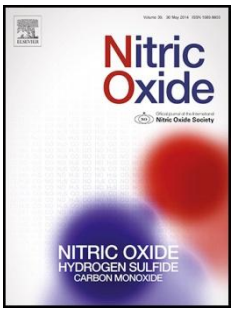
Accepted Manuscript



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PII: S1089-8603(15)00208-6
DOI: http://dx.doi.org/doi:10.1016/j.niox.2015.04.006
Reference: YNIOX 1491

To appear in: *Nitric Oxide*

Received date: 7-1-2015
Revised date: 14-4-2015
Accepted date: 22-4-2015

Please cite this article as: Philip E. James, Gareth R. Willis, Jason D. Allen, Paul G. Winyard, Andrew M. Jones, Nitrate pharmacokinetics: taking note of the difference, *Nitric Oxide* (2015), http://dx.doi.org/doi:10.1016/j.niox.2015.04.006.

Nitrate Pharmacokinetics: Taking Note of the Difference

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Abbreviated title: Nitrate pharmacokinetics

Word Count: 3393

Number of tables: 1

Number of figures: 4

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Highlights

- We summarize a collection of findings and approaches by researchers working in the field, highlighting key considerations for new and current researchers and intended as a prologue to this special issue of the Nitric Oxide Journal.
- We attempt to strike an informative and cautionary note, by highlighting key considerations as opposed to specific study recommendations.
- We include suggestions on what to focus on and/or do better.
- New data is provided to illustrate by example.

Abstract

It is now recognised that administration of oral nitrate (NO_3^-), in its various forms, increases the level of nitric oxide (NO) metabolites in the circulation of humans. Its application to modulate physiology and alleviate cardiovascular dysfunction in some patients is now recorded and shows particular promise in hypertension, in modifying platelet activation/aggregation, and in conditions where tissue ischemia prevails. The potential of oral NO_3^- to modify exercise/performance via elevation of plasma nitrite concentration ($[NO_2^-]$) has been applied across a range of human test systems. Herein we discuss how the choice of NO_3^- source, route of administration and resulting pharmacokinetics might influence the outcome of physiological measures and potentially contribute to discrepancies in performance trials. There are but a few examples of detailed pharmacokinetic data on which the majority of researchers base their test protocols in different cohorts/settings. We compare and contrast the results of key publications with the aim of highlighting a consensus of our current understanding and critical considerations for those entering the field.

Keywords: Nitrite; Nitrate; Pharmacokinetics; Exercise.

Introduction

Administration of oral nitrate (NO_3^-), in its various forms, increases the level of nitric oxide (NO) metabolites in blood. Its application to modulate physiology and alleviate cardiovascular dysfunction shows particular promise in certain clinical conditions and patient cohorts, including reduction of mean arterial pressure in hypertension [1, 2], effective quiescencing of platelet activation/aggregation [3], and reduce risk in conditions where tissue ischemia prevails [4]. However, it is the potential of oral NO_3^- to modify exercise/performance via elevation of plasma nitrite concentration ($[NO_2^-]$) that has been applied across a range of human test systems, from the effects of walking distance in a sedentary population [5] to enhancing performance in elite athletes [6].

There is a general consensus on the principal pathways and primary mechanisms involved in the conversion of oral NO_3^- to circulating NO_2^- and NO. However, there remains significant variation in the levels of NO metabolites attained in the plasma and it is presently not known which levels are sufficient to enhance physical performance in a particular setting. Most investigators (but not all) are aware of the many controllable factors that introduce variance and take measures to counter these (e.g. cohort choice and dietary restriction). We must also acknowledge other, potentially less obvious sources of variation in our research designs or otherwise risk misinterpreting our performance findings.

Herein we discuss how the choice of NO_3^- source, route of administration and resulting pharmacokinetics might influence the outcome of physiological studies and potentially contribute to discrepancy in performance trials. There are but a few examples of detailed pharmacokinetic data on which the majority of researchers base their test protocol in different cohorts/settings. We compare and contrast the results of key publications with the aim of highlighting to this readership a consensus of our current understanding and critical considerations for those entering the field. Perhaps opportunity exists in exploiting these observations.

Nitrate (NO_3^-) source

The source of NO_3^- influences the concentration, bioavailability, subject compliance and route of administration. Foodstuffs contain varying concentrations of NO_3^-, with leafy vegetables and beetroot (BR) a relatively rich source [7]. The awareness and consumption of NO_3^- has been enhanced by the recent commercialisation of BR, chard and rhubarb concentrates in products such as Beet it® (James White Drinks) and Go+Nitrate® gel (Science in Sport). With a burgeoning awareness of the potential importance of NO_3^- in sport and exercise, an ever-increasing variety of NO_3^- fortified products are becoming commercialised, ranging from sport bars and lozenges to BR capsules. Importantly, NO_3^- salts (in solution or via capsule) are readily available for both oral and intravenous administration and

as such provide a convenient and essential pharmacologic NO_3^- standard with which to compare results from human studies in various cohorts/settings. However, as outlined below, this may not translate to studies involving BR, or chard concentrate. In order for investigators to compare the effect of NO_3^- supplementation on exercise and performance, it is first necessary to consider, are we comparing like-for-like? New products include a NO_3^- rich flapjack bar and NO_3^- / NO_2^- crystals which dissolve to produce a beverage. While all of these products likely have physiological effects, they have not been directly compared and it is possible that a given NO_3^- content in these products may differentially influence the peak (and time to peak) plasma [NO_3^-] and [NO_2^-], with implications for performance outcomes.

A further consideration is what other bio-active components exist within each product – a question rarely addressed. An interesting healthcare product is being marketed by Neogenis (Neo40) which contains NO_3^- and NO_2^- but also concentrate from berry and other components aimed at enhancing delivery. Certainly early clinical studies show peak plasma [NO_2^-] is attained within ~20 min which is considerably shorter than that required for BR preparations (typically ~2hrs) [8]. A further product being developed is in the form of a lozenge which also shows early plasma increases in [NO_3^-] and [NO_2^-]. This may suggest that inclusion of a modest amount of NO_2^- in the preparation may be an advantage to facilitate the early onset of physiological effects, and holding the NO_3^- source in the buccal cavity may also speed up NO_3^--NO_2^-NO metabolism. *In vitro* modelling of NO_3^- reduction in simulated gastric and buccal medium however shows that most bacterial reductases exhibit limited function at lower pH. It is therefore interesting to note that commercial products of BR, or chard, exhibit acidic pH (typically 3-5) which can enhance shelf life. This in itself is unlikely to affect the first pass absorption of nitrite and nitrate from the stomach and proximal small intestine into blood, but with second or subsequent administration could hinder second pass buccal NO_3^- reduction (via the enterosalivary circulation). It is also critical to consider the availability of "free" inorganic NO_3^- contained within the organic source (such as BR, or chard). Whilst NO_3^- analysis using VCl_3/HCl at 85˚C or other chemical cleavage reagents are able to cleave the total NO_3^- content for detection by ozone based chemiluminescence, application of *in vitro* NO_3^- reductases under conditions expected in the buccal cavity or stomach yields very little NO_2^- from BR, or chard, despite 100% yield from KNO_3 under the same laboratory conditions (P.E.J. unpublished observations). This further confirms the need for first pass digestion prior to sequestration of NO_3^- already in plasma.

Nitrate (NO_3^-) pharmacokinetic parameters

The variety of NO_3^- sources present different challenges, ranging from subject compliance / palatability, pharmacovigilance and differing pharmacokinetic profiles. Moreover, these challenges

are coupled with a complex NO_3^- biochemistry, where inter-biological variation, oral and gut bacteria capacity and saliva flow rate are but a few variables determining the fate of NO_3^- metabolism.

Velzen *et al*, [9] demonstrated the source of NO_3^- dictates the pharmacokinetic profile of plasma NO_3^-. They found bolus administration of either $NaNO_3$ (365 mg NO_3^-), spinach (564 mg NO_3^-), lettuce (1014 mg NO_3^-) or BR (643 mg NO_3^-), resulted in different peak NO_3^- attained and time to reach maximal level, consistent with the extent of NO_3^- loading from different dietary sources. Interestingly, the half-life was consistent at 6 hrs irrespective of source (Table 1). These findings remain as reference values, but in-depth insight is gained from more recent studies. Wylie *et al*, [10] recently considered the impact of dose and volume load, whereby the bolus administration of Beet it (70 ml, 140 ml and 280 ml) provided 4.2 mmol, 8.4 mmol and 16.8 mmol NO_3^-, a range encompassing most performance/exercise studies to date. The peak in plasma [NO_3^-] and [NO_2^-] was noticeably delayed with increasing dose/volume, with a corresponding increase in time in the circulation (t½). This is in good agreement with the work of Kapil *et al* [11]. The increase in plasma [NO_2^-] relative to the increase in plasma [NO_3^-] ($\Delta[NO_2^-]/\Delta[NO_3^-]$ ratio, as defined in Table 1) was relatively consistent (1.0-1.2) and is in keeping with the work of most using BR [11-14], but inconsistent with Webb *et al* [3] (~0.5). Of those groups administering KNO_3^- (capsule or in solution) the results are also very consistent (0.8-1.0) other than the work of Velmuragan *et al*, [12] which is modestly higher (1.4-2.3) in comparison. There is some consistency in terms of time to peak plasma [NO_3^-], being between 1 - 3 hrs, and a plasma t½ of >6 hrs is found irrespective of KNO_3 dose (4 - 24 mmol).

In addition, taken together there is good evidence that time to peak NO_3^- may depend on the source of NO_3^-, although there are notable exceptions [11]. In general, a KNO_3 solution/capsule and NO_3^- containing gel (SiS Go+Nitrate) appear to exhibit an earlier peak (~1hr) when compared with BR at a similar loading dose. Conversely, there is some evidence to indicate that the overall yield from BR may be higher in comparison to other NO_3^- sources such as swiss chard (SiS Go+Nitrate, based on area under curve analysis over 24 hrs, Figure 1). However, care must be taken in over-interpreting this early observation. It is entirely possible that different forms of nitrate delivery (whether gel, food, or liquid) may in itself dictate plasma pharmacokinetics. This can only be resolved by appropriate cross-over studies in the same individuals.

Importantly, there is discrepancy in the level of plasma [NO_2^-] attained per unit amount of NO_3^- administered between groups (0.5 – 2.5) and even within individual subjects over a prolonged period of time. Of those utilising a similar BR source, in one or two repeated tests there is an apparent split between those exhibiting high relative yield [12-14] and those finding significantly lower yield [3, 11]. In these examples, volume of NO_3^- administration does not appear to be the cause of the

discrepancy, neither does this appear to be methodological (since all studies essentially use ozone-based chemiluminescence to measure plasma NO metabolites).

Remaining questions are whether studies aimed at testing exercise performance should either ensure tests are carried out at the peak plasma [NO_2^-] and/or whether it might be more appropriate to aim for a minimal effective increase (for example 200 nM above baseline or X-fold change). These factors are important to consider when comparing results between groups and in chronic administration studies where it may be extremely difficult to monitor plasma [NO_2^-] as blood samples would have to be collected prior to, and following, each dosing to identify the change. Moreover, as a rule of thumb, classic pharmacological studies suggest >50% of experimental measures should be undertaken around the point of interest in order to yield accurate data on peak plasma [NO_2^-] which may be impractical unless pre-determined in that particular subject.

Individual versus Group Variation

This is difficult to interpret from grouped data expressed as mean±error. Certainly in our hands [13] there remains noticeable variation in both time to peak, and peak plasma [NO_2^-] per [NO_3^-] between individuals in an otherwise closely matched cohort; this despite implementing a 12 hr fast (thus reducing baseline variance) and maintenance of an essentially NO_3^- free diet over 24 hrs (Figure 2). It is important to note that the study conditions represent a 'best case' scenario that adhered to the key considerations suggested by this manuscript. Interestingly, there is overall a consistent area under the curve for plasma [NO_2^-] and plasma [NO_3^-], comparing individuals across 24 hrs, which implies the total yield may be similar. One individual (out of seven) did appear to exhibit an enhanced AUC for ΔNO_2^- compared to the other individuals. It is reasonable to speculate that this individual variation could be due to genetic variation, chronobiology and/or variation in oral/buccal hygiene (see below for a fuller discussion of these factors). Further to this, baseline plasma [NO_3^-] is remarkably consistent across most research groups, whereas baseline plasma [NO_2^-] varies considerably (~80-400 nM). It is tempting to speculate this reflects mainly differences in dealing with and/or storing the plasma sample as we and others have demonstrated [15]. There is no observable trend to indicate higher baseline plasma levels influence the peak yield, but this remains to be tested directly. Figure 3 shows the scatter between measured plasma [NO_3^-] and [NO_2^-] in seven subjects with peripheral arterial disease tested on at least 5 different occasions (many as random surprise tests) as they received 3 separate doses/week for 3 months of 70 ml (4.2 mM) BR in the form of Beet it. Blood samples were consistently taken 3 hrs post-ingestion. It is apparent that the within-subject responses can be quite variable with no apparent trend with time or repeat dosing. Currently, the effects of source/mode of NO_3^-, dosage and frequency of NO_3^- administration on the conversion rate or the maintenance of a minimum "active" plasma [NO_2^-] over prolonged periods is untested.

The influence of diet is complicated. There is evidence that there is considerable cross-talk between dietary nitrate-nitrite-NO and the endogenous NOS pathways [16]. Certainly diet influences baseline plasma [NO_2^-] and [NO_3^-] levels. However Miller *et al*, [14] showed convincingly this has only a minimal (but measurable) overall effect on the peak and t½ of plasma [NO_2^-]/[NO_3^-] when measured following an acute NO_3^- load across 24 hrs. Yet another factor which should be considered is chronobiological variation. It has been shown that chronobiological variations in systemic NO production and urinary nitrate excretion do occur [17]. It is unknown whether a contributor to such variation might be a chronobiological fluctuation in human nitrate uptake and/or chronobiological variation in nitrite reduction. Nevertheless, future trials involving multiple human participants can (to some extent) control for this potential confounder by ensuring that the study design allows for individuals to ingest nitrate, and to have biofluids/tissues sampled, at the same times of day. Of relevance, Velmuragan *et al*, [12] observed a significant difference with gender, where females typically exhibit significantly higher yields for the same given dose of KNO_3/BR, compared to their male counterparts. This also aligned with different physiological effect (e.g. blood pressure reduction). This has not been tested in terms of exercise performance with the majority of studies having been conducted in male subjects.

Several other factors have been implicated and are particularly relevant to a trained cohort who are invariably on a controlled diet and have a healthy lifestyle. These include (but are not limited to) demographics of the cohort being studied, genetic variation, chronobiology and variation in oral/buccal hygiene. The latter has not been studied directly in terms of variation in a normal buccal regimen, but the early work of Kapil *et al*, [18] demonstrated how mouthwash effectively decreased NO_3^- reducing capacity. More recently, we have shown that this is completely dependent on the mouthwash being used – whether antibacterial or antiseptic (typically antibacterial mouthwashes kill or hinder bacterial reproduction whereas antiseptic mouthwashes kills or hinders the reproduction of multiple microorganisms, including bacteria, fungi and viruses. J.D.A. unpublished observations). The levels of [NO_2^-] and [NO_3^-] in saliva and in plasma are completely dependent on the efficacy of the mouthwash. With such biological variation, coupled with the study variables determining the fate of NO_3^- metabolism, to enable inter-study comparison future studies must consider:

1) normalising NO_3^- load to body mass (as opposed to administering a set NO_3^- load), or
2) ensuring a minimum effective increase in plasma [NO_2^-]/ [NO_3^-] in a particular cohort, or
3) adjusting the NO_3^- load in each subject to attain a pre-set physiologically effective plasma level.

Nitrate pharmacokinetic parameters: Impact on exercise performance

In recent years, there has been a plethora of studies published on the effect of NO_3^- supplementation on exercise performance. These have manipulated a variety of factors to elucidate the ergogenic potential of dietary NO_3^- supplementation including: the 'dose' of NO_3^- administered; the duration of NO_3^- supplementation (acute versus more chronic); the duration and intensity of the exercise performance trial; the nature of the exercise (continuous or intermittent); the environmental conditions (hypoxia vs. normoxia); and the characteristics of the participants, particularly in relation to training status.

The bolus or daily amount of NO_3^- administered in exercise performance studies has ranged from about 4 mmol to about 12 mmol, with 'positive' effects most often reported with higher NO_3^- doses. Wylie *et al*, [10] investigated the influence of various doses (4.2, 8.4 and 16.8 mmol) of ingested NO_3^- in BR juice on plasma [NO_3^-] and [NO_2^-], and also the 'dose-response' relationship between acute NO_3^- ingestion and exercise performance. Higher doses of NO_3^- resulted in higher peak values of plasma [NO_3^-] and [NO_2^-], with the peak in plasma [NO_3^-] occurring earlier (1-2 hours post-ingestion) than the peak in plasma [NO_2^-] (2-3 hours post-ingestion). With the assumption that greater plasma [NO_2^-] indicates greater NO bioavailability, these results have been utilised to investigate the influence of NO_3^- dose on exercise performance. An amount of 4.2 mmol NO_3^- did not significantly improve high-intensity exercise performance. In contrast, 8.4 and 16.8 mmol NO_3^- doses both resulted in improved exercise performance, but 16.8 mmol was no more effective than 8.4 mmol. These results indicate that there may be a 'threshold' amount of NO_3^- that should be ingested (with a corresponding 'threshold' elevation of plasma [NO_2^-]) for exercise performance benefits to be obtained, but also that, beyond that threshold, extra NO_3^- ingestion will not further improve performance. This latter result may be important in informing the development of both safe and efficacious NO_3^- supplementation regimens. Based on the results of Wylie *et al*, [10], 'negative' results in studies utilising relatively low doses of NO_3^- should not be considered surprising.

The early studies on NO_3^- ingestion and exercise performance involved subjects consuming NO_3^- salts or BR juice for ~5-7 days [19, 20]. However, later studies [21] indicated that the O_2 cost of sub-maximal exercise could be reduced within just 2-3 hours of NO_3^- ingestion with this effect being maintained if supplementation was continued for 15 days. The finding that exercise efficiency could be improved acutely stimulated a number of research groups to investigate whether exercise performance could also be improved following a single 'bolus' of NO_3^-. Notwithstanding the issue of sufficient NO_3^- dosing alluded to earlier, the results from this approach have been mixed. The mechanistic basis for the early physiological effects of NO_3^- is unclear but could include effects of NO_2^- or NO on mitochondrial function, O_2 delivery to and within muscle, and substrate utilisation. It has been reported that longer-term NO_3^- supplementation (5-7 days) can result in changes in

mitochondrial [22] and contractile [23] proteins that would be expected to enhance skeletal muscle metabolic and mechanical efficiency. It would seem unlikely that these changes could be fully effected within a few hours of NO_3^- ingestion and therefore the duration of NO_3^- supplementation is likely to introduce variability into the potential efficacy of NO_3^- on the physiological responses to exercise.

The influence of NO_3^- supplementation on a wide range of exercise activities has been investigated, ranging from 500 m kayaking [24] to 50 miles cycling [25], with predictably mixed results. While the majority of studies to date have assessed the influence of NO_3^- supplementation on continuous exercise, a few have considered possible effects on intermittent high-intensity exercise tests which are reflective of the pattern of exertion during team sports. While, once again, the results of these studies have been mixed, owing at least in part to differences in dosing and duration of supplementation, there is evidence that NO_3^- can be ergogenic in such activities [10]. With such discrepancies in the efficacy of NO_3^- supplementation on exercise performance, several activity-specific factors need to be considered.

Environmental conditions

Conditions of low O_2 availability would be expected to lower NO synthase (NOS) function and therefore to potentially augment NO production via the reduction of NO_2^-. Consistent with this, it appears that ambient O_2 influences the ability of NO_3^- ingestion to enhance exercise performance. In hypoxia, intramuscular perturbation of phosphate-linked substrates and metabolites is blunted when exercise is preceded by NO_3^- ingestion [26] and cycle time trial [13] and incremental exercise [27] performance in hypoxia is improved following NO_3^- supplementation. The recovery of muscle phosphocreatine concentration, an index of oxidative capacity, is enhanced in hypoxia but not normoxia following NO_3^- supplementation and this effect appears to be related to improved muscle oxygenation [28]. To date, improved exercise performance following NO_3^- supplementation has been more consistently observed in hypoxia compared to normoxia, and environmental conditions are therefore another factor worthy of consideration when assessing differences between studies.

Fitness status

Another important consideration is the fitness/training status of the participants in NO_3^- supplementation studies. Subjects who are aerobically trained, and especially elite endurance athletes, will have experienced numerous adaptations that may limit the possible benefits of NO_3^- supplementation. These adaptations include: greater NOS activity; greater muscle oxygenation owing to greater muscle capillary density; mitochondrial proliferation and possibly improved mitochondrial efficiency; higher baseline plasma [NO_2^-]; and lower proportion of type II muscle fibres. Conversely,

subjects with compromised cardiovascular and skeletal muscle systems often show peripheral tissue maladaptations to these chronic disease conditions which may include capillary density rarefaction, a reduction in mitochondrial number and efficiency, and a higher proportion of type II muscle fibres. Numerous exercise performance studies in disease states such as peripheral arterial disease [29], chronic heart failure [30] and pulmonary diseases [31] are starting to emerge.

Although there is evidence that the exercise performance of elite athletes can be improved by NO_3^- supplementation [23], it does appear that better trained athletes benefit less compared to lesser trained individuals, at least when a similar NO_3^- dose is ingested. It is important to note, however, that it is possible that elite athletes will respond better to NO_3^- supplementation with larger doses or more protracted supplementation regimens. Irrespective of training status, it has been suggested that there may be 'responders' and 'non-responders' to NO_3^- supplementation [24], and this also may explain variability between studies as alluded to earlier. It remains unclear, at what stage in the NO_3^--NO_2^--NO pathway, 'non-responders' are being 'limited' in their response. Identifying reasons for the inter-individual variability in the response to NO_3^- supplementation must be an important future research goal.

Final perspectives

We have selectively drawn on the work of several groups to highlight important similarity but also noticeable discordance in results. These key studies were typically undertaken using a back-to-basics pharmacological approach, and it seems worthwhile for investigators comparing new cohorts, exercise programs or patients to at least first ensure that the axis of NO_3^--NO_2^--NO metabolism is as expected in their particular cohort or model. It will also be critical to first establish whether individual variation could be greater than the effect being tested. It is unlikely that we will be able to advance our understanding and significantly exploit potentially ergogenic and therapeutic properties of oral NO_3^- supplementation until these processes are elucidated.

Some useful future studies could include a comprehensive assessment of nitrate pharmacokinetics, comparing different sources and routes of administration of NO_3^- in the same individual(s) in a carefully controlled crossover study. The focus of this manuscript was to highlight subtle differences in the pharmacokinetic profile of plasma NO metabolites following either KNO_3 solution/salt, NO_3^- gel (chard or rhubarb), or BR. These various NO_3^- sources may each offer an advantage in a particular setting. Once the kinetic profile is deciphered for the particular ergogenic aid in the study cohort, timing of a bolus / serial dose can be recommended and should be based on achieving either a minimum effective increase or a pre-defined plasma [NO_3^-] or [NO_2^-]. We suggest that it should then be considered whether an individual would benefit from initiating exercise at peak plasma [NO_3^-] or

[NO_2^-] and that it should be kept mind that this is synergistically related to the environmental conditions and the individual's variation in pharmacokinetic parameters. Essential considerations are summarised in Figure 4.

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Figure Legends

Figure 1 – Representative data showing (A) plasma $\Delta[NO_2^-]$ and (B) plasma $\Delta[NO_3^-]$ in humans for 24 hrs following a single bolus dose of BR or SiS Go+Nitrate. Δ refers to plasma $[NO_2^-]$ or $[NO_3^-]$ minus basal plasma $[NO_2^-]$ or $[NO_3^-]$, respectively. Data adapted from [10, 13].

Figure 2 – Plasma $[NO_2^-]$ (A) and plasma $[NO_3^-]$ (B) measured in 6 healthy individuals (age 34 ± 6 years) following administration of 2 x 2.9 mmol $[NO_3^-]$ in the form of SiS Go+Nitrate, showing individual results across 24 hrs. Δ refers to plasma $[NO_2^-]$ or $[NO_3^-]$ minus basal plasma $[NO_2^-]$ or $[NO_3^-]$, respectively. Adapted from [12].

Figure 3 – Measurement of plasma $[NO_2^-]$ and $[NO_3^-]$ in 7 individuals on 5 separate occasions. Subjects received 3 weekly doses of Beet it (4.2 mmol) over a 3 month period. Blood measures were undertaken 3 hrs post administration. All administrations were early in the day (am) - 3 hours prior to blood sampling. All subjects were fasted and remained so during the course of the study. NOx; Nitric oxide metabolites.

Figure 4 – Summary of factors to consider prior to embarking on a NO_3^- supplementation research study.

eference	**NO_3^- source**	**Dose/ volume**	**Plasma NO_3^- Δ (baseline – max)**	**Plasma NO_2^- Δ (baseline – max)**	**ΔNO_2^- /ΔNO_3^- (x 10^{-3})**	**T-max plasma $[NO_3^-]$**	**T-max plasma $[NO_3^-]$**	**T ½ $[NO_3^-]$**	**T ½ $[NO_2^-]$**
undberg *et al*, [32]	Oral (liquid)	$NaNO_3$ ~8.5 mmol	302 µM (30 – 432)	269 nM (123 – 392)	0.891	0.5 hr	1.5 hrs	-	-
elzen *et al*, 9]	KNO_3 IV	4.2 mmol	-	-		0.6 hr	-	5	-
	oral food	6.5 mmol	-	-		1.8 hr	-	5	-
	oral food	11.7 mmol	-	-		1.54 hr	-	6.7	-
	oral food	7.4 mmol	-	-		1.75 hr	-	6.1	-

apil *et al*, 11]	Oral (Capsule)	KNO_3: 24 mmol 12mmol 4mmol	1300 µM 670 µM 170 µM	1100 nM 600 nM 150 nM	0.846 0.896 0.882	3 hr 2 hr 1.5 hr	2.5 hr 1 hr 1 hr	~ 6 hr	>24 hr
elmuragan *et al*, [12]	Oral	KNO_3 8mmol: Male female	180 µM (20-200) 215 µM (35-250)	250 nM (100-350) 500 nM (50-550)	1.389 2.326	3 hr 3 hr	3 hr 3 hr	- -	- -
apil *et al*, 11]	Oral (liquid)	BR 250ml ~ 5.5mmol	175 µM (25 to 200)	200 nM (400 - 600)	1.142	1 hr	2.5 hr	-	-
enjale *et al*, [29]	Oral (liquid)	BR 500ml ~ 5.2mmol	-	791 nM (151 – 942)	-	2 hr	2 hr	-	-
ansley *et al*, [5]	Oral (liquid)	BR – 500 ml ~ 6.2 mmol	-	334 nM (241 – 575)	-	2 hr	2 hr	-	-
arsen *et al*, 19]	Oral (liquid)	$NaNO_3$ 0.1 mmol	55 µM (27 – 182)	102 nM (124 - 226)	1.854	45 min	45 min	-	-
Vebb *et al*, 3]	Oral (liquid)	BR ~11.2 mmol, 500ml	350 µM (25 - 375)	180nM (420 – 600)	0.514	1.5 hr	3 hr	~ 6.5 hr	~5.5 hr
Muggeridge *et al*, [33]	Oral (liquid)	BR, ~ 5 mmol, 70 ml	107 µM (42.8 - 150)	270 nM (408 - 678)	2.519	3 hr	3 hr	-	-
Vylie *et al*, 10]	Oral (liquid)	16.8 mmol (in 280 ml)	570 µM (30-600)	645 nM (80-725)	1.132	2 hr	4 hr	9.5 hr	8 hr
		8.4 mmol (in 140 ml)	270 µM (30-300)	395 nM (80-475)	1.463	1 hr	2 hr	7 hr	6 hr
		4.2 mmol (in 70 ml)	160 µM (30-190)	120 nM (80-200)	0.75	1 hr	2 hr	6 hr	7.5 hr
elmuragan *et al*, [12]	Oral (liquid)	BR 3.1 mmol: male female	100 µM (20-120) 125 µM (25-150)	150 nM (200-350) 250 nM (300-550)	1.5 2.0	3 hr -	3 hr -	- -	- -
Miller *et al*, 14]	Oral (liquid)	BR - 8.5 mmol	350 µM	800 nM	2.286	2 hr	2-3 hr	8 hr	>7 hr
Muggeridge *et al*, [33]	SiS gel	Chard gel - 5.8 mmol	350 µM (67-416)	179 nM (155-334)	0.503	1 hr	1-1.5hr	6 hr	5 hr

Table 1 –Pharmacokinetic summary of acute nitrate loading. Summary of NO_3^- pharmacokinetic parameters from key papers. [Blue shade]: inorganic potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$) or food source. [Green shade]: BR, beetroot juice. [White shade]: Swiss chard gel. Δ refers to plasma [NO_2^-] or [NO_3^-] delta. IV, intravenous.