on the activity of NAD linked isocitrate dehydrogenase of muscle.

[62] Williams. J. N, Feigelson. P, Shahinian. S. S and Elvehjem. C. A (1951). Further studies on tryptophan-niacin-pyridine nucleotide relationships.

[63] Williamson. D. H, Lund. P and Krebs. H. A (1967). The redox state of free nicotinamide adenine dinucleotide in the cytoplasm and mitochondria of rat liver.

[64] Wittmann. W, Du Plessis. J. P, Nel. A and Fellingham. S. A (1971). The clinical and biochemical effects of riboflavin and nicotinamide supplementation upon Bantu school children.

[65] Yang. N. C, Song. T. Y, Chang. Y. Z, Chen. M. Y and Hu. M. L (2015). Up-regulation of nicotinamide phosphoribosyltransferase and increase of NAD+ levels by glucose restriction extend replicative lifespan of human fibroblast Hs68 cells.

[66] Yang. N. C, Song. T. Y, Chen. M. Y and Hu. M. L (2011). Effects of 2-deoxyglucose and dehydroepiandrosterone on intracellular NAD(+) level, SIRT1 activity and replicative lifespan of human Hs68 cells.

[67] Yudkoff. M, Nelson. D, Daikhin. Y and Erecinska. M (1994). Tricarboxylic acid cycle in rat brain synaptosomes: fluxes and interactions with aspartate aminotransferase and malate/aspartate shuttle.

[68] Zubay. G. L (1996). Biochemistry fourth edition.