The Effect of Nicotinamide Riboside and Pterostilbene on exercise performance at a simulated altitude of 2500m

By

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A thesis submitted to The University of Birmingham for the degree of MASTERS BY RESEARCH

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University of Birmingham
2019

Abstract

Exercise performance at altitude is impaired via compromised oxygen delivery to the working muscle. Nutritional supplements that improve metabolic pathways and/or vascular function may help diminish such altitude-induced performance impairment. The aim of this study was to examine the combined effects of Nicotinamide Riboside (NR) and Pterostilbene (PT) on exercise performance at altitude. Utilising a between-group design, 20 participants (11 male, 9 female; aged 21±1.7) were randomly assigned, in a double-blind manner, to either a NRPT or placebo group after completing familiarisation and baseline testing. Testing consisted of a 30-minute steady state cycle (65% and 55% Watt Max in normoxia and hypoxia respectively) followed by a 5-km time trial (TT) in both normoxic (21% FiO_2) and hypoxic (15% FiO_2) environmental conditions (separated by 48 hours) before and following a 4-week supplementation period (NRPT: 500mg NR, 100mg PT; Matched placebo). Outcome measures included TT performance, changes in fat oxidation (indexed via RER) and forearm blood flow (rest and post-exercise). The data successfully shows that exercising at a simulated altitude of 2500m resulted in an impaired Time Trial performance with a 4.8%±0.3 increase in Time Trial time (environment main effect p=0.004). However, the data indicated that 5km-TT time and power improved similarly, although not significantly, over the 4-weeks between NRPT and placebo group (Time; 1.2%±0.07 and 0.2%±0.001 in NRPT normoxia and hypoxia respectively and 2.1%±0.1 and 1.9%±0.1 in placebo normoxia and hypoxia respectively; Power; 2.3%±3.3 and 1.4%±1.8 in NRPT normoxia and hypoxia respectively and 4.0%±5.7 and 6.5%±9.0 in placebo normoxia and hypoxia respectively ($p>0.05$)). In the steady state exercise there was no significant difference in the NRPT group between normoxia and hypoxia ($p>0.05$) in RER, however, the placebo group displayed a lower RER in hypoxia in comparison to normoxia. (0.93±.01 and 0.97±0.01, respectively; $p<0.05$). Thus, NRPT did not elicit any beneficial metabolic effects or performance at altitude. In addition,

an overall significant decrease ($p=0.035$) in resting forearm blood flow over the 4-weeks was similar between NRPT and placebo (10.6%±0.15% and 17.7%±0.3% respectively). Both groups showed a significant difference between baseline and post exercise blood flow measures ($p=0.026$). To conclude NRPT did not result in any greater improvements to exercise performance at altitude compared with placebo or sea level. Future studies should investigate the effects of longer submaximal exercise to promote a greater shift in lipid oxidation. In addition, it would be worthwhile to further explore both metabolic and mechanistic implications through blood and muscle samples.

Acknowledgments

I would like to thank my thesis Supervisor Dr Sam Lucas of the University of Birmingham School of Sport, Exercise and Rehabilitation Sciences, for reassuring and building my confidence in lab and testing environments. It has been constant enjoyment.

Additionally, I would like to thank Sam Weaver for his help and guidance throughout the study. The advice has been invaluable and has led to me progressing my academic journey at the University of Exeter at Doctoral level.

Thank you to the Lucas group for welcoming me into the group and always offering their time and knowledge to help me during my MSc. The breadth of knowledge from those I have been involved with over the past year has been educational and more than helpful. Thanks to the four undergraduate students that assisted me greatly during the study, without their help the study would not have been possible to run, I wish them all the success in their future endeavours.

Finally, I would like to thank all my family and friends for their constant support during my Undergraduate and Postgraduate journey.

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List of Abbreviations

NAD+	Nicotinamide adenosine dinucleotide
NRPT	Nicotinamide Riboside + Pterostilbene
ATP	Adenosine Triphosphate
ETC	Electron Transport Chain
TCA	Tricyclic Acid
NADH	Nicotinamide adenonine Dinucleotide Hydrogen
ADP	Adenosine Diphosphate
DNA	Deoxyribnucleic Acid
PARP	Poly ADP-Ribose Polymerase
SIRT	Sirtuin
PPARa	Peroxisome Proliferator Activated Receptor alpha
CHO	Carbohydrate
PGC-1	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
eNOS	endothelial Nitric Oxide Synthase
NA	Nicotinic Acid
NR	Nicotinamide Riboside
NMN	Nicotinamide Mononucleotide
NMNAT	Nicotinamide mononucleotide adenylyltransferase
NAM	Nicotinamide
$\dot{V}O_2$	Maximal Oxygen Uptake
PT	Pterostilbene
AMPK	AMP activated protein kinase
HIIT	High intensity interval training
SOD	Superoxide Dismutase

O2	Oxygen
FFA	Free Fatty Acid
KJ	Kilojoules
KG	Kilogram
G	Grams
IMTG	Intramuscular Triglycerides
RER	Respiratory Exchange Ratio
SS	Steady State
TT	Time Trial
Wmax	Watt Max
AUC	Area under curve

Introduction

Exercise at Altitude

Since the 1968 Mexico Olympics and the increase in accessibility to visit altitude there has been a rise in the number of sporting events held at altitude, therefore, the need to reduce the impairment caused by altitude is crucial to retain the high-class level of elite sport. Evidence has shown that athletes selected a power output 5-6% lower than normal in both sustained exercise and repeated bout efforts when breathing gases simulating an altitude of ~2100m (Brosnan et al, 2000). Thus, acute exposure to altitude can have a detrimental impact on exercise capacity, which is mediated through a reduction in O_2 availability and thus O_2 consumption resulting in compromised O_2 delivery to the working muscles and an ultimately compromised performance capacity (Amann & Cabet, 2008). Peltonen & colleagues (2001) reported that at ~2500m $\dot{V}O_2$ max was reduced nearly twice as much in a rowing ergometer test. This decrement in performance has been further supported with decreases in power, a prominent performance measure. Clark & colleagues (2007) tested a cohort of 10 well trained cyclists and found that 5-minute Time Trial (TT) power decreased with increasing altitude, 5.8±2.9%, 10.3±4.3% and 19.8±3.5% at 1200m, 2200m and 3200m respectively. Clark et al 2007 also found that mean power in 5-min TT at 2200m was 329± 38W a reduction of 38W from an elevation of 200m with an overall 7% decrease per 1000m of altitude, highlighting the negative relationship between performance and increasing altitude.

There is undoubtedly vast evidence confirming the impairment in exercise performance seen at altitude and with an increasing number of sporting competitions held at altitude and the clear altitude-induced decline in performance the need for an intervention is heightened. The mechanistic underpinning of the altitude induced compromise in performance is understood to be due to the lower oxygen concentration at altitude compared to sea level. This lower O_2 content causes varied vascular changes within the body which alter the

capacity of exercise. These changes include; accumulation of NADH, vasoconstriction of vessels, decreased O_2 uptake and decreased O_2 saturation, however, the main determinant for reduced performance at altitude has been defined as the exercise-induced decrease in arterial oxygenation (Faoro et al, 2017). Alongside this, the nature of a hypoxic environment, lower oxygen availability, causes greater strain on vasculature due to the lower skeletal muscle oxygen availability for a given volume of blood, suggesting that the need for an enhanced oxygen carrying capacity is key to performance success at altitude (Balsom et al, 1994).

Altitude and Aging

The physiological responses seen at altitude are closely mirrored in the aged population, such that, for example, mitochondrial function is impaired, which is also seen in the sedentary and unhealthy population (Finck, 2006 & Handschin, 2008). Likewise, when a group of healthy adult men were assessed, it was found that resting whole-leg arterial blood flow and vascular conductance reduces with age, this is independent of the reduction in muscle mass associated with the ageing process. Further to this, there is a reduction in oxygen demand, specifically in the lower limbs, which is thought to be due to a rise in sympathetic vasoconstrictor nerve activity (Dinenno et al,1999) thus, O_2 uptake is lower. Similarly, and also in line with that seen at altitude, ageing is associated with a decline in vascular function, that being, impaired endothelial function/vessel elasticity and increased blood pressure (Seals et al, 2011). Evidence would therefore suggest, that exercise at sea level in an aged population may elicit similar physiological responses to a young healthy population exercising at altitude. Suggesting that altitude is an acceptable model to elicit these desired responses and also presents an opportunity for nutraceuticals to bridge the gap between exercise at sea level and at altitude.

Nutraceuticals ability to bridge the gap between sea level and altitude

The growing demand to maximise physical exercise capacity and subsequently close the disparity in performance between sea level and altitude, by prolonging and/or enhancing these physiological boundaries, has opened an opportunity for the development of ergogenic aids to further push an athlete's natural capacity and/or limit external influences that negatively impact performance. The advancement in knowledge of sports performance and human physiology has also resulted in a correlated advancement in the nutraceutical industry and the development of novel supplement combinations. Natural food derived nutraceuticals have been shown to promote health and well-being and treat ailments and diseases, most commonly cardiovascular risk factors (Moss & Ramji, 2016), thus, hold a valued position in sport especially in comparison to the use of their pharmacological analogs since they have fewer toxic and adverse side effects (Vendrame et al, 2016), giving nutraceuticals the ethical approval required within sport. It is thought that between 40-93% of elite level athletes use dietary ergogenic aids to supplement their performance (Erdman et al, 2007; Sajber et al, 2013; Knapik et al, 2016) highlighting the wide use of nutraceuticals throughout todays sporting world, and consequently, the need for nutritional supplementation to be formally tested and supported is imperative to their growth and success. The nutraceutical industry is vast and nutraceuticals alike have a broad range of ergogenic potential to help maximise an athlete's exercise capacity, whether that be prior to, during exercise or in the recovery phase. Nutraceuticals have influences on physiological processes and pathways in the body from promoting mitochondrial biogenesis (Ostojic 2016), substrate flexibility (Stephens et al, 2013) and control of oxidative stress (Simoni et al, 2018) to stimulation of skeletal muscle protein synthesis and re-synthesis (Tipton et al 2007), thus, certain nutraceuticals with specific actions may play an important role in improving exercise performance at altitude, yet they cannot bridge the gap of inadequate training and are therefore only used to supplement a

quality training and nutrition programme. Two supplements namely, the Vitamin B3, Nicotinamide Riboside (NR) and the Polyphenol, Pterostilbene (PT) have been suggested to have an anti-ageing influence on physiology in the elderly. Therefore, considering the evidence presented linking altitude and aging, it is being put forward that these supplements, when administered as a combination may reduce the decline in performance seen at altitude via a metabolic and vascular influence.

Substrate utilisation during exercise

Exercise performance is greatly determined by substrate availability and flexibility. The capacity to utilise and mobilise muscle glycogen in both moderate and high intensities, to cope with the demands of muscular contractions, has been highlighted as a key factor in prolonging aerobic exercise (Ivy et al 1991; Balsom et al 1999). Consequently, in the absence of sufficient glycogen stores physical exertion is greatly compromised. (Ivy et al 1991; Balsom et al 1999). This provoked an increase in interest and use of nutritional interventions including food, drinks and supplements to optimise substrate utilisation for exercise performance. Traditionally, the fundamental aim of nutritional supplementation was to increase time to fatigue and therefore focused predominantly on endurance exercise (Maughan, 2002). However, with a greater understanding of the pharmacokinetics of compounds and the cellular and molecular adaptations of skeletal muscle to exercise, the ability to manipulate training and exercise performance in a variety of conditions and intensities has seen the development of nutritional ergogenic aids. The primary aim to spare glycogen has been broadly seen by increasing fat oxidation through acute and chronic dietary manipulation and via natural supplementation including, green tea extract (Hodgson et al, 2013) and caffeine (Maughan, 1999; Kurobe et al, 2015). Firstly, in the absence of any exogenous supplementation, it has been shown that endurance training promotes a whole-

body shift in substrate oxidation towards fatty acid oxidation (Hawley et al, 1998) alongside a reduction in glycolysis (Green et al, 1995). This ultimately leads to a sparing of muscle glycogen at a given workload (Green et a,1995). Holloszy et al (1967) supported this idea suggesting that any intervention that promotes the reduction in glycogen breakdown thus, glycogen sparing, with the addition of increased lipid oxidation will promote an optimised fuel utilisation for endurance exercise. Odland et al (1998) supported this idea of increasing lipid oxidation to ultimately spare glycogen. They infused an intralipid and heparin during exercise as an attempt to increase plasma Free Fatty Acids (FFA). They successfully showed that this increase in FFA concentration enhanced lipid oxidation and subsequently reduced CHO oxidation. A study by Burke et al (2000) explored the effects of a high CHO diet against an isoenergetic high fat diet. They found that metabolic changes including greater lipid oxidation (94 ± 6 g vs. 61 ± 5 g) and a reduction in CHO oxidation (271 ± 16 g vs. 342 ± 14 g) were observed even with acute high fat intake and were preserved after CHO restoration. However, this alteration in substrate utilisation did not result in any beneficial performance implications in 7kj/kg body mass time trial performance following a 2-hr cycle at 70% $\dot{V}O_2$ max. In contrast, Achten et al (2003) showed that ingestion of CHO in the hour leading up to exercise and therefore the potential restoration of CHO availability resulted in a reduction of the intensity and rate of maximal lipid oxidation. Although lipid oxidation may have a considerable impact on exercise performance there are large inter-individual differences and a number of factors that influence the ability to utilise fats. Achten & Jeukendrup (2003) published data showing a 4-fold increase in the ability to utilise fatty acids in some individuals showing a 0.91g/min in comparison to others at a remarkably lower rate of 0.23g/min. This individuality has been further reported with peak fat oxidation rates at 0.60 ± 0.07 g/min seen at an exercise intensity of ~64% $\dot{V}O_2$ max (Achten & Jeukendrup) but also an average maximal fat oxidation reported at 0.46 ± 0.01 g/min occurring at a lower $\dot{V}O_2$

max of 48 ± 1 % V·O_2 max (Venables et al, 2004) although this difference is more likely due to the trained status between the cohorts. Indirect calorimetry is a commonly used method to quantify CHO and fat oxidation levels, although it does contain some barriers such as the inability to distinguish between the differing fat sources (Jeukendrup & Wallis 2005). A method known as Fatmax has been developed to determine maximal lipid oxidation. It has been suggested that Fatmax is optimal in low to moderate intensities ranging between 33-65% $\dot{V}O_2$ max (Bergman et al 1999; Broeder et al, 1991; Friedliner et al, 1999; Romijn et al 1993; Van Loon et al, 2001). This has been further clarified by T Meyer who reported Fatmax was ~65% $\dot{V}O_2$ max in constant load trials of 1 hr duration. (T Meyer et al, 2007). Above this intensity of ~75% $\dot{V}O_2$ max lipid oxidation has been shown to decline (Rowlands & Jeukendrup, 2004). This is due to a move towards Fatty acids derived from intramuscular triglycerides (IMTG) and further to this glycogen stores. The mechanisms behind the control of fat oxidation and the decrease during increasing exercise intensity may be attributed to availability of plasma fatty acids (Romijin et al, 2993) and a potential rate limiting step in the pathway of fat oxidation being the entry of fatty acetyl-CoA into the oxidation process (Coyle et al, 1993). Jeukendrup and collegues (1998) proposed that the two steps most likely to impact fat oxidation during increasing exercise intensities are; the mobilisation of fatty acid from adipose tissue and secondly the transportation of fatty acids into the mitochondria where beta oxidation takes place. In addition to other factors including mitochondrial density and the muscles fat oxidation capacity. To note, although lipid oxidation and substrate flexibility may be the influencing factor to improve performance, Jeukendrup and Wallis (2005) reported high fat oxidation rates may not always equate to an improved training ability. This therefore suggests that although fatty acid oxidation is possible to enhance in the majority of individuals, it is not always followed by a subsequent utilisation and performance outcome in every individual.

Nicotinamide Riboside and Pterostilbene (NRPT)

It is yet to be understood the true ergogenic actions of NR and PT when administered as a combination, however, using current evidence it suggests that the combination may have synergistic ergogenic effects compared to administration as individual supplements. It is thought to hold natural anti-aging properties by slowing the degeneration process, enhancing physiological pathways and preventing age-related diseases such as, cardiovascular disease, neurodegeneration and diabetes, via re-establishing NAD+ levels and upregulating Sirtuins. Research has successfully shown that when consumed separately they elicit expected and noteworthy effects within the body in both human and rodent studies such that NR elevates NAD+ levels (Trammel et al, 2016) and PT activates SIRT1 (Nagao et al 2017). NAD+ concentration were quantified through blood samples analysed by liquid chromography mass spectrometry after being split into peripheral blood mononuclear cells and plasma. However, little has been identified in terms of any sporting advancements. Effective utilisation of any supplement or fuel in the body requires the presence of its associated consuming molecules, in this case the presence of SIRT's act as NAD+ consuming molecules. Evidence has shown that with supplementation of NR can increase the levels of NAD+ in the body, however, naturally, this elevation of NAD+ will not be utilised without an equated rise in NAD+ consuming molecules, such as Sirtuins. This suggests the supplementation of a compound that upregulates NAD+ consuming molecules, to fully utilise the artificial rise in NAD+ levels, such as PT, would produce a peak in ergogenic influence from NR, with this the idea that NR and PT may work synergistically together has been produced. These anti-ageing properties have given rise to the potential use in a sporting context and may be rationalised by the possibility that Sirtuins are regulated by diet but more specifically environmental stress (Chang & Guarente, 2014) such as altitude. This suggests the use of hypoxia to

produce this environmental stress may provide a driving stimulus for SIRT1 activation, ultimately observing improved performance at altitude.

Dellinger and colleagues (2017) undertook the first human study supplementing NRPT. They investigated the dose response and safety of a chronic supplementation (8 weeks) in an aged population (60-80 years old) with a control group, recommended single dose (250mg NR and 50mg PT) and double dose (500mg NR and 100mg PT) groups. Whole blood samples were collected at baseline, day 30 and day 60 and were analysed using a GLP-compliant method to analyse NAD+ from human blood lysate by liquid chromatography mass spectrometry. They showed that NR increased NAD+ levels in a dose dependent manner with a ~40% increase with a single dose and ~90% with a double dose in 4-weeks which was retained over the 8-week period. They highlighted that there were no adverse effects within the cohort and a repeated dose is a safe and effective way to increase NAD+ concentration in humans. It is thought, from the current evidence of their individual actions they may provide both vascular and metabolic actions in a hypoxic environment via the upregulation of molecules such as NAD+ and SIRT1. Specifically, metabolic factors are a crucial factor in exercise performance due to the ability to influence intensity and duration via substrate flexibility, such that an upregulation of lipid oxidation occurs during a prolonged bout of exercise mediated by SIRT1 producing a sparing action on glycogen for subsequent higher intensity exercise efforts. The rationalisation of the combination can be explained by looking more closely at the individual actions of each component of the NRPT supplement.

Nicotinamide Riboside (NR) is a potent NAD+ pre-cursor

NR is found in milk, making it highly accessible to the general population. Its influence on health has been widely found as an effective treatment for a variety of age-related diseases including; neurodegeneration, muscle degeneration, diabetes, obesity and ageing (Srivastava,

2016). In addition to health benefits NR has been investigated for its effect on cell and mitochondrial function in a sporting context due to its action on NAD+, a key molecule in mitochondrial function and ATP synthesis. In comparison to other Niacins it has been suggested that NR is one of the most direct NAD+ precursors, having only 2 steps in its metabolism process (Figure 1). It is transported into the cell via nucleoside transporters and is then phosphorylated by NRKinases 1 and 2 producing NMN. Finally, the enzyme NMNAT catalyses the formation of NAD+ (Figure 1). Trammell et al (2016) supported NR use over other Niacins ((Nicotinic Acid (NA) and Nicotinamide (NAM)) in mice, reporting that NR produced an elevated peak concentration in liver NAD+ and mediated NAD+ precursors due to this differentiated pharmacokinetics. NAD+ plays a vital role in redox reactions for cell functioning and is found throughout the cell in the mitochondrion, cytosol and nucleus. Mitochondrial function is dependent on NAD+/NADH redox homeostasis (Jokinen et al, 2017) and thus is key to mitochondrial biogenesis, yet NAD+ has been shown to reduce as we age in mice (Ramsey et al, 2008; Mouchiroud et al, 2013). NAD+ also serves as a crucial coenzyme in the transfer of electrons for ATP synthesis. This occurs via a reduction reaction by gaining a hydrogen ion to produce NADH, notably, a key molecule used throughout the energy systems including the Krebs Cycle and Electron Transport Chain (ETC). Glycolysis requires 2 NAD+ molecules per molecule of glucose enabling the conversion of glucose to pyruvate to assist the initiation of the TCA cycle. Here NAD+ molecules are unable to cross the mitochondria barrier and are converted to NADH and transported into the mitochondrial via the malate-asparate shuttle or glycerol-3-phosphate shuttle (McKenna et al, 2006) before reaching the ETC inside the mitochondria (Canto et al, 2012). Inhibition of NADH dehydrogenase complex 1 in the ETC compromises the flow of electrons and proton gradient back into the matrix and is accountable for ~40% of the formation of mitochondrial ATP (Kühn et al, 2015). It is therefore crucial to maintain adequate levels of NAD+ to allow

sustained physical exertion (Goody & Henry, 2018). Consequently, the redox functions of NAD+ are central to the production of energy and ATP for muscular contractions and in its absence would result in the early onset of fatigue and a reduction in performance. Not only is NAD+ involved in the energy pathways but it also plays a role in a range of pathways relating to cellular homeostasis including; energy metabolism, lifespan regulation, DNA repair, apotheosis and telomere maintenance (White et al, 2012). Thus, the maintenance of NAD+ concentrations in the body are crucial for regular functioning.

Nicotinic Acid 'v' Nicotinamide Riboside

Nicotinic Acid (NA) and Nicotinamide Riboside (NR) are the two most commonly used NAD+ precursors. Tryptophan is another source of NAD+ and can be metabolised as an endogenous source of nicotinamide via the de novo biosynthesis pathway to form NAAD and subsequently NAD+. In mice studies Nicotinamide Riboside Kinase 1 (NRK1) and 2 (NRK2) are not key in the process of endogenous NR salvage to NAD+, however, their action is necessary to utilise exogenous NR and NMN (Fletcher & Lavery, 2018). This is similar in mammalian tissue, with NR availability being the limiting factor for NR salvage to NAD+ via NRK pathway, in addition to minimal endogenous NR concentrations in tissue, whereas supplementation of NR via exogenous sources results in a potent stimulation of NAD+ via NRK (Fletcher *et al*., Ratajczak *et al*. 2016). Prior to NR use, NA was the major NAD+ precursor and found to be one of the most effective treatments of cardiovascular risk factors (Chapman et al 2004). However, a consideration to its validity arose when NA was given in high doses and caused the appearance of painful flushing (Bogan et al, 2008). This flushing response is caused by the binding of NA to the GPR109A receptor, which does not occur with NR supplementation and therefore flushing is not observed. This provides ethical favour towards NR supplementation given its protection against such mild health conditions. It is

important to note that although NR may increase NAD+ levels, without the associated consuming molecules this rise in NAD+ would not be utilised effectively. There are 2 molecules that are categorised as NAD+ consuming molecules; Sirtuins (SIRT) and Poly ADP Ribose Polymerase (PARP's). Sirtuins, a group of proteins consisting of 7 constituents (i.e. SIRT1-7), are NAD+ dependent deacetylases and use NAD+ as a co-substrate to catalyse the acetylation and/or mono-ADP-ribosylation of target proteins (Gupte et al, 2019). A common characteristic throughout all subunits is they depend solely on the presence of NAD+ to function independent of their location and work to retain cellular function and homeostasis (Kupis et al, 2016). Sirtuin subunits have been shown to have distinct cellular functioning actions and the link with NAD+ and other genes associated with substrate utilisation initiated interest into Sirtuins' role in metabolism and has led to suggestions that certain subunits, specifically SIRT1 and SIRT4, are fundamental influencers in exercise-induced mitochondrial biogenesis in skeletal muscle (Gerhart-Hines 2007; Canto 2009; Canto 2010). These metabolic influences tend to be found under calorie-restricted cells, malnutrition, fasting or DNA damage, with SIRT1 and SIRT4 holding key responsibilities in mediating metabolic transcriptional adaptations (Imai & Guarente 2015; Wang et al, 2019). These particular Sirtuins (SIRT1 and SIRT4) have been further supported to have metabolic actions, specifically enhancing fat oxidation by deacetylating and activating Peroxisome Proliferator Activated Receptor alpha (PPARa) (Purushotham et al, 2009). Thus, promoting a potential performance affect via the ability to spare carbohydrate (CHO) stores during steady state exercise efforts. Cox et al (2016) showed that a ketogenic diet in highly trained athletes has the ability to shift substrate utilisation towards fatty acid oxidation leading to an improvement in cycling Time Trial performance, cycling on average 411 ± 162 m further with Ketone Ester (KE) + CHO compared to CHO alone. However, there is limited research to suggest whether these findings are translated in an untrained population. Hawley (2002)

reported that a 15-48% sparing in muscle glycogen compared to control occurred with the consumption of an adequate fat intake. However, this did not result in an improvement in exercise performance. This was supported by Burke (2015) that, although it is widely shown that an increase in dietary fat intake elevated fatty acid availability and consequently a shift towards fat oxidation, evidence to promote a clear performance outcome was lacking. This poses the question as to whether something is missing to utilise the spared glycogen and promote a performance outcome? Sirtuins can play an important role in producing a beneficial outcome when calorie intake is a limiting factor and therefore may provide an aid towards desired performance improvements in terms of metabolic shift. As evidence suggests NAD+ plays a key role in reactions that are mediated by mitochondrial metabolism, SIRTs and PARPs (Jokinen, 2017), with further reports expressing that exogenous NAD+ ingestion or the biosynthesis of NAD+ has a protective ability, in the presence of SIRT1, to dampen processes driven by PARP activation such as cell apotosis. (Pillai et al 2010).

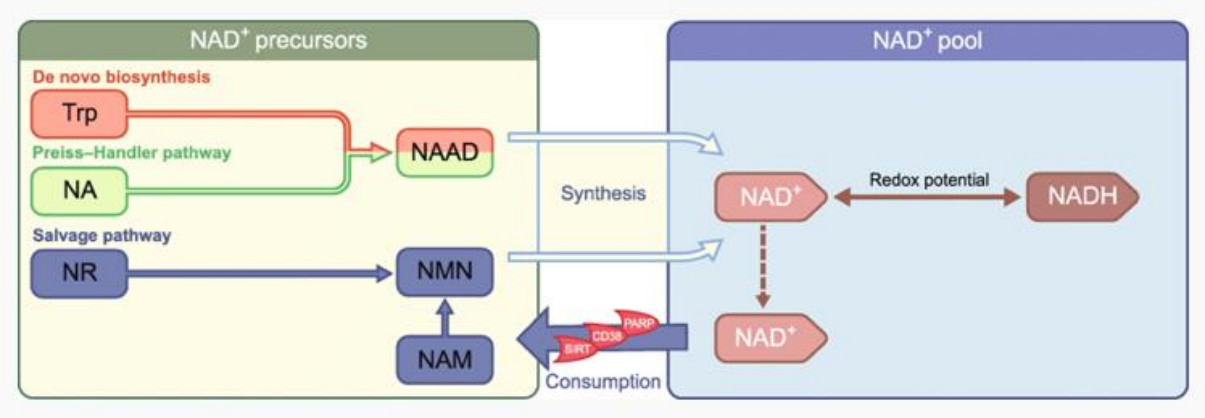

Figure 1. NAD+ metabolism pathway. (Connell et al, 2019)

Performance implications of Nicotinamide Riboside supplementation

The vast majority of research has been undertaken in rodents with research in humans somewhat limited, however, these human studies have shown that chronic supplementation of NR is well tolerated and safe to use in both healthy and clinical populations (Dellinger et al,

2017; Martens et al, 2018). Martens and colleagues produced a 2x 6-week, clinical trial in healthy middle aged and older adults. They assessed NAD+ content in peripheral blood mononuclear cells, as it has been shown that NAD+ in blood and other associated metabolites are not observed in plasma and urine but instead in peripheral blood mononuclear cells (Trammel et al, 2016). They reported that it is well tolerated and showed a 50% increase in NAD+ concentration after 6-weeks NR supplementation of 500mg twice daily, alongside improvements in cardiovascular measures, with a mean difference of >1% in flow mediated dilation (FMD) which they reported as clinically relevant. However, evidence of NR's actions on exercise performance has resulted in contradicting outcomes. A study by Dolopikou et al (2019) on 12 young (22.9 years ± 1.0) and 12 old men (71.5 years ± 1.0) reported that acute (2 hours) NR supplementation (2 x 250mg/day) only elicited beneficial results in individuals with low baseline NADH levels, such as the aged and unhealthy population, but not in the younger healthy population. This rationalised their finding that an improvement in performance was only seen in the older cohort. Specifically, the older group exhibited an 8% increase in isometric peak torque compared to the younger group with NR supplementation alongside an improved ability to prolong physical exertion by resisting fatigue. They concluded that NR had no redox or physiological implication on the young population attributing this to the need for a limitation in the individuals physiology, specifically in this case, a depleted baseline NAD(P)H level, in addition to muscular damage to gain performance benefits from NR supplementation. This has been supported in rodent studies. Canto et al (2012) suggested that NR supplementation may have a positive implication when combined with an obese cohort on a high fat diet. It was found that mice fed on a high-fat diet supplemented with NR (400mg/day for 1 week) showed a reduced weight gain in comparison to the control chow fed mice. They also saw an increase in fatty acid oxidation, insulin sensitivity and energy expenditure with NR supplementation,

suggesting that NR may have a metabolic action in the body alongside the already reported vascular responses and health implications. In terms of exercise performance, they also reported that NR enhanced mitochondrial biogenesis in muscle tissue and resulted in an improved endurance capacity as indexed by the NR fed mice running ~200m further than control. This was suggested to be due to an improved oxidative capacity. This protection against high-fat diets is further supported by Canto et al (2012) and Belenkey et al (2007), highlighting that NR supplementation improved age-related insulin resistance and life span of yeast replication. In contrast, Dollerup et al (2018) found that 12 weeks supplementation of 2000mg/d of NR did not have any effect on insulin sensitivity, endogenous glucose production and glucose oxidation in 40 sedentary obese (BMI>30kg/m2), insulin-resistant men. Nor did supplementation alter resting energy expenditure, lipolysis or lipid oxidation. However, it was concluded to be a safe dosage in this clinical population. The authors suggested that effect size may have been a contributing factor for the lack of difference between groups as the NR group had an initially lower Hepatic lipid content (HLC) by 2.8% compared to the placebo group and when looking at the individual data identified a reduction in HLC throughout the NR group. These findings are in line with other research that found supplementing with NA, an NAD+ precursor and similar to NR in terms of its action on NAD+, was detrimental to exercise performance in 11 trained men, attributing this to a reduction in exercise-induced increase in plasma free fatty acids (FFA). Leading to the hypothesis that NR shifts substrate oxidation by decreasing fatty acid oxidation and causing earlier onset of fatigue (O'Neil et al, 2004). Kourtzidis et al (2016) supported this lack of performance outcome and expressed that NR supplementation (300mg/kg/day for 21 days via gauge) was inhibitory towards exercise performance in rats. The animals underwent an incremental swimming test, completed in the morning after an overnight fast. A load weight was positioned on the base of the tail and increased over 12-minutes. At 4-minute intervals

the load was increased from 2%, 3.5% and 5% of the rat's body weight, following this a 10% load was added and the rats were left to swim to exhaustion (exhaustion = an inability to return to the surface within 10seconds x3). They reported that post 21-day NR supplementation there was a dysfunction in redox and energy metabolism resulting in a deterioration in exercise performance by 35% in the NR supplement group compared to control (94 ± 53 s and 145 ± 59 s respectively). However, it was suggested this result, although unclear, may be contradicting to other studies due to difference in animal model (rat 'v' mice 'v' human), type of exercise using different muscles and differing metabolic demands (swimming in comparison to treadmill running) and/or mode of NR administration, whether ab libitum feeding would result in different absorption rates in comparison to using a gavage technique (force-feeding v mixed into feed). Kourtzidis et al (2018) revisited their study to underpin the mechanisms behind the detriment to performance. They reported increase NADPH and glycogen in the liver but not muscle, decreased antioxidant capacity with increased systemic oxidative stress and finally a reduced peak lactate reduction during exercise. This led them to conclude and satisfy their previous study that exogenous NR administration resulted in an impairment in redox metabolism and redox homeostasis. Although evidence shows inconsistency in directly improving performance in both humans and rodents, NR has been shown to upregulate and influence pathways and processes that are otherwise compromised with aging and at altitude. It would seem that the evidence suggesting a performance decrement is undertaken in a healthy population, with no physical impairment, thus, the trend suggests that to yield a beneficial outcome from NR supplementation a baseline impairment in physiology is required such that can be simulated by aging, disease and possibly altitude.

Polyphenol supplementation in sport

Polyphenols have been widely promoted as natural antioxidants with chemopreventive properties (Zhang & Tsao, 2016; Chong et al, 2010). Their chemical structure has given them properties as potent anti-oxidants and anti-inflammatiories, two factors commonly seen throughout sport. Polyphenols are grouped into 4 different categories based on the degree of oxidation of the oxygenated ring structure (Bowtell & Kelly, 2019); phenolics, flavonoids, lignans and stilbenes, each having varied in vivo effects. Polyphenols derived from foods such as blueberries (McLeay et al, 2012), pomegranate (Matthaiou et al,2014), cherries (Kelley et al 2006) and cocoa (Marten et al, 2016) have all shown to support the antioxidant and anti-inflammatory responses suggested by their structure. In addition, studies using flow mediated dilation (FMD) have found that polyphenols have a vascular influence via nitric oxide dependent, endothelium-dependent dilation. Hooper et al (2012) found that a group of 42 studies consisting of both chronic and acute cocoa supplementation resulted in significant increases in FMD; 1.3% and 3.2% respectively. However, exercise performance responses to polyphenol supplementation have been varied. Time of consumption on subsequent exercise performance is an important factor to consider when administering supplements. Bowtell & Kelly (2019) reported that on average, polyphenol consumption 1hr before commencing exercise elicited beneficial ergogenic responses however, exceeding 2hr+ prior to exercise saw no improvements in performance. This time response is likely to differ depending on exercise duration and half-life of supplement compounds and therefore every supplement should be treated individually. A function of polyphenols to enhance exercise performance is their action on mitochondrial biogenesis. Mitochondrial biogenesis is the fusion and fission of pre-existing mitochondria (Yuan et al, 2016). Ageing and excess accumulation of stress via reactive oxygen species (ROS) during exercise can impair mitochondrial function which can lead to insufficient production of ATP and consequently apotosis of mitochondria and fatigue in exercise (Biala et al, 2015). Ungvari et al (2011) supported this idea and reported

that as a result of impaired mitochondrial biogenesis, mitochondrial turnover decreases, leading to unwanted oxidised lipids, proteins and DNA. Polyphenols action on mitochondrial biogenesis is promoted by the upregulation of a protein known as Sirtuins (SIRT). Specifically, SIRT1 achieves this action via a cascade of protein up-regulation of; AMPK, HIF-1a and PGC-1a (Wenz, 2013; Gomes et al, 2013). These are used in the expression of new mitochondrial genes, the process of natural cell cycle and ATP synthesis (Yuan et at, 2016). Further supported by Menzies and Hood (2012) who suggested that the SIRT1 dependent increase in mitochondrial biogenesis is a highly influencing factor in elongating life span and preventing age-related disease. Polyphenols categorised as Stilbenes namely; Pterostilbene and Resveratrol, which are chemically similar, have gained precedence for their robust nature in preserving cardiac and vascular health, however, they have more recently been found to promote substrate flexibility by upregulating and activating gene expression and target molecules associated with fatty acid oxidation (Lopez-Lluch et al, 2008). It was thought that this metabolic influence was a result of the upregulation of Sirtuin activation via PT/Resveratrol administration. Price et al (2012) suggested that the upregulation of AMPK in a SIRT1 dependent manner promoted this increase in lipid metabolism. Evidence suggests that polyphenols may have a strong metabolic action and therefore promote substrate flexibility to assist exercise performance.

Vascular action of Pterostilbene and Resveratrol via SIRT1 and NO activation respectively

Due to Pterostilbene (PT) being chemically similar to Resveratrol we can assume that the evidence found in Resveratrol will closely mirror results found with Pterostilbene. Resveratrol has been explored extensively and both PT and Resveratrol have been found to have a metabolic action via upregulation of SIRT1. Smith et al (2009) supported this idea and reported that formulated Resveratrol (SIRT501 and SRT1720) diminished the aetiology of

Type 2 diabetes via a specialised signalling pathway, inclusive of enhanced mitochondrial function and improved metabolic signalling. Further evidence by LaGouge et al (2006) has supported these metabolic properties. They found that 15-week supplementation of resveratrol in mice is able to increase skeletal muscle mitochondrial biogenesis and fatty acid oxidation widely due to the activation of the PGC-1 protein. They reported a noticeable increase in mitochondrial activity and an increase in oxidative muscle fibre types, giving scope for improvements in exercise performance with supplementation of Resveratrol. However, these findings were seen more noticeably in high fat diet fed mice than chow-diet mice, suggesting ingestion of Resveratrol in a healthy population may have capped benefits, proposing that performance in trained individuals may not have a great impact. In line with this idea of substrate shifts, Baur et al (2006) showed that Resveratrol increased AMPK, PGC-1a, mitochondrial number and motor function in rats on a high-calorie diet. Although the following evidence shows that Resveratrol results in an enhancement of the concerned gene expression this can only be transferred in an unhealthy rodent population, rather than a 'trained' human population. Dolinsky et al (2012) showed that a 12-week supplementation of Resveratrol, in mice, resulted in an improvement in endurance capacity, which they attributed to an increase in skeletal muscle force, cardiac function and oxidative metabolism. Human studies have shown somewhat similar results with an obese cohort showing reduced weight gain, however, these cellular and mechanistic actions have not been translated into performance outcomes in a healthy population. Surprisingly, a human study of recreationally active men (~22 years old) found that Resveratrol supplementation in combination with exercise training had a detrimental impact on low-volume high intensity interval training (HIIT) exercise through a reduced PGC-1a, SIRT1 and SOD2 gene expression in addition to no fibre type preference alterations (Scribbans et al, 2014). This was taken over a 4-week period consisting of 3 HIIT sessions a week (Scribbans et al, 2014). Voduc et al (2014)

supported this finding by Scribbans et al. They found that a 4-week ramped supplementation of Resveratrol (500g-1000g) resulted in no significant effects between Resveratrol and exercise performance. This was completed in 13 healthy, sedentary males. In contrast, a human study, notably in an obese cohort have found that 30 days of Resveratrol intake (150mg/d) manipulates metabolism to that seen under calorie restriction (Trimmer et al, 2011). In agreement with this observation, Howitz et al (2003) also put forward Resveratrol's ability, in yeast cells, to mimic the signalling responses observed when calories are deprived via SIRT2 activation, improving DNA stability and prolonging lifespan by over 50%. Although evidence supporting Resveratrol in improving exercise performance may be contrasting and produce discrepancies between rodent and human studies, it has been found to be a potent catalyst of SIRT1 (Cheng et al, 2016), and therefore may simply require an imbalance in nutritional/physiological homeostasis to produce the desired responses. Resveratrol has also been found to have an action on endothelial function. The ability for the vascular system to respond to exercise and environmental stress, e.g vasodilation and vasoconstriction is important in the regulation of homeostasis. Vasodilatory dysfunction as we age has been shown to correlate to an age-related reduction in vasodilatory responses to heating and heat stress (Holowatz et al, 2003; Kenney et al, 1997; Minson et al, 2002). This dysfunction has been attributed to the attenuation of vasodilatory mechanisms governed by

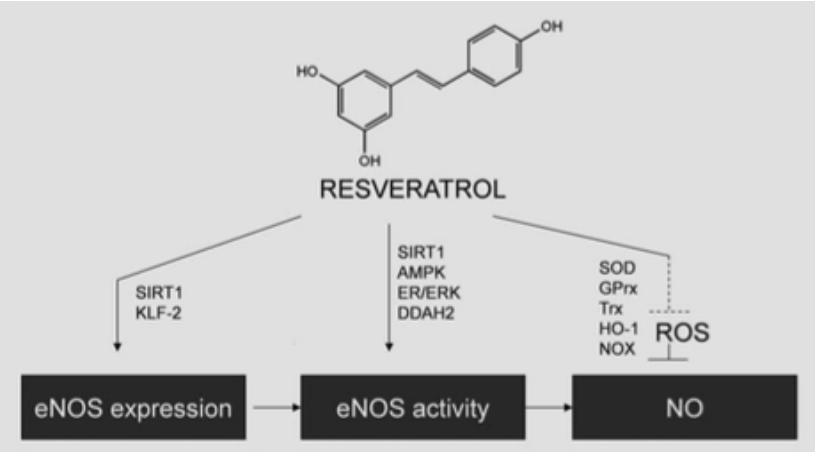

Figure 2. Resveratrol metabolism pathway (Schmitt et al 2010)

the activation of nitric oxide production (Holowatz et al, 2003; Minson et al, 2002). Compromised endothelial function via impaired nitric oxide (NO) production has been shown to be a determining factor in the development of cardiovascular risk factors, thus, it is essential for the maintenance of health (Rajendran et al, 2013). This action in maintaining endothelial and cardiac health and function is achieved via activation of endothelial nitric oxide synthase (eNOS) expression in a SIRT1 dependent manner which then stimulates the production of nitric oxide (NO) (Figure 2) (Mattagajasingh et al, 2007). NO's antiproliferative, anti-inflammatory and antithrombotic influence on the endothelium is partly achieved by its property as a potent vasodilator (Morishima et al, 2019). Although Resveratrol has been found to enhance NO availability in the vasculature, levels are mainly increased via a decline in ROS which would normally contribute towards the decline in NO (Schmitt et al, 2010). This further supports Resveratrol's capacity as an antioxidant and preventative property against oxidative damage. In addition to age-related impairments in endothelial function, exogenous factors including environmental stress can impair activation of endothelial cells which leads to a reduction in NO bioavailability (Schmitt et al, 2010). By maintaining or increasing NO availability in the vasculature, during exercise, it will improve blood flow and therefore increase muscle oxygenation thus delaying fatigue onset.

Safety and Tolerance of Administration

Although both, in differing depths and capacities, have been shown to be safe to administer (Neves et al, 2012) the chemical structure supports the use of PT over Resveratrol. PT contains two methoxy groups and one hydroxyl group whereas Resveratrol has three methoxy groups. This provides PT with a superior lipophilic property and thus increases PT's oral and cellular absorption (Lin et al, 2009). This bioavailability has been quantified in rats with PT reported to have a bioavailability of 95% with a half-life of 105 mins in comparison

to a 20% bioavailability and 14 min and half-life with Resveratrol due to Pterostilbenes superior chemical structure with systemic exposure based on C_o and AUC_{o-inf} (area under curve) values from blood (Asensi et al, 2002; Remsberg et al, 2008; Kapetanovic et al, 2011; Nutakul et al, 2011). McCormack and McFadden (2012) also highlighted that PT is predicted to have a notably greater half-life and therefore bioavailability in humans, however, this is yet to be formally tested. There are currently limited dose response studies that explore the most optimum dose of PT to elicit the desired response but merely an acceptable dose that is deemed safe. Important aspects to consider in any nutritional supplementation include; administration, dose and bioavailability as these are key factors in the effectiveness of supplementation. Riche et al (2013) explored the safety of chronic supplementation of PT in humans but did not measure systemic exposure due to lack of knowledge. They tested 80 participants over 6-8 weeks under 4 conditions; 125mg of PT x2 daily, 50mg PT x2 daily, 50mg PT + 100mg grape extract x2 daily, matching placebo. They reported no adverse hepatic, renal or glucose markers effects, concluding PT of up to 250mg is safe to administer to humans. Interestingly, although safe to be consumed in both male and females, Dellinger et al (2015) reported the possibility of gender differences in terms of the metabolism of both Resveratrol and PT. They found that females were more efficient at metabolising PT than males with a reported 50% increase in catalytic efficiency. They also found a significant increase in the V_{max} for females compared to men HLM (Human Liver Microsomes) 2,706 ± 100 $pmol \cdot min^{-1} \cdot mg^{-1}$, 1,678 ± 123 $pmol \cdot min^{-1} \cdot mg^{-1}$ respectively. They attributed this gender difference to the expression of a gender specific category of enzymes that are crucial in the metabolism of Resveratrol and PT known as UDP-glucuronosyltransferase (UGT). In absence of this information, more research is needed to verify the bioavailability of PT, however, current evidence would suggest it is a feasible supplement to use for its proposed actions.

Pterostilbene- Antioxidative action against Oxidative Stress

The properties of the Polyphenol Pterostilbene suggest that it may work in conjunction with NR to produce a synergistic ergogenic effect, however, in comparison to other polyphenols its actions are not fully understood. Pterostilbene is a natural stilbene found in blueberries and grapes (Riche et al, 2013) and a key property of PT is its action as an anti-inflammatory and antioxidant (AO). During high intensity exercise, muscles are subjected to a process called oxidative stress. Oxidative stress causes the formation of reactive oxygen species (ROS), the body combats these molecules via a natural pool of antioxidants known as the total antioxidant capacity which can be enhanced through supplementation of antioxidants such as Pterostilbene. It was originally thought that this oxidative stress caused by exercise was detrimental to the body, however, this idea has been revised, and it is now thought that without oxidative stress, cells and mitochondria are unable to adapt to stress and damage (Margaritelis et al, 2018) (Figure 3). The repair process from oxidative stress produces an adaptive response whereby cells and mitochondria increase their capacity, via an increase in cristae folding and increase size and number of mitochondria, to cope with the exercise induced stress and thus, are able to minimise the damage on future exposure and ultimately allow the individual to exercise for longer and at high intensity before fatigue onset (Jackson, 2015). Evidence has supported this showing that with increased stress levels (low, medium and high), up to a certain point, resulted in an improved $\dot{V}O_2$ max (12%,17%, 19%), time trial (9%, 24% and 22%) and Wingate test (7%, 12% and 15%) respectively (Margaritelis et al, 2018). It is thought that resting levels of oxidative stress are a good determinant of exercise induced oxidative stress (Margaritelis et al, 2018). Cakir-Atabek et al (2015) reported no significant differences between trained and untrained rowers and exercise intensity, suggesting that there is not one simple solution to AO supplementation and depends on

training status and training intensity. Supplementing using an antioxidant such as Pterostilbene can help counter the oxidative stress caused from physical exertion to maintain an effective balance between oxidative stress and antioxidative supplement and aid ATP production efficiency by enhancing electron transfer and preventing the production of ROS. The formation of ROS implicates exercise performance due to the activation of Poly (ADP-

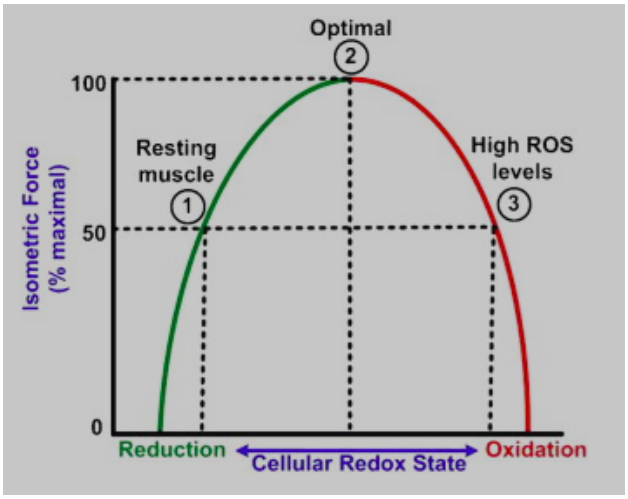

Figure 3. Graph showing proposed 'U' shape of optimal muscular performance. (Reid et al, 1993)

ribose) polymerase, which causes catabolism of NAD+ and has subsequently been suggested that PARP's may have an inhibitor influence on mitochondrial biogenesis, a major contributor towards enhanced endurance tolerance (Lappalainen, 2010). Therefore, by reducing ROS formation NAD+ levels remained elevated and can be used in the re-synthesis of ATP for energy pathways and retain mitochondrial functioning. However interestingly, evidence has proposed that PARP activity and PARP-1 levels are not influenced by NR supplementation suggesting that NR's impact on NAD+ levels has a direct impact on NAD+ biosynthesis instead of an indirect effect on the salvage pathway or consumption (PARP) pathway (Canto et al, 2015). PT has been suggested as a more potent up-regulator of the protein, SIRT1 in comparison to its analogue Resveratrol (Guo et al, 2016). In addition to the activation of SIRT1 as a preventative measure against oxidative stress, as mentioned SIRT1 has been shown to produce metabolic actions in the body by promoting substrate flexibility.

Therefore, suggesting PT promotes a metabolic action which may positively influence sporting performance.

Both NR and PT have been shown to have metabolic actions when administered individually, mediated by the upregulation and activation of SIRT1. Alongside the rise in NAD+ and its influence of ATP re-synthesis and vascular function it is thought they may have a synergistic response to exercise performance when combined with a stimulus to impair physiology or produce an imbalance to baseline functional capacity. The aim of this study is therefore to explore the vascular and metabolic actions elicited by the chronic supplementation of NRPT under hypoxic and normoxic environments. The evidence supplied by current research in the primary and surrounding fields has led to us defining the following hypotheses:

Hypotheses

1. NRPT supplementation will improve Time Trial performance in hypoxic conditions
2. NRPT supplementation will have no impact on Time Trial performance in normoxic conditions.
3. Exercise performance will be compromised at altitude compared to sea level, however, supplementation of NRPT will close the gap between these environmental conditions.

Methods

Participants

A total of 17, 18-24 year old male and female participants were recruited (7 female). Of those 17, n=3 voluntarily completed the second arm of the study making a total of n=20 participants completing the study. Participants were all at least 'recreationally active' and achieved the UK government physical activity guidelines for their age range (at least 150 minutes of moderate intensity activity or 75minutes of vigorous intensity activity). A minimum $\dot{V}O_2$ max was set at >30ml/kg/min for females and >40ml/kg/min for males to ensure participants were physically capable of performing the outlined task. Participants were excluded from the study if; they were currently participating in another study that required them to consume a dietary supplement, high-fat or high/low CHO diet, answered 'yes' to any the question on the health questionnaire (e.g. cardiovascular, metabolic or neurological disease), had allergies to Pterostilbene and/or red wine polyphenols, they had a history of substance abuse or, they smoked, they were taking medication likely to influence metabolism excluding the contraceptive pill.

Ethical approval was granted by the University of Birmingham School of Sport, Exercise and Rehabilitation sciences Ethics Health and Safety committee.

Study Design

An overview of the study design is shown in Figure 4. The study is a randomised, double blind, placebo controlled, parallel groups (accepting n=3 crossover treated as non-crossover), 4-week supplementation exercise intervention. The study consisted of a total of 5 visits all of which were completed in an environmental chamber at the University of Birmingham. Chamber conditions were controlled throughout the visits at ~18^{o}C, ~30% humidity and

altitude during the hypoxic condition was set at 2500m. Participants were blinded from both the environment condition during the testing and pill allocation and the experimenters were blinded from the pill allocation, however, unavoidably they were aware of the environmental condition on each test due to the nature of setting up. Testing visits were completed at a similar time of day (± 2 hours) for individuals and took place between 8am and 8pm. Prior to the 4 testing visits participants were asked to complete a diet diary 48 hours before the test, this was done to justify any possible changes in performance, participants were also advised to eat an isoenergetic meal before each visit. An incremental test on a cycle ergometer and familiarisation of 5km-TT was completed on visit 1. Visits 2 and 3 were the pre-supplement experimental visits consisting of a 30minute Steady State (SS) cycle and 5km-Time Trial (TT) in either normoxia or hypoxia. Visits 4 and 5 followed the 4-week supplementation period and were identical in protocol to visits 2 and 3 but may not have followed the same order of environmental condition. Steady state exercise was completed at 65% and 55% Wmax in normoxia and hypoxia respectively, with these workloads thought to be within the

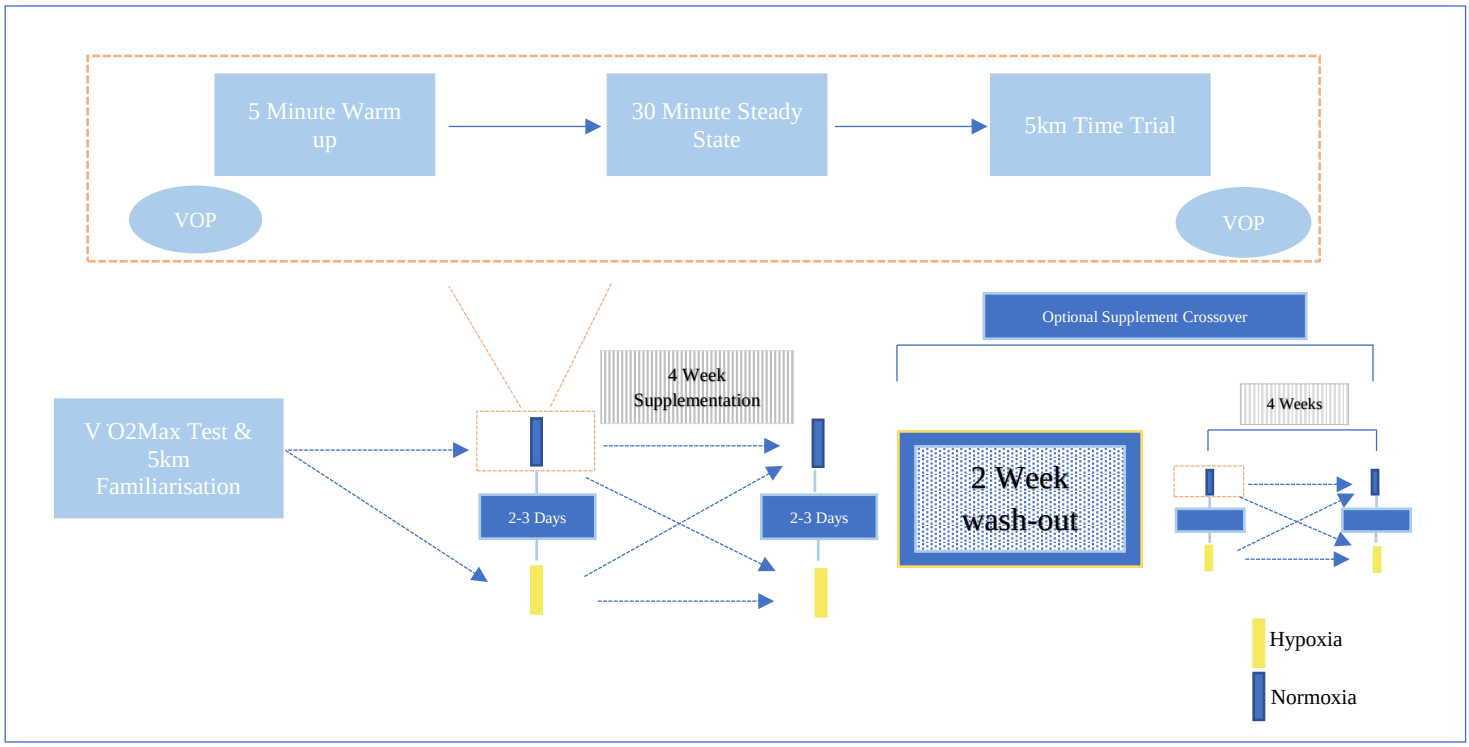

Figure 4. Study design

range that allows physiological steady state (Meyer et al 2017).). The discrepancy in absolute workloads between environmental conditions was due to finding it considerably more difficult and in some cases the inability to complete the outlined task in hypoxia than normoxia. By altering the workloads blinding was successful.

Details of Experimental Visits

Visit 1

On arrival to the laboratory, participants were asked to complete a consent form, health screening questionnaire and allergy form prior to exercising, if the forms did not indicate any reason for exclusion, height and weight were taken and urine osmolality was measured prior to exercise to quantify hydration status. Participants were supplied with a polar heart rate monitor strap which was connected via Bluetooth to the polar app on a phone along with a face mask which was connected through a sealed door in the wall of the chamber to a Vyntus CPX (Jaeger, CareFusion, Germany). A 5-minute warm up was completed with an RPE of 11 achieved prior to commencing the test. Participants then undertook an incremental $\dot{V}O_2$ max test using the Velotron ergometer (Velotron, RacerMate Inc., Seattle, WA) followed by a familiarisation of the 5km-TT in the environmental chamber in normoxic conditions. The $\dot{V}O_2$ protocol and RPE was shown explained to the participant, they were instructed that the bike resistance would increase by 30W in 3-minute intervals when a heart rate and RPE will be taken and to cycle for as long as possible until they could not cycle any further. Standardised encouragement was given to participants so to push themselves as much as possible, along with music that was standardised to either the same or a similar genre. Participants were allowed to recover before commencing the 5km-TT. All data was hidden from the participant except for the distance covered. This was maintained throughout the study. Prior to the 5km-TT participants were instructed they now had control of the bike

gears and not to implement a pacing strategy, rather give 100% throughout the TT and attempt to do it in the quickest possible time. Again, encouragement was given, and music was played to motivate the participant throughout all tests. The participant was not aware of their TT time throughout the study.

Visit 2 and 3

Participants returned at least 48 hours after the initial visit (visit 1) to complete the first pre-supplement experimental test. On arrival a urine sample was taken to quantify hydration status. Following this, resting HR, forearm blood flow, $\dot{V}O_2$, $\dot{V}CO_2$ and RER were taken. Venous Occlusion Plethysmography (VOP) was used to take forearm blood flow. This consists of an upper arm cuff inflated to 40mmHg, a wrist cuff inflated to ~220mmHg and a strain gauge placed around the forearm (Strain gauge size was determined by measuring the diameter of the thickest part of the forearm using a tape measure minus 2cm). The wrist cuff was inflated via a hand pump, after 30 seconds the upper cuff was inflated for 10 seconds and deflated for 20 seconds via a rapid cuff inflation system (E20, Hokanson) this was repeated 3 times (or to produce 3 similar graphs). A heart rate monitor was worn around the chest and connected via Bluetooth to the Polar app. Participants completed a 5-minute warm up on the Velotron with an aim to reach RPE 11. Participants rolled into the test phase of 30-minute steady state (SS) exercise at 65% watt max (Wmax) in the normoxic condition and 55% Wmax in the hypoxic condition. Following the SS participants were allowed a short recovery period to stretch and re-adjust the bike in necessary. To commence the TT participants were asked to ‘get up to pace’, once they acknowledged they were ready a 3 second countdown initiated the 5km TT. During the TT the TV screen was covered displayed only the distance the participant had covered. A post-exercise VOP measurement was taken within a standardised 3-minutes period following the end of exercise. Participants were advised to

stretch and cool down following the end of exercise. All measures were taken in 5-minute intervals during the Steady State and every km during the Time Trial. Participants returned to the chamber at least 48 hours after to complete the other environmental condition, normoxic or hypoxic. Throughout the test music was played and encouragement was given to the subject to motivate the subject to work as maximal as possible. Once both conditions had been completed participants were given the 4-week supplementation of either placebo or NRPT in a randomised order.

Visit 4 and 5

Participants returned in the 4th week of supplementation with the last supplement taken on the morning of the final test day (Visit 5). The test protocols as in visits 2 and 3 were repeated in a randomised order of normoxia and hypoxia. After completion of the final test, participants were given the opportunity to cross-over and complete the other arm of the study after a ~10-day washout period where they would take the opposite supplement to their first round of testing.

Blinding and Randomisation

All participants were recruited and enrolled by investigators directly involved in the study but were randomly allocated to the NRPT or placebo groups. The supplements were labelled ‘A’ and ‘B’ with an additional labelling system of ‘435’ and ‘071’ by an external researcher not involved in the running, data collection or analysis of the study. This blinding remained in place until all data collection and analysis had been completed following this, participants individual data was disclosed via the communication link stated in the consent form. Blinding of environment condition was single only, this was due to the setting up of the equipment. Due to the environmental chamber being used by other researchers it was not possible to

fully randomise the order of the environmental condition. However, there was an attempt to produce an equal divide between the first completed environment where possible.

Sample Size

Evidence has proposed an average drop out of 15-25% in health and exercise sciences, in addition, with evidence also suggesting that a minimum of 15 individual datasets per cohort (condition) is required (Shang et al, 2012). Taking into consideration drop outs, adding 2-3 participants per cohort means statistical power should be maintained. A total of n= 20 was completed, n=3 of which are crossover, making n=10 in each condition. Due to time constraints more participants could not be tested, but are unlikely to have influenced the data positively to form significant results following n=10 in each condition

Supplementation

Participants were allocated to either the experimental (500mg nicotinamide riboside, 100mg pterostilbene; Elysium Health) or placebo (Microcrystalline cellulose, gelatin, magnesium stearate, titanium dioxide and silica. No active ingredient detected) group in which they consumed two capsule tablets. Supplementation began following the 3rd visit for 4-weeks when they returned to the lab to complete the post-supplement testing. Participants were instructed to orally consume the capsules at a similar time of day over the intervention period, consuming both at the same time, with or without food. Participants were also instructed to not take a double dose if they missed a day. A ~10-day washout period was completed for those volunteering to crossover on the second arm of the study following which they would take the other supplement (NRPT or placebo). This length of time was based on Trammel et al (2016) who used a 7 day period in which no supplementation of NR

alone was given. Supporting this, the half life of NR and PT are 8hours and 105 mins respectively (Trammel et al, 2016; Kapetanovic et al, 2011)

Control measures

All participants were asked to refrain from vigorous physical exercise, excessive consumption of alcohol 24 hours prior to each laboratory visit and not consuming caffeine-containing drinks within 24 hours of the testing visit. Participant were asked to consume their habitual diet throughout the study and were asked to complete a diet diary 48 hours before all visits and asked to replicate this diet in the 24-hour period directly before all visits, dietary intake was not analysed other than by eye from diet diaries. Participants were not fasted at any point during this study. Participants were also asked to avoid any drastic changes in dietary intake and exercise routine over the 4-week intervention period.

Performance Measures

Performance

The Velotron ergometer (Velotron, RacerMate Inc., Seattle, WA) was used to perform the steady state and time trial and provided real time power outputs and completion time of the 5km-TT using Velotron Coaching Software (Velotron CS 2008, RacerMate Inc., Seattle,

WA) (Figure 5).

Figure 5. Velotron and equipment set up during 5km-TT

Venous Occlusion Plethsymography (method: Wythe 2015)

Venous Occlusion Plethysmography (VOP) is a simple, short, non-invasive and inexpensive method used to measure limb blood flow. VOP is a commonly used method in human studies specifically in vascular physiology, measuring both brain and limb blood flow (Wilkinson & Webb, 2001; Benjamin et al, 1995) and has been shown to produce highly reproducible results both at rest and under decreased/increased blood flow. VOP has been favoured over other methods such as doppler ultrasound due to its reproducibility (Roberts et al. 1986; Pallares et al. 1994; Thijssen et al. 2005; Kooijman et al. 2007). The response observed from occluding venous outflow from a distal limb such as the hand, is an increase in the associated limb compartment volume which has been suggested to mirror that of arterial inflow. This is created due to the cuff pressure which prevents venous flow but leaves arterial inflow undisrupted, therefore an optimal cuff pressure has been suggested to be that of the highest arterial inflow. Groothius (1985) quantified this and reported a cuff pressure of 30mmHg up to diastolic blood pressure is the most effective to measure arterial inflow via VOP. Therefore, VOP works on the assumption that the increase in limb volume produced by occlusion of venous outflow reflects arterial inflow. In this study VOP was used to measure

forearm blood flow. A standard blood pressure cuff (SC10D, Hokanson, Bellevue, USA) was placed around the upper arm/bicep and connected to a rapid cuff inflator (E20, Hokanson) set

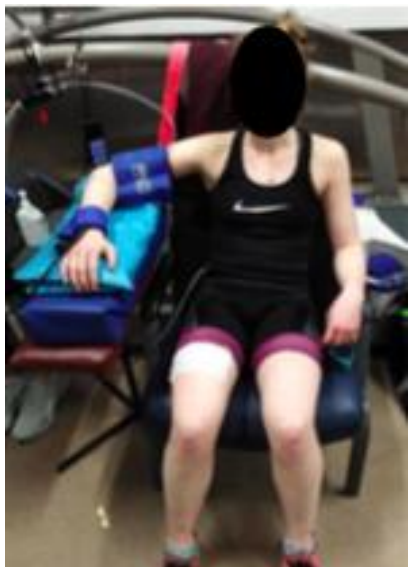

Figure 6. Venous Occlusion Plethysmography set up for measurement of forearm blood flow.

at 40mmHg; above venous but below arterial pressure. A mercury-silastic strain gauge was placed around the widest part of the forearm. The size of the gauge was determined by measuring the circumference of the widest part of the forearm with a tape measure minus 2cm (Wythe, 2105). This was connected to a plethysmograph. Calibrating the gauge prior to commencing the test allowed the change in electrical resistance in the strain gauge tubing to be converted to a change in limb volume (Whitney 1953). A segmental cuff was placed around the wrist (TMC7, Hokanson) and inflated manually to supra-arterial pressure (~220mmHg) via a hand-held inflator preventing hand blood flow which has been found to contain many arterio-venous shunts (Wythe et al, 2015). Participants remained seated in a chair during VOP and positioned their right arm on a side stand with an inflated sand cushion to elevate the arm ~10cm above the heart (Figure 6). Participants were asked to remain as still and relaxed as possible throughout the test. The wrist cuff was fully inflated prior to initiating the protocol, a timer was then started. An initial 'rest' period of 30 seconds was allowed to allow blood flow to settle following this the upper cuff was inflated for 10 seconds

and then released for 20 seconds by pressing the button on the rapid cuff inflator. This was repeated 3-4 times to produce 3 similar graphs and 'stop' was pressed on labchart. This protocol was completed pre and post exercise on all 4 of the testing visits. The initial 2.5s is disregarded due to a non-specific jump in blood flow, the final 2 seconds were also discounted. A segment of 4-8 seconds was used in both pre and post exercise graphs. An average of 3 graphs was taken this was then multiplied by 60 to get a %/min.

Indirect Calorimetry- Vyntus Metabolic Cart

Prior to the initiation of the test protocol, calibration of the apparatus was completed using gases of a known composition. The Vyntus CPX (Jaeger, CareFusion, Germany) used to measure indirect calorimetry provides information on energy expenditure from respiratory gases, O_2 consumption and CO_2 production, at the tissue level (Haughen et al, 2007) in addition to breath by breath gaseous exchange and ventilation measures to be taken during exercise. The cart was attached to the participant via a facemask passed through the wall of the chamber and was secured using a headstrap. Baseline O_2 and RER were taken prior to any exercise and markers were used to identify the start and end of both steady state and time trial efforts, ensuring these values were used during analysis and disregarding all other values outside of the desired times. For analysis 5 minute averages were taken over the 30-minute steady state and an average over each 1km was taken for the 5-km TT. Much of the RER data was disregarded from the TT as values were consistently >1 which is typically seen during non-steady state exercise efforts indirect calorimetry overestimates CHO and underestimates fat oxidation producing inaccurate values (Jeukendrup and Wallis, 2005).

Data Collection and Statistical Analysis

Data was collated using Microsoft excel and analysed in IBS SPSS (Version 22) software. All graphs are produced using GraphPad Prism (Version 8.1.2) with individual data displayed

where appropriate. Values are expressed as means + standard deviation (SD), with the significance reported at $p<0.05$. Between subject repeated measures ANOVA was used to compare the effects of time, condition and environment for the Steady State (SS) and Time Trial (TT) of all measures (time, power, $\dot{V}O_2$, RER, forearm blood flow). Bonferroni adjustment was applied to data where significance was found. During the statistical analysis process, the 3 participants that crossed-over were treated as different people and thus we can presume statistical data is more conservative.

Results

The target $\dot{V}O_2$max to categorise individuals as physically trained (i.e. >30 mL/kg/min female and >40 mL/kg/min male) was met by 12/17 participants, with the remaining five being within 6 ± 5 mL/kg/min of the targeted threshold. The overall mean $\dot{V}O_2$max for females and males was 35.1 ± 5.7 mL/kg/min 44 ± 7.5 mL/kg/min, respectively. Only 3 individuals crossed over and completed both supplement conditions, therefore the 2 groups consisted largely of different people; however, there were no differences in baseline characteristics between NRPT and placebo groups ($p>0.05$; see Table 1). The individuals that completed both arms of the study were treated as different people for the statistical analysis.

Table 1. *Population characteristics; sex, age, height, weight, BMI and* $\dot{V}O_2$max *(n=17, n= 3 crossover) in NRPT (n=10) and Placebo (n=10) groups. M= male F= female.* $\dot{V}O_2$*max determined using incremental cycle test. Values are means ± SD*

	NRPT (n=10)	Placebo (n=10)
Sex	M=5 F=5	M= 6 F=4
Age (years)	21.2 ± 1.7	21.2 ± 1.9
Height (cm)	177.1 ± 11.8	173.8 ± 7.6
Weight (kg)	71.9 ± 12.5	68.9 ± 10.1
BMI (kg/m^2)	22.8 ± 1.8	22.8 ± 2.2
$\dot{V}O_2$max (mL/kg/min)	42.0 ± 5.6	43.4 ± 8.9

TIME TRIAL- No improvement in performance with NRPT supplementation

For both normoxia and hypoxia, there was an on average improvement in 5-km performance time, albeit non-significantly, for both NRPT and placebo groups following the 4-week supplement period (NRPT: by 1.2%±0.07 and 0.2%±0.001 in normoxia and hypoxia respectively; placebo: by 2.1%±0.1 and 1.9%±0.1 in normoxia and hypoxia respectively; time*group interaction effect: p=.426; time main effect: p=.117, see Figure 7). As expected hypoxia resulted in a 4.8%±0.3 increase in TT performance in comparison to normoxia (environment main effect p=0.004). However, contrary to our hypothesis, these changes between environments were not different between NRPT and placebo groups. (time*group*environment interaction effect: p=.784;). Overall, there were no differences between groups for 5km-TT time (group main effect; p=.746

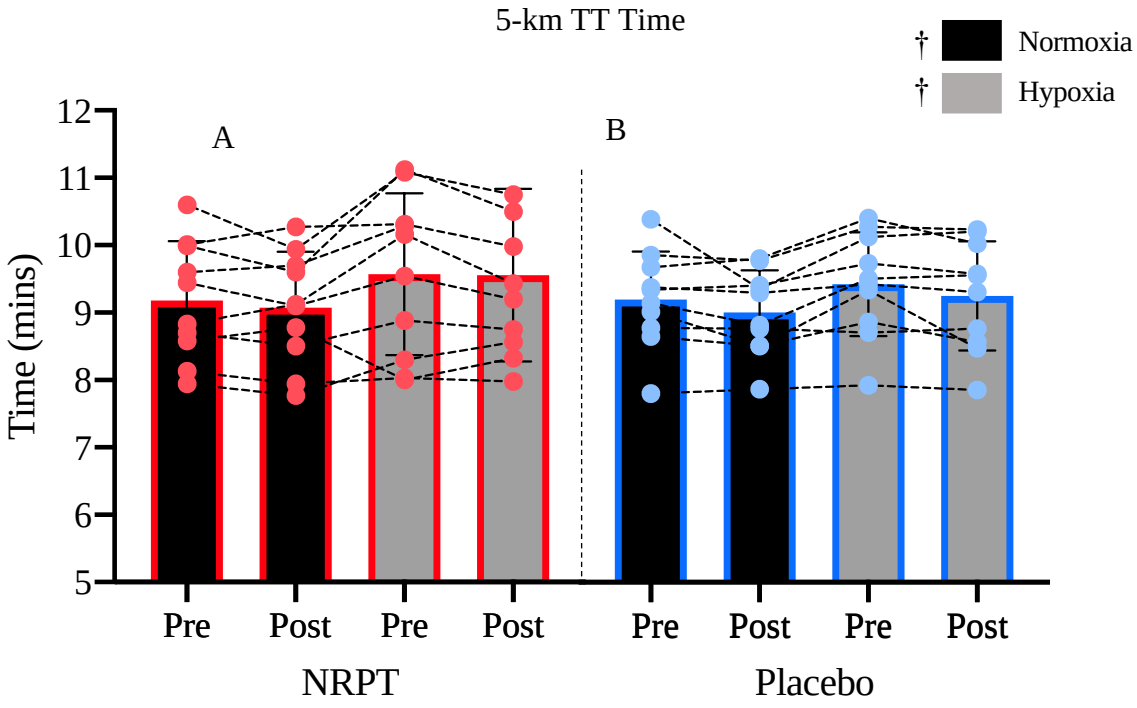

Figure 7. Mean time to complete 5-km Time Trial pre and post 4-week supplementation of NRPT (red) or placebo (blue) in normoxia and hypoxia (~15% FiO2). (A) pre and post 4-week supplementation in NRPT (red) and placebo (blue) group in the normoxic environment, (B) pre and post 4-week supplementation in NRPT (red) and placebo (blue) group in the

hypoxic environment. † significant difference between normoxia and hypoxia ($p<0.05$). All groups n=10. Values are means ± SD with individual data displayed.

POWER OUTPUT- No improvement in power with NRPT supplementation

Power output increased on average by 3.4% over the 4-week supplement period; however, this improvement, in both normoxia and hypoxia, was similar between the NRPT and placebo groups (NRPT: by 2.3%±3.3% and 1.4%±1.8% in normoxia and hypoxia, respectively; Placebo: by 4.0%±5.7% and 6.5%±9.0% in normoxia and hypoxia, respectively; time*group interaction effect p=.333; time main effect p=.037, see Figure 8). As expected, power output was lower in hypoxia than in normoxia (by 6.7%±9.2), however, this did not differ between placebo and NRPT groups (time*group*environment interaction p=.728; environment main effect p=.002). Finally, there was no difference between groups for power output across all time points (group main effect: p=.986.

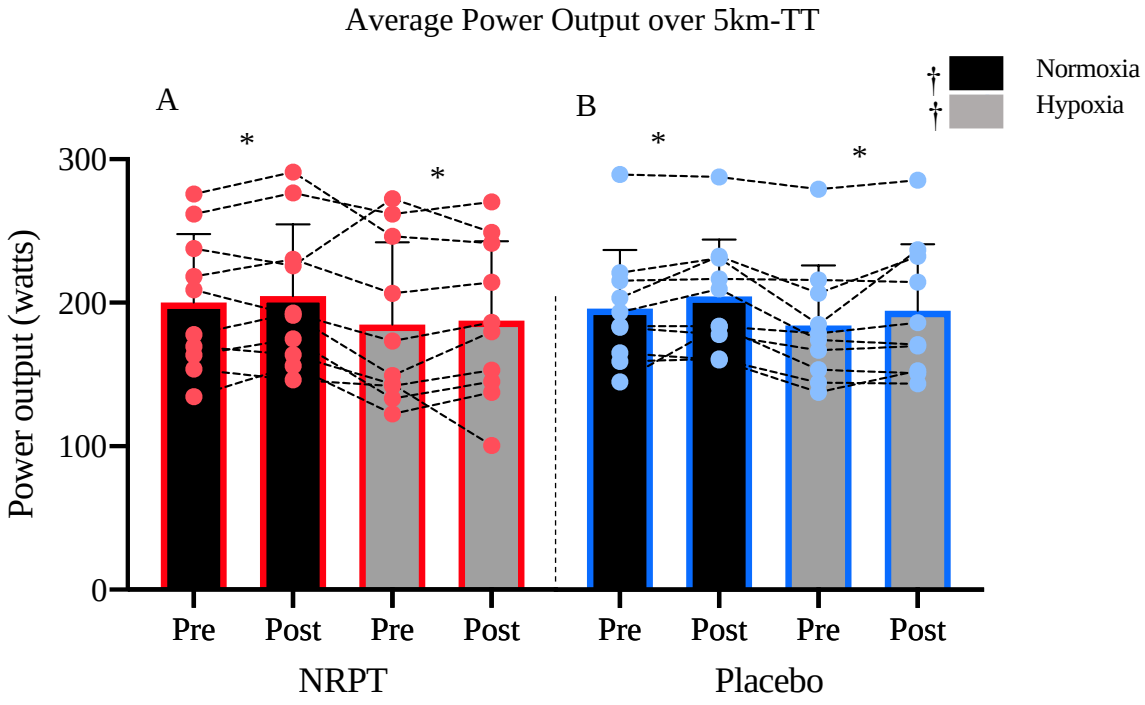

Figure 8. Mean power output over 5km Time Trial pre and post 4-week supplementation of NRPT (red) or placebo (blue) in normoxia and hypoxia (~15% FiO2). (A) pre and post 4-week supplementation in NRPT (red) and placebo (blue) group in the normoxic environment,

(B) pre and post 4-week supplementation in NRPT (red) and placebo (blue) group in the hypoxic environment. † significant difference in between normoxic and hypoxic environments ($p<0.05$); * significant difference between pre and post 4-week supplementation ($p<0.05$). All groups n=10. Values are means + SD with individual data displayed.

Power output per KM

A pacing strategy was observed over the 5-km in all conditions, as evident by a main effect over the time trial distance ($p<0.001$). Specifically, average power output was significantly different between the 1st km and 2nd km, and the 5th km was significantly greater than all preceding distance points (all $p<0.05$) (Figure 9). In line with our hypothesis, power output was lower throughout the hypoxic condition in comparison to normoxia (environment main effect $p=0.02$). However, contrary to the hypothesis, these power differences between each kilometre were similar between placebo and NRPT groups between environments (distance*group*environment interaction effect: $p=.350$).

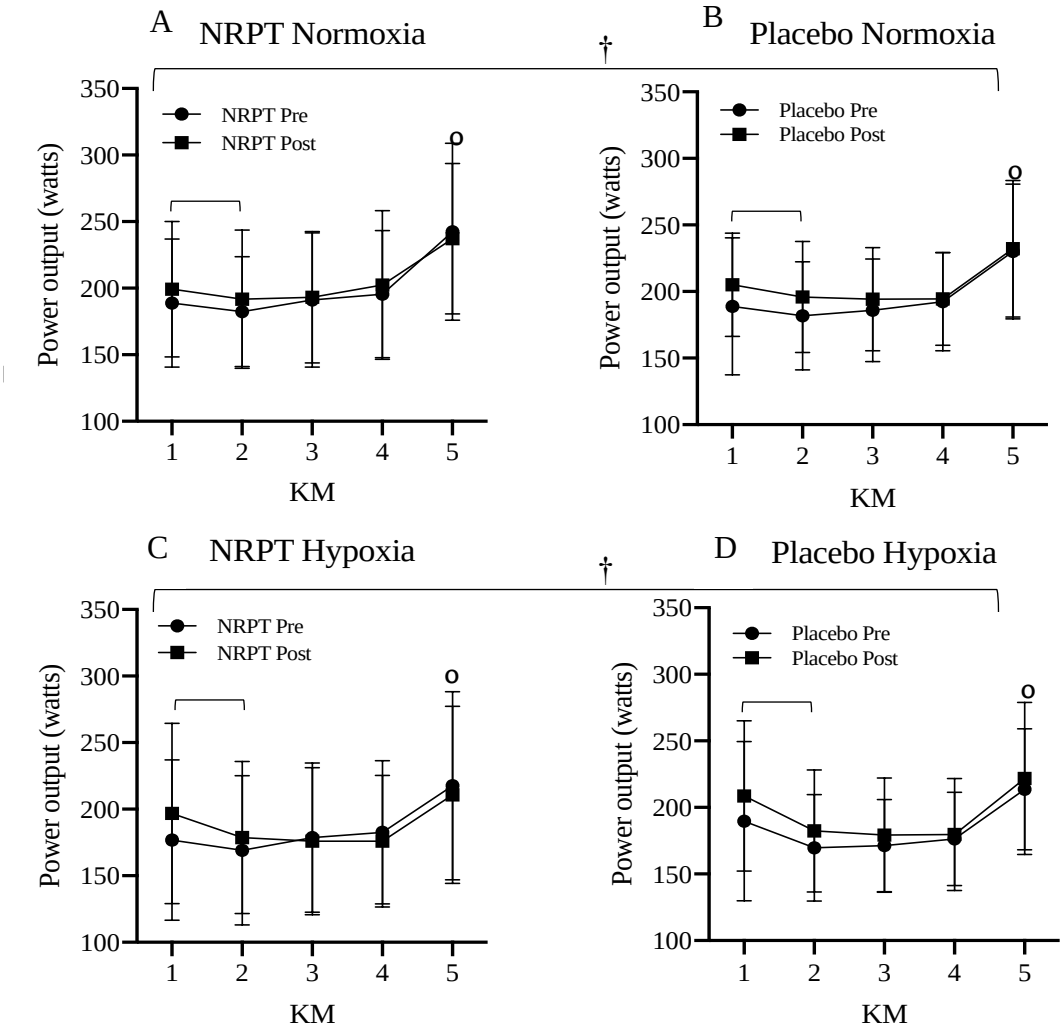

Figure 9. Mean power output taken at each kilometre (km) interval over the 5-km Time Trial pre and post 4-week supplementation of either NRPT or placebo in normoxic and

hypoxic (~15% FiO2) conditions. n=10. (A) Normoxic pre/post supplement NRPT group; (B) Normoxic pre/post supplement placebo group; (C) Hypoxic pre/post supplement NRPT group; (D) Hypoxic pre/post supplement Placebo group. Pre supplement (●) post supplement (■). † significant difference in between normoxic and hypoxic environments ($p<0.05$); º significant difference between 5km and all other distance points ($p<0.05$); ≈ significant difference between 1km and 2km ($p<0.05$). All groups n=10; Values are means ± SD

OXYGEN CONSUMPTION ($\dot{V}O_2$)

Steady state (SS) per 5 minutes

$\dot{V}O_2$ gradually increased over the initial 20 minutes when averaged over 5-minute bouts, with all time points being significantly different ($p<0.05$) until the final 10 minutes (duration main effect $p<0.001$, Figure 10). However, comparison of the averaged final 5 minutes for each session revealed no significant differences between pre/post time points or groups (all main and interaction effects $p>0.05$, Figure 11). As expected, the hypoxic condition resulted in a lower $\dot{V}O_2$ in comparison to normoxia (by 5.7%±17; environment main effect $p=.004$). However, contrary to our hypothesis, when averaged over 5-minute intervals (see Figure 10), the placebo group remained similar between environments ($p>0.05$), whereas the NRPT group displayed a lower $\dot{V}O_2$ throughout the SS in hypoxia compared to normoxia (Figure 10 and 11) (Environment*Group interaction effect $p=0.001$), however, this was not significantly different over the 4-week supplement period.

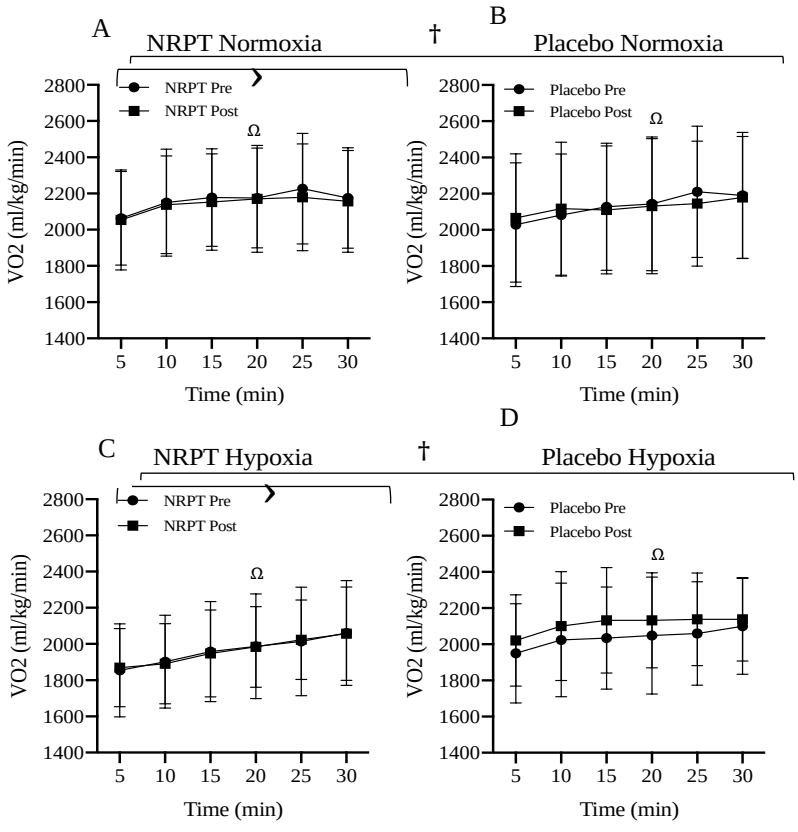

Figure 10. Mean $\dot{V}O_2$ taken over the last 1-minute of each 5-minute over the 30-minute Steady State pre and post 4-week supplementation of NRPT or placebo in normoxia and hypoxia (~15% FiO2). (A) Normoxic pre/post supplement NRPT group; (B) Normoxic pre/post supplement placebo group; (C) Hypoxic pre/post supplement NRPT group; (D) Hypoxic pre/post supplement Placebo group. Pre supplement (●); Post supplement (■). Ω significant difference between all time points excluding final 10 minutes; † significant difference between normoxic and hypoxic environments ($p<0.05$); › signifcant difference between normoxia and hypoxia in the NRPT group ($p<0.05$). All groups n=10 excluding NRPT normoxic post n=9. Placebo hypoxic post missing data points at 25mins and 30mins. Values are means ± SD

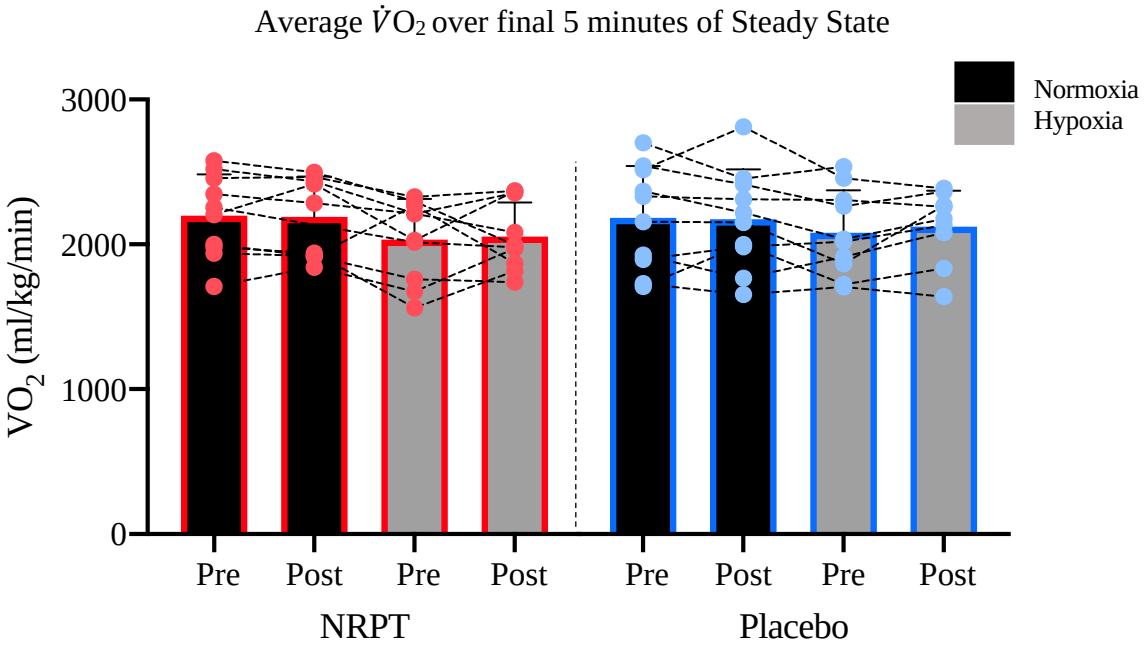

Figure 11. Mean $\dot{V}O_2$ taken from the final 5 minutes of 30-minute steady state exercise pre and post 4-week supplement of NRPT (red) or placebo (blue) in normoxia and hypoxia. (A) Pre and post 4-week supplementation in NRPT (red) and placebo (blue) group in the normoxic environment, (B) Pre and post 4-week supplementation in NRPT (red) and placebo (blue) group in the hypoxic environment. All groups n=10 excluding NRPT normoxic post n=9. Values are means + SD with individual data displayed.

Time trial per KM

All distance points were significantly different to each other over the 5-km TT, with a gradual increase in $\dot{V}O_2$ evident (main effect of distance: $p<0.05$, see Figure 12). As expected, $\dot{V}O_2$ was lower in hypoxia than normoxia (environment main effect $p=0.025$), although this was not different between placebo and NRPT groups (environment*group interaction effect $p=.088$).

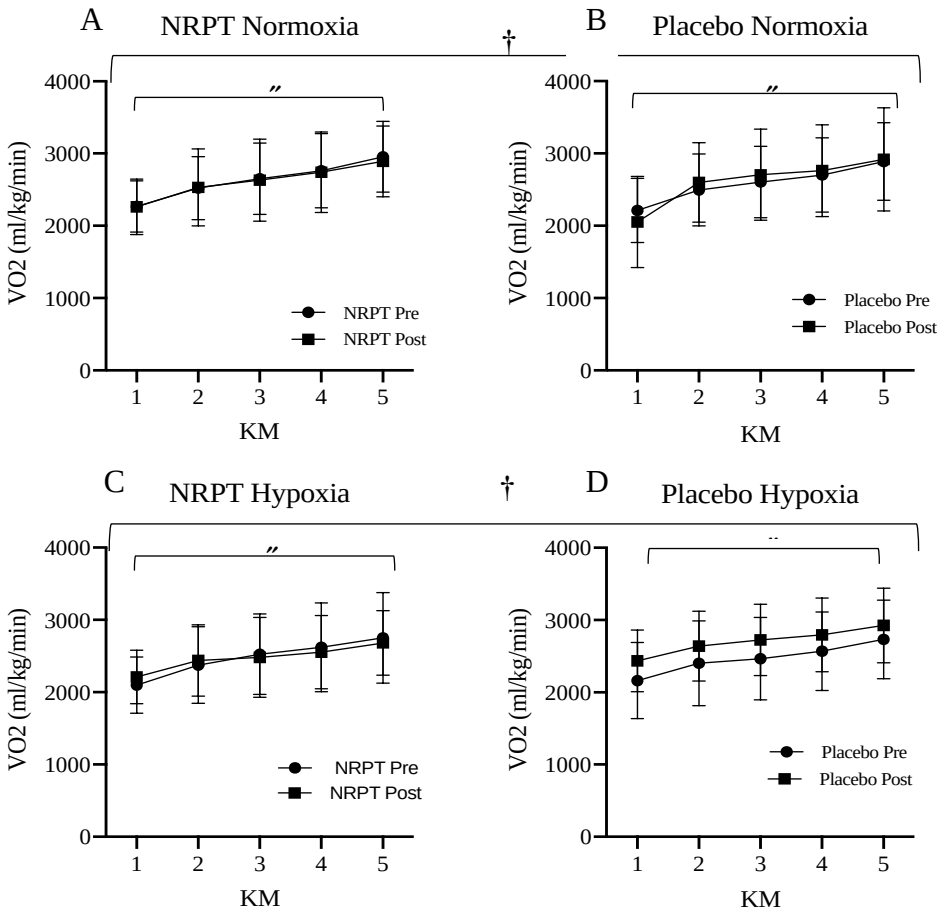

Figure 12. Mean $\dot{V}O_2$ measured from each kilometre over for the 5km Time Trial, pre and post 4-week supplementation of NRPT or placebo in normoxia and hypoxia (~15% FiO2). (A) Normoxic pre/post supplement NRPT group; (B) Normoxic pre/post supplement placebo group; (C) Hypoxic pre/post supplement NRPT group; (D) Hypoxic pre/post supplement placebo group. Pre-supplement (●); Post supplement (■) . ″ significant difference between all distance points ($p<0.05$); † significant difference between normoxia and hypoxia ($p<0.05$).

NRPT n=10, Placebo n=10 excluding normoxia post with a missing data point at 3km. Values are means ± SD.

RESPIRATORY EXCHANGE RATIO (RER)

Steady state per 5 minutes

There was an overall reduction in RER throughout all conditions (by ~5%) over the 30-minutes SS, with all-time points being significantly different except for the final two 5-minute periods where RER stabilised (duration main effect $p<0.001$, see Figure 13). RER was lower in the hypoxic condition (0.93±0.01) compared to the normoxic condition (0.96±0.01) (environment main effect p=.006). This reduction in RER was comparable in the NRPT group between both environments ($p>0.05$), however, in the placebo group the reduction in RER was greater between hypoxia (0.93±.01) and normoxia (0.97±0.01) (group*environment interaction effect p=0.049).

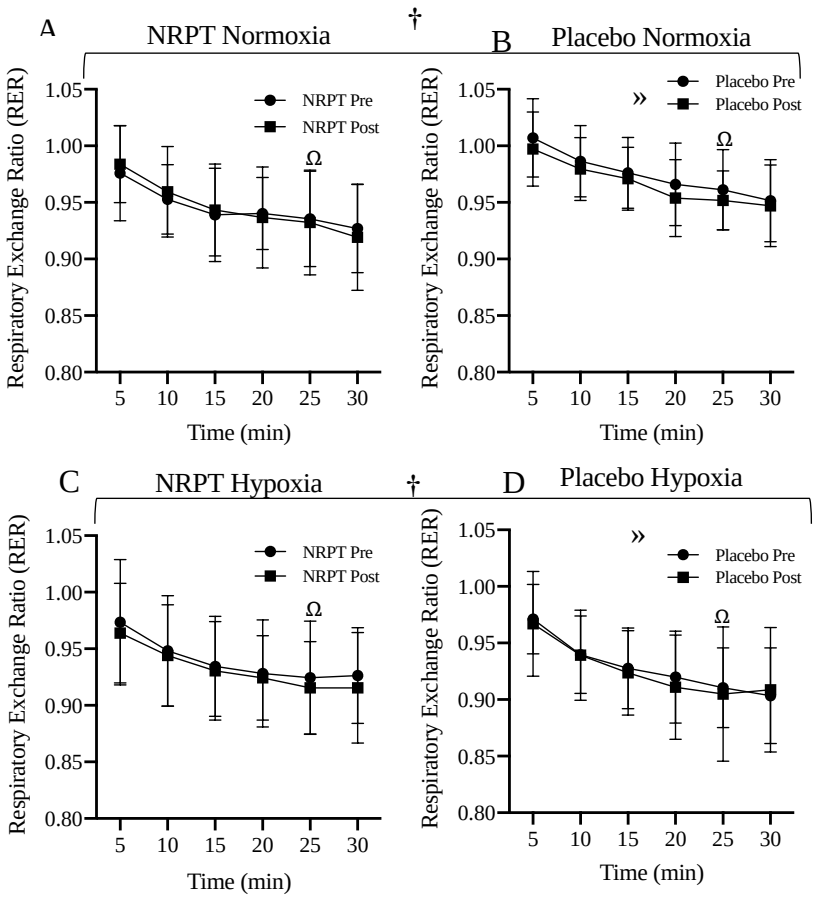

Figure 13. Mean Respiratory Exchange Ratio (RER) taken of the final 1 minute of each 5-minute bout over the 30-minute Steady State, pre and post 4-week supplementation of NRPT or placebo in normoxia and hypoxia (~15% FiO2). (A) Normoxic pre/post supplement NRPT group; (B) Normoxic pre/post supplement placebo group; (C) Hypoxic pre/post supplement NRPT group; (D) Hypoxic pre/post supplement placebo group. Pre supplement (●); Post supplement (■) . † significant difference between normoxia and hypoxia ($p<0.05$); » significant difference between normoxia and hypoxia in placebo group ($p<0.05$); Ω significant difference between all-time points excluding final 5 minutes ($p<0.05$). All groups n=10 excluding NRPT N2 n=9. Placebo H2 missing data points at 25mins and 30mins. Values are means ± SD.

FOREARM BLOOD FLOW (FBF)

There was an overall decrease in resting Forearm Blood Flow (FBF) over the 4-week supplement period from 2.78 ±.19 to 2.47 ±.12 (time main effect p=0.035), which was similar between the NRPT and placebo groups and for both normoxic and hypoxic environments (time*group*environment interaction effect p= .492) (Figure 15). As expected, post-exercise FBF was, on average greater than resting baseline measures, this exercise effect was seen in both NRPT and placebo groups (Exercise*Group interaction effect p=.026; exercise main effect p<.001). Post-exercise FBF (Figure 16) was similar between hypoxia (grey) (3.07± 1.02) and normoxia (black) (3.16±1.14) (p=.539), however, baseline FBF (Figure 15) was greater in hypoxia (2.26±.90) than normoxia (1.88±.67) (p=0.005) (environment*exercise interaction effect p=.022). This did not differ between NRPT and placebo groups (environment*exercise*group p=.958).

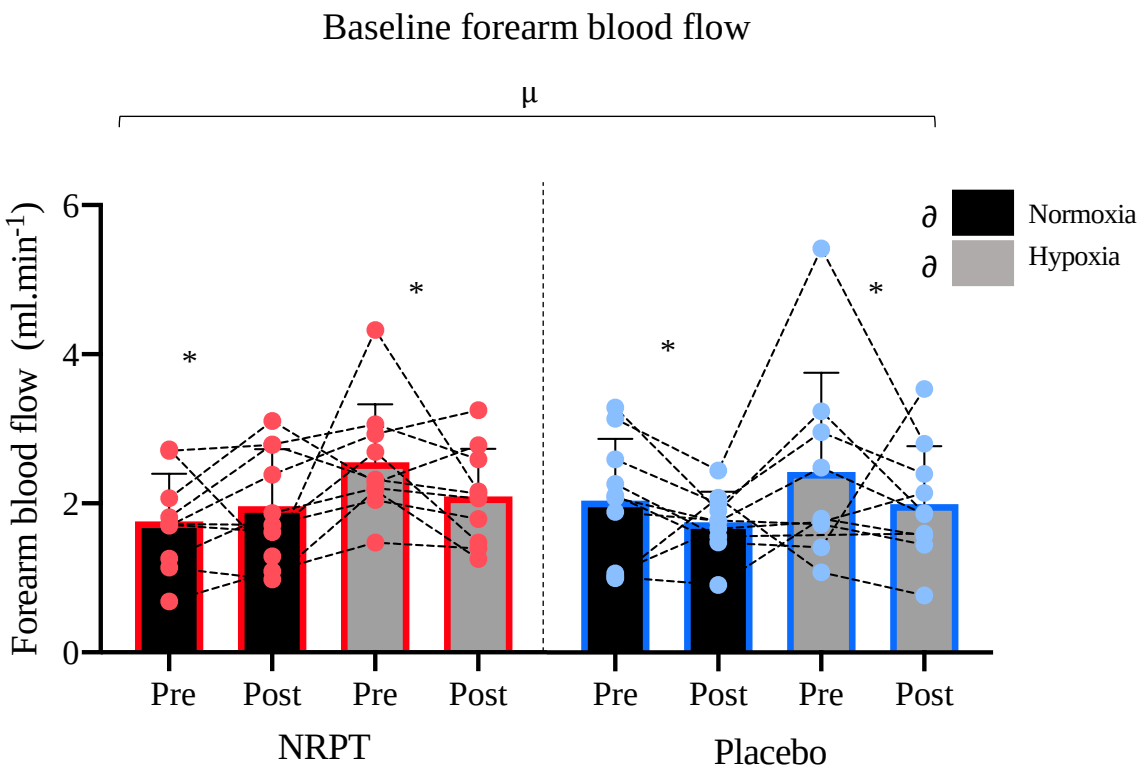

Figure 15. Mean baseline VOP pre and post 4-week supplementation in NRPT (red) and placebo (blue) group normoxia (black) and hypoxia (grey) (15% FiO2). * significant difference between pre-supplement and post -supplement ($p<0.05$); ∂ significant difference between normoxia and hypoxia at baseline ($p<0.05$); µ significant difference between baseline and post exercise blood flow ($p<0.05$). n= 10 NRPT; n=9 placebo. Values are means+ SD with individual data displayed.

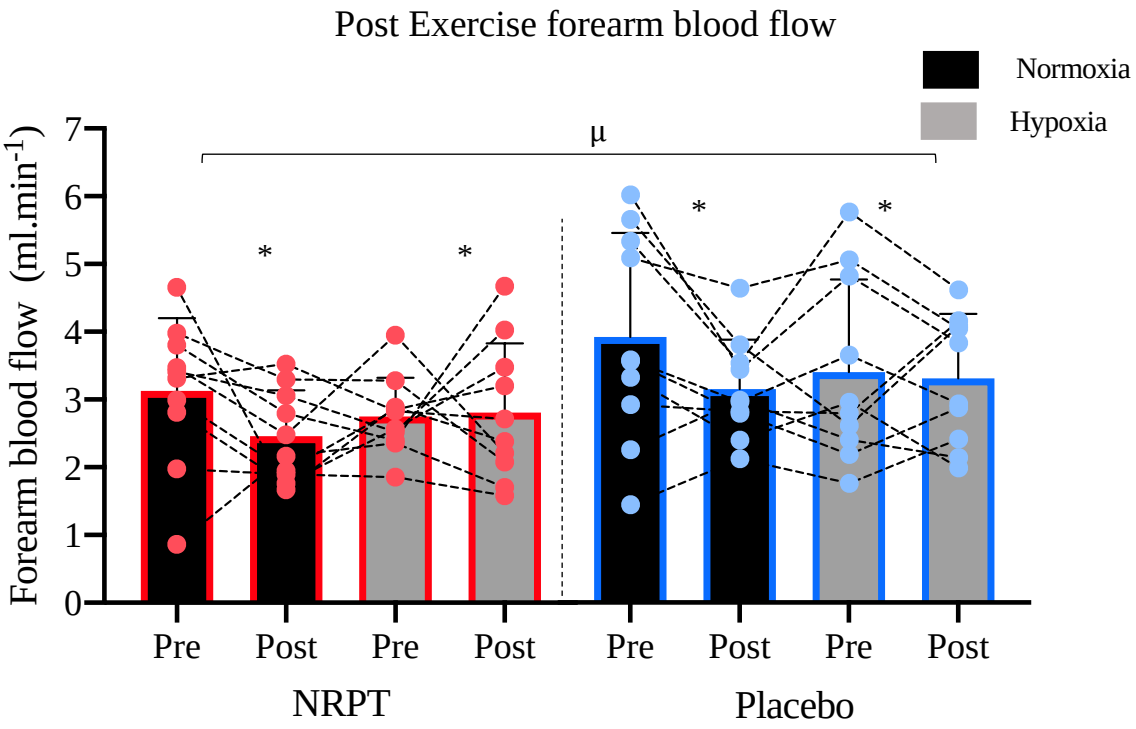

Figure 16. Mean post-exercise forearm blood flow pre and post 4-week supplementation in NRPT (red) and placebo (blue) in normoxia (black) and hypoxia (grey) (15% FiO2). * significant difference between pre-supplement and post-supplement ($p<0.05$); µ significant difference between baseline and post exercise blood flow ($p<0.05$). n= 10 NRPT; n=9 placebo. Values are means+ SD with individual data displayed.

Discussion

We have produced the first study to our knowledge, looking at the effects of chronic (4-week) NRPT supplementation on exercise performance at altitude in a young, healthy population. Current research displays discrepancies on NRPT's true response on improving exercise performance. It seems the need for a depleted baseline NAD(P)H and/or damaged muscle, such that is seen in the aged, obese or unhealthy populations (Dolopikou et al ,2019; Baur et al 2006; Trimmer et al, 2011), may be the key to yield a benefit for NRPT supplementation. Exercise at altitude has shown to produce similar physiological responses to that seen in aging, such that vasculature and mitochondrial function is impaired and NADH is accumulated. To simulate this physiological state, we have used an altitude model to manipulate the physiology of young healthy volunteers to mimic that seen in the aged population and investigate if NRPT supplementation is able to improve exercise performance in a young healthy population at altitude and thus reduce the performance decrement caused by altitude.

Overall, the data in this study suggests that NRPT had no beneficial influence on exercise performance such that there was no increase in blood flow or shift in substrate utilisation at altitude. This is justified by a lack of significant difference in the performance measures; Time, Power, $\dot{V}O_2$ and Blood flow, between the NRPT and placebo group and thus is consistent with current research in young healthy human participants, reporting either no improvement or a detriment to performance. This could be explained by either; being unable to modify physiology to mimic older adults with altitude and therefore unlikely to see a benefit, or, the change caused by altitude did produce the physiology and the supplement is not beneficial in this environment.

Performance Outcomes

A 5km-TT was used as an assessment of performance, specifically looking at time to complete and power output. Comparable improvements were observed in performance between the placebo and NRPT groups in relation to an improved 5km-TT time and enhanced power over the 4-week intervention period, with the marginal improvement in power likely due to a learnt response or improvement in fitness, rather than a direct effect from the supplementation. Thus, the results seem to be in line with current literature reporting that NRPT supplementation does not have a positive influence on exercise performance in a young, healthy, population (Dolopikou et al, 2019; Canto et al, 2012; LaGouge et al, 2006). Consistent with our hypothesis, a difference between environmental conditions (normoxia and hypoxia) was observed throughout both time and power over the TT. A significant compromise was observed in the hypoxic environment compared to normoxia, resulting in an increased time to complete 5km (Figure 7) and a decrease in power output (Figure 8). However, this was not different between groups. This environmental discrepancy is accounted for by the natural reduction in oxygen content in a hypoxic climate. This subjects participants to a greater reduction in skeletal muscle oxygenation in comparison to exercising at sea level (Subudhi et al, 2007), this reduction has also been reported in older adults with a reduction in oxygen demand, thought to be due to a rise in sympathetic vasoconstrictor nerve activity (Dinenno et al,1999). Although the data was unable to show this reduction in a quantitative manner, via muscle oxygenation due to a fault in the NIRS equipment. This reduction in oxygen content therefore meant participants were unable to work at the same given workload as in the normoxic condition or needed to work harder to sustain the same performance outcomes. Work rate initially caused too much impairment between environments and resulted in blinding being unsuccessful and participants struggling to complete the outlined task. This led to the alteration of the SS work rate and TT distance to

produce equilibrium between the environments and more reliable results. However, by reducing the intensity at altitude it may have removed the stimulus needed to produce the necessary physiological detrimental required for NRPT to take effect. Yet, literature widely suggests that exercise capacity is impaired at altitudes considerably below the 2500m used in this study (Gore et al, 1997; Peltonen et al, 2001) thus, considering current evidence based on older adults and the connection between exercising at altitude and aging such that, vasculature becomes compromised (Balsom et al, 1994) and mitochondrial function is impaired (Finck, 2006 & Handschin, 2008), 2500m should have provided a great enough impairment to yield a response from NRPT. Moreover, to support this claim, Figure 7 and 8 display a noticeable detriment to performance due to the environment. This is further justified by the consistent trend of significance throughout measures in both groups and over the 4-weeks between normoxia and hypoxia. Thus, the data displays that hypoxia caused a noteworthy detriment to performance such that, if in line with current evidence in older adults, should have produced a beneficial response from NRPT supplementation. From this the data concludes that due to the performance outcome measures, chronic supplementation of NRPT did not have the expected influence of improving exercise performance at altitude, however, this may not fully disprove the use of NRPT at altitude. A possible explanation may be that, although altitude produces a detriment to vasculature, it may require a compromise at a more cellular level such that has been reported by Dolopikou et al (2019), showing that beneficial responses from supplementation of NRPT were only identified in conditions where baseline NADH was initially compromised.

Metabolic Influence

Metabolic shift in substrate utilisation

No significant shift in substrate utilisation between Steady State and Time Trial performances was observed. This trend was continued between groups and over the 4-week intervention

period, showing no improvement in substrate flexibility. Based on current literature that has reported an increase in lipid oxidation results in a sparing action of CHO, (Odland et al (1998) it was thought that by promoting lipid oxidation via supplementation of NRPT during submaximal steady state exercise the sparing action of CHO may then be utilised in subsequent higher intensity exercise such as the 5km-TT in this study. It was expected that this substrate flexibility would be mediated by the upregulation of SIRT1 via PT consumption (Price et al, 2012). SIRT1 is associated with lipid oxidation and has been linked to the expression of metabolic genes such as AMPK and PGC-1a both of which have been shown to promote substrate shifts in exercise (Wenz, 2013; Gomes et al, 2013). A range of literature supports this metabolic action of NR and PT. Canto et al (2012) showed that NR supplemented, obese mice displayed a reduced weight gain compared to chow fed mice attributing this to metabolic actions via increased lipid oxidation and insulin sensitivity, however, when tested in obese men NR was found to have no response on substrate utilisation or insulin sensitivity such that was seen in rodents (Dollerup et al, 2018). A study by O'Neil et al (2004) even reported a detriment to performance in young trained individuals with supplementation of NA, a niacin similar to NR, suggesting this to be due to a reduction in exercise-induced increase in plasma free fatty acids and a shift in substrate utilisation resulting in an earlier onset of fatigue. This was supported in a rodent study supplemented with NR by Kourtzidis et al (2016). They found a reduced time to exhaustion and a dysfunction in redox and energy metabolism after 21 days supplementation. This highlights the discrepancy within the literature in terms of the metabolic effects and responses caused by NR supplementation on exercise performance. The lack of significance in substrate utilisation in this study may be explained by the lack of impairment in baseline NADH and\or damage to muscle (Dolopikou et al, 2019) to yield a beneficial response from the supplement explained previously, that may not have been simulated by the altitude model. This is further

clarified by the lack of significance between the NRPT and placebo group over the 4-week supplement period in both the performance outcomes outlined above but also the vascular and metabolic measures; $\dot{V}O_2$, RER and forearm blood flow. The data also shows that both $\dot{V}O_2$ and RER begin to stabilise in the final 10 minutes of the 30 minutes steady state cycle (Figure 10 and 13). Although not different between the two conditions (NRPT and placebo), the stabilising of RER and $\dot{V}O_2$ towards the latter part of submaximal exercise does suggest a possible explanation for this similarity and lack of substrate flexibility. It is unlikely the intensity of the steady state exercise solely influenced the stabilising of substrate utilisation instead a major factor that may have considerably influenced the results is the duration of the steady state exercise. According to evidence, 30-minutes may be on the border of producing the desired response of substrate flexibility with Watt et al (2002) showing that a decline in CHO oxidation is only seen after 120-minutes of cycling exercise at 55% $\dot{V}O_2$ max. Thus, the results highlight the possibility that the SS exercise bout used in this study was not long enough to promote a shift in substrate utilisation. It is unlikely to have been due to not achieving physiological steady state, but that to promote a shift in substrate oxidation a longer overall duration is required than that used in this study. An additional influencing factor for the lack of substrate shift, may have been the fitness of the cohort producing an overriding influence on substrate flexibility. Bowden & McMurray (2000) investigated the influence of aerobic training status on fat and CHO metabolism in women at rest. Although they reported that training status had no effect on postprandial metabolic rate or total energy expenditure, they went on to state that trained women had greater fat oxidation following a CHO meal compared to untrained. This possible training status influence is supported by Djelic et al (2015) who suggested metabolic response to a single bout of maximal-intensity is subject to training status such that trained individuals have greater substrate flexibility. Thus, with a relatively low trained cohort the ability to shift substrate utilisation may have not be

achieved. This has been supported by Hetlelid and colleagues (2015) that confirmed well trained individuals performed better with a three-fold higher rates of fat oxidation, also reporting that elevated fat oxidation had a strong correlation with $\dot{V}O_2$max. The mean $\dot{V}O_2$max of the cohort was 30mL/min/kg and 40mL/min/kg for female and male respectively, considering the research above, may have had an impact on the ability to complete the outlined task and thus implicated the data produced. Specifically, not having the cardiovascular capacity to comfortably complete the outlined task and thus having to work harder, resulting in a drive towards a mix of both fat and CHO utilisation rather than predominately fat oxidation during the submaximal exercise. The RER data displayed no significant change over the 4-week intervention period and with the cohort being generally untrained, it is likely that the training status of the participants meant that we may not have been able to alter substrate utilisation and thus not observed a shift between lipid and CHO oxidation. However, homogeneity was sustained between the groups as seen in Table 1 and therefore it can be assumed that the data produced can be rightfully compared. Taking these factors into consideration, it may be advised in future studies to use a more trained cohort and increase the steady state period to produce a greater and more obvious stimulus for fat oxidation. According to Watt et al (2002) this could be achieved with a duration above 120 minutes. Additionally, the data also displayed a significantly lower $\dot{V}O_2$ in the SS in the NRPT group in both normoxia and hypoxia (Figure 10, graph C). However, this did not change over the 4-week intervention period and is therefore unlikely to be a direct response from NRPT supplementation. Rather, an expected environmental response that resulted in a reduced $\dot{V}O_2$ from exercise in normoxia that was not re-established from supplementation from NRPT. However, interestingly the placebo group showed a non-significant increase in $\dot{V}O_2$ ($p=0.08$) over the 4-week supplementation period in the hypoxic environment (Figure 10, graph D). Additionally, as mentioned above there was an alteration to exercise intensities,

that being a reduced intensity during the hypoxic environment. There is relatively limited metabolic research on exercise at altitude with current recommendations to mirror those suggested for sea level (Burke et al., 2019; Stellingwerff, 2013; Stellingwerff et al., 2019a). However, it is propsed that altitude results in an increase CHO oxidation (Brooks et al, 1991). Contrary to this, a lower RER, related to fat oxidation, was seen in the hypoxic environment in the data in this study. The reduction of exercise intensity at altitude may be the explanation behind this discrepancy with current evidence, suggesting that the alteration in intensity may have removed the stimulus of hypoxia and consequently may not have provided the necessary catalyst to promote the proposed metabolic properties of NRPT."

Supplement Ingestion and Food Consumption

Timing of supplement ingestion and type and timing of food consumption prior to visits may have also contributed towards this lack of significance in substrate utilisation. Due to the attempt to mirror a real-life situation it was decided not to provide a controlled diet, however, participants were asked to consume a similar diet prior to all testing visits and were not fasted, this was verbally checked. There is however, a possibility that substrate metabolism data may have reflected individual dietary habits which could have been eliminated by a fully controlled diet. It is important to note that due to the differences in testing times between participants some participants who exercised at midday may have been fasted for 4/5 hours in comparison to someone exercising in the morning ~1 hour after eating a potentially high CHO breakfast. The contribution of macronutrients within a meal has the ability to promote specific substrate utilisation, such that a high CHO meal would promote greater CHO utilisation in comparison to a high fat diet which produces a drive towards an increase in fat oxidation (Christensen and Hansen 1939; Griffiths et al, 1994a). Consequently, this would have skewed RER data and underrepresented or overrepresented any impact from NRPT supplementation. The use of a controlled diet and fasted exercise would have eliminated any discrepancy that is produced from macronutrient consumption and therefore, allowed the opportunity to make a more informed conclusion on the metabolic influence on NRPT. Although, mirroring a real-life situation is a valuable tool in nutrition studies, to fully understand the actions from NRPT supplementation it would be useful to standardise the time that exercise testing took place in relation to consumption of food, for example a meal 1 hour before exercise or fasted exercise completed in the morning.

Furthermore, an important consideration when using nutraceuticals is their appearance and metabolism in the body (half-life). Trammel et al (2016) reported that NAD+ and NADP+

were elevated by the ingestion of NR however they then went on to state that these levels remained elevated for the subsequent 8hours post NR supplementation. In this study participants were instructed to take both tablets together at the same time of day each day. Upon verbal questioning all participants took the tablets in the morning on waking/with breakfast. Taking into consideration this 8hr window and the time of exercise testing it may have caused some discrepancy where the morning and afternoon sessions may have differed in the appearance of NAD+ in the body. Although this cannot be justified in this study, it would be worthwhile in future studies to consider taking venous blood samples and/or muscle biopsies to confirm the appearance of NAD+ in relation to supplement ingestion. A less invasive technique to overcome this difference in appearance would be to instruct participants to take 1 tablet upon waking in the morning and the other before bed in the evening, this would provide a more sustained elevation of NAD+ and overcome any discrepancy between testing times between participants. As mentioned above, this factor could have been measured by the use of more invasive techniques such as venous blood samples and muscle biopsies to provide a more cellular level of analysis and justification. However, the data clarifies that the chronic supplementation (4-weeks) of Nicotinamide Riboside (500mg) and Pterostilbene (100mg) in combination is a safe dosage to use in a young, healthy population from the appearance of no acknowledged adverse effects from participants.

Vascular Influence

Exercising at altitude has been shown to produce an impairment at altitudes as low as 600m (Gore et a, 1997) and with the accessibility of higher altitudes and the increase in world competitions held at altitude the need to reduce this impairment is crucial to retain the high-class level of elite sport. Exercising at altitude causes a strain on the vasculature such that has

been characterised by a lower availability of oxygen resulting in lower arterial oxygenation causing vasoconstriction and reduced oxygen to the working muscles. In addition, Wengrowski (2013) highlighted that under hypoxic conditions the heart becomes ischemic and results in an accumulation of NADH, the presence of oxygen is required in this process to oxidise NADH to NAD+, a key molecule in the maintenance of mitochondrial function and other processes, such as ATP synthesis, required to sustain physical exertion. These impairments subsequently result in a reduced capacity to perform exercise. The need to bridge the gap between exercise at altitude and sea level has produced a demand for an intervention such that can possibly be filled by nutraceuticals. These physiology responses seen at altitude are similar to those observed during the ageing process such as decreased NAD+ levels, vascular dysfunction, impaired vasculature in terms of vasoconstriction and reduced O_2 intake partly due to a reduction in muscle mass and therefore an overall reduction in demand for O_2 (Dinenno et al,1999; Seals et al, 2011).

Evidence has shown that altitude and aging results in vasoconstriction of blood vessels meaning an increase in blood pressure and blood flow (Faoro et al, 2017). Exercise is a natural stimulus that causes an increase in blood flow (Hellström & Wahlgren, 1993). The data in this study clearly shows a significant increase in forearm blood flow from baseline to post-exercise forearm in both conditions and environments as expected (Figure 15 and 16). However, a study by Martens et al (2018) in both healthy middle aged and elderly adults over a 6-week intervention reported an improvement in cardiovascular measures using FMD and a 50% increase in NAD+, however, these were only seen in the elderly group and not in the middle-aged group, thus, we hypothesised that NRPT would then result in an increase in blood flow in comparison to the placebo group in hypoxia, due to its action on the vasculature via upregulation of SIRT1 and NO, however, this was not shown in this study. In fact, over the 4-week supplementation period overall blood flow decreased in both groups. In

addition, and noteworthy, a greater increase in blood flow from baseline to post-exercise was observed in the placebo group (73%) in comparison to the NRPT group (33%) suggesting that NRPT may have had an inhibitory effect on blood flow in both normoxia and hypoxia (Figure 15 and 16). This would support research reporting an impairment in redox reactions and energy metabolism in rodent (Kourtzidis et al, 2016) and in humans an impairment in exercise performance due to reduced PGC-1a, SIRT1 and SOD2 gene expression (Scribbans et al, 2014).

Considerations

The use of altitude in this study was used as an attempt to create the physiological responses similar to those seen in ageing and in an unhealthy population, such that physiology and exercise performance is impaired. A simulated altitude of 2500m was produced at an attempt to blind the participants from the environmental condition they were in during each visit. It has been suggested that this is a safe altitude with ~0.01% susceptibility to high altitude pulmonary oedema at 2500m (Hall et al, 2003), allowing participants to be blinded throughout each visit. Burtscher and Koch (2016) outlined a safe acclimatisation protocol that is necessary when reaching altitudes greater than 2500m, suggesting that <2500m may not require the same level of awareness before exposure. This therefore seemed to be the upper limit of an acceptable and suitable altitude to produce the desired exercise responses without eliciting any health or physiological identification to the participants. The lack of performance improvement may provide an argument that 2500m was not high enough to cause the necessary detriment to physiology to elicit a beneficial response with NRPT supplementation, however, literature widely suggests that exercise capacity is impaired at altitudes considerably below the 2500m used in this study and therefore, should provide a great enough stimulus to impair performance (Gore et al, 1997; Peltonen et al, 2001). The

data supports this idea and successfully shows that exercise at a simulated altitude of 2500m caused a detriment to performance in comparison to the normoxic environment, such that $\dot{V}O_2$, time and power output were all lower in the hypoxic environment compared to the normoxic environment, providing justification in the model produced to test the effect of supplementation. The use of W_{max} in comparison to $\dot{V}O_2$ max sets the intensity at a marginally higher level and therefore could have also contributed to a lack of significance. Using $\dot{V}O_2$ max may have meant that those in the lower range of fitness may have been more able to complete the outlined task to a stronger standard. Time Trial performance may have been compromised by the lack of fitness and cycling experience of the cohort. Although previous cycling experience was not a desired characteristic for the study, the range of ability to cycle effectively may have added to the discrepancy in the results between participants. Further to this, initially, relatively fit individuals were to be used to lower potential training effects on performance outcomes. However, due to time restrictions, sample size was prioritised over excluding participants that didn't quite meet inclusion criteria, with 12/17 individuals achieving the desired $\dot{V}O_{2\ max}$. Under no time restraint the exclusion of these participants would have been upheld. Nevertheless, all participants were at least moderately trained, and the fitness of participants was similar between groups. Due to the novel scope of this supplement under the proposed environmental conditions the compromise seemed justified. These are areas to consider in future studies in addition to the those already discussed, such as a prolonged submaximal exercise bout, venous blood samples, muscle biopsies and standardising macronutrient influence via fasting. Finally, the use of a cross over design, allows the participants to be their own control and thus allows for greater reliability and accuracy of results. As only 3 participants crossed over in this study, completing the other arm of the study, it was decided that they would be treated as individuals in the statistical analysis process. Thus, it is likely that this will have produced more conservative

results, and therefore, the reader can presume that stated values in this study are an under-estimation of actual significance.

Conclusion

The results formed in this study suggest that chronic supplementation of NRPT in young, healthy participants improved exercise performance over 4-weeks, similarly to placebo. There was no obvious metabolic action between the NRPT and placebo group such that showed a shift in substrate utilisation to produce a performance outcome. Forearm blood flow measures suggested that NRPT supplementation may have resulted in a detriment to blood flow which could have had implicated exercise performance by further compromising the perfusion of working muscles and ultimately causing earlier onset of fatigue. However, the data successfully shows that exercise performance is impaired at a simulated altitude of 2500m. From this study, the data concludes that NRPT supplementation in a population of 20 young, health humans and at 2500m of simulated altitude does not produce vascular or metabolic actions to improve exercise performance at altitude. Future studies should consider increasing the duration of submaximal exercise to promote a greater shift in substrate utilisation, in addition to taking blood and muscle samples.

References

Achten, J. and Jeukendrup, A. (2003). Maximal Fat Oxidation During Exercise in Trained Men. *International Journal of Sports Medicine*, 24(8), pp.603-608.

Amann. M., Calbet. J,A. (2008). "Connective oxygen transport and fatigue". J Apply Physiol.Vol. 104., pp.861-870.

Asensi, M., Medina, I., Ortega, A., Carretero, J., Baño, M., Obrador, E. and Estrela, J. (2002). Inhibition of cancer growth by resveratrol is related to its low bioavailability. *Free Radical Biology and Medicine*, 33(3), pp.387-398.

BALSOM, P., GAITANOS, G., EKBLOM, B. and SJÖDIN, B. (1994). Reduced oxygen availability during high intensity intermittent exercise impairs performance. *Acta Physiologica Scandinavica*, 152(3), pp.279-285.

Balsom, Gaitanos, Söderlund and Ekblom (1999). High-intensity exercise and muscle glycogen availability in humans. *Acta Physiologica Scandinavica*, 165(4), pp.337-345.

Baur, J., Pearson, K., Price, N., Jamieson, H., Lerin, C., Kalra, A., Prabhu, V., Allard, J., Lopez-Lluch, G., Lewis, K., Pistell, P., Poosala, S., Becker, K., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K., Spencer, R., Lakatta, E., Le Couteur, D., Shaw, R., Navas, P., Puigserver, P., Ingram, D., de Cabo, R. and Sinclair, D. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 444(7117), pp.337-342.

Belenky, P., Racette, F., Bogan, K., McClure, J., Smith, J. and Brenner, C. (2007). Nicotinamide Riboside Promotes Sir2 Silencing and Extends Lifespan via Nrk and Urh1/Pnp1/Meu1 Pathways to NAD+. *Cell*, 129(3), pp.473-484.

Bergman, B. and Brooks, G. (1999). Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *Journal of Applied Physiology*, 86(2), pp.479-487.

Biala, A., Dhingra, R. and Kirshenbaum, L. (2015). Mitochondrial dynamics: Orchestrating the journey to advanced age. *Journal of Molecular and Cellular Cardiology*, 83, pp.37-43.

Bogan, K. and Brenner, C. (2008). Nicotinic Acid, Nicotinamide, and Nicotinamide Riboside: A Molecular Evaluation of NAD+Precursor Vitamins in Human Nutrition. *Annual Review of Nutrition*, 28(1), pp.115-130.

Bowden, V. and McMurray, R. (2000). Effects of Training Status on the Metabolic Responses to High Carbohydrate and High Fat Meals. *International Journal of Sport Nutrition and Exercise Metabolism*, 10(1), pp.16-27.

Bowtell, J. and Kelly, V. (2019). Fruit-Derived Polyphenol Supplementation for Athlete Recovery and Performance. *Sports Medicine*, 49(S1), pp.3-23.

Broeder, C., Brenner, M., Holman, Z., Paijame, I., Thomas, E. and Wilmore, J. (1991). THE METABOLIC CONSEQUENCES OF LOW AND MODERATE INTENSITY EXERCISE

WITH OR WITHOUT FEEDING IN LEAN & BORDERLINE OBESE MALES. *Medicine and Science in Sports and Exercise*, 15, pp.95-104.

Burke, L. (2015). Re-Examining High-Fat Diets for Sports Performance: Did We Call the 'Nail in the Coffin' Too Soon?. *Sports Medicine*, 45(S1), pp.33-49.

Burke, L., Angus, D., Cox, G., Cummings, N., Febbraio, M., Gawthorn, K., Hawley, J., Minehan, M., Martin, D. and Hargreaves, M. (2000). Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. *Journal of Applied Physiology*, 89(6), pp.2413-2421.

Burke, L.M., A.E. Jeukendrup, A.M. Jones, and M. Mooses (2019). Contemporary nutrition strategies to optimize performance in distance runners and race walkers. Int. J. Sport Nutr. Exerc. Metab. 29:117-129

Burtscher, M. and Koch, R. (2016). Effects of Pre-acclimatization Applying the ''Climb High and Sleep Low'' Maxim: An Example of Rapid but Safe Ascent to Extreme Altitude. *Journal of Human Performance in Extreme Environments*, 12(2).

Brooks, G.A., G.E. Butterfield, R.R. Wolfe, B.M. Groves, R.S. Mazzeo, J.R. Sutton, E.E. Wolfel, and J.T. Reeves (1991). Increased dependence on blood glucose after acclimatization to 4,300 m. J. Appl. Physiol. 70:919-927.

Brosnan, M., Martin, D., Hahn, A., Gore, C. and Hawley, J. (2000). Impaired interval exercise responses in elite female cyclists at moderate simulated altitude. *Journal of Applied Physiology*, 89(5), pp.1819-1824.

Cantó, C., Houtkooper, R.H., Pirinen, E., Youn, D.Y., Oosterveer, M.H., Cen, Y., Fernandez-Marcos, P.J., Yamamoto, H., Andreux, P.A., Cettour-Rose, P., Gademann, K., Rinsch, C., Schoonjans, K., Sauve, A.A., Auwerx, J. (2012). "The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet induced obesity". Cell Metabolism. Vol. 15., 838-847.

Cantó, C., Jiang, L., Deshmukh, A., Mataki, C., Coste, A., Lagouge, M., Zierath, J. and Auwerx, J. (2010). Interdependence of AMPK and SIRT1 for Metabolic Adaptation to Fasting and Exercise in Skeletal Muscle. *Cell Metabolism*, 11(3), pp.213-219.

Cantó, C., Gerhart-Hines, Z., Feige, J., Lagouge, M., Noriega, L., Milne, J., Elliott, P., Puigserver, P. and Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature*, 458(7241), pp.1056-1060.

Cakir-Atebek, H., Özdemir, F., Colak, R. (2015). "Oxidative stress and antioxidant responses to progressive resistance exercise intensity in trained and untrained males". Biol Sport. Vol. 32., pp.321-8

Chang, H. and Guarente, L. (2014). SIRT1 and other sirtuins in metabolism. *Trends in Endocrinology & Metabolism*, 25(3), pp.138-145.

Chapman, M., Assmann, G., Fruchart, J., Shepherd, J. and Sirtori, C. (2004). Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid

– a position paper developed by the European Consensus Panel on HDL-C*. *Current Medical Research and Opinion*, 20(8), pp.1253-1268.

Cheng, Y., Di, S., Fan, C., Cai, L., Gao, C., Jiang, P., Hu, W., Ma, Z., Jiang, S., Dong, Y., Li, T., Wu, G., Lv, J. and Yang, Y. (2016). SIRT1 activation by pterostilbene attenuates the skeletal muscle oxidative stress injury and mitochondrial dysfunction induced by ischemia reperfusion injury. *Apoptosis*, 21(8), pp.905-916.

Chong, M., Macdonald, R. and Lovegrove, J. (2010). Fruit polyphenols and CVD risk: a review of human intervention studies. *British Journal of Nutrition*, 104(S3), pp.S28-S39.

Clark, S., Bourdon, P., Schmidt, W., Singh, B., Cable, G., Onus, K., Woolford, S., Stanef, T., Gore, C. and Aughey, R. (2007). The effect of acute simulated moderate altitude on power, performance and pacing strategies in well-trained cyclists. *European Journal of Applied Physiology*, 102(1), pp.45-55.

Connell, N., Houtkooper, R. and Schrauwen, P. (2019). NAD+ metabolism as a target for metabolic health: have we found the silver bullet?. *Diabetologia*, 62(6), pp.888-899.

Cox, P., Kirk, T., Ashmore, T., Willerton, K., Evans, R., Smith, A., Murray, A., Stubbs, B., West, J., McLure, S., King, M., Dodd, M., Holloway, C., Neubauer, S., Drawer, S., Veech, R., Griffin, J. and Clarke, K. (2016). Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metabolism*, 24(2), pp.256-268.

Coyle, E., Jeukendrup, A., Wagenmakers, A. and Saris, W., 1997. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *American Journal of Physiology-Endocrinology and Metabolism*, 273(2), pp.E268-E275.

Dellinger, R., Santos, S., Morris, M., Evans, M., Alminana, D., Guarente, L. and Marcotulli, E. (2017). Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD+ levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. *npj Aging and Mechanisms of Disease*, 3(1).

Dinenno, F., Jones, P., Seals, D. and Tanaka, H. (1999). Limb Blood Flow and Vascular Conductance Are Reduced With Age in Healthy Humans. *Circulation*, 100(2), pp.164-170.

Djelic, M., Mazic, S., Lazovic, B., Zikic, D., Sumarac-Dumanovic, M. and Micic, D. (2015). Carbohydrate and fatty acid metabolism responses to a graded maximal exercise test and recovery period in athletes and sedentary subjects. *Science & Sports*, 30(6), pp.321-327.

Dollerup, O., Christensen, B., Svart, M., Schmidt, M., Sulek, K., Ringgaard, S., Stødkilde-Jørgensen, H., Møller, N., Brenner, C., Treebak, J. and Jessen, N. (2018). A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. *The American Journal of Clinical Nutrition*, 108(2), pp.343-353.

Dolopikou, C., Kourtzidis, I., Margaritelis, N., Vrabas, I., Koidou, I., Kyparos, A., Theodorou, A., Paschalis, V. and Nikolaidis, M. (2019). Acute nicotinamide riboside supplementation improves redox homeostasis and exercise performance in old individuals: a double-blind cross-over study. *European Journal of Nutrition*.

Erdman, K., Fung, T., Doyle-Baker, P., Verhoef, M. and Reimer, R. (2007). Dietary Supplementation of High-performance Canadian Athletes by Age and Gender. *Clinical Journal of Sport Medicine*, 17(6), pp.458-464.

Esposito, D., Chen, A., Grace, M., Komarnytsky, S. and Lila, M. (2014). Inhibitory Effects of Wild Blueberry Anthocyanins and Other Flavonoids on Biomarkers of Acute and Chronic Inflammation in Vitro. *Journal of Agricultural and Food Chemistry*, 62(29), pp.7022-7028.

Faoroo, V., Deboeck, G., Vicenzi, M., Gaston, A., Simaga, B., Doucende, G., Hapkova, I., Roca, E., Subirats, E., Durand, F. and Naeije, R. (2017). Pulmonary Vascular Function and Aerobic Exercise Capacity at Moderate Altitude. *Medicine & Science in Sports & Exercise*, 49(10), pp.2131-2138.

Finck, B., Gropler, M., Chen, Z., Leone, T., Croce, M., Harris, T., Lawrence, J. and Kelly, D. (2006). Lipin 1 is an inducible amplifier of the hepatic PGC-1α/PPARα regulatory pathway. *Cell Metabolism*, 4(3), pp.199-210.

Fletcher, R. and Lavery, G. (2018). The emergence of the nicotinamide riboside kinases in the regulation of NAD+ metabolism. *Journal of Molecular Endocrinology*, 61(3), pp.R107-R121.

Friedlander, A., Casazza, G., Horning, M., Usaj, A. and Brooks, G. (1999). Endurance training increases fatty acid turnover, but not fat oxidation, in young men. *Journal of Applied Physiology*, 86(6), pp.2097-2105.

Gerhart-Hines, Z., Rodgers, J., Bare, O., Lerin, C., Kim, S., Mostoslavsky, R., Alt, F., Wu, Z. and Puigserver, P. (2007). Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *The EMBO Journal*, 26(7), pp.1913-1923.

Gomes, A., Price, N., Ling, A., Moslehi, J., Montgomery, M., Rajman, L., White, J., Teodoro, J., Wrann, C., Hubbard, B., Mercken, E., Palmeira, C., de Cabo, R., Rolo, A., Turner, N., Bell, E. and Sinclair, D. (2013). Declining NAD+ Induces a Pseudohypoxic State Disrupting Nuclear-Mitochondrial Communication during Aging. *Cell*, 155(7), pp.1624-1638.

Goody, M. and Henry, C. (2018). A need for NAD+ in muscle development, homeostasis, and aging. *Skeletal Muscle*, 8(1).

GORE, C., CLARK, S. and SAUNDERS, P. (2007). Nonhematological Mechanisms of Improved Sea-Level Performance after Hypoxic Exposure. *Medicine & Science in Sports & Exercise*, 39(9), pp.1600-1609.

Green, H., Ball-Burnett, M., Jamieson, G., Cadefau, J. and Cussó, R. (1995). Metabolic adaptations to short-term training are expressed early in submaximal exercise. *Canadian Journal of Physiology and Pharmacology*, 73(4), pp.474-482.

Guo, Y., Zhang, L., Li, F., Hu, C. and Zhang, Z. (2016). Restoration of sirt1 function by pterostilbene attenuates hypoxia-reoxygenation injury in cardiomyocytes. *European Journal of Pharmacology*, 776, pp.26-33.

Gupte, R., Liu, Z. and Kraus, W. (2017). PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes. *Genes & Development*, 31(2), pp.101-126.

Hall, D.P., Duncan, K., Baillie, J.K. (2011). "High altitude pulmonary oedema". J R Army Med Corps. Vol. 157., pp. 68-72.

Handschin, C. and Spiegelman, B. (2008). The role of exercise and PGC1α in inflammation and chronic disease. *Nature*, 454(7203), pp.463-469.

Hawley, J. (2002). Adaptations Of Skeletal Muscle To Prolonged, Intense Endurance Training. *Clinical and Experimental Pharmacology and Physiology*, 29(3), pp.218-222.

Hawley, J., Brouns, F. and Jeukendrup, A. (1998). Strategies to Enhance Fat Utilisation During Exercise. *Sports Medicine*, 25(4), pp.241-257.

Hellström, G. and Wahlgren, N. (1993). Physical exercise increases middle cerebral artery blood flow velocity. Neuro Surgical Review, 16(2), pp.151-156.

Hetlelid, K., Plews, D., Herold, E., Laursen, P. and Seiler, S., 2015. Rethinking the role of fat oxidation: substrate utilisation during high-intensity interval training in well-trained and recreationally trained runners. *BMJ Open Sport & Exercise Medicine*, 1(1), p.e000047.

Hodgson, A., Randell, R. and Jeukendrup, A. (2013). The Metabolic and Performance Effects of Caffeine Compared to Coffee during Endurance Exercise. *PLoS ONE*, 8(4), p.e59561.

Hooper, L., Kay, C., Abdelhamid, A., Kroon, P., Cohn, J., Rimm, E. and Cassidy, A. (2012). Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *The American Journal of Clinical Nutrition*, 95(3), pp.740-751.

Holowatz, L.A., Houghton, B.L., Wong, B.J., Wilkins, B.W., Harding, A.W., Kenney, W.L. and Minson, C.T (2003). "Nitric oxide and attenuated reflex cutaneous vasodilation in aged skin". Am. J. Physiol. Heart Circ. Physiol. Vol. 284., pp. H1662–H1667

Holloszy, J.O. (1967). "Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle". J Biol Chem. Vol. 242., pp. 2278-82.

Howitz, K., Bitterman, K., Cohen, H., Lamming, D., Lavu, S., Wood, J., Zipkin, R., Chung, P., Kisielewski, A., Zhang, L., Scherer, B. and Sinclair, D. (2003). Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature*, 425(6954), pp.191-196.

Hubbard, B.P., Sinclaur, D. A. (2013). "Measurement of sirtuin enzyme acitivty using a substrate-agnostic flurometric nicotinamide assay. Methods Mol Biol. Vol. 1077., pp.167-177.

Guo, Y., Zhang, L., Li, F., Hu, C. and Zhang, Z. (2016). Corrigendum to “Restoration of sirt1 function by pterostilbene attenuates hypoxia-reoxygenation injury in cardiomyocytes” [Eur. J. Pharmacol. 776 (2016) 26–33]. *European Journal of Pharmacology*, 782, p.120.

Imai, S. and Guarente, L. (2014). NAD+ and sirtuins in aging and disease. *Trends in Cell Biology*, 24(8), pp.464-471.

Ivy, J. (1999). ROLE OF CARBOHYDRATE IN PHYSICAL ACTIVITY. *Clinics in Sports Medicine*, 18(3), pp.469-484.

Jackson, M. (2015). Redox regulation of muscle adaptations to contractile activity and aging. *Journal of Applied Physiology*, 119(3), pp.163-171.

Jeukendrup, A., Saris, W. and Wagenmakers, A., 1998. Fat Metabolism During Exercise: A Review. Part I: Fatty Acid Mobilization and Muscle Metabolism. *International Journal of Sports Medicine*, 19(04), pp.231-244.

Jeukendrup, A. and Wallis, G. (2005). Measurement of Substrate Oxidation During Exercise by Means of Gas Exchange Measurements. *International Journal of Sports Medicine*, 26, pp.S28-S37.

Jokinen, R., Pirnes-Karhu, S., Pietiläinen, K. and Pirinen, E. (2017). Adipose tissue NAD+-homeostasis, sirtuins and poly(ADP-ribose) polymerases -important players in mitochondrial metabolism and metabolic health. *Redox Biology*, 12, pp.246-263.

Kapetanovic, I., Muzzio, M., Huang, Z., Thompson, T. and McCormick, D. (2010). Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemotherapy and Pharmacology*, 68(3), pp.593-601.

Karlsen, A., Paur, I., Bøhn, S., Sakhi, A., Borge, G., Serafini, M., Erlund, I., Laake, P., Tonstad, S. and Blomhoff, R. (2010). Bilberry juice modulates plasma concentration of NF-κB related inflammatory markers in subjects at increased risk of CVD. *European Journal of Nutrition*, 49(6), pp.345-355.

Kelley, D., Rasooly, R., Jacob, R., Kader, A. and Mackey, B. (2006). Consumption of Bing Sweet Cherries Lowers Circulating Concentrations of Inflammation Markers in Healthy Men and Women. *The Journal of Nutrition*, 136(4), pp.981-986.

Kenney, W.L., Morgan, A.L., Farquhar, W.B., Brooks, E.M., Pierzga, J.M. and Derr, J.A. (1997). “Decreased active vasodilator sensitivity in aged skin”. Am J. Physiol. Vol. 272., pp. H1609–H1614.

Knapik, J., Steelman, R., Hoedebecke, S., Austin, K., Farina, E. and Lieberman, H. (2015). Prevalence of Dietary Supplement Use by Athletes: Systematic Review and Meta-Analysis. *Sports Medicine*, 46(1), pp.103-123.

Kourtzidis, I., Stoupas, A., Gioris, I., Veskoukis, A., Margaritelis, N., Tsantarliotou, M., Taitzoglou, I., Vrabas, I., Paschalis, V., Kyparos, A. and Nikolaidis, M. (2016). The NAD+

precursor nicotinamide riboside decreases exercise performance in rats. *Journal of the International Society of Sports Nutrition*, 13(1).

Kourtzidis, I., Dolopikou, C., Tsiftsis, A., Margaritelis, N., Theodorou, A., Zervos, I., Tsantarliotou, M., Veskoukis, A., Vrabas, I., Paschalis, V., Kyparos, A. and Nikolaidis, M., (2018). Nicotinamide riboside supplementation dysregulates redox and energy metabolism in rats: Implications for exercise performance. *Experimental Physiology*, 103(10), pp.1357-1366.

Kühn, K., Obata, T., Feher, K., Bock, R., Fernie, A.R., Meyer, E.H. (XXXX). "Complete mitochondrial complex I deficiency induces an up-regualtion of respiratory fluxes that is abolished by traces of cuntional complex I". XXXX. Vol. XX., pp.

Kupis, W., Pałyga, J., Tomal, E. and Niewiadomska, E. (2016). The role of sirtuins in cellular homeostasis. *Journal of Physiology and Biochemistry*, 72(3), pp.371-380.

Kurobe, K., Nakao, S., Nishiwaki, M. and Matsumoto, N. (2015). Combined effect of coffee ingestion and repeated bouts of low-intensity exercise on fat oxidation. *Clinical Physiology and Functional Imaging*, 37(2), pp.148-154.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P. and Auwerx, J. (2006). Resveratrol Improves Mitochondrial Function and Protects against Metabolic Disease by Activating SIRT1 and PGC-1α. *Cell*, 127(6), pp.1109-1122.

Lappalainen, Z. (2010). Sirtuins: A Family of Proteins With Implications for Human Performance and Exercise Physiology. *Research in Sports Medicine*, 19(1), pp.53-65.

Lin, H., Yue, B. and Ho, P. (2009). Determination of pterostilbene in rat plasma by a simple HPLC-UV method and its application in pre-clinical pharmacokinetic study. *Biomedical Chromatography*, 23(12), pp.1308-1315.

López-Lluch, G., Irusta, P., Navas, P. and de Cabo, R. (2008). Mitochondrial biogenesis and healthy aging. *Experimental Gerontology*, 43(9), pp.813-819.

Margaritelis, N., Theodorou, A., Paschalis, V., Veskoukis, A., Dipla, K., Zafeiridis, A., Panayiotou, G., Vrabas, I., Kyparos, A. and Nikolaidis, M. (2017). Adaptations to endurance training depend on exercise-induced oxidative stress: exploiting redox interindividual variability. *Acta Physiologica*, 222(2), p.e12898.

Martens, C., Denman, B., Mazzo, M., Armstrong, M., Reisdorph, N., McQueen, M., Chonchol, M. and Seals, D. (2018). Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD+ in healthy middle-aged and older adults. *Nature Communications*, 9(1).

Martín, M., Goya, L. and Ramos, S. (2016). Preventive Effects of Cocoa and Cocoa Antioxidants in Colon Cancer. *Diseases*, 4(1), p.6.

Martín, M. and Ramos, S. (2016). Cocoa polyphenols in oxidative stress: Potential health implications. *Journal of Functional Foods*, 27, pp.570-588.

Mattagajasingh, I., Kim, C., Naqvi, A., Yamamori, T., Hoffman, T., Jung, S., DeRicco, J., Kasuno, K. and Irani, K. (2007). SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proceedings of the National Academy of Sciences*, 104(37), pp.14855-14860.

Matthaiou, C., Goutzourelas, N., Stagos, D., Sarafoglou, E., Jamurtas, A., Koulocheri, S., Haroutounian, S., Tsatsakis, A. and Kouretas, D. (2014). Pomegranate juice consumption increases GSH levels and reduces lipid and protein oxidation in human blood. *Food and Chemical Toxicology*, 73, pp.1-6.

Maughan, R. (2002). The athlete's diet: nutritional goals and dietary strategies. *Proceedings of the Nutrition Society*, 61(1), pp.87-96.

McCormack, D. and McFadden, D. (2012). Pterostilbene and Cancer: Current Review. *Journal of Surgical Research*, 173(2), pp. e53-e61.

McKenna, M., Waagepetersen, H., Schousboe, A. and Sonnewald, U. (2006). Neuronal and astrocytic shuttle mechanisms for cytosolic-mitochondrial transfer of reducing equivalents: Current evidence and pharmacological tools. *Biochemical Pharmacology*, 71(4), pp.399-407.

McLeay, Y., Barnes, M., Mundel, T., Hurst, S., Hurst, R. and Stannard, S. (2012). Effect of New Zealand blueberry consumption on recovery from eccentric exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, 9(1), p.19.

Meyer, T., Gäßler, N. and Kindermann, W. (2007). Determination of "Fatmax" with 1 h cycling protocols of constant load. *Applied Physiology, Nutrition, and Metabolism*, 32(2), pp.249-256.

Minson, C.T., Holowatz, L.A., Wong, B.J., Kenney, W.L. and Wilkins, B.W. (2002). "Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age during local heating.". J. Appl. Physiol. Vol. 93., pp. 1644–1649

Morishima, T., Lemitsu, M., Ochi, E. (2019). "Short-term cycling restores endothelial dysfunction after resistance exercise". Medicine and science in sports and exercise. Vol. 51., pp. 665.

Moss, J.W.E, Ramji, D.P. (2016). "Nutraceutical therpaies for atherosclerosis". Nature Reviews Cardiology. Vol.13., pp. 513-532

Nagao, K., Jinnouchi, T., Kai, S., Yanagita, T. (2017). "Pterostilbene, a demethylated analog of resveratrol, promotes energy metabolism in obese rats". The journal of nutritional biochemistry. Vol. 43., pp. 151-155.

Neves, R., A., Lucio, M., L.C. Lima, J. and Reis, S. (2012). Resveratrol in Medicinal Chemistry: A Critical Review of its Pharmacokinetics, Drug-Delivery, and Membrane Interactions. *Current Medicinal Chemistry*, 19(11), pp.1663-1681.

Noratto, G., Angel-Morales, G., Talcott, S. and Mertens-Talcott, S. (2011). Polyphenolics from Açaí (Euterpe oleracea Mart.) and Red Muscadine Grape (Vitis rotundifolia) Protect

Human Umbilical Vascular Endothelial Cells (HUVEC) from Glucose- and Lipopolysaccharide (LPS)-Induced Inflammation and Target MicroRNA-126. *Journal of Agricultural and Food Chemistry*, 59(14), pp.7999-8012.

Nutakul, W., Sobers, H., Qiu, P., Dong, P., Decker, E., McClements, D. and Xiao, H. (2011). Inhibitory Effects of Resveratrol and Pterostilbene on Human Colon Cancer Cells: A Side-by-Side Comparison. *Journal of Agricultural and Food Chemistry*, 59(20), pp.10964-10970.

Odland, L., Howlett, R., Heigenhauser, G., Hultman, E. and Spriet, L. (1998). Skeletal muscle malonyl-CoA content at the onset of exercise at varying power outputs in humans. *American Journal of Physiology-Endocrinology and Metabolism*, 274(6), pp.E1080-E1085.

O'Neill, M., Watt, M., Heigenhauser, G. and Spriet, L. (2004). Effects of reduced free fatty acid availability on hormone-sensitive lipase activity in human skeletal muscle during aerobic exercise. *Journal of Applied Physiology*, 97(5), pp.1938-1945.

Ostojic, S.M. (2016). "Mitochondria-targeted nutraceuticals in sports medicine: a new perspective". Research in sports medicine. Vol.25., pp. 91-100.

Peltonen, J., Tikkanen, H. and Rusko, H. (2001). Cardiorespiratory responses to exercise in acute hypoxia, hyperoxia and normoxia. *European Journal of Applied Physiology*, 85(1-2), pp.82-88.

Pillai, J., Isbatan, A., Imai, S. and Gupta, M. (2005). Poly(ADP-ribose) Polymerase-1-dependent Cardiac Myocyte Cell Death during Heart Failure Is Mediated by NAD+Depletion and Reduced Sir2α Deacetylase Activity. *Journal of Biological Chemistry*, 280(52), pp.43121-43130.

Price, N., Gomes, A., Ling, A., Duarte, F., Martin-Montalvo, A., North, B., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J., Hubbard, B., Varela, A., Davis, J., Varamini, B., Hafner, A., Moaddel, R., Rolo, A., Coppari, R., Palmeira, C., de Cabo, R., Baur, J. and Sinclair, D. (2012). SIRT1 Is Required for AMPK Activation and the Beneficial Effects of Resveratrol on Mitochondrial Function. *Cell Metabolism*, 15(5), pp.675-690.

Purushotham, A., Schug, T., Xu, Q., Surapureddi, S., Guo, X. and Li, X. (2009). Hepatocyte-Specific Deletion of SIRT1 Alters Fatty Acid Metabolism and Results in Hepatic Steatosis and Inflammation. *Cell Metabolism*, 9(4), pp.327-338.

Rajendran, P., Rengarajan, T., Thangavel, J., Nishigaki, Y., Sakthisekaran, D., Sethi, G., Nishigaki, I. (2013). "The vascular endothelium and human diseases". Int. J. Biol. Sci. Vol. 9., p.1057-69.

Ramsey, K., Mills, K., Satoh, A. and Imai, S. (2008). Age-associated loss of Sirt1-mediated enhancement of glucose-stimulated insulin secretion in beta cell-specific Sirt1-overexpressing (BESTO) mice. *Aging Cell*, 7(1), pp.78-88.

Reid, M., Khawli, F. and Moody, M. (1993). Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *Journal of Applied Physiology*, 75(3), pp.1081-1087.

Remsberg, C., Yáñez, J., Ohgami, Y., Vega-Villa, K., Rimando, A. and Davies, N. (2008). Pharmacometrics of pterostilbene: preclinical pharmacokinetics and metabolism, anticancer, antiinflammatory, antioxidant and analgesic activity. *Phytotherapy Research*, 22(2), pp.169-179.

Riche, D., McEwen, C., Riche, K., Sherman, J., Wofford, M., Deschamp, D. and Griswold, M. (2013). Analysis of Safety from a Human Clinical Trial with Pterostilbene. *Journal of Toxicology*, 2013, pp.1-5.

Romijn, J., Coyle, E., Sidossis, L., Gastaldelli, A., Horowitz, J., Endert, E. and Wolfe, R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology-Endocrinology and Metabolism*, 265(3), pp.E380-E391.

Rowlands, D.S., Jeukendrup, A.E. (2004). "Fat-Oxidation during Exercise: Comparison of RER vs. 13 C - Glycogen Enrichment Method. Clermont Ferrand; Congress Presentation European College of Sport Science. Vol. XX., pp.

Saiver, D., Rodek, J.,. Escalente, Y., Olujic, D. (2013). "Sports nutrition and doping factors in swimming; parallel analysis among athletes and coaches". Coll Antropol. Vol.37., pp.179-86

Scribbans, T., Ma, J., Edgett, B., Vorobej, K., Mitchell, A., Zelt, J., Simpson, C., Quadrilatero, J. and Gurd, B. (2014). Resveratrol supplementation does not augment performance adaptations or fibre-type–specific responses to high-intensity interval training in humans. *Applied Physiology, Nutrition, and Metabolism*, 39(11), pp.1305-1313.

Seals, D.R., Jablonski, K.L., Donato, A.J. (2011). "Aging and vascular endothelial function in humans". Clin Sci (Lond). Vol. 120., pp. 357-75.

Shang, S., Zhou, Q., Liu, M. and Shao, Y., 2012. Sample Size Calculation for Controlling False Discovery Proportion. *Journal of Probability and Statistics*, 2012, pp.1-13.

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., & Neri, L. M. (2018). "Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging". *Oncotarget*. Vol. 9., pp.17181-17198.

Srivastava, S. (2016). Emerging therapeutic roles for NAD+ metabolism in mitochondrial and age-related disorders. *Clinical and Translational Medicine*, 5(1).

Stephens, F.B., Wall, B.T., Marimuthu, K., Shannon, C.E., Constantin-Teodosiu, D., Macdonald, I.A., Greenhaff, P.L. (2013). "Skeletal muscle carnitine loading increases energy expenditure, modulates fuel metabolism gene networks and prevents body fat accumulation in humans". *J Physiol.* Vol. 591., pp. 4655-4666.

Stellingwerff, T. (2013). Contemporary nutrition approaches to optimize elite marathon performance. Int. J. Sports Physiol. Perform. 8:573-578.

Stellingwerff, T., I.M. Bovim, and J. Whitfield (2019a). Contemporary nutrition interventions to optimize performance in middle-distance runners. Int. J. Sport Nutr. Exerc. Metab. 29:106-116

Subudhi, A., Dimmen, A. and Roach, R. (2007). Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. *Journal of Applied Physiology*, 103(1), pp.177-183.

Tipton, K.D., Elliot, T.A., Cree, M.G., Aarsland, A.A. (2007). "Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise". American Journal of physiology- Endocrinology and metabolism. Vol. 292., pp. E71-E76

Trammell, S.A.J., Schmidt, M.S., Weidermann, B.J., Redpath,P., Jaksch, F., Dellinger, R.W., Li, Z., Abel. D.E., Migaud, M.E., Brenner, C. (2016). "Nicotinamide riboside is uniquely and orally bioavailable in mice and humans". Nature Communications. Vol. 7.

Ungvari, Z., Sonntag, W., de Cabo, R., Baur, J. and Csiszar, A. (2011). Mitochondrial Protection by Resveratrol. *Exercise and Sport Sciences Reviews*, 39(3), pp.128-132.

Van Loon, L., Greenhaff, P., Constantin-Teodosiu, D., Saris, W. and Wagenmakers, A. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology*, 536(1), pp.295-304.

Venables, M.C., Achten, J., Jeukendrup, A.E. (2004). "Determinants of fat oxidation during exercise in healthy men and women: A cross-sectional study". J Appl physiol. Vol. 98., pp.160-167

Vendrame, S., Tsakiroglou, P., Kristo, A., Schuschke, D. and Klimis-Zacas, D. (2016). Wild blueberry consumption attenuates local inflammation in the perivascular adipose tissue of obese Zucker rats. *Applied Physiology, Nutrition, and Metabolism*, 41(10), pp.1045-1051.

Voduc, N., la Porte, C., Tessier, C., Mallick, R. and Cameron, D. (2014). Effect of resveratrol on exercise capacity: a randomized placebo-controlled crossover pilot study. *Applied Physiology, Nutrition, and Metabolism*, 39(10), pp.1183-1187.

Wang, Y., He, J., Liao, M., Hu, M., Li, W., Ouyanh, H., Wang, X., Ye, T., Zhang, Y., Ouyang, L. (2019). "An overview of Sirtuins as potential therapeutic target: Structure, function and modulators". European Journal of Medicinal Chemistry. Vol. 161., pp. 48-77.

Wenz, T. (2013). Regulation of mitochondrial biogenesis and PGC-1α under cellular stress. *Mitochondrion*, 13(2), pp.134-142.
White, A. and Schenk, S. (2012). NAD+/NADH and skeletal muscle mitochondrial adaptations to exercise. *American Journal of Physiology-Endocrinology and Metabolism*, 303(3), pp.E308-E321.

Yuan, Y., Cruzat, V., Newsholme, P., Cheng, J., Chen, Y. and Lu, Y. (2016). Regulation of SIRT1 in aging: Roles in mitochondrial function and biogenesis. *Mechanisms of Ageing and Development*, 155, pp.10-21.

Zhang, H. and Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science*, 8, pp.33-42.