Does dietary nitrate supplementation
improve performance in cardiopulmonary exercise testing and
post-operative recovery
in patients with colorectal cancer?
A randomised controlled trial.

A thesis submitted for the degree of
Doctor of Medicine at the University of Surrey

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Declaration

The candidate confirms that the work submitted is her own, and has been carried out in the Minimal Access Therapy Training Unit, University of Surrey and The Royal Surrey County Hospital, Guildford.

Appropriate credit has been acknowledged and reference made to the work of others.

The work in this thesis has been carried out in accordance with the regulations of the University of Surrey. The work is original, and in no part of this thesis has been submitted for any other degree.

A grant was awarded for the work performed by The Royal College of Surgeons, England. They had no input into the design of this study, nor the presentation and publication of the results.

Views expressed are that of the author and not of the University of Surrey.
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**Summary**

Major surgery generates a stress response which increases oxygen demand and consumption post-operatively. Patients with low cardiopulmonary reserve may not be able to meet the increased oxygen demand and are at risk of increased morbidity. Dietary nitrate reduces the amount of oxygen required to perform a set amount of exercise and enhances exercise tolerance and performance in athletes by improving oxygen utilisation.

Cardiopulmonary exercise testing (CPX) is an established method of assessing patients’ cardiopulmonary reserve prior to surgery. The anaerobic threshold (AT) is the oxygen uptake at which anaerobic metabolism supplements aerobic metabolism; is calculated during CPX and can predict short and long term outcomes after surgery and postoperative complications.

We conducted a single centre, randomised, double-blind, placebo controlled trial to determine if dietary organic nitrate supplementation with beetroot juice (BRJ) improves pre-operative performance in CPX in patients with colorectal cancer (CRC). Eligible patients were adults undergoing elective laparoscopic resection for CRC. Patients were randomised to receive BRJ or nitrate-depleted BRJ (Placebo). Initial CPX was performed to a standard ramped incremental exercise protocol. Patients then received BRJ or placebo every day for 7 days followed by a second CPX. The primary outcome measure was the change in AT.

There was a statistically significant increase in the mean AT in the nitrate group (+0.706, 95% CI 0.130 to 1.281; p=0.018) but not in the placebo group. There was a significantly lower length of stay in nitrates group. There was no significant difference in rate of complications between the two groups.

Just 7 days of dietary nitrate supplementation results in a significant improvement in oxygen utilisation in an elderly population with colorectal cancer. This is a novel finding and this study is the first in patients undergoing surgery. This trial provides evidence that dietary nitrate supplementation is beneficial in a preoperative setting.
# Table of contents

- Declaration .......................................................................................................................... 2
- Acknowledgements .............................................................................................................. 3
- Summary ............................................................................................................................... 4
- Table of contents .................................................................................................................. 5
- List of Figures ....................................................................................................................... 9
- List of Appendices ................................................................................................................ 10

1. Introduction ......................................................................................................................... 11
   - 1.1 Colorectal cancer ........................................................................................................ 11
   - 1.2 Enhanced Recovery after Surgery ........................................................................... 11
      - History of peri-operative care .................................................................................. 11
      - Enhanced Recovery after Surgery ........................................................................... 12
   - 1.3 Prehabilitation ........................................................................................................... 15
      - 1.3.1 Exercise prehabilitation ................................................................................... 16
      - 1.3.2 Malnutrition and Nutritional prehabilitation ................................................... 17
   - 1.4 Nitrates ....................................................................................................................... 18
      - 1.4.1 History of nitrates ........................................................................................... 19
      - 1.4.2 Sources of nitrates ........................................................................................... 21
      - 1.4.3 Nitrate Absorption – Entero-salivary circulation .............................................. 23
      - 1.4.4 Effects of dietary nitrate supplementation in healthy volunteers ...................... 26
      - 1.4.5 Nitrates mechanism of action ............................................................................ 32
      - 1.4.6 Effects of dietary nitrate supplementation in Clinical populations ....................... 38
      - 1.4.7 Effects of Dietary nitrate supplementation in disease ........................................... 44
      - 1.4.8 Conclusion - Effects of Dietary nitrate supplementation in healthy volunteers, clinical populations and patients with disease ......................................................................................... 50
   - 1.5 The Ageing population ............................................................................................... 51
   - 1.6 Assessment of risk prior to surgery ............................................................................ 52
      - 1.6.1 Questionnaires / Risk scores .............................................................................. 53
      - 1.6.2 Formal Exercise tests .......................................................................................... 55
   - 1.7 Conclusion – assessing risk prior to surgery ............................................................ 60

2. Trial aims and hypothesis .................................................................................................. 62
   - 2.1 Aims ............................................................................................................................ 62
   - 2.2 Design ........................................................................................................................ 62
   - 2.3 Hypotheses ................................................................................................................ 62
2.4 Primary Outcomes ........................................................................................................62
2.5 Secondary outcomes ....................................................................................................62
3. Methods ..........................................................................................................................63
   3.1 Trial design ..................................................................................................................63
   3.2 Patient Selection .........................................................................................................64
   3.3 Recruitment ...............................................................................................................65
      3.3.1 Patient preparation ..............................................................................................65
   3.4 Protocol .......................................................................................................................66
   3.5 Measurements ............................................................................................................67
      Cardiopulmonary Exercise testing ...............................................................................67
   Adherence .......................................................................................................................68
   Surgical technique .........................................................................................................68
   Post-operative measurements .......................................................................................68
   3.6 Randomisation ..........................................................................................................69
   3.7 Blinding ......................................................................................................................70
   3.8 Statistics ....................................................................................................................70
      Sample Size calculation ...............................................................................................70
      Statistical analysis .......................................................................................................70
   3.9 Approvals and Registrations .....................................................................................71
      Health Research Authority regulations and approval ................................................71
      Ethics Approval / Research and development sponsorship ....................................71
      Randomised Clinical Trial Registration ....................................................................71
4. Results ............................................................................................................................72
   Patient Flow ....................................................................................................................72
   Figure 5 Consort diagram .............................................................................................72
Demographics .....................................................................................................................73
   Figure 6 Demographics .................................................................................................74
   Exclusions .......................................................................................................................74
   Figure 7 Clinical and operative data ............................................................................76
      Surgical Procedure, Operative Time and Histology ................................................76
   Histology .......................................................................................................................77
   Laboratory values ..........................................................................................................77
   Juice Compliance .........................................................................................................77
   Figure 8 Juice compliance ............................................................................................77
Cardiopulmonary exercise test variables ........................................................................78
Main CPX Variables .................................................................................................................. 78
Figure 9 Main CPX variables ........................................................................................................ 78
Figure 10 Change in AT with Nitrato Supplementation ............................................................... 79
Figure 11 Change in AT with placebo ............................................................................................. 80
Figure 12 AT before and after 7 days supplementation ............................................................... 81
Ramp / Watts ................................................................................................................................ 81
Other CPX Variables ................................................................................................................... 82
Spirometry data ........................................................................................................................... 82
Figure 13 Spirometry results ......................................................................................................... 82
Blood pressure ............................................................................................................................. 83
All patients ..................................................................................................................................... 83
Non hypertensive patients only ..................................................................................................... 83
Figure 14 Blood pressure ............................................................................................................... 84
Post operative outcomes .............................................................................................................. 84
Length of stay ................................................................................................................................. 84
Figure 15 Median Length of Stay .................................................................................................. 84
Figure 16 Post operative outcomes .............................................................................................. 85
Grip Strength ................................................................................................................................. 85
Figure 17 Grip Strength (T Test) .................................................................................................. 86
ERAS Compliance .......................................................................................................................... 86
Figure 18 ERAS Compliance ........................................................................................................ 86
Complications ............................................................................................................................... 87
Clavien Dindo classification ........................................................................................................... 87
Figure 19 Clavien Dindo ................................................................................................................. 87
Complications within 30 days ......................................................................................................... 87
Figure 20 Specific complications within 30 days ......................................................................... 88
Overall complications .................................................................................................................... 88
Figure 21 Overall complications .................................................................................................. 89
5. Discussion .................................................................................................................................. 90
5.1 Summary of Results .................................................................................................................. 90
5.2 Changes in Oxygen consumption with dietary nitrate supplementation ................................. 90
5.3 Variable response to dietary nitrate supplementation .............................................................. 90
5.4 Blood pressure changes with dietary nitrate supplementation ................................................. 91
5.5 Post-operative outcomes ........................................................................................................ 91
5.6 Possible underlying mechanisms for findings .......................................................................... 92
5.7 Limitations and strengths of study ................................................................. 93
5.8 Future research directions ................................................................................. 95
6 Conclusion ........................................................................................................ 96
7. Glossary ........................................................................................................... 97
8. Appendix ......................................................................................................... 100
9. Bibliography .................................................................................................... 166
List of Figures

Figure 1 Summary of ERAS recommendations ................................................................. 13
Figure 2 Nitrate content vegetables .............................................................................. 22
Figure 3 Enterosalivary absorption pathway ................................................................. 25
Figure 4 DASI score ...................................................................................................... 54
Figure 5 Consort diagram ............................................................................................. 72
Figure 6 Demographics ............................................................................................... 74
Figure 7 Clinical and operative data ............................................................................ 76
Figure 8 Juice compliance ............................................................................................. 77
Figure 9 Main CPX variables ....................................................................................... 78
Figure 10 Change in AT with Nitrate Supplementation ................................................ 79
Figure 11 Change in AT with placebo) .......................................................................... 80
Figure 12 AT before and after 7 days supplementation ................................................ 81
Figure 13 Spirometry results ......................................................................................... 82
Figure 14 Blood pressure .............................................................................................. 84
Figure 15 Median Length of Stay .................................................................................. 84
Figure 16 Post operative outcomes ............................................................................. 85
Figure 17 Grip Strength ................................................................................................. 86
Figure 18 ERAS Compliance ......................................................................................... 86
Figure 19 Clavien Dindo ............................................................................................... 87
Figure 20 Specific complications within 30 days ......................................................... 88
Figure 21 Overall complications .................................................................................. 89
List of Appendices

Appendix 1 Nitrate content vegetables ........................................................................................................ 101
Appendix 2 Summary Effects of dietary nitrate supplementation in healthy volunteers ......................... 102
Appendix 3 Summary Effects of dietary nitrate supplementation in older volunteers .............................. 114
Appendix 4 Summary Effects of dietary nitrate supplementation in clinical populations .................... 118
Appendix 5 Plasma nitrate and nitrite levels in clinical populations after dietary nitrate supplementation ..................................................................................................................... 137
Appendix 6 Blood pressure changes in clinical populations after dietary nitrate supplementation .... 138
Appendix 7 CPX report variables ................................................................................................................. 141
Appendix 8 Patient Information leaflet ...................................................................................................... 144
Appendix 9 GP Information leaflet ............................................................................................................. 153
Appendix 10 Food Diary ............................................................................................................................... 154
Appendix 11 Patients without cancer on histology ...................................................................................... 158
Appendix 12 Laboratory values .................................................................................................................... 158
Appendix 13 Ramp data ............................................................................................................................... 158
Appendix 14 Summary CPX results ........................................................................................................... 159
Appendix 15 Summary Spirometry results ................................................................................................. 160
Appendix 16 Summary blood pressure results ............................................................................................ 161
Appendix 17 Clavien Dindo scoring system ............................................................................................... 162
Appendix 18 All inpatient complications classified by Clavien Dindo ...................................................... 163
Appendix 19 Complications after discharge not requiring readmission .................................................. 165
Appendix 20 Readmissions ......................................................................................................................... 165
1. Introduction

1.1 Colorectal cancer

Colorectal (bowel) cancer is the second most common cause of cancer death in the UK, with over 40,000 new diagnoses a year. Every year approximately 16,000 people die from colorectal cancer (1, 2). Colorectal cancer (CRC) is a disease of the elderly, with incidence rates rising from age 50-54 years, with the highest rates in the 85-89 year old group. In 2012-2014, 44% of CRC were diagnosed in people age 75 and over in the UK (1). Ten year survival after being diagnosed with CRC is relatively high at 57% (2010-2011). However in England, 5 year survival rates for CRC in both men and are below average when compared with the rest of Europe (1).

Surgery is the most effective way of curing CRC and can be also used for palliation to avoid late complications such as perforation and obstruction. The majority of patients undergo an operation to treat their CRC, with 62% undergoing surgery in 2006-2010. Despite occurring more commonly in the elderly, only 39% of those aged 85 and above undergo surgery for their CRC compared with 64% of those aged 15-54 (1). Why those who have the highest incidence of CRC are the least likely to have surgery is multifactorial. Elderly patients are more likely to have multiple comorbidities which in turn increase surgical risk. The elderly may also be more reluctant to undergo surgery. Cancer biology is felt to be less aggressive in older patients and so the decision not to operate may be made on the basis that it could detrimentally affect quality of life and may not improve survival. However with an aging population the number of patients suffering from and requiring surgery for colorectal cancer is increasing. Efforts are therefore being made to reduce the risks of major surgery and thereby improve outcomes.

1.2 Enhanced Recovery after Surgery

History of peri-operative care

Major surgery generates a stress response comprising metabolic and inflammatory changes which increase oxygen demand and consumption postoperatively. Patients who have low cardiopulmonary reserve may not be able to meet these increased oxygen demands and therefore are at risk of increased morbidity and mortality post-operatively.

Traditionally, patients undergoing elective surgery for CRC were fasted preoperatively and given bowel preparation. Surgery was performed via a large incision and post-operatively bed rest was
encouraged whilst foods were reintroduced slowly. This resulted in a 7-10 day post-operative stay and a 15-48% complication rate (3, 4).

Standard practice was to fast patients from midnight to ensure an empty stomach prior to anaesthesia to reduce the risk of pulmonary aspiration despite there being little scientific reason to do so (5). This level of fasting increased catabolism by depleting glycogen stores pre-operatively leading to the intra-operative breakdown of muscle to meet energy requirements (5).

Patients were given bowel preparation to ‘clean’ the bowel prior to surgery in the belief that it would prevent infection by decreasing the spillage of bowel contents, both during surgery and if an anastomotic leak (see glossary for explanation) occurred (5). However this caused dehydration and electrolyte imbalances which were exacerbated in the elderly population (3). The large incision stimulated a significant inflammatory response including the development of insulin resistance and also caused pain with associated pulmonary dysfunction and ileus.

Intraoperatively patients were given liberal amounts of intravenous fluids to replace third space losses, and to combat the dehydration from bowel preparation and starvation. This caused salt water overload and oedema and impaired tissue oxygenation. Postoperatively foods were reintroduced slowly and oral opiates were the main form of pain relief. Patients were advised to rest in bed after an operation and the average length of stay for an open appendicectomy was 8 days in 1980 (6). This period of prolonged bed rest increased insulin resistance, the risk of pneumonia, thromboembolism, and muscle wasting.

It is now widely recognised these interventions were not only unhelpful in aiding recovery but were in fact ‘outdated and harmful’ (4).

**Enhanced Recovery after Surgery**

The majority of patients undergoing surgery for CRC nowadays follow an enhanced recovery after surgery (ERAS) pathway which aims to minimise the stress response generated by surgery. These pathways comprise a multimodal combination of evidence based peri-operative interventions aimed at improving oxygen delivery and maintaining homeostasis (3). Initially adopted in a few centres for mainly colorectal surgery, ERAS programmes have become the standard of care in most surgical specialities (7). For a summary of ERAS recommendations see Figure 1.
Pre-operative recommendations

1. Pre-operative counselling and training.
2. A curtailed fast (6 hours to solids and 2 hours to clear liquids) and pre-operative carbohydrate loading.
5. A single dose of prophylactic antibiotics covering both aerobic and anaerobic pathogens

Peri-operative recommendations

1. High (80%) inspired oxygen concentration in the peri-operative period
2. Prevention of hypothermia
3. Goal directed intra-operative fluid therapy
4. Preferable use of short and transverse incisions for open surgery
5. Avoidance of post-operative drains and nasogastric tubes
6. Short duration of epidural analgesia and local blocks

Post-operative recommendations

1. Avoidance of opiates and the use of paracetamol and non steroidal anti-inflammatory drugs (NSAIDS)
2. Early commencement of post-operative diet
3. Early and structured post-operative mobilisation
4. Administration of restricted amounts of intravenous fluid
5. Regular Audit

Figure 1 Summary of ERAS recommendations (8)

The stress response after surgery can be harmful in excess (7) and reduction of the stress response enhances immunity and may impact on short and long term survival (7). By reducing the stress response following surgery, oxygen demand is decreased and the development of insulin resistance curtailed. Central to the principles of ERAS protocols is improving oxygen delivery and maintaining homeostasis.

Peri-operatively the use of high concentration (80%) inspired oxygen is recommended as it has been shown that anastomotic healing depends upon adequate tissue perfusion and oxygenation (9). Administering 80% oxygen during and after an operation improves perfusion at the anastomotic site and has also been shown to reduce the rate of wound infections (8).
Postoperatively, the use of epidural anaesthesia decreases oxygen demand by directly inhibiting the stress response and blocking sympathetic activity (10). Studies have shown that particularly thoracic epidurals reduce the sympathetic and pituitary/adrenocortical response to surgery (7). Meta-analyses have shown that epidural anaesthesia in major surgery reduces the rate of thromboembolism, respiratory depression and consequent pneumonia, and reduces the need for opiate analgesia. This allows early mobilisation and feeding, less post-operative nausea and vomiting and a quicker return of gastrointestinal (GI) function (7).

Spinals provide limited duration blockade and so transiently suppress the hypophyseal-pituitary-adrenal axis reducing the stress response (10). As the blockade is limited they are associated with less hypotension and immobilisation. In one RCT comparing PCA with spinal anaesthesia, spinal anaesthesia was associated with less post-operative complications mainly due to the decreased rates of ileus (10).

**Benefits of ERAS**

As well as reducing length of stay by more than 2 days and therefore being economical, ERAS has been shown to lead to early return of gut function, improve post-operative recovery and to reduce post-operative morbidity and complication rates by as much as 50% for patients undergoing open surgery (4) with no concomitant increase in readmission rates or mortality.

Complications negatively impact on survival. Postoperative complications in particular are a significant cause of avoidable morbidity and mortality (11) and adversely affect long term survival (12). In Khuri’s study which looked at 105,951 patients after non-cancer surgery, the occurrence of any complication decreased 5 year survival from 52.3% to 34.8%. A simple urinary tract infection in the postoperative period was associated with a decrease in 5 year survival from 47.9% to 33.3% (12). In the same study any complication in the first 30 days after surgery was the most important factor in determining long-term survival, even more important than patient risk preoperatively. The occurrence of a complication in the first 30 days reduced median survival by 69%.

More recently high adherence to ERAS protocols in patients with CRC has been proven to be associated with increased rates of 5-year cancer specific survival (13). Any complication post-operatively can decrease long term survival post colorectal resection (12) and anastomotic leak is associated with higher local and distant recurrence rates (14). Therefore by decreasing complication rates ERAS protocols improve long term survival.
This is thought to be because surgical trauma causes a transient post-operative suppression of immune function which may in turn lead to higher rates of recurrence (15). The mechanism by which this occurs is thought to be by suppression of the immune system post-operatively causing increased adhesion of exfoliated tumour cells or enhanced growth of residual tumour cells (15) thereby increasing metastases and recurrence. It has also been hypothesised that inflammation associated with an operation or complications may accelerate apoptosis and therefore negatively impact upon survival (12).

If a patient does not experience any complications after colorectal surgery the main factor in determining recovery is the time it takes for bowel function to return (4). This is adversely affected by prolonged fasting, the use of bowel preparation, opiate analgesia, overuse of intravenous fluids and pre-existing comorbidities. Avoiding these in an ERAS setting therefore also improves recovery.

**Conclusion**

ERAS pathways minimise immune dysfunction and reduce morbidity rates by reducing the stress response and improving oxygen delivery. This may in turn improve cancer survival rates. **The key therefore post-operatively is meeting the increased oxygen demand, thereby avoiding complications and improving long term survival.**

**1.3 Prehabilitation**

Whilst ERAS has optimised peri-operative care, focus has now shifted to the pre-operative period. Prehabilitation is the process of improving an individual’s functional and mental capacity before an operation. The aim of prehabilitation is to increase physiological reserves to withstand the stress of surgery and thereby improve post-operative recovery and outcomes (16).

In the past interventions were aimed at improving recovery post-operatively, otherwise known as rehabilitation. However with the development of ERAS pathways has come the recognition that intervention in the pre-operative period may be more successful as (17)-

1. Patients are more receptive / motivated pre-operatively and the date of surgery can act as a goal to which to work towards
2. Functionally patients are fitter pre-operatively and therefore more able to engage in exercise
3. Prevention is better than cure - Improving fitness pre-operatively may reduce complications and avoid reduction in post-operative functional capacity

4. The window between the decision to operate and the operation date provides a time period for intervention

Prehabilitation programmes are multimodal encompassing exercise, nutritional and psychological support and behavioural modification to reduce modifiable risk factors and thereby decrease surgical risk. In an RCT of prehabilitation versus rehabilitation Gillis et al found that a trimodal programme of exercise, nutritional and psychological therapy led to a significant increase in the proportion of patients who recovered to or above baseline exercise capacity at 8 weeks when given preoperatively than when given postoperatively (18).

However it should be noted that patients with colorectal cancer often undergo surgery within 3-4 weeks of diagnosis within a 64 day treatment pathway and so there is a limited window in which to perform prehabilitation.

1.3.1 Exercise prehabilitation

It is known that patients who are fitter have better outcomes (17, 19). Both unsupervised and supervised exercise training programmes have been shown to be beneficial nevertheless inconsistencies with methodology have led to varying results in trials (17) and compliance is a problem.

West et al found that a 6 week exercise programme of 3 supervised sessions per week improved the anaerobic threshold (AT); a marker of fitness measured by CPX; by 2.1 ml/kg/min (20). This duration of exercise may be feasible in patients who are having neoadjuvant treatment for their cancer but in patients who proceed straight to surgery there may not be enough time for this.

A recent systematic review of elderly patients; perhaps those who would benefit the most; proved that prehabilitation can improve fitness (21). However despite an increase in physical fitness there was no significant reduction in length of stay or rate of complications. The authors noted the heterogenous nature of the studies which precluded a meta-analysis and a variable compliance rate of 16-97%, and postulated that supervised exercise may be better at improving fitness.
Currently exercise prehabilitation is not recommended as part of an ERAS pathway (22) but results have been inconsistent and more studies are needed.

1.3.2 Malnutrition and Nutritional prehabilitation

More recently attention has been drawn to prehabilitation with nutrition, so called ‘nutritional prehabilitation’. In studies conducted mainly on the 1970’s and 80s malnutrition occurred in 40-50% of surgical and medical inpatients (23). In one UK study, 20% of preoperative colorectal cancer patients were malnourished (24).

Malnutrition in surgical patients

Malnutrition is the nutritional state of deficiency of protein, energy and nutrients which results in adverse postoperative outcomes. It has been defined as - a BMI of less than 20 and weight loss of greater than 5% in 3-6 months, or a BMI of less than 18.5, or unintentional weight loss more than 10% in 3-6 months (25). Malnutrition is associated with increased surgical morbidity and mortality and prolonged length of stay (5, 26).

Surgical patients are often malnourished due to multifactorial disease processes including alimentary tract dysfunction and starvation, operative induced catabolism and complications (17). Patients with cancer can be malnourished due to local effects of the tumour, the host response to the tumour, anticancer treatment and increased in nutrient metabolism (27). The tumour itself can cause pain, anorexia, alterations in taste and smell, and cachexia and tiredness, all of which lead to decreased food intake (27) further exacerbating the imbalance. Patients with cancer have been shown to have an increased resting energy expenditure (27).

The stress response of surgery causes proteolysis and lipolysis to enable gluconeogenesis (17), resulting in insulin resistance and a hypermetabolic state which can last for several weeks postoperatively. The protein loss occurs primarily from skeletal muscle leading to a decline in functional capacity and post-operative fatigue (17). In patients with cancer the hypermetabolic state is more common due to the increased gluconeogenesis (27), and therefore fasting in cancer patients leads to further protein depletion and muscle atrophy.

Postoperatively in particular, food consumption is reduced whilst nutritional requirements increase leading to a catabolic state (27). GI surgery can also cause direct problems with dietary intake and
absorption and it has been shown that there is a decline in nutritional status for 2 months after discharge (28).

Malnutrition can impair performance status, immune, organ, and muscle function, and quality of life leading to increased morbidity and length of stay (28). It can negatively affect the response to anticancer treatment and overall survival (27). Preoperative weight loss and malnutrition has been shown to increase the risk of anastomotic leaks (29, 30). Malnourished patients have limited nutritional stores and it has been shown that pre-operative nutritional supplementation in malnourished patients decreases infectious complications and the rate of anastomotic leaks postoperatively (3). In a multicentre prospective cohort study Jie et al found that a minimum of 7 days parenteral or enteral nutritional support for patients deemed high risk for malnutrition resulted in a 50% reduction in complication rates and a significantly shorter length of stay (31).

Malnourished patients are more frail with limited physiological reserves and therefore even within ERAS pathway they are still at increased risk of complications and prolonged LOS (32). Studies have shown that after surgery for colorectal cancer malnourished patients managed within an ERAS pathway have a longer stay, more complications, and delayed GI recovery and that the these correlate with degree of malnutrition (32).

**Carbohydrate loading and ERAS**

Pre-operative carbohydrate loading; with a dietary supplement; is now an established part of all ERAS pathways including colorectal (22) with the belief that patients thus loaded undergo surgery in a ‘metabolically fed’ state. Studies have shown it can reduce pre-operative hunger and thirst and postoperative insulin resistance (22). However a Cochrane review published in 2014 was less supportive concluding that whilst carbohydrate preloading was associated with a small but significant decrease in length of stay there was no associated reduction in complications (33).

**1.4 Nitrates**

We have shown that ERAS pathways have improved post-operative outcomes by improving oxygen delivery, but what if patients could use the oxygen that is delivered more efficiently? In the last decade, multiple trials have been performed by sports scientists which have shown that dietary nitrate supplementation can improve the efficiency with which the body uses oxygen and therefore reduce the oxygen cost of exercise by enabling nitric oxide (NO) production in hypoxic conditions.
Traditional exercise physiology states that the oxygen consumption per unit submaximal work rate is a physiological constant at approximately 10 ml/min/watt, regardless of training, age or diet (34, 35). Pulmonary oxygen uptake (\( \text{VO}_2 \)) increases linearly in relation to work rate until gas exchange threshold (GET) is met. (For further explanation see Glossary and Section 1.6.2.3 Cardiopulmonary Exercise testing)

However in 2007 Larsen et al performed a ground-breaking trial in nine healthy young men and found that dietary nitrate supplementation with sodium nitrate 0.1mmol/kg/day could alter pulmonary \( \text{VO}_2 \) dynamics by reducing \( \text{VO}_2 \) during submaximal exercise, thereby decreasing oxygen consumption during exercise (36). They also found a significant increase in calculated muscular efficiency with nitrate supplementation. There was no significant change in peak \( \text{VO}_2 \), heart rate (HR), RER or maximal work rates.

Following this multiple studies were conducted in athletes and patients confirming a decrease in oxygen consumption during exercise with the use of nitrate supplements (34, 37-44). Dietary nitrate supplementation has consequently become very popular in the sporting world and the use of beetroot juice as a high nitrate nutritional supplement has become widespread, with Team GB Olympics, British cycling, premiership footballers and rugby being regular users (45, 46). Proponents of beetroot juice claim it can enhance exercise tolerance and performance (35).

However nitrates have not always been so positively regarded. In fact, historically dietary nitrate intake has been limited by fears over carcinogenesis and levels of nitrate and nitrite are closely regulated in food and drinking water (47).

1.4.1 History of nitrates

Traditionally both nitrite and nitrate were used in meat preserving (48) to enhance flavour and appearance and prevent meat going rancid by inhibiting microorganisms. However if nitrate was used as a preservative a variable conversion of nitrate to nitrite during curing could lead to inadequate or excessive amounts of nitrite in the finished product. In 1923 studies were performed to determine the minimum amount of sodium nitrite required to cause curing and it was established that nitrate was not needed for the curing process. In 1925 USDA approved nitrite alone as a meat curing agent (48).
**Cancer risk**

In the 1960s Nitrosamines were identified as carcinogenic. In the 1970s the potential for nitrites in combination with amines or amides under acidic or heat conditions to form nitrosamines was noted and it was realised that nitrosamines were formed when bacon was fried at high temperatures (48). It was also noted that exposure to nitrates led to the development of lymphoma and hepatic tumours in rat populations, and a nitrosamine in food NDMA (N-nitrosodimethylamine) was noted to be a potent carcinogen (49). This led to widespread concerns over potential carcinogenicity of a diet high in nitrates due to cured and processed meats and it became difficult to separate nitrate and processed meat as the cause of cancer. Extensive negative media coverage about nitrates at the time may be partially responsible for the negative perceptions of nitrites and nitrates today.

A recent meta-analysis in 2015 with a total of 650,826 combined patients found that consumption of a diet rich in nitrates was actually associated with a decreased risk of gastric cancer whereas a diet high in nitrites and NDMA carried an increased risk of cancer (49). They also noted that whilst other studies (50) had proven an association between a high nitrate / low antioxidant diet and gastric cancer, their pooled data did not, and that more work was needed in this area.

However with the increasing recognition of the importance of Nitric Oxide (NO) in recent years, and the discovery of the entero-salivary pathway; an innate pathway which actively absorbs nitrate from the diet; this has been called into question. Current expert consensus is that the ingestion of nitrates is safe (48, 49, 51, 52).

**A healthy diet**

A consistent finding of nutritional research is that a diet high in fruit and vegetables is protective against the development of cardiovascular disease, cancer and diabetes. The mechanism underlying this is unclear and further research is required to identify the specific nutrients and components of vegetables and fruit associated with a decrease risk of cardiovascular disease and cancer (51). It is however, widely recognised, that the benefits of high fruit and vegetable consumption significantly outweigh any perceived risk of developing cancer from consumption of nitrate and nitrite (51).

The DASH diet (DASH = dietary approaches to stop hypertension) is an eating plan designed to lower blood pressure (BP) without medication (53). As well as lowering BP it has been shown to decrease the incidence of gout (54). Hord et al (51) modelled a diet based on low and high nitrate choices of the DASH diet and measured nitrate and nitrite content. They found that the DASH diet is naturally
high in nitrates, providing between 174mg-1222mg of dietary nitrate and 0.351-0.041mg nitrite. They postulated that the high nitrate content of diet acts as the substrate for NO which acts to cause vasodilation and decreased BP thereby supporting cardiovascular function (51). Dejam et al postulated whether nitrate conversion to nitrite underlies the cardiovascular protective effects of a Mediterranean diet which is also rich in vegetables (55).

**Gastroprotective effects of nitrate**
Further evidence of the benefit of nitrates came from animal studies. Nitrate supplementation in rats led to a 20% increase in the thickness of the mucous layer in the stomach, which did not occur in the rats given nitrate and treated with mouthwash, but was replicated in the rats given oral nitrite (56). Rats given diclofenac and nitrate supplementation also showed inhibited diclofenac induced P selectin upregulation, leading to a decreased inflammatory response (56). Petersson et al therefore postulated that the supplementary dietary nitrate was converted to nitrite by bacteria in the mouth (56). The nitrite produced was then reduced to NO in the acidic stomach, which led to increased gastric mucosal blood flow and mucous formation thereby protecting against ulcer formation. In rats treated with mouthwash the initial conversion of nitrate to nitrite in the mouth was abolished and therefore the mucous layer thickness did not change. Removing this step by supplying dietary nitrite restored this pathway. This study therefore implicates a role for salivary nitrite in regulation of mucous secretion (56) and gastroprotection (57).

**1.4.2 Sources of nitrates**
In humans the main sources of nitrates and nitrites are food, with approximately 80-85% of dietary nitrate coming from vegetable consumption, especially green leafy vegetables (51, 58). Leaves contain the highest levels of nitrates whereas seeds and tubers contain low levels (59). Cured meats and cereals are also naturally high in nitrates (52). Nitrate from sources other than vegetables i.e. meat and water has been estimated at 35-44mg/day for a 60kg person (51). Cured meats are treated with sodium nitrate but these levels are also strictly limited.

**Sources of nitrates - Vegetables**
Vegetables are the primary source of nitrates in the human diet. Farmers use nitrogen based fertilisers to boost crops therefore nitrate can accumulate, particularly in leaves of vegetables. Higher concentrations of nitrate are found vegetables grown in lower light exposure, for example in winter and in more northern latitudes where daylight hours are shorter (59).
Beetroot and green leafy vegetables including lettuce, rocket, and spinach contain high concentrations of inorganic nitrate at over 250mg (>4mmol) nitrate per 100g fresh weight (35). Higher concentrations of nitrate are found in the outer oldest leaves (59). One serving of lettuce, spinach or beetroot therefore contains more nitrate than is generated by entire NOS system in one day (47). Studies have measured the nitrate concentration of cooked spinach at 1880mg/kg (frozen, heated in microwave for 20 mins at 90 watts), lettuce 3017mg/kg, and cooked beetroot 3017mg/kg (boiled in tap water for 35 mins then cubed) (58). Bioavailability was approximately 100%. For more specific details of the nitrate content of common vegetables see Figure 2 and Appendix 1.

<table>
<thead>
<tr>
<th>vegetable</th>
<th>Nitrate content mg/kg</th>
<th>g containing 400mg nitrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rocket</td>
<td>4677</td>
<td>85</td>
<td>(60)</td>
</tr>
<tr>
<td>lettuce</td>
<td>3017</td>
<td>133</td>
<td>(58)</td>
</tr>
<tr>
<td>cooked beetroot</td>
<td>3017</td>
<td>133</td>
<td>(58)</td>
</tr>
<tr>
<td>Chervil</td>
<td>&gt;2500</td>
<td>&lt;160</td>
<td>(61)</td>
</tr>
<tr>
<td>Cress</td>
<td>&gt;2500</td>
<td>&lt;160</td>
<td>(61)</td>
</tr>
<tr>
<td>cooked spinach</td>
<td>1880</td>
<td>213</td>
<td>(58)</td>
</tr>
<tr>
<td>Endive</td>
<td>1,475</td>
<td>271</td>
<td>(60)</td>
</tr>
<tr>
<td>beans</td>
<td>&lt;200</td>
<td>&gt;2000</td>
<td>(61)</td>
</tr>
<tr>
<td>cucumber</td>
<td>185</td>
<td>2,162</td>
<td>(60)</td>
</tr>
<tr>
<td>cauliflower</td>
<td>148</td>
<td>2,703</td>
<td>(60)</td>
</tr>
<tr>
<td>peppers</td>
<td>108</td>
<td>3704</td>
<td>(60)</td>
</tr>
<tr>
<td>garlic</td>
<td>69</td>
<td>5,797</td>
<td>(60)</td>
</tr>
<tr>
<td>tomato</td>
<td>43</td>
<td>9,302</td>
<td>(60)</td>
</tr>
<tr>
<td>carrots</td>
<td>10</td>
<td>40,000</td>
<td>(61)</td>
</tr>
</tbody>
</table>

**Figure 2 Nitrate content vegetables**

Nitrate levels can however vary considerably, depending on where vegetable grown (48). Organic vegetables are likely to contain less nitrate than those grown with nitrogen fertilisers. Nitrate losses can also occur from washing, peeling and cooking.

**Sources of nitrates - Water**

Groundwater contains below 10mg/l of nitrates. However nitrate is used as a fertiliser in agriculture (59) and can contaminate groundwater (52). Groundwater contaminated by fertiliser therefore can
have significantly higher levels of nitrate and exceptionally levels over 500mg/L have been reported (59). Levels of nitrate and nitrite are closely regulated in food and drinking water (47). In Europe the amount of nitrate permitted in water is 50mg nitrate ion/litre and in the US is 44mg nitrate ion/litre. Water nitrate levels are measured and treated to ensure nitrate content does not rise above these levels.

1.4.3 Nitrate Absorption – Entero-salivary circulation

It was previously believed NO was solely generated in vivo via the L-arginine pathway through oxidation of L-arginine by the nitric oxide synthase (NOS) family of enzymes (62). It is now recognised that via the entero-salivary pathway nitrite and nitrate absorbed from the diet can be reduced in vivo to NO. Dietary nitrate/nitrite may account for approximately 50% of NO at steady state and body stores can be increased by ingestion of dietary nitrate (51).

The absorption pathway for dietary nitrate has been recently well established (Figure 3). Ingested nitrate is absorbed in proximal intestine with a bioavailability of 100% and circulates in the plasma (51, 58). Approximately 25% of the circulating nitrate is concentrated nearly 10 fold in the saliva which is then secreted in the mouth (59, 63). In the mouth the 20% of the salivary nitrate (i.e. 5-8% of total nitrate intake) is reduced to nitrite by commensal facultative anaerobic bacteria which are found in crypts on the surface of the tongue (63).

Some of the swallowed nitrite in the saliva is converted to NO in the acid environment of the stomach however most is absorbed and enters the circulation as plasma nitrite. Nitrate is also actively secreted by the gastric parietal cells into the stomach where it can be reduced to nitrite by bacteria (59). Consumption of oral nitrite has been shown in vitro to have an antimicrobial effect on common gut pathogens including salmonella and E Coli (64). Therefore the concentration of nitrite in the saliva has been postulated as form of innate immunity as it may protect non-specifically against pathogens in GI tract (48).

Human colostrum contains high concentrations of nitrite and nitrate. In the first few days of life; before colonisation of GI tract by nitrate reducing bacteria; breast milk is high in nitrite. After day 3 breast milk nitrite levels drop and nitrate levels rise (65).

Endogenous NO is converted to nitrate in blood and is also taken up by salivary glands and secreted in saliva. Govoni et al noted fasting salivary nitrite levels of 0.26mM and calculated that saliva is
probably a major source of circulating nitrite (66). Following a bolus of dietary nitrate supplement plasma nitrate levels peak at 2 hours and then plasma nitrite levels peak at 3 hours (67). Plasma nitrite is reduced to NO in resistance vessels where the oxygen content is lower causing vasodilatation thereby reducing total peripheral resistance (TPR) and BP (68).

**Nitrate Excretion**

The majority of the remaining nitrate not concentrated in the saliva is excreted in urine, with a minimal amount being found in stool (48, 69). However approximately 80% of the nitrate in the urine is actively transported back into the blood. This active urinary scavenging as well as the recycling from saliva and bile suggests that nitrate is an important substance that the body is trying to conserve (59).

**Effects of Mouthwash on nitrate absorption**

Antibacterial mouthwash kills all types of bacteria and so prolonged use may reduce the number of nitrate reducing bacteria in the mouth and GI tract (56). If antibacterial mouthwash is used alongside ingestion of sodium nitrate thus removing the oral bacteria, systemic nitrite formation is significantly reduced (66). However there is still a significant (70%, likely to be enough for biological effect) rise in plasma nitrite after mouthwash use leading to speculation that either microflora in the stomach and small intestine or mammalian cells are capable of reducing nitrate to nitrite (66).

As mentioned previously, the concomitant use of antiseptic mouthwash with oral nitrate supplementation has been shown in rats to decrease the number of ‘nitrate reducing bacteria’ in the mouth (56) and as a consequence the concentration of plasma nitrite levels. Mouthwash also abolished the gastroprotective effect of nitrate supplementation but if nitrite was supplemented with mouthwash bypassing the need for the ‘nitrate reducing bacteria’ (56) then the damage from diclofenac to the stomach was reduced.
Entero-salivary circulation

Dietary nitrate/nitrite
- account for approximately 50% of NO at steady state
- body stores can be increased by Ingestion

Urine
80% of the nitrate in the urine actively transported back into blood

Plasma - Nitrate
Ingested nitrate is absorbed in proximal intestine, bioavailability 100%

Mouth –
- 25% circulating nitrate is concentrated x10 in saliva, secreted in the mouth
- Nitrate to nitrite -20% of the salivary nitrate is reduced to nitrite by bacteria in crypts tongue
- NO to nitrate - Endogenous NO is converted to nitrate in blood and is also taken up by salivary glands and secreted in saliva
- Nitrate excretion - Rest nitrate not concentrated into saliva is excreted in urine

Resistance vessels - nitrite to NO
Plasma nitrite is reduced to NO

Plasma - nitrite
Acts as a pool of NO

Stomach –
- Nitrite to NO -Some nitrite in saliva is converted to NO in the acid environment of the stomach
- Plasma nitrite -Salivary nitrite absorbed and enters the circulation
- Nitrate to nitrite - Nitrate is also actively secreted by the gastric parietal cells into the stomach where it can be reduced to nitrite by bacteria

Figure 3 Enterosalivary absorption pathway (Adapted from James et al (70))
In human studies the use of antibacterial mouthwash has also been shown to lower circulating nitrite and is associated with a rise in BP (71). In patients who consumed beetroot juice but then spat out all saliva, the rise in plasma nitrate occurred but plasma nitrite did not rise supporting the enterosalivary circuit theory. The BP reduction that occurs after beetroot juice ingestion was also lost in patients who spat. (68)

1.4.4 Effects of dietary nitrate supplementation in healthy volunteers

Over recent years the use of dietary nitrate supplements in the sporting world has become widespread. This is because multiple studies performed in athletes have shown that supplementation with dietary nitrate decreases the oxygen cost of exercise. The majority of studies performed in athletes are unfortunately generally characterised by small sample sizes, with all but 2 studies having 14 participants or less. The studies which are reliable, of good quality and that have further added to knowledge in this area are discussed below, the key findings of which are summarised in Appendix 1 - Summary Effects of dietary nitrate supplementation in healthy volunteers.

Effects of dietary nitrate supplementation on Submaximal exercise in healthy volunteers

The majority of studies in healthy volunteers with dietary nitrate supplementation have been performed using submaximal exercise protocols. Eight studies used a cycle ergometer to determine if nitrate supplementation would affect oxygen consumption at submaximal exercise. In their ground-breaking trial in 2007 Larsen et al gave participants 0.1mmol/kg of sodium nitrate split into 3 doses, for 3 days or placebo in a double blind crossover trial (36). They found a significantly reduced the oxygen cost of exercise; measured by a reduced VO$_2$ (p<0.02) during submaximal exercise.

Following this several other studies in healthy volunteers also confirmed a significant reduction in the VO$_2$ cost of submaximal exercise (34, 39, 41, 43, 44) using beetroot as a source of nitrate supplementation (34, 39, 42-44). Three studies also noted a significantly increased time to task failure with nitrate supplementation (34, 38, 42).

The acute effects of nitrate supplementation were studied by Wylie 2013 who performed the largest study with 34 participants, with a randomised balanced crossover design trial which aimed to investigate the acute dose-response relationship between nitrate and the physiological response to exercise (42). Participants were given a single dose of 4.2, 8.4, 16.8mmol of nitrate in a denomination of a 70ml concentrated sports shot. At moderate intensity exercise they found a
significantly decreased end exercise VO₂ after 16.8mmol but not 4.2 or 8.4 mmol of nitrate compared with placebo. They also noted an improvement in time to task failure with 8.4mmol nitrate, which was not further improved by 16.8mmol, but did not occur with 4.2mmol nitrate supplementation.

Vanhatalo et al studied the acute and chronic effects of nitrate supplementation and confirmed a 4% reduction in steady state pulmonary oxygen uptake with 5.2mmol nitrate supplementation but not placebo supplementation which occurred after only 2.5 hours ingestion (39). The effect was maintained with 15 days supplementation, even with a normal diet.

In one of the key studies into the effects of chronic supplementation, Wylie et al gave patients dry beetroot extract containing 3 or 6mmol of nitrate in an independent group matched placebo for 30 days to investigate whether long term low dose nitrate supplementation would change oxygen dynamics (43). They found that 6mmol but not 3mmol nitrate supplementation significantly reduced the steady state VO₂ at submaximal exercise after 2 hours, 7 days and 28-30 days of supplementation. The reduction in VO₂ associated with the 6mmol supplementation lasted 24 hours after the last nitrate dose despite a decreased plasma nitrite and therefore was the first demonstration of decreased submaximal VO₂ after chronic supplementation without an associated increase in systemic plasma nitrate. Furthermore they noted that the addition of another nitrate dose after the 24 hour gap in supplementation whilst causing an increase in plasma nitrite did not reduce oxygen consumption further.

Interestingly, despite a significant increase in plasma nitrite and nitrate with the 3mmol nitrate dose there was no reduction in the oxygen cost of submaximal exercise after 7 days or almost 4 weeks of supplementation. The authors therefore concluded that extended supplementation with low dose 3mmol nitrate could not reduce submaximal exercise VO₂, whereas supplementation with 6mmol nitrate reduced the oxygen cost of exercise after only 7 days and the effect was maintained for up to 4 weeks with no reduction in sensitivity.

Several studies used alternatives to cycle ergometers and also found a decrease in the oxygen cost of exercise (37, 38, 40). Larsen et al tested healthy volunteers on an arm and leg ergometer and confirmed a significant decrease in VO₂ after a single dose of nitrate at submaximal exercise (37). In 2010 Bailey et al used a knee extension ergometer and found a 25% reduction in pulmonary VO₂ during low intensity exercise with an 11 % reduction overall after 6 days of 5.1mmol nitrate
supplementation (38). At high intensity exercise nitrate supplementation led to a significantly 25% decreased slow component and 25% improvement in time to task failure.

Lansley 2011 used a treadmill and found a significant reduction in the VO2 during walking of 12%, end exercise VO2, the amplitude of pulmonary VO2 response and the oxygen cost of running 1km with nitrate supplementation (40). There was no significant change in end-exercise VCO2, Ve, RER, HR and blood lactate between the two groups. They noted that the improvements after 4 days of nitrate supplementation were comparable to 6-9 weeks of training.

Therefore at submaximal exercise the majority of studies in young healthy volunteers have found a significant decrease in oxygen consumption with nitrate supplementation (34, 36-44, 72).

Effects of dietary nitrate supplementation on Maximal exercise in healthy volunteers

Only a few of studies in healthy volunteers with dietary nitrate supplementation have been performed using maximal exercise protocols. The majority have found a significant improvement oxygen utilisation after more than one dose of supplementation (37, 39, 40, 43).

In their seminal paper in 2007 Larsen et al found no change in the VO2peak or VEmax (maximal measurement for VO2 attained during CPX, for further explanation see Glossary and Section 1.6.2.3 Cardiopulmonary Exercise testing) during maximal exercise using a cycle ergometer after 3 days supplementation (36). However in 2010 the same group exercised with combined arm and leg ergometers thereby aiming to use more than 50% of the total muscle mass for a true VO2max and restudied the effects of acute dietary nitrate supplementation (2 days) on maximal exercise (37). They found a significant reduction in VO2max after nitrate supplementation at maximal exercise, with no associated change in heart rate, pulmonary ventilation or lactate formation. There was also a trend (p=0.13) to an increase in the time to exhaustion in the nitrate group with a positive correlation between the change in VO2max and the change in time to exhaustion (p=0.042).

After 15 days supplementation Vanhatalo et al confirmed a small but significant increase in VO2max which was associated with a significant increase in peak power output and work rate at GET (39). The S1 slope of the ∆VO2/∆WR was also significantly reduced and the S2 slope was significantly increased (for further explanation see Glossary).
After 6 days supplementation; at maximal exercise; Lansley et al also reported a significant reduction in VO\(_2\) during walking of 14%, a 7% reduction in the end exercise VO2, and a 6% reduction in the VO\(_2\) at task failure (40). There was also a significantly increased time to task failure during running of 15%, with all subjects in the nitrate group exercising for longer. There was no significant change in the baseline and end exercise VCO\(_2\), Ve, RER, HR, blood lactate.

Wylie et al (42) found that after a single dose of nitrate there was no change in VO\(_2\) at task failure in severe-intensity exercise but at the two higher dosages (8.4mmol and 16.8mmol of nitrate) there was a significant increase in time to task failure which significantly correlated with the change in plasma nitrite.

Therefore at submaximal and maximal exercise the majority of studies with nitrate supplementation have confirmed a decrease in the oxygen cost of exercise.

Effects of dietary nitrate supplementation on plasma nitrite / nitrate in healthy volunteers
Multiple studies have proven significantly elevated levels of plasma nitrate and / or nitrite after dietary nitrate supplementation in young healthy volunteers with both sodium nitrate (36, 37, 41) and beetroot juice (34, 38-40, 42, 44, 73).

Nitrite is thought to be the bioactive compound. Larsen et al (36) measured plasma nitrate and nitrite during exercise and found that whilst plasma nitrate did not change during exercise, plasma nitrite levels decreased. Wylie et al found that the change in the nitrate dose correlated with the change in plasma nitrite and with the change in end exercise VO\(_2\) (42).

Plasma nitrate levels peak at 1-2 hours post ingestion (68); dependant on the nitrate dose (42); and remain significantly elevated at 24 hours. In contrast plasma nitrite levels peak at 2-4 hours and remained elevated up to 5 hours after ingestion and returned to near baseline at 24 hours (68), again dependant on nitrate dose. After a very high dose of 16.8mmol nitrate, plasma nitrite remains significantly elevated above baseline at 12 and 24 hours (42). After 7 days supplementation Wylie et al found no further increase in plasma nitrate or nitrite levels (43). Plasma levels remain elevated with prolonged supplementation and there appears to be no tachyphylaxis, with plasma levels remaining elevated with up to 30 days supplementation (43).
Nitrate supplementation in the Elderly

Whilst the majority of studies were initially performed in healthy young volunteers and athletes, several studies have been conducted in older healthy populations to confirm an intact enterosalivary nitrate pathway (67, 72, 74).

In older, mean age of 72.5 years, healthy adults, Miller et al found that plasma nitrate and nitrite were significantly elevated throughout the day after a beetroot juice supplement; containing 8.5mmol (527mg) nitrate; but surprisingly not a high nitrate diet alone (containing 155mg / 2.5mmols nitrate) (67). Patients with a disease or on medications which might confound treatment effect were excluded from the study, including those with a history of diabetes, cancer, hypertension or hypotension, thyroid disease, COPD, IBD and cardiovascular disease. Patient on medications to treat angina, phosphodiesterase type 5 inhibitors, antihypertensives and proton pump inhibitors (PPIs) were also excluded as these may affect nitrate absorption or metabolism.

The high nitrate diet alone did significantly elevate plasma nitrate / nitrite as expected but the nitrate content was only 2.5mmol which is lower than the dose 4mmol known to have an effect, and was spread across 3 meals rather than a bolus. They also found that plasma nitrite had a secondary rise after the evening meal and hypothesised this could be due to an increase in saliva at evening meal and swallowing of saliva when eating.

Investigators therefore postulated that the timing of nitrate ingestion was therefore not important as plasma nitrate levels remained raised throughout day. Therefore despite 100% bioavailability, a high nitrate dietary intake alone may not be sufficient to cause a physiologically significant rise in plasma nitrate / nitrite in the elderly.

Variability / non responders

Several studies have noted a wide interindividual variability in the rise in plasma nitrite from the different nitrate supplementation regimes with some participants requiring larger doses of nitrate to improve performance. This could be due to variations in salivary flow rate or body mass (42, 75).

Wylie et al defined ‘non responders’ as patients who had normal baseline plasma nitrite levels and a significant increase in plasma nitrite with supplementation but no improvement in time to task failure. They observed these ‘non-responders’ who did not improve performance at lower doses of supplementation, did improve performance at higher doses.
In 2015 Siervo et al performed a meta-analysis of 4 trials in older participants (75) and proposed 2 phenotypes, the “reducers” and “non-reducers” classified by their efficiency at reducing nitrate to nitrite in vivo. They postulated that differences in oral microbiota, gastric acidity, plasma pH, enzyme efficiency and ageing may all contribute to the variable rise in plasma nitrite. They also proposed that higher doses of nitrate might overcome the inefficiency in nitrate reduction and the insensitivity of vascular smooth muscle in an older population.

**Effects of dietary nitrate supplementation on Blood pressure in healthy volunteers**

Dietary nitrate supplementation can reduce BP in healthy young volunteers and is cardioprotective (68). The majority of studies have found a significant decrease in systolic BP (SBP) (34, 36, 38-40, 42) with longer periods or high doses of supplementation also leading to a decrease in diastolic BP (DBP) (36, 38, 39, 42).

Systolic blood pressure seems to be more amenable to nitrate induced change as studies have shown the reduction persists at 24 hours whereas DBP returns to baseline (42, 68). Bailey et al found that after only 6 days supplementation of 5.1mmol of nitrate, SBP but not DBP was significantly reduced (34). As with plasma nitrate the change in BP is dose dependant, with higher doses of nitrate supplementation leading to a longer effect (42). Vanhatalo et al noted the reduction in BP was more pronounced after 12 days supplementation (39).

The reduction in BP is likely to be due to nitrite vasodilatation as Larsen et al measured plasma renin, and aldosterone levels and noted there was no significant change (37). Lansley et al were one of the first groups to use a true placebo therefore establishing that the nitrate in beetroot juice was responsible for the reduced BP and improved oxygen utilisation (40).

In a meta-analysis performed in 1991, Law et al concluded that a 5mmHg reduction in BP could reduce stroke incidence by 22% and CHD by 16% thereby preventing 75,000 deaths per year in UK (76). Vanhatalo et al noted that although the reduction in BP with nitrate supplementation in their trial was small, it was therefore likely to make a significant difference to outcomes given the findings of Law et al (39).
**Effects of dietary nitrate supplementation on older healthy adults**

Whilst the majority of studies in young healthy volunteers showed a reduction in BP with nitrate supplementation studies into older adults have not been as promising. In older healthy volunteers Kelly et al confirmed an intact nitrate-nitrite pathway with significantly elevated plasma nitrite with nitrate supplementation and reduced SBP and DBP but no significant change in the oxygen cost of exercise (72).

In overweight older adults populations others found a progressive decline in daily SBP measured at home; which returned to baseline within one week of stopping; but no significant decrease in 24 hour ambulatory BP monitoring (ABPM) (74). Jajja et al suggested the return to baseline within one week of stopping supplementation implied the need for continuous nitrate supplementation to maintain cardiovascular effects.

A recent meta-analysis of 4 trials in older participants that used 24 hour ABPM found no significant effect beetroot juice on systolic or diastolic 24 hour ABPM (75). Sub-group meta-analysis found that older age and higher BMI decreased the reduction effect of nitrate on BP. For further details of individual trials and their findings please see Appendix 2- Summary Effects of dietary nitrate supplementation in older volunteers.

**Conclusion - Effects of dietary nitrate supplementation in healthy volunteers**

Whilst the above studies were small, they were well conducted and do show concurrence. Therefore it is clear that dietary nitrate supplementation has beneficial effects in healthy individuals, and can reduce the oxygen cost of exercise.

**1.4.5 Nitrates mechanism of action**

The mechanisms by which dietary nitrate supplementation decreases oxygen consumption during exercise have been extensively investigated. The decreased oxygen consumption during exercise is not thought to be central adaptation from the heart or lungs as several studies have shown no associated change in the heart rate, Ve (Ventilatory Equivalent, for further explanation see Glossary) or oxygen pulse at a given oxygen uptake (34). Larsen et al postulated that the improved efficiency was therefore either due to changes in skeletal muscle or mitochondria (36), (38).

Others considered whether the decreased oxygen consumption associated with nitrate supplementation was due to a lack of NO inhibition of cytochrome oxidase C. NO synthase (NOS)
inhibitors block NO production and in dogs have been shown to increase VO$_2$ during submaximal exercise (77). However Larsen et al noted that if the effects of nitrate supplementation were due solely to a lack of NO inhibition of cytochrome oxidase C, this would lead to an increase in anaerobic metabolism with a resultant increase in lactate which did not occur in their study or others. They therefore concluded the effects of dietary nitrate were not due solely decreased inhibition of cytochrome c oxidase (36).

**Dietary nitrate supplementation and improvements in muscle oxygenation**

During skeletal muscle contraction the sarcoplasmic reticulum pumps calcium requiring a significant proportion of Adenosine Triphosphate (ATP) turnover. Bailey postulated that NO from BRJ may prevent an excess of calcium release thereby reducing the ATP cost of re-sequestration (34). Using near-infrared spectroscopy (NIRS) to assess muscle oxygenation they found that nitrate supplementation improved muscle oxygenation; demonstrated by elevated oxyhaemoglobin concentration (HbO$_2$) and total haemoglobin concentration (Hb$_{tot}$) suggesting increased blood flow; and reduced oxygen extraction demonstrated by a reduction in the HHb (deoxyhaemoglobin concentration) amplitude.

Myocytes far from capillaries have poorer oxygen supply which creates a hypoxic acidic environment which, may in turn, stimulate conversion of nitrite to NO causing vasodilatation and thereby increasing oxygen delivery. Bailey et al postulated NO may also inhibit mitochondrial oxygen consumption in nearby myocytes thus allowing the oxygen to penetrate further, enabling better matching. They concluded that as nitrate supplementation also reduced VO$_2$ at submaximal exercise, along with the reduction in oxygen extraction, there must have also been a ‘reduction in aerobic energy turnover or muscle energy utilisation’.

In 2010 the same group used phosphorous-31 magnetic resonance spectroscopy to non-invasively assess tissue changes in phosphocreatine (PCr) and pH to estimate skeletal muscle oxygen consumption and changes in total ATP turnover rate (38). They found that nitrate supplementation was associated with a reduced PCr degradation and ATP$_{total}$ and therefore concluded that nitrate supplementation reduces the total ATP cost of muscle force production thereby reducing the oxygen cost of exercise. They postulated that this was due to a reduced rate of ATP turnover due to an NO modulated reduction in calcium cycling or handling. The reduction in ATP turnover led to PCr sparing and therefore improved exercise tolerance.
Another study also used NIRs to measure gastrocnemius oxygenation during CPX and demonstrated a reduction in fractional oxygen extraction following nitrate supplementation despite increased performance (63). They concluded that as the symptoms of intermittent claudication are caused by ischaemia, it was logical that plasma nitrite improved exercise tolerance by providing NO which caused vasodilatation and increased oxygenation.

During intense constant exercise the rate of ATP turnover in myocytes increases as the exercise proceeds and therefore becomes less efficient. Bailey et al postulated that nitrate supplementation overcomes this loss in efficiency thereby reducing VO$_2$ and PCr (34). They observed that at termination of high intensity exercise PCr and pH and Pi reach consistent critical values which they postulated was the limit to further muscle function. Therefore supplementation with nitrate enabled exercise to be sustained for longer before these critical values were reached.

Effects of dietary nitrate supplementation on Mitochondria
Several groups have conducted mitochondrial experiments with the aim of establishing if the reduction in oxygen consumption associated with nitrate supplementation is due to an improvement in mitochondrial efficiency.

Proton leak is a term for proton cycling over the inner mitochondrial membrane that does not produce ATP and can be responsible for 15-20% of oxygen consumption. It has been postulated that the nitrate driven improved oxygen efficiency might be due to a decreased mitochondrial proton pump leak or slippage (34, 36). In their initial experiments Larsen et al noted that NO inhibits proton leakage which could improve muscular efficiency at submaximal work rates, and that this would fit best with their results (36). After their 2010 experiments they postulated that the increase in VO$_{2\text{max}}$ may be due to a decrease in a ‘non-ATP-producing but O2-consuming process’ such as proton leakage, cellular transport, heat production or ion channel leaks (37).

Previous studies had found that sustained NO exposure can induce mitochondrial biogenesis (78) and others therefore postulated that sustained nitrate supplementation might increase mitochondrial content leading to an increase in VO$_{2\text{max}}$ or GET (39). Vanhatalo et al hypothesised that the increase VO$_{2\text{max}}$ and peak power output after 15 days supplementation at maximal exercise in their study was either due to NO mediated changes in skeletal muscle perfusion and cardiac output or increased mitochondrial mass / biogenesis (39).
Larsen et al performed a series of well conducted experiments to determine if the improvement in oxygen utilisation with three days of nitrate supplementation was due to changes in mitochondrial function by harvesting skeletal muscle mitochondria from muscle biopsies after nitrate supplementation (41). They found a 19% increase in effective P/O ratio (amount of oxygen consumed per ATP unit production, see definitions) during submaximal ADP stimulation and concluded this was likely due to improved mitochondrial efficiency. They also found a significantly increased rate of ATP production after nitrate supplementation and noted a clear correlation between the degree of increase in P/O ratio and the degree of reduction in oxygen consumption in subjects. Giving nitrite directly to isolated mitochondria had no effect therefore they postulated that prolonged exposure was needed to induce changes.

Larsen et al also measured basal mitochondrial oxygen consumption and found a significant reduction in LEAK and state 4 respiration (for explanation see Glossary) and concluded that the improvement in the P/O ratio was due to less mitochondrial wastage. They estimated mitochondrial respiratory efficiency by measuring thermodynamic coupling and found an improvement in thermodynamic efficiency after nitrate supplementation. As this was not associated with a change in stage 3u respiration they postulated the increase in thermodynamic efficiency was due to a lower basal mitochondrial respiration rate. They also measured the expression of the mitochondrial proteins responsible for proton leak and found a significant downregulation of ANT protein levels after only 3 days supplementation. To prove this was not due to reduced numbers of mitochondria or less biogenesis they measured mitochondrial DNA levels, and found that mitochondrial density and biogenesis were unchanged after 3 days supplementation. They therefore concluded that nitrate induced improvement in intrinsic mitochondrial efficiency with decreased proton leak / spillage due to a decreased expression of ANT.

Lansley et al used phosphorous-31 magnetic resonance spectroscopy to estimate muscle oxidative mitochondrial capacity (Qmax) to establish if nitrate supplementation caused mitochondrial biogenesis (40). Four days of supplementation led to enhanced aerobic performance; measured by increased time to task failure; but there was no associated change in Qmax. They postulated that the improvements in total body oxygen consumption during exercise they found after 4 days supplementation may be due to improvements in muscle contractile function and speculated that their supplementation regime may not have been long enough to cause mitochondrial biogenesis.
After 7 days supplementation with high dose beetroot juice containing 26mmol nitrate Whitfield et al confirmed a significant reduction in oxygen consumption during submaximal exercise (44). However they found no change in mitochondrial leak or maximal respiration, no alteration in P/O or Respiratory Control Ratio (RCR) and protein levels of ANT and UCP3 were unaltered. They concluded the reduction in oxygen consumption associated with supplementation was not due to improvements in mitochondrial respiration / coupling, in stark contrast to Larsen et al (41) and that ‘alterations in mitochondrial efficiency are not required for the observed whole body responses’. They acknowledged their study had several limitations - whole body oxygen was measured 48 hours prior to mitochondrial efficiency (at day 7), and at day 8 plasma nitrite levels were no longer significantly raised suggesting it may be possible to become resistant to prolonged high dose supplementation. They also had a very small sample size of 5 for some of the isolated mitochondrial experiments.

**Effects of dietary nitrate supplementation on blood vessels**

Several groups have studied whether the beneficial effects of nitrate supplementation are due to NO mediated vasodilatation. One study found that nitrate supplementation enhances cardiac function at rest and during exercise by significantly increasing stroke volume (SV) and cardiac output (CO) measured using impedance cardiography (73). They postulated the increase in SV / CO was due to reduced afterload caused by reductions in total peripheral resistance (TPR ) and BP with nitrate supplementation. This was supported by the fact that the resting pulse pressure (RPP), an index of myocardial oxygen consumption also decreased. They noted that both CO and stroke volume did not increase at the highest workloads, and speculated this was because the BP decreased proportionally less than the BP response to exercise and so there was less overall reduction in afterload.

Lee et al postulated the reduction in TPR could be either due to increased NO-induced vasodilation in contracting muscle, or due to NO inhibition of sympathetic nerve mediated exercise associated vasoconstriction (73). They also measured endothelial function by measuring flow mediated dilatation (FMD, a measure of vascular dysfunction) and found that supplementation with nitrate caused significant increases FMD. The degree of FMD was positively correlated with the concentration of plasma nitrate and nitrite (measured as NO₃⁻).

Wylie et al that the effects of nitrate supplementation on task failure at maximal exercise may be independent from the effects of supplementation on the oxygen cost of submaximal exercise, as a
single dose of beetroot juice led to a significant dose dependant decrease in end exercise VO₂ at moderate intensity exercise but no change in VO₂ at task failure in severe-intensity exercise despite a significantly increased time to task failure in the 8.4mmol and 16.8mmol groups (42).

They postulated the acute improvements in submaximal exercise may be due to NO impacting on protein function to reduce the oxygen cost of exercise which could occur within the 2.5 hour timeframe. They hypothesised the increase in time to task failure at maximal exercise may be due to improvements in blood flow or NO mediated matching of blood flow to more metabolically active areas.

**Effects of dietary nitrate supplementation on Protein expression**

Wylie et al postulated that chronic nitrate supplementation caused alterations in protein expression thereby improving mitochondrial efficiency and / or reduced the ATP cost of skeletal muscle contraction (43). They proposed these changes in protein expression were dependent on both duration and magnitude of dose as there were no changes in the oxygen cost of exercise with 3mmol supplementation despite prolonged exposure.

After chronic supplementation the reduction in steady state VO₂ was maintained for 24 hours after the last dose; even after plasma nitrite has returned to baseline. They concluded the continued improved efficiency was due either to protein expression changes which are maintained for a period of time after supplementation is stopped or due to persistent elevation in NO bioavailability due to elevated nitrogen intermediates. They postulated the improvement with acute supplementation was either due to improvements in perfusion and oxygenation and / or changes in protein function as 2-3 hours was not enough time to alter protein expression.

**Conclusion - Nitrates mechanism of action**

Whilst supplementation has been shown to enhance cardiac function the majority of studies agree that the benefits of nitrate supplementation are not solely due to inotropic effects or central adaptation from the heart or lungs. Most studies have also found no increase in lactate levels despite a sometimes increased time to exhaustion so it is unlikely the aforementioned improvements are due to increased anaerobic metabolism.

Nitrates improve oxygen utilisation by tissues. There are probably different mechanisms for the effects of acute and chronic nitrate supplementation. The mechanism of action is probably
multifactorial, with acute supplementation leading to microvascular blood flow changes to improve blood flow to contracting muscle and chronic supplementation leading to changes in protein expression and improved mitochondrial efficiency and biogenesis.

1.4.6 Effects of dietary nitrate supplementation in Clinical populations

Whilst the majority of studies with dietary nitrate supplementation were initially performed in athletes, several studies have now been performed in clinical populations. These trials are generally of larger sample sizes than the trials in athletes with only 3 trials having less than 14 patients. The studies which are of good quality and reliable and that have further added to knowledge in this area are discussed below. For further details of individual trials and their findings please see Appendix 3 Summary Effects of dietary nitrate supplementation in clinical populations.

Effects of dietary nitrate supplementation on Submaximal exercise in Clinical populations

In patients with type 2 diabetes; after 4 days of nitrate supplementation; Shepherd et al found no significant difference in the oxygen cost of walking, baseline or end exercise pulmonary VO\textsubscript{2} and no improvement in distance covered in the six minute walk test (6MWT, for further explanation see Section 1.6.2.1 Six min walk test) (62).

In patients with COPD, Berry et al found that a single dose of beetroot juice containing 7.58mmol of nitrate reduced oxygen consumption and extended time to exhaustion during submaximal exercise (79). However 2 other studies in patients with COPD did not find a significant improvement with nitrate supplementation (80, 81). One of these studies however, which recognised they were underpowered, did find a non-significant increase in submaximal exercise endurance with a 11% increase in endurance shuttle walk test distance and a 6% increase in time to fatigue (80).

The results with nitrate supplementation in patients with heart failure at submaximal exercise have also been mixed. Coggan et al found a small but non-significant improvement in the distance covered in the 6MWT after a single dose of 11.2mmol of nitrate (82). Hirai et al found no significant reduction in the oxygen cost of exercise after 9 days of nitrate supplementation in patients with heart failure with reduced ejection fraction (HFrEF) (83).

In contrast, two studies found a benefit of nitrate supplementation in patients with heart failure. Borlaug et al found that a single dose of sodium nitrite iv significantly increased VO\textsubscript{2} during submaximal exercise with associated improvements in cardiac output, normalising the delta
CO/delta VO\textsubscript{2} slope (For further explanation see Glossary) (84). Eggebeen et al gave patients with heart failure with preserved ejection fraction (HFrEF) 7 days of supplementation and found a significant improvement in submaximal aerobic endurance of 24%, there was no significant change after just one dose.

**Effects of dietary nitrate supplementation on Maximal exercise in Clinical populations**

Dietary nitrate supplementation has been shown to enhance exercise tolerance and performance in professional athletes and physically active males. There have however only been a few studies using maximal exercise in clinical populations.

In patients with peripheral arterial disease Kenjale et al found that a single dose of beetroot juice increased claudication time by 18% (32 seconds) and walking time by 17% (65 seconds) (63). There was a significant correlation between the change in walking time and plasma nitrate. They noted that the improvements were clinically significant and provided approximately one third of the benefits of a three month supervised exercise training programme which led to a 66% increase in claudication time and a 51% increase in peak walking time (85).

In patients with COPD, at maximal exercise, Kerley et al noted a significantly improved in the incremental shuttle walk test (ISWT) distance of 25m after a single dose of 12.9mmol nitrate or placebo (86). However in patients with heart failure results at maximal exercise have been less positive with two groups finding no significant improvements after a single dose (82) or 9 days of nitrate supplementation (83) at maximal exercise.

**Effects of dietary nitrate supplementation on Plasma nitrite / nitrate in Clinical populations**

In clinical populations multiple studies have proven significantly elevated levels of plasma nitrate and / or nitrite after dietary nitrate supplementation with beetroot juice in patients with peripheral arterial disease (63), hypercholesterolaemia (87), hypertension (88, 89), diabetes (62, 90), COPD (79-81, 86) and heart failure (82-84, 91) proving a functional enterosalivary pathway in patients with comorbidities on medications.

Kenjale et al were one of the first groups to study the effects of dietary nitrate supplementation in a clinical population (63). They conducted a small study in patients with peripheral vascular disease (PVD) and intermittent claudication, all who were on antiplatelet and lipid lowering medications. They found that a single dose of 500ml of nitrate rich beetroot juice raised plasma nitrate six-fold and plasma nitrite three-fold and that levels remained elevated for the duration of testing. They
noted a peak plasma nitrite of approximately 900nM three hours after supplementation which was higher than in some healthy adults. They postulated this could be due to differences in oral bacteria, saliva production and digestion (63).

One study, in patients with heart failure, also noted plasma nitrite levels fell more than expected during exercise, consistent with active consumption, again implicating nitrite as the bioactive compound (84). Others noted a lower than expected level of increase of plasma nitrate after supplementation than in healthy volunteers and postulated this may be due to decreased NO synthase-mediated synthesis, decreased nitrate absorption and / or increased NO destruction (82).

More recently it has been proven that patients on medications for blood pressure and diabetes have an intact enterosalivary pathway. Shepherd et al performed a subset analysis on baseline nitrite and found no significant difference in baseline nitrite for patients on angiotensin converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers (ARBs), metformin, insulin, statins and sulfonylureas (62).

It has also been proven that prolonged supplementation does not cause tachyphylaxis in a clinical population (87). Velmurugan et al gave patients a prolonged supplementation period of 6 weeks and found that the serum nitrite further increased after 6 weeks and identified an increased number of bacteria in the oral cavity that denitrify nitrate e.g. *N Flavescens*. They postulated this could be the mechanism for the further rise at 6 weeks supplementation.

For further details please see Appendix 4 Plasma nitrate and nitrite levels in clinical populations after dietary nitrate supplementation.

**Variability / non responders**

Although different patient populations and analytic approaches make direct comparisons between trials difficult several studies have commented on the variability of baseline plasma nitrate and nitrite in clinical populations (79, 80, 86) and of a heterogenous response to supplementation (79, 80, 86, 91).

In patients with COPD, Berry et al noted that baseline plasma nitrite levels were similar to healthy subjects and they and others noted a variable response to nitrate supplementation (79, 80, 86). Berry et al postulated that lower baseline levels may be expected in an older comorbid population.
due to the oxidative stress of aging, changes in gastric pH and changes in oral bacteria (79). Kerley et al found there was no correlation between change in exercise performance and change in serum nitrite, but they did note that patients had multiple comorbidities and medication regimes which could act as confounding factors (86). Of the two of 11 patients who did not show an increase in exercise capacity, one had severe COPD (FEV1 15%) and was the only current smoker, the second was the only patient on a PPI and had a decrease in the serum nitrite after supplementation. They postulated the decrease in acidity in the stomach caused a decrease in serum nitrite and therefore a lack of improvement in exercise capacity.

In patients with heart failure on multiple cardiac medications, Eggebeen et al noted a variable increase in plasma nitrite with nitrate supplementation and postulated this may be due to individual variation in oral nitrate to plasma nitrite conversion which therefore may be overcome by increasing doses (91).

In patients with diabetes, Shepherd et al noted a much higher baseline plasma nitrate at 680 +/- 256nM compared with other studies in similar populations (232nm/L (90) and that of Berry et al (62, 79). They did however note that timing and circumstances of measurement were likely to be critical, as Gilchrist et al measured after an overnight fast, 16 hours after juice ingestion compared with 3 hours in their study. Shepherd et al postulated that the elevated baseline plasma nitrite could be due to disease pathology perhaps due to the upregulation of inducible NOS, circadian variation in eNOS activity, increased oxidative stress and NO scavenging or due to a change in the oral bacteria due to repeated steroid or antibiotic use. They proposed elevated baseline plasma nitrite as an explanation as to why there was no improvement in pulmonary oxygen uptake in their trial as levels were already running high in their population.

**Effects of dietary nitrate supplementation on Blood pressure in Clinical populations**

In healthy individuals it has been well proven that dietary nitrate decreases BP however in clinical populations dietary nitrate supplementation has shown varying effects on BP. This may in part be due to medications in clinical populations directly interfering with either the absorption of nitrate (e.g. antacids, PPIs) or the reduction of nitrate to nitrite (e.g. xanthine oxidase inhibitors).

However several studies have shown significant reductions in SBP and / or DBP both at rest and / or during exercise in patients with PVD, hypercholesterolaemia, COPD and heart failure (63, 79, 80, 84, 86, 87, 91). After 6 weeks of supplementation one study found a significant decrease in SBP proving
no tachyphylaxis with prolonged supplementation (87). In patients with heart failure with preserved ejection fraction (HFP EF) Borlaug et al found significantly reduced central resting pressures and exercise DBP, but no significant change in SBP after a single dose of sodium nitrite iv (84). However they recognised it would not be possible to achieve the plasma nitrite levels achieved in their study with oral supplementation and patients on organic nitrates or phosphodiesterase inhibitors were excluded.

Other studies have not shown a significant reduction in BP with nitrate supplementation (62, 81). In patients with diabetes two studies found there was no significant change in resting SBP or DBP (62, 90). Shepherd et al postulated the increased oxidative stress in patients with type 2 diabetes might lead to an increased scavenging of NO, attenuating the effect of dietary supplementation (62).

In another study in patients with COPD, 5 doses of 6.77mmol of nitrates did not cause a significant change in resting SBP or DBP (81). The authors noted that 38% of patients in their study were antihypertensives and postulated the ability of nitrate to reduce BP may be reduced in patients already on treatment. In another study the authors noted that the one patient on a PPI did not show any significant changes in BP or exercise capacity and in fact plasma nitrite levels decreased (86).

In patients with heart failure Coggan et al found no significant reduction in SBP or DBP with a single dose of 11.2mmol of nitrates but did recognise that the size of BP reduction was inversely related to baseline BP (82). All patients were on a beta blocker and 7 of the 9 were on an ACE inhibitor / ARB. They postulated the lack of blood pressuring lowering with nitrate may be due to a blunted endothelial vasodilatory response due to dysfunction (82). They did however note that therapeutically this could be beneficial as patients with heart failure often have low / normal BP and so treatment hypotension could limit potential therapy with nitrates supplements.

Hirai et al also and found no significant changes in BP in patients with heart failure with reduced ejection fraction (HFrEF) after 9 days of twice daily 6.45mmol of nitrate (83). Their population were all on optimal treatment including beta blockers, ACE inhibitors or ARBs, with 90% of patients also on diuretics. They postulated that perhaps there was less room for improvement in BP in their stable, well treated population.

For further details see Appendix 5 Blood pressure changes in clinical populations after dietary nitrate supplementation.
Effects of dietary nitrate supplementation on patients with hypertension

Perhaps the most interesting effects of dietary nitrate supplementation on BP are from those trials in whom patients are already on treatment for hypertension. Hypertension is a multifactorial disease, the final common pathway of which is thought to be caused by endothelial dysfunction of vascular smooth muscle cells and failure of arterial dilatation in response to increased flow (92).

Meta-analysis of studies in clinical populations has shown that in middle to older age BP is ‘strongly and directly related to vascular (and overall) mortality, without any evidence of a threshold down to at least 115/75 mmHg’ (93). Lewington et al noted that even small changes in BP carried a significant effect, with every 2mm increase in SBP in middle age associated with an increase in mortality from IHD or other vascular causes by 7%; and stroke by 10% (93).

In the largest study Kapil et al gave 64 patients with hypertension, half of which were on treatment, 4 weeks of beetroot juice containing 6.4mmol of nitrate or placebo (88). Despite patients remaining on a normal diet they found a significant reduction in BP measured in clinic, at home and with ambulatory monitoring; with no tachyphylaxis over 4 weeks; in the nitrate group. Subgroup analysis of those on treatment for hypertension also showed a significant reduction in BP in the nitrate group. Kapil et al also noted that the change in plasma nitrite was inversely correlated with the change in SBP at home and in clinic. Peak decreases in BP occurred after 4 weeks of supplementation with SBP decreasing by 8.1mmHg and DBP decreasing by 3.8mmHg. They compared supplementation with standard monotherapy antihypertensive treatment which has been shown to reduce BP by 9.1/5.5 mmHg and therefore felt the hypotensive effect of beetroot juice to be significant. Kapil et al postulated that the BP lowering effect might be larger with the degree of hypertension, or may be reduced in patients on multi-antihypertensives due to established vascular damage.

In patients with COPD; 55% of who were already on antihypertensives; a single high dose of beetroot juice containing 12.9mmol of nitrate led to a significantly decreased resting SBP and DBP (86). In two smaller trials both with 27 patients there were no significant changes in BP after 7 and 14 days supplementation (89, 90). Bondonno et al postulated that because many antihypertensives influence NO production the lack of BP reduction may be due to the antihypertensive medications (89). They also noted their patients had a lower baseline BP than the patients in Kapil’s study and postulated there was no further fall in BP with nitrate supplementation in their population as the BP was
already well controlled. Gilchrist conducted a study on 27 patients with diabetes, 98% of whom were on antihypertensives (90). They postulated the lack of BP reduction may be due to stiffened vasculature due to diabetes or a decreased response of vessels to NO due to age.

The duration of supplementation may also be significant (89). Bodonno et al found no change in BP after 7 days supplementation (89), Gilchrist et al found no change after 2 weeks (90), Jajja found a significant decrease after 3 weeks (74) and Kapil found a significant decrease after 4 weeks (88).

1.4.7 Effects of Dietary nitrate supplementation in disease

Effects of Dietary nitrate supplementation on flow mediated dilatation and in patients with Peripheral vascular disease

One of the first trials with nitrate supplementation in a clinical population was conducted in patients with peripheral arterial disease (63). After a single dose of 9.1mmol of nitrate they found a significant increase in claudication time and walking time and a trend towards decreased oxygen consumption at rest (p=0.06). They postulated this was due to improvements in tissue oxygenation as it was associated with a lower BP and oxygen extraction. There was no change in ankle brachial pressure index (ABPIs), and they therefore concluded there were no changes in stenosis, and no difference in brachial artery resting diameters implying no change in eNOS production of NO.

Patients with cardiovascular disease have dysfunction of the L-arginine pathway and increased NO scavenging leading to reduced NO bioavailability and consequent vascular dysfunction. Flow mediated dilatation is a measure of vascular dysfunction and pulse wave velocity is the gold standard way of measuring arterial stiffness; both are markers of extent of atherosclerosis and predictors of cardiovascular events. Several studies have measured flow mediated dilatation to assess the effect of nitrate supplementation on vascular function, with significant results in patients with hypercholesterolaemia and hypertension (87, 88) but not diabetes (90).

In patients with hypercholesterolaemia, Velmurugan et al found that 6 weeks of supplementation significantly increased flow mediated dilatation and therefore improving vascular function (87). They noted the effect was evident at 3 hours post supplementation so suggested it may be due to modification of existing pathways and that the effect persisted at 6 weeks. They postulated patients with hypercholesterolaemia had increased oxidative stress leading to reduced NO bioavailability and increased NO scavenging triggering systemic inflammation. FMD is thought to be caused by the release if NO from the endothelium, therefore the improvement in FMD with nitrate
supplementation might be due to decreased NO scavenging and oxidative stress. To support this Velmurugan et al noted that in patients with high baseline CRP ‘a trend for reduction was evident’. They also noted a reduction in the markers of platelet activation; implicated in the progression of atherosclerosis; with a small but significant reduction in platelet-monocyte aggregates (PMA which play a role in inducing endothelial dysfunction) and a significant reduction in stimulated P-selectin expression (a marker of platelet activation) (87).

In patients with hypertension, Kapil et al found significantly improved endothelial function (approx. 20%) and significantly reduced arterial stiffness measured by reduced pulse wave velocity, augmentation index and increased peak flow mediated dilatation (88). However in patients with stable type 2 diabetes, Gilchrist et al found no significant change in macrovascular endothelial function measured by FMD, or microvascular endothelial function measured by perfusion response after 2 weeks supplementation despite proving a significant rise in plasma nitrate / nitrite. They postulated this could be because of age related stiffening of vasculature or decreased responsiveness of vasculature to NO in patients with diabetes.

**Effects of Dietary nitrate supplementation in patients with Heart failure**

Heart failure (HF) is a condition characterised by a decreased functioning of the heart as a pump, characterised by shortness of breath which is worse on exertion, fatigue and palpitations (94). Disease severity is determined by severity of symptoms, although echocardiogram of the heart can be helpful in determining the degree of cardiac dysfunction by measuring the ejection fraction (fraction of fluid ejected from heart with each heartbeat).

Whilst the majority of patients with heart failure have a reduced EF of below 40% (HFrEF) caused by decreased ventricular contraction, some patients have heart failure with preserved ejection fraction (HFrEF) (94). This is also known as diastolic heart failure and is caused by inadequate ventricular filling in diastole (the period of time the heart is at rest between each heart beat) due to increased left ventricular filling pressures; which is often worse during exercise; leading to decreased cardiac output (84). Patients with both types of heart failure have impaired exercise capacity, primarily due to reduced oxygen delivery and tissue utilisation therefore supplementation with dietary nitrate which has been shown to improve oxygen delivery and efficiency of utilisation has the potential to benefit (82).
Organic nitrates, which enhance NO-cGMP production are an established treatment for heart failure, however tolerance and renal sodium retention are a problem. Multiple trials have therefore been conducted in patients with both HFrEF and HFpEF to see if dietary nitrate supplementation has beneficial effects in this population.

After a single large (11.2mmol) dose of nitrate in patients with heart failure there was a significant increase in muscle contractile function measured by isokinetic dynamometry, which was comparable to 2-3 months of exercise training (82). Coggan et al postulated NO stimulates soluble guanylyl cyclase and thereby increases cGMP levels in skeletal muscle which increases maximal shortening velocity. There was also a non-significant improvement in 6MWT from 517m to 528m, however post hoc analysis revealed the study was underpowered to detect a significant change in 6MWT.

After a single dose of sodium nitrite iv, in patients with HFpEF, there was a significant reduction in pulmonary capillary wedge pressure (PCWP) and resting PCWP which are surrogate markers for left heart filling pressures which are abnormally raised in HPpEF. They was also a significant increase in VO₂, cardiac output and stroke volume. The abnormalities associated with HFpEF are thought to be due to limitation in NO-cGMP production. The use of phosphodiesterase inhibitors e.g. Viagra / sildenafil which inhibit cGMP catabolism do not enhance exercise capacity in HFpEF, because the limitation is one of production not catabolism (84). Borlaug et al therefore postulated that the reduction in PCWP and increase in VO₂, cardiac output and stroke volume with nitrate supplementation were due to enhanced NO production in hypoxic acidotic conditions such as those found in exercise.

In patients with HFpEF after 7 days supplementation there was a significant increase in submaximal aerobic endurance with no significant changes in VO₂, heart rate or gas exchange at volitional exhaustion (91). Although a submaximal work rate of 75% was used, the authors noted a mean respiratory exchange ratio (RER, for further information see Glossary) of >1.15, consistent with severe intensity exercise. Eggebeen et al postulated the increase in endurance may be due to an increase in perfusion and not an increase in mitochondrial efficiency, as VO₂ did not change. They suggested the increase in perfusion was either due to decreased systemic vascular resistance causing increased cardiac output or more effective diversion of blood flow to exercising muscle.

However in the most recent study in patients with HFrEF after 9 days twice daily nitrate supplementation there was no significant improvement in exercise tolerance, central
haemodynamics (including stroke volume, heart rate and cardiac output), pulmonary oxygen uptake, or the oxygen cost of exercise (83). Hirai et al postulated the lack of improvement may be due to medication interference, increased NO scavenging, or perhaps a less acidic environment compared with previous studies which did not promote NO formation. They noted that infusion studies such as Borlaug et al could have resulted in supraphysiological concentrations of plasma nitrite which were unachievable with dietary supplementation. They also noted that patients HFpEF may have more peripheral oxygen extraction abnormalities which were therefore more amenable to nitrate intervention.

**Effects of Dietary nitrate supplementation in patients with COPD**

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease caused by damage to the lung airways and parenchyma leading to airflow limitation. Impairments in gas exchange cause tissue hypoxia and patients often complain of shortness of breath and decreased exercise tolerance.

Diagnosis of disease is determined by the FEV1/FVC ratio, (FEV1 is the forced expiratory volume in 1 second and FVC is the forced vital capacity), with patients with a diagnosis of COPD having a FEV1/FVC ratio of less than 0.7 (95). Severity of disease is determined by FEV1 with patients with mild disease having FEV1 ≥80% of predicted, moderate disease 50-79% of predicted, severe disease 30-49% of predicted and very severe <30% of predicted (95). Patients with COPD are therefore chronically hypoxic and reducing the oxygen cost of exercise with nitrate supplementation could potentially allow for symptomatic benefit. Study results in clinical populations with COPD have however been mixed, four well conducted studies are discussed here, however it should be noted that all had very short supplementation periods of 1-3 days only.

Two studies used a single dose of nitrate supplementation. In moderate COPD (Mean FEV1 % predicted 61.8%), Berry et al found that after 7.58mmol of nitrate there was a significantly longer exercise time during submaximal constant work rate of almost 29 seconds despite no significant change in oxygen consumption, heart rate, arterial oxygen saturation or dyspnoea ratings (79). They noted a variable response with 11 patients improving and five of the fifteen patients having an improvement of more than the minimum clinically important difference (MCID) of 33% of baseline. They postulated this could be due to a reduction in pulmonary artery pressure, improved blood flow and oxygen delivery or increased force production and calcium handling in type 2 muscle fibres which are increased in COPD.
In patients with severe COPD (mean FEV1 43.4%) a single dose of 12.9mmol significantly improved incremental shuttle walk test (ISWT, for further information see Section 1.6.2.2) distance of 25m when compared to placebo (86). After nitrate supplementation seven patients increased their walk distance from baseline, with 3 exceeding the MCID for the ISWT which has been set at 47.5m (30). In the placebo group no patients increased on their baseline walk. There was no correlation between the change in ISWT distance and the percentage rise in serum nitrate and the authors postulated this may be due to a heterogenous population with multiple comorbidities and medication regimes. Despite the increase in ISWT there were also no significant differences in oxygen saturation, HR or dyspnoea between the placebo and the nitrate groups, even though those on nitrates walked further.

In moderate COPD; after 5 doses supplementation over 3 days; Leong et al (80) found an increase of 79m distance (11%) on endurance shuttle walk test (ESWT, for explanation see glossary); just below the MCID for ESWT of 60-115m (96); and an improvement of 36 seconds on walking time (6%) both of which were statistically insignificant. However they recognised their study might be underpowered due to a large number of dropouts (n=16). They postulated that COPD may cause deconditioning and exercise may be limited by pulmonary and cardiac factors, thus limiting a significant improvement in submaximal exercise endurance in their population.

In the most recent study in patients with moderate COPD, Shepherd et al found no significant change in the oxygen cost of exercise or the distance covered in the six minute walk test (6MWT, for further information see Section 1.6.2.1) (81). They postulated this may be due to increased oxidative stress and inflammatory driven increased reactive oxygen species in patients with COPD causing a reduction in NO bioavailability. They compared their 6MWT results to those of Kenjale et al and postulated that patients with PVD may have had a more hypoxic environment which therefore led to more NO conversion.

**Effects of Dietary nitrate supplementation in patients with Type 2 diabetes**

Type 2 diabetes (T2DM) is a metabolic disorder caused by insulin resistance leading to hyperglycaemia and the micro and macrovascular complications thereof (90). In patients with stable type 2 diabetes; despite finding no significant changes with nitrate supplementation; Gilchrist et al noted after ingestion of 250ml juice there was no change in monitored capillary glucose for small number who monitored proving that the beetroot juice supplementation was safe in patients with T2DM (90).
A second much larger study was therefore conducted by Shepherd et al with 48 patients with T2DM. Despite confirming a significant rise in plasma nitrate / nitrite with supplementation they also did not find a decrease in the oxygen cost of exercise or an improvement the distance covered in the six minute walk test (6MWT).

Type 2 diabetes is associated with reduced bioavailability of NO, in part caused by elevated levels of ADMA, an analog of L-arginine (97). ADMA is known to inhibit all 3 NOS isoforms and causes eNOS uncoupling and superoxide radial release leading to increased oxidative stress. The superoxide radicals generated react with NO, quenching activity. Shepherd et al hypothesised that long term elevation in oxidative stress; associated with elevated ADMA levels in T2DM; might cause damage to mitochondrial membranes and therefore reduce mitochondrial efficiency (62). They postulated that the reduction in mitochondrial efficiency may be the reason patients with T2DM do not achieve a decreased oxygen cost of exercise with nitrate supplementation (62). They hypothesised that NO may be overconsumed during exercise in patients with T2DM, either due to decreased NO production or due to increased scavenging, therefore leading to a lack of effect on oxygen consumption with dietary nitrate supplementation in this population.

**Effects of Dietary nitrate supplementation in overweight patients**

The role of increased amounts of adipose tissue has been postulated as a as yet undefined modifying factor for the response to nitrate supplementation (80). Attention has been drawn to the fact that with increasing BMIs the beneficial effects of dietary nitrate supplementation are lost.

For example for trials which investigated vascular function Velmurugan’s patients with hypercholesterolaemia had a BMI of 26.8 and a significant increase in FMD with 6 weeks supplementation, whilst Gilchrist’s patients with T2DM had a BMI 30.8 and had no change in FMD with 2 weeks of supplementation (87, 90).

In trials with patients with COPD, Kerley et als patients had a BMI of 27.3 and they found a significantly lower resting SBP and increase in ISWT. Berry et als patients had a BMI of 29.2 and they found a significantly lower SBP and increased submaximal exercise time (79). Leong et al patients’ had a BMI of 29.1 and had a significantly lower resting SBP and increased HR but no significant increase in submaximal exercise endurance, change in walking time or distance on ESWT (80). Shepherd et als patients were heavier still with a BMI of 29 and they found no significant change BP
and no effect on pulmonary VO$_2$ or 6MWT (81). They postulated that in overweight patients the adiposity may attenuate the response to dietary nitrate supplementation.

In 2015 in their meta-analysis, Siervo et al performed a subgroup meta-analysis and found that higher BMI decreased the reduction effect of nitrate on BP (75).

1.4.8 Conclusion - Effects of Dietary nitrate supplementation in healthy volunteers, clinical populations and patients with disease

Despite the promising results in athletes there have been mixed results in clinical populations. These studies have however confirmed an intact enterosalivary pathway in patients with comorbidities who are on medications. The effects of nitrate supplementation on BP were mixed but in general supplementation periods were short.

However, notwithstanding the larger sample sizes; out of the 14 studies in clinical populations discussed; 4 of the larger studies did not exercise their patients and chose to focus on the BP and flow mediated dilatation effects of nitrate supplementation. The studies which did exercise had variable methods of exercise and inaccurate forms of measuring exercise capacity and oxygen consumption. Only six studies used CPX or a derivative thereof, and out of these only 2 supplemented patients with nitrate for more than 4 days. To date no studies have been published in a pre-operative population.

Post-operatively oxygen consumption has been shown to increase by more than 50% from 110 ml/min/m$^2$ at rest to approximately 170 ml/min/m$^2$ which lasts for several days (98). Patients who are unable to meet the increased oxygen demand because of decreased cardiopulmonary reserve are at increased risk of morbidity and mortality. ERAS pathways have improved post-operative outcomes by decreasing oxygen demand (by decreasing stress response) and increasing oxygen delivery. Studies in healthy and clinical populations with dietary nitrate supplementation have shown a decreased oxygen cost of exercise by improving oxygen utilisation.

We therefore decided to study if 7 days of nitrate supplementation would decrease oxygen utilisation and thereby improve outcomes after surgery in patients with colorectal cancer.

The ability to increase oxygen delivery and therefore meet the increased oxygen demand after surgery may be predicted by exercise tolerance which is in turn a measure of functional capacity and
physiological reserve. But how do we measure functional capacity and physiological reserve? And how do we assess fitness for surgery?

1.5 The Ageing population
Colorectal cancer is a disease of the elderly and is a significant cause of morbidity and mortality in this population (99). It has been estimated that between 2015 and 2050 the proportion of those over 60 years will nearly double from 12% to 22% (100). As the population ages, the number of cases of colorectal cancer occurring in the elderly will also increase. A significant proportion of patients undergoing colorectal cancer resections are classed as elderly and this is only set to rise (1).

For elderly patients with a limited life expectancy it is often difficult to decide if major surgery is justified (99). As age increases, life expectancy decreases, and clinicians often have the difficult decision of predicting whether the colorectal cancer will become symptomatic enough to require treatment before another comorbidity limits life. The elderly have a higher risk of complications post colorectal surgery; particularly respiratory complications; and 30 day mortality is three times higher in the 75-84 year olds and six times higher in the over 85s when compared with patients under 65 (99).

However the general consensus is that the elderly ‘should not be denied surgery on the basis of age alone’ (99). A significant proportion of patients over 65 will survive for at least 2 years after surgery (99) and so if the primary tumour is not resected then symptoms such as pain, bleeding, change in bowel habit and complications from the tumour are likely to adversely affect quality of life.

In fact the outcomes for some elderly patients, even in the 85+ age range can be good (99). Assessment of risk preoperatively in elderly patients is often inaccurate as physiological reserves are difficult to measure (101) and the tools used by surgeons to identify those at high risk from surgery are often unreliable and subjective (102). Studies have shown that fitter patients have better outcomes after major surgery and that fitness is more important than age when assessing peri-operative risk (19).

Decision making in the elderly population is challenging due to the variability in health (101). Elderly patients lie on a spectrum of risk and a substantial proportion lie in the middle of the spectrum with seemingly minimal comorbidities and normal markers of biochemical and physiology (103).
What is clear is that the elderly are still able to partake and gain benefit from care within an ERAS pathway (7) and therefore it is the differentiation between the elderly who are ‘fit’ for surgery and those who are ‘frail’ and likely to suffer complications that is key.

1.6 Assessment of risk prior to surgery

Preoperatively, accurate risk stratification is necessary to identify patients at high risk of poor outcomes who may benefit from optimisation (17) and so that strategies to reduce mortality post-operatively can be offered. If a patient is identified as high risk pre-operatively this can be used to inform decision making, guide postoperative care and further inform patients. Additional counselling can then be offered to enable shared decision making, reconsideration of surgery and improve the quality of informed consent (17, 104-106).

It is known that the patients most at risk are elderly patients undergoing major abdominal surgery who have pre-existing cardiac disease (105). However it is also accepted that chronologic age is poor predictor of risk and that patients age physiologically at different rates (107). Current international guidelines suggest that patients undergoing elective noncardiac surgery should proceed directly provided they can perform the equivalent of four metabolic equivalents of activity (METs) without symptoms (108). However, the subjective assessment of four METs can be difficult and often inaccurate.

Current approaches to risk evaluation include history, physical examination, clinical acumen, patient questioning / risk prediction scores, plasma biomarkers and formal exercise testing. Whilst clinical acumen is good at identifying those who are very low or very high risk, it is not good at accurately stratifying the majority of patients who fall in-between and has been shown to be a poor predictor of mortality and morbidity postoperatively (106).

Routine blood tests such as serum albumin, creatinine (or estimated glomerular filtration rate (eGFR)), and HbA1c (a marker or long term blood sugar control) can provide information on organ dysfunction which is then added to risk prediction models such as P-POSSUM. Novel biomarkers are measured with the aim of predicting outcome risk (109), the two most promising are B-type natriuretic peptide (BNP) and C-reactive protein (CRP).

BNP is released when the ventricles of the heart are overstretched and high levels are associated with poorer outcomes in patients with heart failure with pre-operative levels of >40pg/ml in one
study being associated with an increased risk of death and cardiac events (110). A meta-analysis concluded that although raised levels were predictive of adverse events the threshold for discrimination in studies was very variable and the further studies were needed to identify the optimal cut-off (111). CRP is a marker of systemic inflammation and in one study involving patients undergoing vascular surgery pre-operative levels above 6.5mg/L were associated with increased cardiac complications and 30-day mortality (112). Whilst elevation of both markers are associated with cardiac complications and increased mortality they do not predict other complications and further studies are needed to identify cut-off values which are likely to be different for different patient groups.

The majority of pre-operative investigations are tests of individual systems and do not test how the cardiovascular, respiratory and metabolic systems work together. There are multiple investigations for cardiac disease which can determine the extent of ischaemic heart disease but were not designed for use in the pre-operative setting (107). In particular whilst cardiac investigations such as echocardiography and ventriculography are accurate at assessing wall motion and ejection fraction, their results do not correlate with functional capacity (107).

Preoperative functional capacity is a good predictor of post-operative outcome as it reflects physiological reserve and the ability of a patient to undergo the physiological stress of major surgery and recovery (17). However patients themselves are not good at accurately describing exercise capacity. In several studies the majority of patients with postoperative morbidity and mortality had poor exercise tolerance pre-operatively (104).

Prior to the introduction of ERAS pathways, most colorectal resections were managed post-operatively in an HDU environment. Since the introduction of ERAS pathways the majority of patients can be managed on a normal surgical ward. The results of risk assessment can therefore be used to triage higher risk patients to the correct level of care post-operatively.

The key pre-operatively therefore, is to accurately identify risk and correctly manage high risk patients postoperatively. This allows optimisation to ensure the increased oxygen demand post-operatively is met, thereby avoiding complications and improving long term survival.

1.6.1 Questionnaires / Risk scores
There are multiple clinical scoring systems and risk prediction scores such as the ASA,
Portsmouth physiological and operative severity score for the enumeration of mortality and morbidity (P-POSSUM), DASI (see below) and performance status (PS) which aim to identify high risk patients pre-operatively.

The ASA (American society of anaesthesiologists) grade is a simple bedside five point scale which aims to assess the physical status of patients prior to elective surgery (109). Prause et al studied 16227 patients having elective surgery and found a significant correlation between ASA grade and post-operative mortality (113). However some studies have shown significant inter-observer inconsistency when assessing the score (109) and in 2013 West et al (114) found in their study of 136 patients undergoing major colonic surgery that the ASA was not independently related to outcome.

<table>
<thead>
<tr>
<th>Can you-</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Take care of yourself, that is, eat, dress, bathe or use the toilet?</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>2 Walk indoors, such as around your house?</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>3 Walk 200 yards on level ground?</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>4 Climb a flight of stairs or walk up a hill?</td>
<td>5.50</td>
<td></td>
</tr>
<tr>
<td>5 Run a short distance?</td>
<td>8.00</td>
<td></td>
</tr>
<tr>
<td>6 Do light work around the house like dusting or washing dishes?</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>7 Do moderate work around the house like vacuuming, sweeping floors, or carrying groceries?</td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td>8 Do heavy work around the house like scrubbing floors or lifting or moving heavy furniture?</td>
<td>8.00</td>
<td></td>
</tr>
<tr>
<td>9 Do yard work like raking leaves, weeding or pushing a power mower?</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>10 Have sexual relations?</td>
<td>5.25</td>
<td></td>
</tr>
<tr>
<td>11 Participate in moderate recreational activities like golf, bowling, dancing, doubles tennis, or throwing a ball?</td>
<td>6.00</td>
<td></td>
</tr>
<tr>
<td>12 Participate in strenuous sports like swimming, singles tennis, football, basketball, or skiing?</td>
<td>7.50</td>
<td></td>
</tr>
</tbody>
</table>

Duke Activity Status Index (DASI) = sum of ‘Yes’ replies ……………………..

\[
\text{VO}_2\text{peak} = (0.43 \times \text{DASI} + 9.6) \quad \text{VO}_2\text{peak} = \text{……………………. ml kg}^{-1} \text{ min}^{-1}
\]

Figure 4 DASI score (115)

The POSSUM score was published in 1991 and incorporates physiological and operative parameters to provide a prediction of morbidity and mortality. It is the most widely used and validated risk
prediction score in the UK. It has been subsequently modified by Whitely et al to create the P-POSSUM which is more accurate for low risk patients and several groups have developed speciality specific POSSUM for elective surgery. These scores have been well validated in their populations but lose accuracy when applied in different populations (109). The Duke Activity Status Index (DASI) aims to estimate functional status by asking patients a series of questions based on activity (115) (Figure 4). However it was developed with cardiology patients and has not been validated in a general surgical population (104).

Whilst many questionnaires and risk scores have shown promise in identifying those patients at higher risk of complications and mortality post-operatively, some scores include subjective assessments from patient or doctors which can make them inaccurate. They aim to predict but not formally measure functional capacity and as such do not identify the specific cause of the increased risk. To date there is no single model that can be used for all patient populations. Therefore formal exercise tests were developed to measure functional capacity and predict surgical risk.

1.6.2 Formal Exercise tests

The ability to increase oxygen delivery and therefore meet the increased oxygen demand after surgery may be predicted by exercise tolerance. It is known that fitter patients have better outcomes (17) and there is a strong association between measured cardiopulmonary fitness and post-operative outcomes (116, 117). There are several tests which aim to measure functional capacity and therefore predict surgical risk.

1.6.2.1 Six min walk test

The six minute walk test (6MWT) is a submaximal test and is a simple, basic objective test of patients functional capacity (106). Patients are asked to walk a course for 6 mins and the distance walked is recorded. It is frequently used to pulmonary rehabilitation to guage improvement to interventions. It is a global assessment of functional capacity and is an accurate measurement of aerobic capacity.

1.6.2.2 Incremental shuttle walk test

The incremental shuttle walk test involves patients walking between two cones; 9m apart; forming a 10m course. Chimes are played to determine the time allowed to walk between shuttles, and each minute the time between chimes reduces thereby increasing the walking pace required to complete the 10m. The test continues until the patients are unable to complete the 10m distance in the time between chimes. The ISWT is therefore designed to simulate maximal exercise such as that
performed in CPX. It is easy to perform but its accuracy may be limited in elderly patients as comorbidities may limit their ability to walk (104). The results from ISWT have been shown to correlate well with peak oxygen consumption in patients with cardiac disease and COPD (104).

Whilst the 6MWT and ISWT are measures of functional capacity they do not establish the cause of poor exercise tolerance and do not give a repeatable measure of oxygen consumption during exercise (118). Their effectiveness in predicting surgical morbidity is therefore limited and those with poor outcomes in questionnaires or walk tests require further testing (119).

1.6.2.3 Cardiopulmonary exercise testing

Cardiopulmonary exercise testing (CPX) is an established, non-invasive and safe method of assessing patients’ cardiopulmonary reserve prior to surgery. It is an objective measure of functional capacity (17, 104) and is repeatable with a low inter-patient standard deviation between tests. CPX takes approximately an hour to perform and can be performed at short notice with minimal preparation (107).

Struthers et al compared the DASI questionnaire, ISWT and CPX in a prospective cohort trial (104). Whilst they found significant correlation between all 3 tests they noted that many patients who did not fare well on the questionnaire or ISWT had satisfactory CPX results and therefore concluded the ability of the DASI questionnaire or ISWT to discriminate between high and low risk patients was poor with both tests finding a large number of patients with equivocal results who then required further testing. Snowden et al concluded that the AT (anaerobic threshold, calculated from CPX (see later)) was more predictive of outcome in patients undergoing major elective surgery than POSSUM, a VASI (Validated activity) questionnaire or a cardiac risk scoring system (116). Wilson et al concluded that clinical risk factors alone would only identify a small proportion of those patients who are high risk for surgery and so selecting a population for further testing based on these would not be helpful (117).

CPX tests the cardiovascular, respiratory, muscular and metabolic systems together to see how they cope with stress of exercise, mimicking stress of major surgery (106, 120). CPX is able to detect subclinical cardiac failure and myocardial ischaemia (107). The risk of death during CPX has been quoted to be between two and 5 per 100,000 tests (121). CPX therefore provides a safe but comprehensive assessment of cardiorespiratory fitness and has been shown to predict complications and length of stay in patients undergoing major elective surgery (104).
CPX requires increased cardiac output to meet the increased oxygen demand during exercise and we know that postoperatively oxygen demand increases due to stress response (105). Those who have poor oxygen delivery whilst exercising are therefore predicted to have poor ability to increase oxygen demand postoperatively (105). CPX enables identification of the cause of reduced exercise tolerance enabling tailored optimised intra and post-operative management. Abnormalities detected; for example in the cardiovascular and respiratory systems can be further investigated and the referral to specialists made for pre-operative optimisation.

CPX results are also very reliable at detecting those not at risk of mortality from cardiopulmonary causes who can therefore be fast-tracked through critical care areas (105). Conversely CPX is also accurate at identifying those patients at high post-operative risk allowing efficient use of resources such as ITU beds and enabling targeted review of outreach services once discharged from critical care (114).

CPX on a cycle ergometer uses large muscle groups for a total body workout with a high metabolic requirement (106). However those with diseases affecting the legs such as arthritis and PVD may not be able to effectively exercise on the bike and reach peak exercise. Arm ergometers provide an alternative means of exercising for those with lower limb dysfunction. However in healthy volunteers the values obtained on arm ergometers are lower than cycle ergometers, because; it is postulated; of the lower metabolic requirement of the smaller muscle groups used (106). Therefore tests on arm and cycle ergometers are not comparable.

CPX testing is expensive and requires specialist equipment and personnel to conduct the tests and interpret the results. However when compared to questionnaires and the incremental shuttle walk test, CPX is considered the most precise and objective method of evaluating fitness pre-operatively and is currently the gold standard (114).

CPX variables

The anaerobic threshold

The anaerobic threshold (AT) is the oxygen uptake (VO₂) at which anaerobic ATP synthesis supplements aerobic ATP synthesis; is calculated during CPX. At AT, the increased lactate production is buffered by bicarbonate leading to an increase in carbon dioxide (104), the AT is identified at the
point where there is an increase in carbon dioxide excretion relative to oxygen uptake (120). The AT is the most repeatable measurement of CPX and can be determined with high accuracy (107).

Traditional exercise physiology states that the oxygen consumption per unit of submaximal work rate is a physiological constant (approx. 10 ml/min/watt), regardless of training, age or diet. Pulmonary VO$_2$ increases linearly in relation to work rate until gas exchange threshold (GET) or AT is met. During this period ATP catabolism and re-synthesis are in equilibrium (38). At exercise above GET, pulmonary VO$_2$ dynamics are more complex as a delayed onset pulmonary VO$_2$ slow component is added and this elevates pulmonary VO$_2$ above 10. Training can decrease the oxygen cost of exercise supra-GET by reducing the pulmonary VO$_2$ slow component, but steady state pulmonary VO$_2$ remains unaffected.

The AT is a reproducible measure of functional capacity (104), and has no volitional component and so is not possible to alter by trying harder or pretending to be exhausted. The AT can predict short and long term outcomes after surgery and postoperative complications (122, 123). The AT occurs well before maximum aerobic capacity and can be easily and safely achieved in the elderly as it does not require high physical stress (105). It is more dependent on fitness and disease than age (105). Interestingly, patients with colorectal cancer have a lower AT, which increases significantly post resection (124). So there is something about the cancer itself which changes the way the body uses oxygen.

**Peak VO$_2$**

Peak VO$_2$ is the highest value for oxygen uptake attained during CPX, and generally occurs near peak exercise. It is a prognostic marker of postoperative morbidity (123). Unlike AT it is volitional and depends upon a participants willingness to exert themselves to their limit (120). When Peak VO$_2$ plateaus and no longer increases despite increments in workload, Peak VO$_2$ becomes VO$_2$ max, which is one of the most accurate and reproducible measures of cardiopulmonary fitness (125). VO$_2$ max is higher in men than women due to a larger muscle mass, greater stroke volume and higher haemoglobin concentrations, and also declines with age. A VO$_2$ max of less than 85% of predicted is considered reduced (125).

Several other measurements are measured and derived during CPX testing. For their explanation and further explanation and definition of those discussed above see Appendix 6 Other CPX report variables.
**Cardiopulmonary exercise testing - The evidence**

Over the years multiple trials validating CPX as a risk predictor have been published. The key papers and their findings are summarised here. CPX results have been shown to predict complications and length of stay in patients undergoing major elective surgery (114, 116). The majority agree that an AT of <11 ml/kg/min is an independent predictor of complications.

In 1999 Older et al published one of the seminal papers on CPX. They studied 548 patients over the age of 60 who were undergoing major abdominal surgery including aortic and oesophageal surgery. They found that an AT of less than 11 ml/kg/min was a predictor of mortality from cardiorespiratory causes in the post-operative period and concluded that the AT could be used to guide the venue for post-operative management (105). They also commented that the majority of patients admitted to the intensive care unit with AT less than 11 ml/kg/min required considerable postoperative support.

In 2010 Wilson et al published their study involving 847 patients all of whom were all 55 years and older and undergoing major elective intra-abdominal surgery including colorectal resection, radical nephrectomy or cystectomy. They found that an AT <10.9 ml/kg/min was a significant predictor of hospital and 90 day mortality after major elective intra-abdominal surgery (117).

In their study looking at 116 patients with a low subjective METs undergoing major elective hepatobiliary, oesophagogastric and vascular surgery, Snowden et al found that an AT of <10.1 ml/kg/min was an independent predictor of post-operative complications and length of stay (LOS) (116).

In 2012 Colson et al published the largest study to look at long term survival in patients undergoing major surgery using CPX (122). They used a submaximal exercise regime to collect CPX data on 1725 patients, with a median age of 71 years, who then underwent major abdominal or thoracic surgery. They found that 5 year survival was independent of patient age and that multivariate analysis using CPX data could be used to predict 5 year survival after major surgery.

For patients specifically undergoing colorectal surgery, the main studies have been performed by West et al (114, 119, 123). In 2013 they performed a prospective study using CPX in 198 patients (62 patients excluded) undergoing major colonic surgery (114). By day 5 48% of patients had a complication. They found that AT, Peak VO₂ and VO₂/VCO₂ at AT were all significantly lower in patients with complications at day 5 post-operatively. At multivariate analysis an AT of >10.1
ml/kg/min and female gender were independent predictors of post-operative complications. In 2014 the same group performed a study on 95 patients with rectal cancer, the majority of whom had undergone neoadjuvant treatment and identified optimal cut off points of AT<10.6 ml/kg/min and Peak VO\textsubscript{2} 18.6 ml/kg/min for identifying patients at increased risk of post-operative morbidity (123). In 2016 West et al studied performed a multi-centre trial including 733 patients at 6 UK centres who were undergoing major colorectal surgery (119). 85 patients had benign disease, the rest had cancer. They identified optimal values of AT <11.1 ml/kg/min and peak VO\textsubscript{2} <800 as able to independently identify patients at risk of postoperative complications.

Two trials have studied the reliability and variability associated with CPX testing and the determination of the AT. In 2009 Kothman et al performed four CPX tests over a 6 week period in order to establish the reliability and reproducibility of the AT (126). There was a within-patient error of 10% (95%CI 8-13%) across the four tests; approx. 1.3ml/kg/min; with a high intraclass correlation coefficient of 0.74 (95%CI 0.55-0.89). They found ‘no significant or clinically substantial change’ in the mean AT across all 4 tests and concluded there was no learning effect with repeated testing with good reliability. Sinclair et al performed a study to quantify the inter-reader variability when determining the AT (127). They found a technical error measurement of 8.1% (approx. 0.9ml/kg/min) with a mean absolute difference of 4.5% between interpreters and a typical random error of 6.5% (127).

Therefore CPX is a safe, objective measure of functional capacity which gives repeatable measure of oxygen consumption during exercise.

1.7 Conclusion – assessing risk prior to surgery
Major surgery generates a systemic inflammatory response that places extra metabolic demands on the body and increases oxygen demand and consumption postoperatively. Dietary nitrate supplementation has been shown to enhance exercise tolerance and reduce oxygen consumption in professional athletes and clinical populations. The majority of interventions in enhanced recovery have focused on improving oxygen delivery and decreasing oxygen demand by minimising the stress response. **Nitrates however improve oxygen utilisation by tissues.**

CPX is an established, non-invasive and safe method of assessing patients’ cardiopulmonary reserve prior to surgery. It has been shown to predict complications and length of stay in patients.
undergoing major elective surgery. The AT can predict short and long term outcomes after surgery and postoperative complications.

We therefore decided to investigate if the ingestion of inorganic nitrate rich beetroot juice improves efficiency of oxygen use by the body by determining if nitrate loading with BRJ improves the AT and Peak VO₂ in patients with colorectal cancer and thereby improves recovery after surgery using CPX as a measure of oxygen consumption and functional capacity.
2. Trial aims and hypothesis

2.1 Aims
To study the effect of dietary nitrate supplementation on pre-operative CPX results and on recovery from surgery in patients having laparoscopic surgery for colorectal cancer, and whether the ingestion of inorganic nitrate rich beetroot juice improves efficiency of oxygen use by the body and thereby, recovery after surgery for colorectal cancer.

2.2 Design
This study is a randomised, double-blind, placebo controlled trial, in which patients were randomised in a 1:1 ratio to receive nitrate supplement drink or placebo.

2.3 Hypotheses
Pre-operative dietary supplementation with Beetroot juice (BRJ) will decrease oxygen consumption by improving Anaerobic Threshold (AT) and Peak Oxygen consumption (Peak VO₂) measured at CPX in patients undergoing elective surgery for colorectal cancer.

2.4 Primary Outcomes
The primary outcome of the study was the change in AT before and after nitrate supplementation.

2.5 Secondary outcomes
Secondary outcomes included twice daily grip strength, return of gastrointestinal function, length of stay, post-operative complications, weight, incidence of readmissions and mortality.
3. Methods

3.1 Trial design

Surgery generates a stress response which increases oxygen demand and consumption postoperatively. Patients who have low cardiopulmonary reserve may not be able to meet the increased oxygen demand and therefore are at risk of increased morbidity and mortality post-operatively. The time between decision to perform surgery and operation date is a window which allows optimisation of the patient (17).

To date, no studies have been performed with dietary nitrate supplementation in surgical patients to see if they improve recovery after surgery. We therefore decided to perform a double blind randomised controlled trial to see if dietary nitrate supplementation with beetroot juice would improve oxygen utilisation (measured by CPX testing) and improve post-operative outcomes within an enhanced recovery setting.

We decided to perform a trial on pre-operative patients with colorectal cancer as it is a common cancer and the majority of ERAS measures have been developed in this population. The research was performed at the Royal Surrey County hospital Guildford which is a high volume laparoscopic surgical centre. Pre-operatively patients have a short time between diagnosis and operation limiting the window for exercise prehabilitation but allowing nutritional prehabilitation with dietary nitrate which could be added to the existing carbohydrate loading regime.

From their baseline subset analysis Shepherd et al found that patients on ACE inhibitors, ARBs, metformin, insulin, statins and sulfonylureas had an intact enterosalivary pathway and normal baseline plasma nitrite levels (62). We therefore decided to include patients with comorbidities such as type 2 diabetes and hypertension as these are conditions common in older adults, to make data generalisable. Patients on PPIs and other medications which may affect nitrate absorption and processing were also included.

We decided to place patients on a low nitrate diet with advice to avoid nitrate rich foods for 10 days prior to the start of supplementation (please see Appendix 8 Patient information sheet) in order to standardise the baseline plasma nitrite / nitrate levels between the groups. Patients were also advised to abstain from mouthwash and chewing gum based on the work of Govonni et al and Petersson et al (56, 66).
We selected dose as within the lower ranges that have been shown to reduce resting MAP in order to minimise safety issues that might arise with high doses and hypotension in an older population with colorectal cancer (73). James White company produce a 70ml sport shot containing 6.5mmol nitrate, and a nitrate free placebo which looks and tastes exactly the same, making a double blind RCT possible. We therefore decided to give patients one shot of juice a day.

Given that changes in mitochondrial function were detected after 3 days supplementation and changes in muscle oxygenation occurred after 6 days supplementation in healthy volunteers, and in clinical populations the majority of positive results occurred after 7 days supplementation we decided to supplement for 7 days which also allowed testing in weekly CPX clinics.

We calculated that the estimated average wait for an operation after the second CPX test would be 7.16 days (based on lists available at 1, 3, 7, 8, 10 and 14 days). Given an average length of stay of 3 days post op we therefore estimated each patient would need on average 17 days supply of juice.

We decided to use CPX as it is a safe, objective measure of functional capacity which gives repeatable measure of oxygen consumption during exercise. This allowed weekly CPX clinics to be held on a Friday, supplementation then continued over the weekend and the majority of operations were then performed the next week after 10 days supplementation.

We decided not to measure plasma nitrate / nitrite as an intact entero-salivary pathway had already been established in clinical populations, and because of technical difficulties with its measurement.

The trial was conducted at a single centre to study the effects of dietary nitrate supplementation on performance in cardiopulmonary exercise testing and recovery after surgery for colorectal cancer.

### 3.2 Patient Selection

Eligible patients were those due for elective laparoscopic colonic or rectal resection for colorectal cancer between ages 18-99. Patients were excluded if they were physically unable to perform CPX (e.g. lower limb dysfunction / amputation), had a known contraindication to CPX (e.g. unstable cardiac disease), did not have capacity to consent or were allergic to beetroot. Patients who were pregnant or planning to become pregnant were also excluded.

The study took place at the Royal Surrey County hospital Guildford between August 2015 and February 2017. The trial was registered with [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT02319356). Ethical approval
for the trial was obtained from the South East Coast Surrey Research Ethics committee and the Royal Surrey County Hospital Research and Development department. The study was conducted in compliance with the declaration of Helsinki and the principles of the international conference on harmonization of Good Clinical Practice.

3.3 Recruitment
Potentially eligible patients were approached at an outpatient appointment by the chief investigator or colorectal nurse specialist and invited to participate in the study. If interested, the clinician explained the study to the patient and gave them a copy of the patient information sheet to review. (Appendix 8 Patient Information leaflet). Interested patients were asked to complete a contact details form and confirm they were happy to be contacted by the research team via telephone. An information leaflet was then sent to their General practitioner to inform them of the trial (Appendix 9 GP information leaflet). After at least 24 hours patients were contacted by telephone by the research team and any questions were answered. If they consented to be part of the study, verbal consent was taken by a suitably qualified person in accordance with international GCP guidelines.

3.3.1 Patient preparation
Patients were given a list of foods that are rich in nitrates and asked to abstain from them for 10 days before the first CPX and up until their discharge from hospital after surgery. Once verbally consented patients were instructed when to start a ‘low nitrate’ diet and given their appointment times for their first and second CPXs. All tests were scheduled and performed at the same time of day (+/- 2hours) to minimise diurnal variation.

Patients were also asked to abstain from using antibacterial mouthwash and chewing gum for the duration of the study. A food diary was issued in which to record their dietary intake for 24 hours preceding the first CPX, they were asked to replicate this diet prior to the second CPX (Appendix 10 Food Diary).

Patients were asked to arrive for each CPX in a rested and fully hydrated state, more than 3 hours post-prandial and to avoid alcohol and strenuous exercise for 24 hours preceeding each CPX. Patients were invited to give written consent to the trial when they attended clinic for their first CPX.
3.4 Protocol

**Protocol**

**Duration of study**
- Abstain from nitrate rich foods for 10 days before first CPX and duration of study
- Abstain from mouthwash and chewing gum for duration of the study

**CPX 1**
- Record dietary intake for 24 hours preceding first CPX
- Avoid alcohol and strenuous exercise for 24hrs preceding
- Refrain from caffeine 6 hours before
- Arrive rested and hydrated state, more than 3 hours post-prandial

**Patient randomised to beetroot juice / placebo group**

**Drink assigned 70ml shot at 2.5 hours before appointment time every day for the 7 days between CPXs**

**CPX 2**
- Second CPX (to be performed at same time of day +/- 3 hours of first CPX)
- Replicate 24 hours of dietary intake prior to second test

**Operation**
- Standard laparoscopic surgery
- Enhanced recovery pathway

**Post-operatively**
- Continue to drink assigned 70ml shot along with first nutritional drink every morning until discharge
- Measure grip strength with dynamometer on day of surgery and every morning and evening until discharge
- Record return of GI function

Figure 5 Protocol
3.5 Measurements

Immediately prior to the first CPX the following measurements were collected:

- weight (kg), height (cm), resting blood pressure (BP, mmHg) and heart rate (HR, beats per minute)
- ASA (American Society of Anaesthesiologists’ (ASA) classification of Physical Health) and performance status
- Grip strength measured in the dominant hand using a hand-held dynamometer (Jamar), 3 measurements were recorded.

Patients, surgeons, nurses, investigators and other researchers were blinded as to the treatment allocation throughout the study. The investigators did not have access to the code until after the patients had completed the study. The decision to exclude a patient on the basis of their CPX results was made prior to breaking the code.

Cardiopulmonary Exercise testing

The first CPX was performed according to a standard ramped incremental exercise protocol according to American society guidelines (128). All tests were performed with the participants in an upright position on an electronically braked cycle ergometer. Ramp and Saddle height were recorded and replicated for the second CPX. The protocol began with 2-3 minutes unloaded pedalling to acclimatise patients and to obtain baseline data. Ramp gradient was set to 5-25 watts per minute based on the individual patient’s predicted peak VO2, aiming for a total exercise duration of approximately 10 minutes.

Patients were instructed to pedal at a cadence of 60 rpm. Standard verbal encouragement was provided to enable the patients to exercise to their symptom limited maximum. Pulmonary gas exchange and ventilation were measured on a breath by breath basis. Heart rate and rhythm were monitored continuously during the test using an electrocardiogram. Blood pressure was measured at rest and then every 3 minutes throughout the test or earlier if clinically indicated. The test was terminated when cadence fell below 55rpm for more than 15 seconds or patients signalled that they were unable to continue. Other criteria for test termination included ST depression greater than 3mm on ECG, a new arrhythmia, systolic blood pressure of greater than 240 mmHg on 2 measurements, or symptoms such as chest pain, breathlessness or dizziness.
Standard measurements including AT, peak VO$_2$, (highest value for oxygen uptake attained during the test) VO$_2$/WR slope, Ve/VO$_2$, O$_2$ pulse at AT, work rate at GET and watts of work achieved at peak VO$_2$ were calculated and recorded. VO2 peak was taken as the highest value achieved within 60 seconds of exhaustion. Calculation of the AT was by using ventilatory equivalent plots and the V slope method (83). In order to minimise inter-reader variability as found by Sinclair et al (127) all tests were interpreted by one clinician. Patients were excluded after the first CPX if the AT was indeterminate or was not reached. Other reasons for exclusion included findings during CPX that mandated further investigation rendering a second test unsafe.

On successfully completing the first CPX patients received a full supply of supplement drinks as determined by the randomisation code. Patients were advised to drink their 70ml shot at 2.5 hours before their second CPX appointment time every day for the 7 days between CPXs. They received verbal and written explanations about beeturia and red stools. A second CPX was performed under identical conditions after 7 days’ supplementation. Weight, blood pressure, heart rate and grip strength were all retested and recorded. After this visit patients were advised to continue drinking their assigned 70ml shot at 10.30am until the day of their surgery.

Patients were reminded to bring their full supply of supplement drinks with them when they attended the hospital for their surgery. On the day of surgery, they were advised to drink their shot with their morning dose of preload and finish drinking by 6am (morning list) or 10.30am (afternoon list).

**Adherence**

Adherence to study protocol was verified at each visit using a standard questionnaire. Adherence to the trial supplement was checked by self-report and remaining bottle count on admission and discharge on a predetermined form.

**Surgical technique**

Surgery was performed in a standard manner; laparoscopically; with the patient in the Lloyd Davies position with steep head down for left sided resections, and supine for right sided resections.

**Post-operative measurements**

After surgery patients were asked to drink their assigned 70ml shot along with their first nutritional drink every morning until discharge.
Patients who underwent emergency surgery or in whom the operation was converted to an open procedure (defined as an incision larger than that required to extract the specimen) were analysed separately from the laparoscopic elective patients.

Patients followed the standard post-operative enhanced recovery care pathway for colorectal patients and were discharged as per standard protocol when their clinical observations were normal, they were comfortable with oral analgesia, were tolerating a normal diet and self-caring with minimal / pre-surgery level of assistance. Standard post-operative measurements were recorded including:

- Daily temperature, blood pressure, heart rate and saturations
- Time until patient actually leaves the hospital
- The incidence of readmissions

The following addition information was collected during the patients hospital stay:

- Grip strength measured twice daily until patient discharge
- Time until patient is able to tolerate a normal diet
- Time until patient is able to pass flatus
- Time until patient opens their bowels
- Time patient is deemed medically fit for discharge

Morbidity / post-operative complications were recorded using the Clavien Dindo (129) systems. Patients were asked to record their weight at home on day 14 and 28 post surgery. 30 and 90 day mortality were recorded. The incidence of readmissions was recorded.

### 3.6 Randomisation

Patients were randomised to receive either a 70ml “Beet It concentrated beetroot juice shots” (containing nitrate 6.5 mmol or c400mg) or "Beet It nitrate depleted shots" (Placebo) from James White Drinks, Ipswich, United Kingdom on a 1 to 1 basis. The randomisation sequence was computer generated via https://www.sealed envelope.com using random block sizes of 2, 4, 6, 8 and 10. Allocations were then placed in sealed opaque envelopes by a doctor not involved in the study.
3.7 Blinding
Randomisation was performed by a designated member of staff not involved in the patients care and the assigned juice placed in identical bags with patient labels. The nitrate was removed by the manufacturer (James White Drinks, Ipswich, United Kingdom) to make the placebo juice, otherwise the BRJ and placebo were identical in appearance, taste, smell and nutrient composition. Efficacy of blinding was not formally assessed.

3.8 Statistics
Sample Size calculation
Studies have shown up to a 20% improvement in the measures of oxygen consumption during exercise after nitrate supplementation in young physically active males. To allow for confounding factors in an older population with cancer a more conservative estimate was used and we therefore estimated nitrate supplementation would produce a 7.5% difference. Other studies have estimated an 8% reduction in O2 cost of exercise (62). To achieve a power of 80% and a significance level of 5% we calculated 58 patients (total) undergoing laparoscopic surgery needed to be studied to detect a 7.5% difference between mean AT (Mean 14.40, inter-patient SD 1.44, CV 10%[10]) before and after BRJ supplementation (independent t test).

However, to allow for patient drop out due to conversion to open (rate of 5.6%, obtained from a prospectively kept database for the colorectal unit), inability to complete CPX and intolerance to juice / dietary restrictions, approximately 40 patients will be required for each treatment group (for a total recruitment of 80).

Statistical analysis
Statistical analysis was performed with the help of a statistician from the University of Surrey. Continuous data were assessed for normality using Kolmogorov-Smirnov test. The majority of data were normally distributed and therefore the two groups were compared using either paired or unpaired t-tests. Normal data were expressed as mean (SD) or 95% CI as appropriate. Categorical variables were analysed with either Mann Whitney U or Fishers exact test as appropriate and were expressed as median +/- interquartile range. Statistical analysis was performed using SPSS10.
3.9 Approvals and Registrations

Health Research Authority regulations and approval
The study has been reviewed and received a favourable opinion by South East Coast-Surrey Research Ethics Committee

Ethics Approval / Research and development sponsorship
Ethical approval was obtained from the South East Coast Surrey Research Ethics committee (REC) and the Royal Surrey County Hospital Research and Development department. The study was conducted in compliance with the declaration of Helsinki and the principles of the international conference on harmonization of Good Clinical Practice.

Randomised Clinical Trial Registration
The trial was registered with www.clinicaltrials.gov (NCT02319356).
4. Results

Patient Flow
Consecutive patients were identified at the weekly MDT and approached at their Outpatients appointment. Between August 2015 and February 2017, 228 patients were screened. 141 Patients were excluded, 60 because they did not meet the inclusion criteria, 21 because there was not enough time prior to surgery for the trial, and 13 for other reasons (went private n=10, or did not meet exclusion criteria - not fit for CPX n=2, emergency surgery n=1). 134 patients were eligible for inclusion and approached, 47 patients declined to participate leaving 87 patients randomised.

Assessed for eligibility (n=228)

Excluded (n=141)
- Not meeting inclusion criteria, n=60
- Declined to participate, n=47
- Not enough time, n=21
- Other reasons, n=13

Enrollment

Randomised (n=87)

Beetroot Juice
- Allocated to intervention (n=44)
- Received allocated intervention, n=44

Excluded (n=6)
- No second CPX

Allocation

Placebo
- Allocated to intervention (n=43)
- Received allocated intervention, n=42

Excluded (n=8)
- No second CPX, n=2
- Discontinued intervention, n=1
- Intervention, n=5

Analysis

Analysed (n=38)
1. Primary outcomes
   - Excluded – tests not comparable, n=3
   - AT, n=34
     - Excluded – indeterminate AT, n=1
   - Peak VO2, n=32
     - Excluded – submaximal, n=3
2. Secondary outcomes, n=36
   - No operation, n=1
   - Converted to open, n=1

Analysed (n=34)
1. Primary outcomes
   - Excluded – tests not comparable, n=3
   - AT, n=31
   - Peak VO2, n=26
     - Excluded - submaximal, n=5
2. Secondary outcomes, n=33
   - No operation, n=1

Figure 5 Consort diagram
After allocation 15 patients were excluded altogether (8 no second CPX, 5 had another intervention, 1 discontinued and 1 refused the intervention) leaving 72 patients analysed. A further 7 patients were excluded from the AT analysis as they did not reach AT (n=1) or the tests were not comparable (n=6) leaving 65 patients analysed for AT, 34 in the nitrate group. 57 patients performed a maximal test, 32 in the nitrate group. Out of the 72 analysed 69 patients were analysed for secondary outcomes, as 2 did not have an operation and 1 operation was converted to open. (Figure 5 Consort diagram).

Demographics

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>72</td>
<td>38</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.3 (11.2)</td>
<td>68.2 (10.5)</td>
<td>68.5 (12.0)</td>
<td>0.913a</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>38:34</td>
<td>23:15</td>
<td>16:18</td>
<td>0.252b</td>
</tr>
<tr>
<td>(54.2%:45.8%)</td>
<td>(60.5%:39.5%)</td>
<td>(47.1%:52.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.5 (13.5)</td>
<td>79.5 (13.9)</td>
<td>75.2 (12.9)</td>
<td>0.183a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 (4.5)</td>
<td>28.1 (4.5)</td>
<td>26.2 (4.3)</td>
<td>0.062a</td>
</tr>
<tr>
<td>ASA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15 (20.8%)</td>
<td>10 (26.3%)</td>
<td>5 (14.7%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>52 (72.2%)</td>
<td>26 (68.4%)</td>
<td>26 (76.5%)</td>
<td>0.205c</td>
</tr>
<tr>
<td>3</td>
<td>5 (6.9%)</td>
<td>2 (5.3%)</td>
<td>3 (8.8%)</td>
<td></td>
</tr>
<tr>
<td>Morbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>9 (12.5%)</td>
<td>4 (10.5%)</td>
<td>5 (14.7%)</td>
<td>0.727d</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23 (31.9%)</td>
<td>11 (28.9%)</td>
<td>12 (35.3%)</td>
<td>0.564b</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>8 (11.1%)</td>
<td>3 (7.9%)</td>
<td>5 (14.7%)</td>
<td>0.463d</td>
</tr>
<tr>
<td><strong>Cardiac disease</strong></td>
<td><strong>7 (9.7%)</strong></td>
<td><strong>1 (2.6%)</strong></td>
<td><strong>6 (17.6%)</strong></td>
<td><strong>0.047d</strong></td>
</tr>
<tr>
<td>Stroke / TIA</td>
<td>3 (4.2%)</td>
<td>2 (5.3%)</td>
<td>1 (2.9%)</td>
<td>1.000d</td>
</tr>
<tr>
<td>Smoker</td>
<td>7 (9.7%)</td>
<td>3 (7.9%)</td>
<td>4 (11.8%)</td>
<td></td>
</tr>
<tr>
<td>Ex smoker</td>
<td>15 (20.8%)</td>
<td>7 (18.4%)</td>
<td>8 (23.5%)</td>
<td>0.403c</td>
</tr>
<tr>
<td>Never smoked</td>
<td>50 (69.4%)</td>
<td>28 (73.7%)</td>
<td>22 (64.7%)</td>
<td></td>
</tr>
<tr>
<td>Planned side of resection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>26 (36.1%)</td>
<td>17 (44.7%)</td>
<td>9 (26.5%)</td>
<td>0.128b</td>
</tr>
<tr>
<td>Left</td>
<td>45 (62.5%)</td>
<td>21 (55.3%)</td>
<td>24 (70.6%)</td>
<td></td>
</tr>
</tbody>
</table>
Preoperative Chemo-radiotherapy

<table>
<thead>
<tr>
<th></th>
<th>Placebo (86)</th>
<th>Test (86)</th>
<th>χ² (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoadjuvant chemotherapy</td>
<td>8 (11.1%)</td>
<td>3 (7.9%)</td>
<td>5 (14.7%)</td>
<td>0.463\textsuperscript{d}</td>
</tr>
<tr>
<td>Neoadjuvant radiotherapy for CRC</td>
<td>9 (12.5%)</td>
<td>4 (10.5%)</td>
<td>5 (14.7%)</td>
<td>0.727\textsuperscript{d}</td>
</tr>
<tr>
<td>Any pelvic radiotherapy</td>
<td>12 (16.7%)</td>
<td>6 (15.8%)</td>
<td>6 (17.6%)</td>
<td>0.832\textsuperscript{b}</td>
</tr>
<tr>
<td>Did not have resection</td>
<td>2 (2.8%)</td>
<td>1 (2.6%)</td>
<td>1 (2.9%)</td>
<td>0.936\textsuperscript{b}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Placebo (86)</th>
<th>Test (86)</th>
<th>χ² (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous abdominal surgery</td>
<td>14 (19.4%)</td>
<td>6 (15.8%)</td>
<td>8 (23.5%)</td>
<td>0.407\textsuperscript{b}</td>
</tr>
<tr>
<td>Previous colorectal resection</td>
<td>5 (6.9%)</td>
<td>1 (2.6%)</td>
<td>4 (11.8%)</td>
<td>0.182\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Laboratory values

<table>
<thead>
<tr>
<th></th>
<th>Placebo (86)</th>
<th>Test (86)</th>
<th>χ² (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Preop Hb</td>
<td>128 (18.2)</td>
<td>126 (18.0)</td>
<td>131 (18.5)</td>
<td>0.267\textsuperscript{a}</td>
</tr>
<tr>
<td>Mean Preop Alb (n=65)</td>
<td>43 (3.2)</td>
<td>42 (3.4)</td>
<td>43 (3.0)</td>
<td>0.809\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Disease

<table>
<thead>
<tr>
<th></th>
<th>Placebo (86)</th>
<th>Test (86)</th>
<th>χ² (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>66 (91.7%)</td>
<td>35 (92.1%)</td>
<td>31 (91.2%)</td>
<td>1.000\textsuperscript{d}</td>
</tr>
<tr>
<td>No cancer</td>
<td>6 (8.3%)</td>
<td>3 (7.9%)</td>
<td>3 (8.8%)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 6 Demographics** (Quantitative variables expressed as Arithmetic mean plus standard deviation, qualitative variables expressed as absolute numbers and percentages, a=T test, b= chi squared, c= mann whitney, d=fishers exact). Cardiac disease includes PMH of myocardial infarction (MI), coronary artery bypass grafts (CABG) and ischaemic heart disease (IHD).

There were more patients with any cardiac disease in the placebo group. There were no other significant differences in baseline characteristics between treatment groups (**Figure 6 Demographics**).

**Exclusions**

Out of 72 patients, 70 patients had a colorectal resection. Two patients had all pre-operative tests and supplementation but did not have a colorectal resection. Their data was used in the primary outcomes but excluded from the secondary outcomes including postoperative recovery and complications as they had not had a resection.
The first exclusion was in the placebo group. The patient had previously had a subtotal gastrectomy in 2012 for T4aN3aMO adenocarcinoma of stomach, and had synchronous right and left colon cancers on staging CT / colonoscopy. Unfortunately at laparoscopy widespread metastatic disease was identified and therefore no resection was performed. The second exclusion was in the nitrate group. The patient had a past medical history of liver disease with portal hypertension and was therefore deemed high risk for surgery. The staging CT was reviewed and re-reported as multiple varices encasing the colon, which made a colorectal resection not technically possible and the patient was referred to a liver unit for consideration of treatment prior to surgery.

One patient in the placebo group had two CPXs but at different ramps and was therefore excluded from primary outcomes as the CPX results were not comparable. This was not identified until after collection of the secondary outcomes these were therefore included in the secondary outcome analysis.

<table>
<thead>
<tr>
<th>Surgical Procedure</th>
<th>n</th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Hemicolectomy</td>
<td>25</td>
<td>16 (43.2%)</td>
<td>9 (27.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmoid / left hemi</td>
<td>11</td>
<td>6 (16.2%)</td>
<td>5 (15.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior Resection</td>
<td>22</td>
<td>10 (27.0%)</td>
<td>12 (36.4%)</td>
<td>0.411b</td>
<td></td>
</tr>
<tr>
<td>TME</td>
<td>7</td>
<td>4 (10.8%)</td>
<td>3 (9.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APER</td>
<td>5</td>
<td>1 (2.7%)</td>
<td>4 (12.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surgical Outcomes</th>
<th>n</th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Stoma</td>
<td>70</td>
<td>15 (21.4%)</td>
<td>6 (16.2%)</td>
<td>9 (27.2%)</td>
<td>0.260b</td>
</tr>
<tr>
<td>Conversion to open</td>
<td>70</td>
<td>1 (1.4%)</td>
<td>1 (2.7%)</td>
<td>0</td>
<td>0.727d</td>
</tr>
<tr>
<td>Intraoperative problems</td>
<td>70</td>
<td>12 (17.1%)</td>
<td>8 (21.6%)</td>
<td>4 (12.1%)</td>
<td>0.292b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Operative Time</th>
<th>n</th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation time (mins)</td>
<td>69</td>
<td>150 (55)</td>
<td>144 (55)</td>
<td>157 (55)</td>
</tr>
<tr>
<td>Anaesthetic time (mins)</td>
<td>44</td>
<td>44 (12)</td>
<td>44 (14)</td>
<td></td>
</tr>
<tr>
<td>Overall operation time (mins)</td>
<td>194</td>
<td>188 (57)</td>
<td>201 (56)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histology</th>
<th>n</th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>69</td>
<td>8 (11.6%)</td>
<td>3 (8.1%)</td>
<td>5 (15.6%)</td>
</tr>
<tr>
<td>T1</td>
<td>5</td>
<td>3 (8.1%)</td>
<td>2 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>10</td>
<td>4 (10.8%)</td>
<td>6 (18.8%)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>39</td>
<td>22 (59.5%)</td>
<td>17 (53.1%)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>7</td>
<td>5 (13.5%)</td>
<td>2 (6.2%)</td>
<td></td>
</tr>
</tbody>
</table>
### Surgical Procedure, Operative Time and Histology

There were no significant differences in the type of operation, operative time or histology between the two groups.  **(Figure 7 Clinical and operative data)**

One of the 70 patients who had a colorectal resection had a conversion to open surgery. They had had a previous open cholecystectomy and had a right sided cancer. On laparoscopy they had dense adhesions and a small bowel injury occurred and therefore the operation was converted to open. Therefore they were therefore not included in the operating time / post-operative outcome data (n=69).

---

<table>
<thead>
<tr>
<th>T0-T2</th>
<th>23 (33.3%)</th>
<th>10 (27.0%)</th>
<th>13 (40.6%)</th>
<th>x</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>63</td>
<td>41 (65.0%)</td>
<td>20 (58.8%)</td>
<td>21 (72.4%)</td>
</tr>
<tr>
<td>N1</td>
<td>15 (23.8%)</td>
<td>10 (29.4%)</td>
<td>5 (17.2%)</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>7 (11.1%)</td>
<td>4 (11.8%)</td>
<td>3 (10.3%)</td>
<td></td>
</tr>
<tr>
<td>N1-2</td>
<td>22 (34.9%)</td>
<td>14 (41.2%)</td>
<td>8 (27.6%)</td>
<td>x</td>
</tr>
<tr>
<td>M0</td>
<td>58 (92.1%)</td>
<td>30 (88.2%)</td>
<td>28 (96.6%)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>5 (7.9%)</td>
<td>4 (11.8%)</td>
<td>1 (3.4%)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Dukes</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11 (18.3%)</td>
<td>5 (14.7%)</td>
<td>6 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>26 (43.3%)</td>
<td>14 (41.1%)</td>
<td>12 (46.2%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>60</td>
<td>22 (36.7%)</td>
<td>14 (41.1%)</td>
<td>8 (30.8%)</td>
</tr>
<tr>
<td>D</td>
<td>1 (1.7%)</td>
<td>1 (2.9%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

| Dukes A-D | 60         | 60 (83.3%) | 34 (89.5%) | 26 (76.5%)     | 0.229<sup>c</sup> |

<table>
<thead>
<tr>
<th>Others</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Other cancer</td>
<td>2 (2.8%)</td>
<td>0</td>
<td>2 (5.9%)</td>
<td>0.19&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>No operation / resection</td>
<td>2 (2.8%)</td>
<td>1 (2.6%)</td>
<td>1 (2.9%)</td>
<td>1.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>No cancer</td>
<td>6 (8.3%)</td>
<td>3 (7.9%)</td>
<td>3 (8.8%)</td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>2 (2.8%)</td>
<td>0</td>
<td>2 (5.9%)</td>
<td>0.231&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

| Totals   | 72         | 38         | 34         |               |

**Figure 7 Clinical and operative data** (Quantitative variables expressed as Arithmetic mean with standard deviation in brackets, qualitative variables expressed as absolute numbers and percentages, a=T test, b= chi squared, c= mann whitney, d=fishers exact. Other cancer = Lymphoma, signet cell cancer)
Histology
A total of 72 patients had histology. Sixty six patients had a cancer at initial biopsy or final histology, 6 patients did not have cancer at final histology or initial biopsy. Three patients out of 72 did not have a T stage. Two patients did not have a resection and one patient had lymphoma on histology leaving 69 patients analysed. Eight patients were T0 as they had had a complete response (n=2) or did not have a cancer at histology (n=6). For further information see Appendix 10 - Patients without cancer on histology.

Sixty patients had a T stage. Eight patients did not have a dukes stage as they had had a complete response (n=2) or did not have a cancer at histology (n=6). Two patients did not have a resection, and two patients did not have a dukes stage as they had another type of cancer (one patient had lymphoma on histology and another had Signet cell cancer (T stage but no dukes)).

Laboratory values
All patients had a preoperative haemoglobin. 7 patients did not have a preoperative albumin. There were no significant differences in the preoperative haemoglobin or albumin between the two groups. For further information see Appendix 11 – Laboratory values.

Juice Compliance

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative compliance</td>
<td>100</td>
<td>100%</td>
<td>100%</td>
<td>1.00a</td>
</tr>
<tr>
<td>Postoperative compliance</td>
<td>88</td>
<td>83%</td>
<td>93%</td>
<td>0.021a</td>
</tr>
<tr>
<td>Overall compliance</td>
<td>96</td>
<td>95%</td>
<td>97%</td>
<td>0.312a</td>
</tr>
</tbody>
</table>

Figure 8 Juice compliance (Qualitative variables expressed as percentages, a=T test)

Juice compliance was good (see Figure 8 Juice compliance). Before the operation there was 100% compliance with the beetroot juice consumption (n=71, 37 in nitrate group (1 patient had no operation)). Postoperatively 2 patients were excluded from juice compliance, 1 did not have a resection and one was converted to open (n=69, 36 in nitrate group)
### Cardiopulmonary exercise test variables

Seventy two patients performed 2 comparable CPX tests. Sixty five of these reached AT. Fifty eight patients performed a maximal test, 32 in the nitrate group, 26 in the placebo group.

1 patient in the nitrate group had a maximal test but an indeterminate VO2 WR and VeVCO2 slope.

#### Main CPX Variables

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline mean</th>
<th>95% CI</th>
<th>Change</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anaerobic threshold (AT) (ml/kg/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td>34</td>
<td>12.3</td>
<td>11.33 to 13.34</td>
<td>+0.70</td>
<td>0.13-1.28</td>
<td>0.018*1</td>
</tr>
<tr>
<td>Placebo</td>
<td>31</td>
<td>12.6</td>
<td>11.43 to 13.85</td>
<td>-0.37</td>
<td>-1.09-0.36</td>
<td>0.309*1</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.021*2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VO₂ at AT (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td>34</td>
<td>972.6</td>
<td>886.3 to 1059.0</td>
<td>+56.2</td>
<td>9.96 to 102.4</td>
<td>0.019*1</td>
</tr>
<tr>
<td>Placebo</td>
<td>31</td>
<td>956.8</td>
<td>841.1 to 1072.4</td>
<td>-21.9</td>
<td>-76.9 to 33.0</td>
<td>0.421*1</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.029*2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak VO₂ (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td>32</td>
<td>1584</td>
<td>1424 to 1744</td>
<td>-20.6</td>
<td>-83.3 to 42.1</td>
<td>0.507*1</td>
</tr>
<tr>
<td>Placebo</td>
<td>26</td>
<td>1532</td>
<td>1351 to 1714</td>
<td>-35.0</td>
<td>-121.1 to 51.1</td>
<td>0.411*1</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.778*2</td>
</tr>
</tbody>
</table>

*Figure 9 Main CPX variables* (Arithmetic mean with 95% confidence intervals, a1= paired t test, a2= unpaired t test)

There was no significant difference in the baseline means of the placebo and nitrate groups for any of the CPX variables including ATs, VO₂ at AT and Peak VO₂ (AT p=.69, VO₂ at AT p=.82, Peak VO₂ p=.662) (*Figure 9 Main CPX variables*).
Figure 10 Change in AT (ml/kg/min) with Nitrate Supplementation, (Mean in black)

There was a statistically significant increase in the AT and the VO2 at AT in the nitrates group before and after supplementation (AT p=.018, VO2 at AT p=.019) (Figure 10 Change in AT with Nitrate Supplementation).
There was no significant change in the mean AT and VO2 at AT in the placebo group before and after supplementation (**Figure 11 Change in AT with placebo**).
Figure 12 AT (ml/kg/min) before and after 7 days supplementation, with standard error bars (**= statistically significant change)

There was a statistically significant increase in change in the AT and VO2 at AT in the nitrates group compared to the placebo group before and after supplementation (AT p=0.021, VO2 at AT p=0.029) **(Figure 12 AT before and after supplementation)**. There was no significant change in the mean AT and VO2 at AT in the placebo group before and after supplementation.

There was no significant change in the Peak VO2 in the nitrates or placebo groups before and after supplementation (nitrates p=.507, p=0.411). There was no significant change in the Peak VO2 in the nitrates group compared to the placebo group before and after supplementation (p=.691).

**Ramp / Watts**

There was no significant difference in the ramp or baseline watts of the placebo and nitrate groups (Ramp p=0.988, Watts p=0.408). There was a statistically significant increase in the Watts in both the nitrates group (3.2, 95% CI 0.10 to 6.27; p=0.043) and the placebo group (3.4, 95% CI 0.72 to 6.13; p=0.015) before and after supplementation. However there was no significant difference in the change in watts before and after supplementation between the two groups (p=0.909). For further information see Appendix 12– Ramp data and Appendix 13 – Summary CPX results.
Other CPX Variables

VeVCO₂, Peak VE, ΔVO₂/ΔWR, VeCO₂ at AT, VO₂, Peak O₂ Pulse, VO₂ peak/m²

There was a significant increase in the VeVCO₂ in the placebo group before and after supplementation (1.164, 95% CI 0.429-2.285; p=0.042). There was no significant difference in the change in VeVCO₂ before and after supplementation between the two groups (p=0.347).

There was no significant changes in any of the other CPX variables in the nitrates and placebo groups with supplementation and no significant difference in the change in any of the other CPX variables before and after supplementation between the two groups. For further information see Appendix 13 – Summary CPX results.

Spirometry data

72 patients performed spirometry before and after the intervention. 1 patient was excluded as they had poor technique despite coaching leaving 71 patients analysed, 37 patients in the nitrate group and 34 patients in the placebo group.

There was no significant difference in the baseline FVC, FEV₁ or PEFR of the placebo and nitrate groups (FVC p=0.706, FEV₁ p=.290, PEFR p=.218). There was no significant change in the FVC, FEV₁ or PEFR in the nitrates or the placebo groups before and after supplementation (Figure 13 Spirometry results). For further information see Appendix 14 – Summary Spirometry results

<table>
<thead>
<tr>
<th>Mean change</th>
<th>nitrates</th>
<th>P value</th>
<th>placebo</th>
<th>P value</th>
<th>Nitrates vs placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>0.030</td>
<td>0.588¹</td>
<td>0.035</td>
<td>0.451¹</td>
<td>0.939²</td>
<td></td>
</tr>
<tr>
<td>FEV</td>
<td>0.0441</td>
<td>0.286¹</td>
<td>0.047</td>
<td>0.129¹</td>
<td>0.894²</td>
<td></td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>0.003</td>
<td>0.761¹</td>
<td>0.007</td>
<td>0.121¹</td>
<td>0.626²</td>
<td></td>
</tr>
<tr>
<td>PEFR</td>
<td>0.100</td>
<td>0.654¹</td>
<td>0.285</td>
<td>0.179¹</td>
<td>0.545²</td>
<td></td>
</tr>
</tbody>
</table>

Figure 13 Spirometry results (Arithmetic mean change, a1= paired t test, a2= unpaired t test, for full data including 95% CI see Appendix 15)
Blood pressure

All patients

Seventy two patients had blood pressure recorded before and after intervention, 1 patient did not have a resting blood pressure recorded and so was excluded from the BP analysis leaving 71 patients analysed. Twenty two patients were on treatment for hypertension, 11 in each group.

There was no significant difference in the baseline resting systolic or diastolic and baseline maximal systolic or diastolic blood pressures of the placebo and nitrate groups (resting systolic p=0.493, resting diastolic p=0.115, maximal systolic p=0.616, maximal diastolic p=0.714) at CPX1. There was no significant change in the resting systolic or diastolic and maximal systolic or diastolic pressures in either group before and after supplementation (resting systolic nitrate p=0.344, resting systolic placebo p=0.060, resting diastolic nitrate p=0.447, resting diastolic placebo p=0.069, maximal systolic nitrate p=0.427, maximal systolic placebo p=0.294, maximal diastolic nitrate p=0.376, maximal diastolic placebo p=0.792).

There was a statistically significant decrease in the resting systolic blood pressure in the placebo group after supplementation (p=0.039) when compared to the nitrates group. After removal of the patients on antihypertensives from this analysis the change remains significant (p=0.045). There was no significant change in the resting diastolic blood pressure when comparing the nitrate and placebo groups (p=0.270).

Non hypertensive patients only

Excluding the 22 patients who were on treatment for hypertension, 49 patients had blood pressure recorded before and after intervention, (nitrates n=27, placebo n=22). The nitrate group had a significantly lower resting baseline systolic pressure compared with the placebo groups (p=0.045). There was no other significant difference in the baseline blood pressures of the placebo and nitrate groups.

There was a statistically significant decrease in the resting systolic blood pressure in the placebo group after supplementation (p=0.037) when compared to the nitrates group (Figure 14 Blood pressure Results). For further information see Appendix 15 – Summary blood pressure results.
<table>
<thead>
<tr>
<th>Mean change</th>
<th>Nitrates Mean (mmHg)</th>
<th>Change (mmHg)</th>
<th>P value</th>
<th>Placebo Mean (mmHg)</th>
<th>Change (mmHg)</th>
<th>P value</th>
<th>Baseline P value</th>
<th>Nitrates vs placebo P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting systolic</td>
<td>135.4</td>
<td>2.0</td>
<td>0.344&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>138.6</td>
<td>-4.6</td>
<td>0.060&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>0.493&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>0.039&lt;sup&gt;a2&lt;/sup&gt;&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Resting diastolic</td>
<td>78.3</td>
<td>-1.2</td>
<td>0.447&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>82.9</td>
<td>-4.0</td>
<td>0.069&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>0.115&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>0.270&lt;sup&gt;a2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maximal systolic</td>
<td>179.9</td>
<td>-2.6</td>
<td>0.427&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>176.9</td>
<td>3.3</td>
<td>0.294&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>0.616&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>0.194&lt;sup&gt;a2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maximal diastolic</td>
<td>83.9</td>
<td>-3.2</td>
<td>0.376&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>85.5</td>
<td>0.8</td>
<td>0.792&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>0.714&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>0.406&lt;sup&gt;a2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 14 Blood pressure (Arithmetic mean change, a1= paired t test, a2= unpaired t test, * = significant, for full data including 95% CI see Appendix 16)

**Post operative outcomes**

**Length of stay**

There was a significantly lower median LOS in the nitrate group when compared to placebo group (Figure 14 Median length of Stay). There were no other significant differences in any of the other measured post-operative outcomes between the two groups (Figure 15 Post operative outcomes).

Figure 15 Median Length of Stay (days) with standard error bars
<table>
<thead>
<tr>
<th>Length of stay (days)</th>
<th>n</th>
<th>overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Length of stay</strong></td>
<td>69</td>
<td>3.0 (5.1)</td>
<td>3.0 (5.6)</td>
<td>4.0 (4.6)</td>
<td>0.060c</td>
</tr>
<tr>
<td><strong>Mean Length of stay</strong></td>
<td>4.5 (5.1)</td>
<td>4.2 (5.6)</td>
<td>4.7 (4.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Post operative outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hours till tolerating normal diet</td>
<td>69</td>
<td>15.3 (10.5)</td>
<td>14.3 (8.7)</td>
<td>16.5 (12.3)</td>
<td>0.601c</td>
</tr>
<tr>
<td>Hours till pass flatus</td>
<td>52</td>
<td>34.6 (18.5)</td>
<td>34.1 (16.2)</td>
<td>35.2 (21.2)</td>
<td>0.955c</td>
</tr>
<tr>
<td>Hours till bowels open</td>
<td>44</td>
<td>58.9 (30.6)</td>
<td>63.3 (30.0)</td>
<td>53.6 (31.3)</td>
<td>0.394c</td>
</tr>
<tr>
<td>CRP day 1</td>
<td>59</td>
<td>60 (35)</td>
<td>61 (37)</td>
<td>58 (34)</td>
<td>0.958c</td>
</tr>
<tr>
<td><strong>Weight change (Kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPX1 to CPX2</td>
<td>72</td>
<td>0.07 (0.61)</td>
<td>0.04 (0.64)</td>
<td>0.11 (0.58)</td>
<td>0.419c</td>
</tr>
<tr>
<td>Day 14 to 28</td>
<td>47</td>
<td>-0.08 (1.39)</td>
<td>-0.51 (1.47)</td>
<td>0.32 (1.22)</td>
<td>0.083c</td>
</tr>
</tbody>
</table>

*Figure 16 Post operative outcomes* (Quantitative variables expressed as Arithmetic mean with standard deviation in brackets, a=T test, b= chi squared, c= mann whitney, d=fishers exact)

**Grip Strength**

There were no significant changes in percentage of baseline AT between the two groups on day 0 to 5 in the morning and afternoon, and on discharge. The nitrate group significantly improved their percentage of baseline grip strength between the first and the second CPX (p=0.0042 t test). There was also a trend towards a significant improvement in the placebo group (p=0.0541 t test) (*Figure 17 Grip Strength*).

On day 0 in the morning the nitrate group significantly improved their percentage of baseline grip strength between the first CPX and the day of operation (p=0.0028), there was no significant change in the placebo group. However on day 0 in the afternoon the placebo group significantly improved their percentage of baseline grip strength between the first CPX and the day of operation (p=0.0215), there was no significant change in the nitrate group.

On day 2 in the morning the placebo group significantly improved their percentage of baseline grip strength between the first CPX and the day of operation (p=0.0314), there was no significant change in the nitrate group.
<table>
<thead>
<tr>
<th>% of baseline Grip Strength</th>
<th>n</th>
<th>Nitrates</th>
<th>placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPX2</td>
<td>71</td>
<td>104</td>
<td>103</td>
<td>0.550</td>
</tr>
<tr>
<td>Day 0 AM</td>
<td>69</td>
<td>102</td>
<td>100</td>
<td>0.345</td>
</tr>
<tr>
<td>Day 0 PM</td>
<td>70</td>
<td>97</td>
<td>94</td>
<td>0.347</td>
</tr>
<tr>
<td>Day 1 AM</td>
<td>70</td>
<td>102</td>
<td>98</td>
<td>0.250</td>
</tr>
<tr>
<td>Day 1 PM</td>
<td>68</td>
<td>101</td>
<td>99</td>
<td>0.523</td>
</tr>
<tr>
<td>Day 2 AM</td>
<td>68</td>
<td>98</td>
<td>94</td>
<td>0.290</td>
</tr>
<tr>
<td>Day 2 PM</td>
<td>51</td>
<td>101</td>
<td>99</td>
<td>0.476</td>
</tr>
<tr>
<td>Day 3 AM</td>
<td>42</td>
<td>102</td>
<td>98</td>
<td>0.425</td>
</tr>
<tr>
<td>Day 3 PM</td>
<td>28</td>
<td>101</td>
<td>100</td>
<td>0.933</td>
</tr>
<tr>
<td>Day 4 AM</td>
<td>27</td>
<td>101</td>
<td>98</td>
<td>0.572</td>
</tr>
<tr>
<td>Day 4 PM</td>
<td>17</td>
<td>102</td>
<td>99</td>
<td>0.656</td>
</tr>
<tr>
<td>% baseline grip on day of discharge</td>
<td>70</td>
<td>102</td>
<td>99</td>
<td>0.237</td>
</tr>
</tbody>
</table>

Figure 17 Grip Strength (T Test)

ERAS Compliance

Out of the 72 patients in the study, for ERAS compliance data from 70 patients were analysed pre-operatively (as two patients did not have an operation). Post-operatively data for 69 patients were analysed as one patient did not have a resection and one patients operation was converted to an open procedure. There was good compliance to ERAS principles in both groups (Figure 18 ERAS Compliance).

<table>
<thead>
<tr>
<th></th>
<th>overall</th>
<th>nitrates</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>70</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Avoid bowel prep</td>
<td>56 (80%)</td>
<td>73% (27)</td>
<td>88% (29)</td>
</tr>
<tr>
<td>CHO loading</td>
<td>66 (94%)</td>
<td>95% (35)</td>
<td>94% (31)</td>
</tr>
<tr>
<td>Spinal</td>
<td>66 (94%)</td>
<td>95% (35)</td>
<td>94% (31)</td>
</tr>
<tr>
<td>Postoperative</td>
<td>69</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>No drains</td>
<td>56 (81%)</td>
<td>89% (32)</td>
<td>73% (24)</td>
</tr>
<tr>
<td>Avoid opiates</td>
<td>63 (91%)</td>
<td>86% (31)</td>
<td>97% (32)</td>
</tr>
<tr>
<td>TWOC day 1</td>
<td>52 (75%)</td>
<td>73% (27)</td>
<td>76% (25)</td>
</tr>
</tbody>
</table>

Figure 18 ERAS Compliance (variables expressed as percentages with absolute numbers in brackets)
Complications

Clavien Dindo classification

The Clavien dindo system is a validated, objective method of grading postoperative complications and therefore assessing surgical outcomes (129). Patients are scored on discharge and if more than one complication are present the highest grade of complication is used. For further information see Appendix 16 – Clavien Dindo scoring system.

<table>
<thead>
<tr>
<th>n</th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>69</td>
<td>36</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2 (2.9%)</td>
<td>1 (2.8%)</td>
<td>1 (3.0%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>8 (11.6%)</td>
<td>2 (5.6%)</td>
<td>6 (18.2%)</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>5 (7.2%)</td>
<td>2 (5.6%)</td>
<td>3 (9.1%)</td>
<td>0.219c</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1 (1.4%)</td>
<td>1 (2.8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Figure 19 Clavien Dindo (qualitative variables expressed as absolute numbers and percentages, a=T test, b= chi squared, c= mann whitney, d=fishers exact)

There was no significant difference in the rate of complications at discharge classified according to the Clavien Dindo system between the two groups (Figure 19 Clavien Dindo). One patient in the placebo group had a ureteric injury which was recognised and dealt with intra-operatively and had no sequelae and so does not score on the Clavien-Dindo system. For further information see Appendix 17 – All inpatient complications classified by Clavien Dindo.

Complications within 30 days

The rate of specific complications occurring within the first 30 days was low, in line with previous studies published at this institution. There was no significant difference in the rate of surgical complications including ileus, anastomotic leak, return to theatre or requirement for a blood transfusion between the two groups. There was no significant difference in the rate of infectious complications including wound infection, lower respiratory chest infection (LRTI) and urinary tract infection (UTI) between the two groups (Figure 20 Specific complications (within 30 days)). For further information see Appendix 18 – Complications after discharge not requiring readmission.
<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
</tbody>
</table>

**Surgical Complications**

- Ileus: 14.5% (10/69), 13.9% (5/36), 15.2% (5/33), P=1.00d
- Leak: 7.2% (5/69), 8.3% (2/36), 6.1% (2/33), P=1.00d
- Return to theatre: 8.7% (6/69), 8.3% (3/36), 9.1% (3/33), P=0.913d
- Blood transfusion: 11.6% (8/69), 8.3% (3/36), 15.2% (5/33), P=0.384d

**Infectious complications**

- Wound infection: 7.2% (5/69), 5.6% (2/36), 9.1% (3/33), P=0.665d
- LRTI: 2.9% (2/69), 5.6% (2/36), 0% (0/33), P=0.494d
- UTI: 10.1% (7/69), 8.3% (3/36), 12.1% (4/33), P=0.702d

**Readmission and mortality**

- 30 day readmission: 14.5% (10/69), 13.9% (5/36), 15.2% (5/33), P=1.00d
- 30 day mortality: 1.4% (1/69), 2.8% (1/36), 0% (0/33), P=1.00d
- 90 day mortality: 1.4% (1/69), 2.8% (1/36), 0% (0/33), P=1.00d

Totals: 69, 36, 33

Figure 20 Specific complications within 30 days (qualitative variables expressed as absolute numbers and percentages, a=T test, b= chi squared, c= mann whitney, d=fishers exact)

There was no significant difference between the two groups in rates of readmission or 30 and 90 day mortality. For further information see Appendix 19 – Readmissions. There was one death in the nitrate group which was due to post-operative ileus and aspiration pneumonia in a frail patient. This was deemed not treatment related and was reported to the REC.

Overall complications

There was a tendency towards a lower rate of overall complications and any complication occurring within the first 30 days in the nitrate group compared with the placebo group (Figure 21 Overall complications).
<table>
<thead>
<tr>
<th>Complication</th>
<th>Overall</th>
<th>Nitrates</th>
<th>placebo</th>
<th>Significance p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Complication whilst an inpatient</td>
<td>23.2</td>
<td>16</td>
<td>13.9</td>
<td>5</td>
</tr>
<tr>
<td>Patients with ANY surgical complication within 30 days</td>
<td>18.8</td>
<td>13</td>
<td>16.7</td>
<td>6</td>
</tr>
<tr>
<td>Patients with ANY infectious complication within 30 days</td>
<td>18.8</td>
<td>13</td>
<td>16.7</td>
<td>6</td>
</tr>
<tr>
<td>Patients with ANY complication within 30 days</td>
<td>40.6</td>
<td>28</td>
<td>30.6</td>
<td>11</td>
</tr>
<tr>
<td>Patients with at least 2 complication within 30 days of surgery</td>
<td>13.0</td>
<td>9</td>
<td>13.9</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 21 Overall complications (Qualitative variables expressed as absolute numbers and percentages, a=T test, b= chi squared, c= mann whitney, d=fishers exact)

One patient in the nitrate group and one patient in the placebo group vomited but continued to eat and did not require an naso-gastric tube so scored a I in the clavien dindo classification but were not included in the complication whilst an inpatient chart.
5. Discussion

5.1 Summary of Results
Pre-operative dietary nitrate supplementation with a 70 ml beetroot juice shot significantly improved AT after 7 days in patients with colorectal cancer. There was a significantly lower median LOS in nitrate group, however this needs to be balanced against the preponderance of right resections in nitrate group which was not statistically significant. There was a significant decrease in the resting systolic blood pressure in the placebo group but not in the nitrate group with supplementation. There was no significant difference in rate of specific complications between the two groups. There was good compliance with ERAS principles and the beetroot juice was well tolerated pre and postoperatively.

This study has shown a significant reduction in the oxygen cost of exercise in a patient group and is the first study that has been performed in a pre-operative population.

5.2 Changes in Oxygen consumption with dietary nitrate supplementation
The majority of studies with dietary nitrate supplementation have been performed in athletes and have shown decreased oxygen consumption. At submaximal exercise dietary nitrate supplementation significantly decreased oxygen consumption (34, 36, 39). During maximal exercise nitrate supplementation significantly reduced oxygen consumption (37, 40).

The majority of studies in athletes have used peak VO$_2$ as a measure of oxygen consumption. In clinical studies AT is often used as a measure of oxygen consumption as it is a physiological defined end point and is easily reproducible. In patients it can be difficult to achieve maximal exercise capacity and so Peak VO$_2$ is less often used.

In our study, there was a significant improvement in AT (and VO$_2$ at AT) in the nitrate group but no significant change in Peak VO$_2$ when compared to baseline and when compared to placebo. Our data therefore provide strong evidence that dietary nitrate supplementation decreases oxygen consumption in an elderly clinical population with comorbidities.

5.3 Variable response to dietary nitrate supplementation
Dietary nitrate supplementation led to a 5.7% improvement in the AT. In our study; as in others (43); it should be noted that individual responses were variable. In the nitrate group 16 had an
improvement (>10%, average 19.3%), 13 showed no change (-10 to 10% change, average 1%), and 5 showed a decrease in AT (> -10%, average -14.6%).

Wylie et al noted there were patients who had normal baseline plasma nitrite levels and a significant increase in plasma nitrite with supplementation but did not improve on time to task failure (43). They also noted these ‘non-responders’ who did not improve performance at lower doses of supplementation did at higher doses.

5.4 Blood pressure changes with dietary nitrate supplementation
The majority of studies in healthy young volunteers have found that dietary nitrate supplementation leads to a significant decrease in systolic blood pressure (34, 36, 38-40, 42) with longer periods or high doses of supplementation also leading to a decrease in diastolic blood pressure (36, 38, 39, 42).

It has been postulated that systolic blood pressure is more amenable to nitrate induced change (34) as studies have shown that the reduction in systolic blood pressure persists at 24 hours, whereas diastolic blood pressure returns to baseline (42, 68). Vanhatalo et al noted that although the reduction in blood pressure was small, it was likely to make a significant difference to outcomes (39).

Our results did not confirm this expectation. There was a significant decrease in the resting systolic blood pressure in the placebo group with supplementation. There was however no significant change in the resting or maximal systolic or diastolic blood pressures in the nitrate group before and after supplementation.

Previous studies in patient populations have excluded patients on nitrate-based medications or phosphodiesterase V inhibitors. In our trial 23 patients were on treatment for hypertension, 11 in the nitrate group, 12 in the placebo group. Our patients also had multiple other comorbidities as well as cancer. After removal of the patients on antihypertensives from the analysis the results did not change.

5.5 Post-operative outcomes
We had a very low median LOS in line with previous publications from our centre. Our overall median LOS for all types of resection was 3 days, and also for right and left sided resections. Traditionally it is felt that left sided resections take longer to recover after surgery, although this may be due to stoma training which occurred pre-operatively in our patients and so did not delay
discharge. Others believe right sided resections are more prone to ileus. There was a significantly lower median LOS in nitrate group, however this needs to be balanced against the preponderance of right resections in nitrate group which was not statistically significant.

There was no significant difference in rate of specific complications between the two groups. It does however deserve mention that although cohort was not large enough to show a significant decrease in complications with dietary nitrate supplementation there was a trend towards significantly less complications whilst an inpatient in the nitrate group (p=.056) and less patients with any complication within 30 days (p=.077) when compared with the placebo group.

5.6 Possible underlying mechanisms for findings

In this study there was a significant improvement in AT with dietary nitrate supplementation when compared to baseline and placebo. However Peak VO\textsubscript{2} did not significantly change with nitrate supplementation. The oxygen consumption per unit work rate (\(\Delta VO_2/\Delta WR\)) is a physiological constant and Peak work and Peak VO\textsubscript{2} are closely therefore related. We were expecting nitrates to reduce the \(\Delta VO_2/\Delta WR\) to enable VO\textsubscript{2}max to occur at a higher work rate. However this did not happen. Instead, patients in this trial achieved a similar or increased work load but on nitrates more of the work was done aerobically leading to a significant increase in AT.

Peak VO\textsubscript{2} has been shown to be increased by training, and the majority of studies with dietary nitrate supplementation have been performed in athletes were performed in a submaximal setting whilst training. In this study patients were advised to continue with their current level of physical activity. A maximal test protocol was used as this is the only protocol validated in the preoperative population. The two CPX tests were performed a week apart so limiting the effect of training. Therefore an improvement in peak VO\textsubscript{2} was not seen.

We therefore postulate that supplementation with dietary nitrate increased the aerobic capacity and delayed the onset on anaerobic metabolism. As aerobic work is sustainable increasing aerobic capacity should improve the ability to withstand complications.

The patients in our study had an overall baseline AT of 12.5, which is comparable to other studies which have shown a baseline AT of between 11.9 (119) and 14.4 (124). For the placebo group the inter-patient variability between tests was comparable with other studies (ICC 0.818) (126). The minimum clinically important difference (MCID) in the AT has not yet been set but has been placed
at about 1.0 kg$^{-1}$ min$^{-1}$ (126, 127). In this study 16 of 34 had an improvement in AT that exceeded the MCID in the nitrate group compared with 10 of 31 in the placebo group (Figure 23). It should be noted that four patients crossed the boundary of 11 kg$^{-1}$ min$^{-1}$ making them low risk for major colorectal surgery (114).

<table>
<thead>
<tr>
<th>Initial AT ml/kg/min</th>
<th>AT after nitrates ml/kg/min</th>
<th>Change in AT ml/kg/min</th>
<th>% change AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3</td>
<td>9.6</td>
<td>1.3</td>
<td>15.7%</td>
</tr>
<tr>
<td>8.4</td>
<td>9.9</td>
<td>1.5</td>
<td>17.9%</td>
</tr>
<tr>
<td>9.1</td>
<td>10.8</td>
<td>1.7</td>
<td>18.7%</td>
</tr>
<tr>
<td>9.3</td>
<td>11.8</td>
<td>2.5</td>
<td>26.9%</td>
</tr>
<tr>
<td>9.4</td>
<td>11.7</td>
<td>2.3</td>
<td>24.5%</td>
</tr>
<tr>
<td>10.3</td>
<td>11.7</td>
<td>1.4</td>
<td>13.6%</td>
</tr>
<tr>
<td>10.5</td>
<td>13.2</td>
<td>2.7</td>
<td>25.7%</td>
</tr>
</tbody>
</table>

Figure 23 Patients with improvement in AT (ml/kg/min) that exceeded MCID

Previous studies have shown that a six week exercise programme of 3 sessions per week increased the AT by 2.12 ml kg$^{-1}$ min$^{-1}$ (20). However this study was performed in a population which had had neoadjuvant chemo radiotherapy and whose AT had already dropped -1.9 ml kg$^{-1}$ min$^{-1}$. Dietary nitrate supplementation was a tolerable, timely intervention which resulted in approximately 30% of results of an exercise programme in one week.

5.7 Limitations and strengths of study

This trial is noteworthy for several reasons. The study was conducted at a single academic surgical centre which make the results consistent, valid and reliable. Recruitment was prospective and the study population was limited to elective colorectal resections for colorectal cancer so the population was homogenous. The experimental design was robust and the attrition rate was low. Seventy one of the seventy two patients in the trial went on to have surgery for presumed colorectal cancer and 92% of patients had cancer on histology, 11% of patients had undergone neoadjuvant treatment for their cancer which has been shown to adversely affect AT (2, 20) and 7% had proven metastatic disease.

We used a true placebo and blinding was therefore not difficult. The benefits of beetroot supplementation therefore can be attributed to the nitrate content only. Patients, surgeons, nurses,
investigators and other researchers were blinded as to the treatment allocation throughout the study. The investigators did not have access to the code until after the patients had completed the study. The decision to exclude a patient on the basis of their CPX results was made prior to breaking the code. However, the success of masking was not formally assessed.

Diet and exercise were standardised between CPXs and diet and fluid (including caffeine) intake were replicated prior to each CPX. All the CPXs were interpreted by a single experienced clinician. Compliance with the juice and ERAS principles was excellent.

We had adequate power to detect significant changes in our primary outcomes AT and peak VO\textsubscript{2}, however our study was not powered to detect the smaller changes in secondary outcomes. Post hoc analysis indicated for inpatient complications, based on a prevailing rate of approximately 25\% in order to detect a difference of 10\% between the two interventions; using a 2 sided test with size 5\% and power 80\%, at least 294 participants would be required in each intervention group. For infectious complications, based on a prevailing rate of approximately 20\%, in order to detect a difference of 10\% between the two interventions; using a 2 sided test with size 5\% and power 80\%, at least 250 participants would be required in each intervention group.

The average stay at our centre is short, with the overall median LOS being 3 days compared to 7 days in other studies (119). In our study 84\% of patients were discharged by 5 days, compared with 17\% at others (119). In the study population 98.6\% of surgery (69 of 70 operations) was conducted laparoscopically compared to 38.1\% at others (119). Perhaps the effect of dietary nitrate supplementation on length of stay would be more apparent at centres with a longer length of stay and lower rates of laparoscopic surgery.

One of the main limitations of our study was that the numbers are relatively small but reflect recruitment over 18 months in a busy centre. At our centre there are approximately 2-3 weeks between MDT decision, clinic review and operation and a proportion of patients were unable take part in trial due to time constraints. We did not measure blood plasma nitrate / nitrite levels to confirm a rise with supplementation as this has been proven exhaustively in healthy volunteers and more recently clinical populations (63, 79).

The improvements in AT occurred in patients who had been instructed to avoid high nitrate foods for the duration of the study to ensure stable baseline values of plasma nitrate / nitrite. It is
therefore possible that the effects of the dietary nitrate supplement were magnified. However studies have shown that dietary restriction lowers plasma levels by approximately 10% (130) and that in the elderly dietary supplementation alone does not increase plasma nitrate and a supplement is required (67).

5.8 Future research directions
Future research should be directed towards seeing if the improvement in AT translates to an improvement in postoperative outcomes, complication rates and long term outcomes in a multicentre district general setting. Larger multicentre trials with more patients would allow minimisation of type II errors and the interaction between comorbidities, medications and nitrate supplementation to be examined in more detail.

Although currently the majority of patient with colorectal cancer proceed directly to surgery, ongoing current neoadjuvant chemotherapy trials may lead to a change in this, with the majority of patients having chemotherapy prior to surgery. This would leave a window for prehabilitation and the addition of exercise training to dietary nitrate supplementation which may increase Peak VO₂ in this population.

Patients were given a list of high nitrate foods to avoid for the duration of the trial. However there is now evidence that in the elderly a high nitrate dietary intake alone may not be sufficient to cause a physiologically significant rise in plasma nitrate / nitrite (67). Dietary restriction therefore may not be necessary and further trials could therefore be conducted on a normal diet.
6 Conclusion

In conclusion, this trial provides evidence that dietary nitrate supplementation can be beneficial in a preoperative setting. In our study dietary nitrate supplementation significantly improved the Anaerobic Threshold in this elderly population with colorectal cancer compared with placebo. With 7 days of dietary nitrate supplementation we have shown a significant improvement in oxygen utilisation in an elderly population.

With this study we have shown that we can prehabilitate with beetroot juice and significantly improve patients fitness before surgery which has widespread implications. Even in the elderly population we studied physical fitness improved without an increase in exercise. Supplementation with beetroot juice is a simple practical nutritional intervention which was well tolerated and could be used before most operations to improve fitness and therefore outcomes.

Enhanced recovery programmes have revolutionised peri-operative care by maintaining homeostasis and improving oxygen delivery peri-operatively. They have been proven to decrease complications and improve 5 year survival. In this trial we have shown that supplementation with dietary nitrate led an increase in the aerobic capacity of patients with colorectal cancer pre-operatively. This increased aerobic capacity should allow patients to meet the increased oxygen demand post-operatively and has the potential to decrease the risk of complications and thereby improve survival.

The beetroot juice was safe, inexpensive and was well tolerated. The supplementation regime was implemented successfully and was acceptable to patients. It seems reasonable therefore to include beetroot juice within existing enhanced recovery programmes.
## 7. Glossary

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPI</td>
<td>ankle brachial pressure index</td>
</tr>
<tr>
<td>ABPM</td>
<td>24-hour ambulatory BP monitoring</td>
</tr>
<tr>
<td>AL</td>
<td>Anastomotic leak failure of the join between two ends of bowel to heal leading to intraabdominal leakage of bowel contents.</td>
</tr>
<tr>
<td>AT</td>
<td>anaerobic threshold For further information see Appendix 6 CPX variables</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BRJ</td>
<td>beetroot juice colorectal surgery surgery on the colon and rectum</td>
</tr>
<tr>
<td>CR</td>
<td>complete response</td>
</tr>
<tr>
<td>CRC</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>DASH</td>
<td>dietary approaches to stop hypertension</td>
</tr>
<tr>
<td>ERAS</td>
<td>Enhanced Recovery after Surgery</td>
</tr>
<tr>
<td>ESWT</td>
<td>endurance shuttle walk test patients walking between two cones; 9m apart; forming a 10m course. Chimes are played to determine the time allowed to walk between shuttles, the speed of which is determined by a protocol. Patients are asked to walk as long as possible until they are unable to continue, it is therefore a measure of submaximal exercise capacity.</td>
</tr>
<tr>
<td>LEAK respiration</td>
<td>Mitochondrial respiration with substrates but no ADP, driven by back leakage of protons through inner membrane.</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>GET</td>
<td>gas exchange threshold</td>
</tr>
<tr>
<td>Hb&lt;sub&gt;tot&lt;/sub&gt;</td>
<td>total haemoglobin concentration</td>
</tr>
<tr>
<td>HHb</td>
<td>deoxyhaemoglobin concentration</td>
</tr>
<tr>
<td>ISWT</td>
<td>incremental shuttle walk test</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>METs</td>
<td>metabolic equivalents of activity</td>
</tr>
<tr>
<td>MCID</td>
<td>minimum clinically important difference</td>
</tr>
<tr>
<td>NDMA</td>
<td>N-nitrosodimethylamine</td>
</tr>
<tr>
<td>NOS</td>
<td>NO synthase</td>
</tr>
<tr>
<td>PCr</td>
<td>phosphocreatine</td>
</tr>
<tr>
<td>PCWP</td>
<td>pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>RPP</td>
<td>resting pulse pressure</td>
</tr>
<tr>
<td>RER</td>
<td>respiratory exchange ratio</td>
</tr>
<tr>
<td>RCR</td>
<td>Respiratory Control Ratio</td>
</tr>
<tr>
<td>state 4</td>
<td>state 4 respiration</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>TPR</td>
<td>total peripheral resistance</td>
</tr>
<tr>
<td>V&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Ventilatory Equivalent</td>
</tr>
<tr>
<td>V&lt;sub&gt;Emax&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>oxygen uptake</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2max&lt;/sub&gt; or VO&lt;sub&gt;2peak&lt;/sub&gt;</td>
<td>maximal measurement for VO&lt;sub&gt;2&lt;/sub&gt; attained during CPX</td>
</tr>
<tr>
<td>∆VO&lt;sub&gt;2&lt;/sub&gt;/∆WR</td>
<td></td>
</tr>
<tr>
<td>VO₂/VCO₂ at</td>
<td>For further information see Appendix 6 CPX variables</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>PPIs</td>
<td>proton pump inhibitors</td>
</tr>
<tr>
<td>P/O ratio</td>
<td>measure of mitochondrial oxidative phosphorylation efficiency and is the amount of oxygen consumed per ATP unit production.</td>
</tr>
</tbody>
</table>
8. Appendix

Appendix 1 Nitrate content vegetables

Appendix 2 Summary Effects of dietary nitrate supplementation in healthy volunteers

Appendix 3 Summary Effects of dietary nitrate supplementation in older volunteers

Appendix 4 Summary Effects of dietary nitrate supplementation in clinical populations

Appendix 5 Plasma nitrate and nitrite levels in clinical populations after dietary nitrate supplementation

Appendix 6 Blood pressure changes in clinical populations after dietary nitrate supplementation

Appendix 7 CPX report variables

Appendix 8 Patient information leaflet

Appendix 9 GP information leaflet

Appendix 10 Food diary

Appendix 11 Patients without cancer on histology

Appendix 12 Laboratory values

Appendix 13 Ramp / watts data

Appendix 14 Summary CPX results

Appendix 15 Summary Spirometry results

Appendix 16 Summary blood pressure results

Appendix 17 Clavien Dindo scoring system

Appendix 18 All inpatient complications classified by Clavien Dindo

Appendix 19 Complications after discharge not requiring readmission

Appendix 20 Readmissions
<table>
<thead>
<tr>
<th>vegetable</th>
<th>Nitrate content mg/kg</th>
<th>g containing 400mg nitrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rocket</td>
<td>4677</td>
<td>85</td>
<td>(60)</td>
</tr>
<tr>
<td>lettuce</td>
<td>3017</td>
<td>133</td>
<td>(58)</td>
</tr>
<tr>
<td>cooked beetroot</td>
<td>3017</td>
<td>133</td>
<td>(58)</td>
</tr>
<tr>
<td>Chervil</td>
<td>&gt;2500</td>
<td>&lt;160</td>
<td>(61)</td>
</tr>
<tr>
<td>Cress</td>
<td>&gt;2500</td>
<td>&lt;160</td>
<td>(61)</td>
</tr>
<tr>
<td>cooked spinach</td>
<td>1880</td>
<td>213</td>
<td>(58)</td>
</tr>
<tr>
<td>Endive</td>
<td>1,475</td>
<td>271</td>
<td>(60)</td>
</tr>
<tr>
<td>celery</td>
<td>1,024</td>
<td>391</td>
<td>(60)</td>
</tr>
<tr>
<td>Greens inc Collard, Mustard and Turnip</td>
<td>1,160</td>
<td>345</td>
<td>(51)</td>
</tr>
<tr>
<td>Fennel</td>
<td>1,024</td>
<td>390</td>
<td>(60)</td>
</tr>
<tr>
<td>Leek</td>
<td>1000-2500</td>
<td>&gt;160g</td>
<td>(61)</td>
</tr>
<tr>
<td>kohlrabi</td>
<td>987</td>
<td>405</td>
<td>(60)</td>
</tr>
<tr>
<td>Parsley</td>
<td>958</td>
<td>417</td>
<td>(60)</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>933</td>
<td>428</td>
<td>(60)</td>
</tr>
<tr>
<td>pumpkin</td>
<td>894</td>
<td>447</td>
<td>(60)</td>
</tr>
<tr>
<td>Curly kale</td>
<td>537</td>
<td>745</td>
<td>(60)</td>
</tr>
<tr>
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<td>416</td>
<td>962</td>
<td>(60)</td>
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<tr>
<td>Celeriac</td>
<td>390</td>
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<td>(60)</td>
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<tr>
<td>aubergine</td>
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<td>cabbage</td>
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<tr>
<td>broccoli</td>
<td>279</td>
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<tr>
<td>beans</td>
<td>&lt;200</td>
<td>&gt;2000</td>
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<tr>
<td>cucumber</td>
<td>185</td>
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<td>(60)</td>
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<td>cauliflower</td>
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<tr>
<td>peppers</td>
<td>108</td>
<td>3704</td>
<td>(60)</td>
</tr>
<tr>
<td>garlic</td>
<td>69</td>
<td>5,797</td>
<td>(60)</td>
</tr>
<tr>
<td>tomato</td>
<td>43</td>
<td>9,302</td>
<td>(60)</td>
</tr>
<tr>
<td>carrots</td>
<td>10</td>
<td>40,000</td>
<td>(61)</td>
</tr>
</tbody>
</table>

Appendix 1 Nitrate content vegetables
**Appendix 2 Summary Effects of dietary nitrate supplementation in healthy volunteers**

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larsen 2007</td>
<td>Sodium nitrate</td>
<td>Randomised</td>
<td>Cycle ergometer</td>
<td>9 pts</td>
<td>Well trained</td>
<td>• Does dietary nitrate lead to increased systemic pools of nitrate&lt;br&gt;• Does dietary nitrate supplementation change physiological parameters during exercise&lt;br&gt;Diet – low nitrate 3 days&lt;br&gt;Washout – 10 days</td>
<td></td>
</tr>
<tr>
<td>(36)</td>
<td>0.1 mmol/kg     vs Placebo</td>
<td>Double blind</td>
<td>Submax and max</td>
<td>28 (+/-6) years VO2 peak 55 +/- 3.7 ml/kg/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>Crossover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Plasma**
- Significantly elevated plasma nitrate, no change during exercise
- Elevated plasma nitrite, decreased during exercise, non-significant

**Blood pressure**
- Decreased resting systolic and diastolic BP

**Oxygen consumption**
- Significantly reduced VO2 during submaximal work (p<0.02)
- No change Peak VO2, HR, VeVO2, Vemax, max WR, RER, oxygen pulse at absolute O2 uptake
- No significant effects of nitrate supplementation on variables during maximal exercise
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Nitrate dose</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Webb 2008 (68)</td>
<td>500ml BRJ</td>
<td>17-22.5mmol</td>
<td>Open label crossover</td>
<td>1</td>
<td>Spitting BP</td>
<td>14 pts (5F)</td>
<td>BP Spitting</td>
<td>6 pts (1F)</td>
</tr>
<tr>
<td>Vs Placebo 500mls water</td>
<td>Vs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.5 +/- 4.5 yrs</td>
<td>FMD</td>
<td>31 +/- 1.8 yrs.</td>
</tr>
</tbody>
</table>

- Increased gross efficiency GE (Work rate / energy expenditure (EE) and delta efficiency (increase in work rate / increase in EE)
- No change in Hb or haematocrit
- Significantly increased calculated muscle efficiency

**Plasma**
- Significantly raised plasma nitrate and nitrite
- Changes in systolic BP significantly inversely correlated to change in plasma nitrite

**Blood pressure**
- Significantly decreased systolic and diastolic BP with supplementation
- Peak decrease in systolic BP at 2.5 hours, diastolic and MAP 3 hours post supplementation

**Others**
- Effects of dietary nitrate supplementation on blood pressure, platelet aggregation, endothelial dysfunction and Flow mediated dilatation (FMD)
- Diet - normal Washout – minimum 7 days between phases

**Others**
- Oxygen consumption – not measured
**Summary Effects of dietary nitrate supplementation in healthy volunteers**

<table>
<thead>
<tr>
<th>Author, year</th>
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<th>Study design</th>
<th>Exercise / max vs submax</th>
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<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
</table>
| Bailey 2009  | BRJ 500mls      | 5.5mmol      | Randomised   | Cycle ergometer          | 8 pts  | Healthy recreationally active | • No changes in HR  
• Spitting all saliva after supplementation interrupted the enterosalivary circulation and blocked the rise in plasma nitrite  
• Platelet aggregation inhibited 2.5ours after nitrate supplementation but not after spitting  
• Prevention of ischaemia induced endothelial dysfunction as measured by FMD |
|              | vs Placebo      |              | Double blind  | Submax                   | 26 (+/-7) years | VO2 max 49 +/-5 ml/kg/min |
|              | vs Low calorie  |              | Placebo       |                          | (0F)    |                   |      | Plasma                           |
|              | vs Blackcurrant juice cordial |         | Crossover     |                          |         |                   |      | Blood pressure                   |
|              |                 |              |               |                          |         |                   |      | Reduced systolic BP              |
|              |                 |              |               |                          |         |                   |      | Oxygen consumption                |
|              |                 |              |               |                          |         |                   |      | • Significant reduction in pulmonary Vo2 of submaximal exercise p<0.05 |
|              |                 |              |               |                          |         |                   |      | • Severe exercise                |
|              |                 |              |               |                          |         |                   |      | - Significant elevated primary VO2 amplitude p<0.05 |

- Effect of beetroot juice supplementation on plasma nitrite, BP  
- Effect of beetroot juice supplementation on muscle oxygenation (assessed with NIRS)  
- Effect of beetroot juice supplementation on oxygen cost of submaximal and maximal exercise
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>days</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Fitness vs disease</th>
<th>Aims</th>
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</tr>
</thead>
</table>
| Larsen 2010 (37) | Sodium nitrate 0.1mmol/kg split into 3 doses vs placebo | Randomised Double blind Crossover | 2 | Arm and leg ergometers maximal | 9 (2F) | healthy | • "Investigate effect of dietary nitrate supplementation on maximal combined arm and leg exercise" | - Significant reduction in VO\(_2\) slow component  
- No change in VO\(_2\) at task failure  
- Therefore, more efficient oxygen utilisation, VO\(_2\)\(_{\text{max}}\) reached more slowly and sustained for longer  
- 16% improvement in time to task failure in nitrate supplemented group during severe exercise.  
- No change in VO\(_2\)\(_{\text{max}}\), Ve, RER, blood [lactate]  
- No change HR, diastolic BP, MAP  
**Others**  
- Improved muscle oxygenation  
- NIRS Hbot elevated at baseline, Hb02 elevated at baseline  
**Plasma**  
- Elevated plasma nitrate, no change during exercise  
- Elevated plasma Nitrite  
**Blood pressure** |
## Summary Effects of dietary nitrate supplementation in healthy volunteers

<table>
<thead>
<tr>
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<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Sodium Chloride</td>
<td>Randomised Double blind</td>
<td>Knee extension ergometer</td>
<td>7 pts (0F)</td>
<td>Healthy</td>
<td>• No change resting BP, significant decrease diastolic BP 2 min after maximal exercise</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet – low nitrate 2 days prior</td>
<td>Washout –7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oxygen consumption</td>
</tr>
<tr>
<td></td>
<td>BRJ 500mls 5.1mmol nitrate</td>
<td>Randomised Double blind</td>
<td>6</td>
<td>6</td>
<td>Healthy</td>
<td>• Mechanism by which nitrate reduces oxygen cost of submaximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Knee extension ergometer</td>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 pts (0F)</td>
<td></td>
<td></td>
<td>• Elevated plasma Nitrite</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Oxygen consumption**
  - Maximal exercise significantly decreased vo2max 3.72 (90.33) to 3.62 (0.31)
  - Significant decrease in VO2 at submaximal exercise after single dose of nitrate
  - Acute dose caused a decrease in VO2 from 1.45 (0.08) to 1.37 (0.09)
  - No change in VE, Max HR, RER, lactate

- **Others**
  - Non-significant increase in time to exhaustion
  - No difference between nitrate and placebo for plasma [cGMP], [renin] and [aldosterone] at rest or after exercise
<table>
<thead>
<tr>
<th>Author, year</th>
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<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bailey 2010</td>
<td>Placebo vs Blackcurrant juice cordial</td>
<td>Placebo Crossover</td>
<td>submaximal</td>
<td>28 (+/-7) years</td>
<td>Recreationally active</td>
<td>VO2 peak 1647-1726 ml/min</td>
<td>exercise and improved tolerance to maximal exercise</td>
<td>Blood pressure</td>
</tr>
<tr>
<td></td>
<td>vs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Used P-MRS to assess muscle metabolism</td>
<td>Reduced systolic, diastolic BP and MAP</td>
</tr>
<tr>
<td></td>
<td>Placebo Low calorie</td>
<td>Crossover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oxygen consumption</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• Reduced pulmonary VO2 during low intensity by 25%, 11% absolute reduction</td>
<td>Reduced pulmonary VO2 during low intensity by 25%, 11% absolute reduction</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>• VO2 slow component reduced 25%</td>
<td>VO2 slow component reduced 25%</td>
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<td></td>
<td></td>
<td>• 25% improvement in time to task failure at high intensity exercise</td>
<td>25% improvement in time to task failure at high intensity exercise</td>
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<td></td>
<td></td>
<td>• No change VE, HR, Peak VO2</td>
<td>No change VE, HR, Peak VO2</td>
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<td></td>
<td></td>
<td>Others</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduced PCr degradation, Pi and ADP accumulation, no change muscular Ph</td>
<td>Reduced PCr degradation, Pi and ADP accumulation, no change muscular Ph</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Calculated muscle ATP_{total} reduced</td>
<td>Calculated muscle ATP_{total} reduced</td>
</tr>
<tr>
<td>Vanhatalo 2010</td>
<td>BRJ 500mls vs Balanced Placebo Crossover</td>
<td>Cycle ergometer</td>
<td>submax and max</td>
<td>8 pts (3F)</td>
<td>Healthy</td>
<td>Vo2max 3.36 +/- 0.84 l/min</td>
<td>Investigate acute (2.5hrs) and chronic (15 days) effects of dietary nitrate supplementation</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>5.2mmol nitrate</td>
<td>Submax and max</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Elevated plasma Nitrite</td>
<td>Reduced systolic, diastolic BP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oxygen consumption</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Nitrate dose</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lansley 2011</td>
<td>Beetroot juice</td>
<td>500ml 6.2mmol nitrate</td>
<td>Randomised Double blind Crossover</td>
<td>Treadmill + knee extension for Qmax</td>
<td>9 pts (OF)</td>
<td>Physically active</td>
<td>Washout –10 days</td>
<td>• Lower o2 cost of moderate exercise maintained when supplementation continued for 15 days, even with normal diet  • Significant increase vo2max after 15 days supplementation  • After 15 days significantly elevated peak power and WR at GET</td>
</tr>
<tr>
<td></td>
<td>Blackcurrant juice cordial</td>
<td>Low calorie</td>
<td>Placebo</td>
<td>Placebo nitrate deplete beetroot juice</td>
<td>6</td>
<td></td>
<td></td>
<td>Others</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Plasma</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood pressure</td>
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<td>Oxygen consumption</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Used true placebo (nitrate free juice) and normal diet  • Mitochondrial biogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduced systolic BP (4%)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 12% (moderate) 14% (severe) reduction VO2 walking  • Reduced VO2 (7%) moderate / severe intensity running  • Small decrease in Peak vo2 / VO2 max  • Increased time to task failure running 15%, knee extension 5%</td>
</tr>
<tr>
<td>Author, year</td>
<td>Type supplement</td>
<td>Nitrate dose</td>
<td>Study design</td>
<td>days</td>
<td>Exercise / max vs submax</td>
<td>No pts</td>
<td>Fitness vs disease</td>
<td>Aims</td>
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<td>-----</td>
</tr>
<tr>
<td>Larsen 2011</td>
<td>Sodium nitrate</td>
<td>0.1 mmol/kg split into 3 doses</td>
<td>Randomised Double blind Placebo Crossover</td>
<td>3 submaximal</td>
<td>14 pts 25 (+/- 1) years Healthy Vo2 peak 56 (+/- 3) ml/kg/min</td>
<td></td>
<td></td>
<td>• Muscle biopsies to study effects on mitochondria</td>
</tr>
<tr>
<td>vs</td>
<td>Placebo Sodium Chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Summary Effects of dietary nitrate supplementation in healthy volunteers**

- No change VCO₂, Ve, RER, HR, blood lactate

**Others**
- No change in Qmax therefore effect unlikely to be due to mitochondrial biogenesis

**Plasma**
- Elevated plasma nitrate (p<0.01) and nitrite (p<0.01)
- No change in cGMP

**Blood pressure**

**Oxygen consumption**
- Significantly decreased whole body VO₂ oxygen consumption (p=0.02)
- Significantly increased mechanical work output to oxygen uptake (watt/VO₂ ratio) p=0.01
- More efficient metabolism after nitrate supplementation

**Others**
- Nitrate induced improvement in intrinsic mitochondrial efficiency”
### Summary Effects of dietary nitrate supplementation in healthy volunteers

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<thead>
<tr>
<th>Author, year</th>
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<th>Nitr ate dose</th>
<th>Study design</th>
<th>days</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wylie 2013</td>
<td>Beetroot juice</td>
<td>70ml = 4.2mmol</td>
<td>Randomised</td>
<td></td>
<td>Cycle ergometer</td>
<td>10pts</td>
<td>Healthy</td>
<td></td>
<td>• Mitochondria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>140ml = 8.4mmol</td>
<td>Double blind</td>
<td></td>
<td>submax</td>
<td></td>
<td></td>
<td></td>
<td>o 19% increase in effective P/O ratio during submaximal ADP stimulation p=0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>280ml = 16.8mmol</td>
<td>Placebo</td>
<td>1</td>
<td>submaximal and maximal</td>
<td></td>
<td>Healthy</td>
<td></td>
<td>o 23% increase in Maximal rate ATP production p=0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crossover</td>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td></td>
<td>o LEAK respiration = back leakage of protons via inner mitochondria membrane, reduced by 45%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td></td>
<td>o 48% reduction state 4 respiration</td>
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<td></td>
<td></td>
<td>Healthy</td>
<td></td>
<td>o Significant downregulation of ANT protein levels, unchanged amount of mitochondrial DNA</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>Randomised</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• Normal diet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Randomised</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Characterise plasma nitrate and nitrite pharmacokinetics and BP change with 3 different doses of nitrate supplementation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Double blind</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Assess acute dose dependant physiological response to exercise after nitrate supplementation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Double blind</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Dose dependant peak reduction in BP up to 8.4mmol</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>Crossover</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- • Normal diet
- • Characterise plasma nitrate and nitrite pharmacokinetics and BP change with 3 different doses of nitrate supplementation
- • Assess acute dose dependant physiological response to exercise after nitrate supplementation
- • Dose dependant peak reduction in BP up to 8.4mmol
<table>
<thead>
<tr>
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<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee 2015 (73)</td>
<td>Beetroot juice vs Placebo nitrate deplete beetroot juice</td>
<td>140mls water</td>
<td>Randomised Double blind Placebo Crossover</td>
<td>Cycle ergometer submaximal</td>
<td>14pts (0F) healthy</td>
<td></td>
<td>Diet = normal diet Washout ~3 days</td>
<td>Oxygen consumption • Dose dependant reduction in the oxygen cost of moderate intensity exercise • Improved time to task failure with 8.4mmol not further improved by 16.8mmol, no change with 4.2mmol</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Others Plasma • Chronic supplementation increases plasma nitrate and nitrite (measured together as NOx)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Blood pressure • lowers systolic and diastolic BP, MAP and TPR at rest and during exercise Oxygen consumption not measured Others • no effect on HR • reduces RPP (HRxSBP) and increased CO at rest and 30% workload • augments endothelial function,</td>
<td></td>
</tr>
</tbody>
</table>
## Summary Effects of dietary nitrate supplementation in healthy volunteers

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</thead>
</table>
| Wylie 2016   | Dry beetroot extract | 3mmol or 6mmol nitrate in 50mls water vs Placebo 9.5g sucrose and red colouring in 50mls water | Independent Group matched placebo | Cycle ergometer Submaximal | Healthy Recreationally active | 34 pts (15F) 21 (+/-3) | • Investigate whether long term supplementation with low dose nitrate would change oxygen dynamics | • enhances cardiac function – increases SV and CO, probably due to decreased systemic vascular resistance
|               |                 |              |              |                          |        |                   |      | Plasma                        |     |
|              |                 |              |              |                          |        |                   |      | • significant rise in plasma nitrate / nitrite with 3mmol nitrate supplementation |     |
|              |                 |              |              |                          |        |                   |      | Blood pressure                |     |
|              |                 |              |              |                          |        |                   |      | Oxygen consumption             |     |
|              |                 |              |              |                          |        |                   |      | • Acute and chronic supplementation with 3mmol nitrate does not significantly reduce O2 cost submaximal exercise |     |
|              |                 |              |              |                          |        |                   |      | • Acute and chronic supplementation with 6mmol nitrate reduced oxygen cost of exercise after 2hrs, 7 days and 4 weeks |     |
|              |                 |              |              |                          |        |                   |      | • reduction in VO2 at submaximal exercise preserved up to 24 hours after last nitrate dose |     |
|              |                 |              |              |                          |        |                   |      | Others                        |     |

- Diet – low nitrate no crossover so no washout

- Plasma
  - Significant rise in plasma nitrate / nitrite with 3mmol nitrate supplementation

- Blood pressure
  - Oxygen consumption
    - Acute and chronic supplementation with 3mmol nitrate does not significantly reduce O2 cost submaximal exercise
    - Acute and chronic supplementation with 6mmol nitrate reduced oxygen cost of exercise after 2hrs, 7 days and 4 weeks
    - Reduction in VO2 at submaximal exercise preserved up to 24 hours after last nitrate dose

- Others
  - Diet – low nitrate no crossover so no washout
**Summary Effects of dietary nitrate supplementation in healthy volunteers**

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Nitrate dose</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Fitness vs disease</th>
<th>Aims</th>
<th>Nitrate supplementation findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitfield</td>
<td>Concentrated</td>
<td>Beetroot juice</td>
<td>280ml Pre-post 26mmol in 2 doses</td>
<td>Cycle ergometer</td>
<td>10pts (0F)</td>
<td>Healthy Recreationally active</td>
<td>• does dietary nitrate supplementation with beetroot juice alter mitochondrial bioenergetics Diet – low nitrate 7 no crossover so no washout</td>
<td>Plasma Blood pressure Oxygen consumption • Decreased oxygen consumption at 70% of VO2 peak • No change in RER Others • Mitochondria • No change in leak or maximal respiration • Levels ANT and UCP3 unaltered • No alteration in P/O ratio, RCR • Increase in mitochondria ROS emission • ? sodium nitrate and BRJ act via diff mechanisms</td>
</tr>
<tr>
<td>2016</td>
<td>23 (+/- 0.6) years VO2 peak 49.6 +/- 1.4 ml/min/kg</td>
<td>7 Submax</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
### Summary Effects of dietary nitrate supplementation in older volunteers

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Age</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller et al (67)</td>
<td>High nitrate diet 2.5mmol</td>
<td>Nitrate supplement BRJ 500ml 8.5mmol</td>
<td>Randomised four period cross-over controlled</td>
<td>8pts (5F)</td>
<td>72.5 +/- 4.7yrs</td>
<td>Older, age &gt;65</td>
<td>Systemic uncontrolled disease e.g. diabetes, recent cancer treatment, hypertension, hypotension, thyroid</td>
<td>• Does high nitrate diet elevate plasma nitrate / nitrite and decrease BP</td>
<td>Plasma • Significantly Elevated plasma nitrate and nitrite after any supplement but not high nitrate diet alone • Secondary rise in plasma nitrite with supplement after evening meal</td>
</tr>
<tr>
<td></td>
<td>Control diet 0.7mmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medications – nitrates, PDE5 inhibitors, PPIs, antihypertensive medication</td>
<td>Washout – 3-12 days Medications – see exclusions</td>
<td></td>
</tr>
</tbody>
</table>

**Blood pressure**
- Significant reduction systolic and diastolic BP for all treatments, no difference between treatments
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Age</th>
<th>No medication</th>
<th>Diet</th>
<th>Washout</th>
<th>Medications - nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelly et al (72)</td>
<td>BRJ 140ml (one in am, one in pm)</td>
<td>Double blind randomised crossover</td>
<td>Treadmill submax</td>
<td>12 pts (6F)</td>
<td>63M +/- 4yrs, 64F +/- 2yrs</td>
<td>Older (age 60-70), healthy</td>
<td>No caffeine 6 hours, No alcohol 24 hours</td>
<td>Diet – normal diet, Washout – 72 hours</td>
<td></td>
</tr>
</tbody>
</table>

**Type**

Nitrate dose

**Study design**

Double blind randomised crossover

**Exercise / max vs submax**

Treadmill submax

**No pts**

12 pts (6F)

**Age**

63M +/- 4yrs, 64F +/- 2yrs

**Aims**

• Effect on BP, oxygen consumption, others

**Exclusions**

• No medication

**Blood pressure**

• Significantly reduced resting systolic and diastolic BP, oxygen consumption

**Plasma**

• Significantly elevated plasma nitrite, oxygen consumption

**Others**

• Effect on BP, o2 uptake kinetics and muscle and cognitive function in older adults
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / min vs submax</th>
<th>No pts</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jajja 2014 (74)</td>
<td>Beetroot juice 70ml 6.2mmol/kg</td>
<td>28 day 2 arm Parallel placebo</td>
<td>21 No exercise</td>
<td>21 pts (9F)</td>
<td>Overweight BMI 25-40 kg/m2</td>
<td>Non-smoking No medical conditions</td>
<td>• Does dietary nitrate decrease systolic BP in Plasma</td>
<td>• Elevated urinary and salivary nitrate Blood pressure</td>
</tr>
</tbody>
</table>

Summary Effects of dietary nitrate supplementation in older volunteers

- No improvement in 6MWT
- No significant changes in muscle metabolic responses
- No significant difference on cognitive function
- No significant difference in brain metabolite concentrations

Nitrate dose

<table>
<thead>
<tr>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / min vs submax</th>
<th>no pts</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beetroot juice 70ml 6.2mmol/kg</td>
<td>28 day 2 arm Parallel placebo</td>
<td>21 No exercise</td>
<td>21 pts (9F)</td>
<td>Overweight BMI 25-40 kg/m2</td>
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<td>• Elevated urinary and salivary nitrate Blood pressure</td>
</tr>
<tr>
<td>Author, year</td>
<td>Type supplement</td>
<td>Study design</td>
<td>Exercise / max vs submax</td>
<td>No pts Age</td>
<td>disease</td>
<td>exclusions</td>
<td>Aims</td>
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</tr>
<tr>
<td>Vs placebo caprisun blackcurrant 200ml</td>
<td>Randomised control trial</td>
<td>62.7 (+/- 1.5) years</td>
<td>Older 55-70 years</td>
<td>Excluded if on corticosteroids, organic nitrates diuretics, hormonal therapies, weight loss medication</td>
<td>overweight older participants?</td>
<td>Diet – low nitrate 28 days Medications – see exclusions</td>
<td>• No significant change resting clinic BP • No significant change 24-hour BP • Significantly lower systolic BP at home monitoring in week 3 of supplementation</td>
</tr>
</tbody>
</table>
### Appendix 4 Summary Effects of dietary nitrate supplementation in clinical populations

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Age</th>
<th>Disease</th>
<th>Exclusions</th>
<th>Aims</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(63) Beetroot juice</td>
<td>500ml</td>
<td>Open label Crossover Placebo controlled</td>
<td>CPX Treadmill</td>
<td>8 pts (4F)</td>
<td>BMI 28.6</td>
<td>Peripheral arterial disease</td>
<td>Stable intermittent claudication &gt;3/12</td>
<td>ABPI 0.64 +/-0.2</td>
<td><strong>Investigate acute effect of beetroot juice vs placebo on plasma nitrate and nitrite levels, exercise tolerance</strong></td>
</tr>
<tr>
<td>9.1mmol nitrate vs placebo orange juice</td>
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<td></td>
<td><strong>Investigate acute effect of beetroot juice vs placebo on tissue oxygenation (assessed with near infrared spectroscopy)</strong></td>
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<td></td>
<td><strong>Significantly lower DBP post ingestion, lower HR and SBP during recovery</strong></td>
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<td><strong>Significantly lower VO2 at first stage of exercise</strong></td>
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<td><strong>NIRS significantly lower fractional oxygen extraction in beetroot group, despite significantly increased performance.</strong></td>
</tr>
<tr>
<td>Study design</td>
<td>Type supplement</td>
<td>Study design</td>
<td>Nitrate dose</td>
<td>Exercise / max vs submax</td>
<td>No pts</td>
<td>Aims</td>
<td>findings</td>
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<tr>
<td>Double blind, randomised, placebo controlled parallel</td>
<td>Beetroot juice 250ml (6.0mmol nitrate) vs placebo</td>
<td>US assessment of flow mediated dilatation (FMD)</td>
<td>65 pts</td>
<td>Hypercholesterolaemia (Serum cholesterol &gt;6.0mmol/L or any elevation LDL / triglycerides + QRISK2 &gt;15%)</td>
<td>Off statins or cholesterol lowering meds for 2 months, Medications - 14 pts on antihypertensives, no mention PPIs</td>
<td>Effect of 6 weeks BRJ on vascular and platelet function in untreated patient with hypercholesteremia</td>
<td>Others</td>
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<td></td>
<td>18% / 32 second increase in exercise time before onset of claudication pain (COT)</td>
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<td>17% / 65 second increase in time to exhaustion (PWT)</td>
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<td></td>
<td>No change in ABPI, no difference in brachial artery resting diameters</td>
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<td>Plasma</td>
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<td></td>
<td>Elevated serum, urinary and salivary nitrate and nitrite</td>
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<td>Blood pressure</td>
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<td></td>
<td>No significant change in BP</td>
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</tr>
<tr>
<td>Author, year</td>
<td>Type supplement</td>
<td>Study design</td>
<td>Exercise / max vs submax</td>
<td>No pts</td>
<td>disease</td>
<td>exclusions</td>
<td>Aims</td>
<td>findings</td>
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</tr>
<tr>
<td>BRJ 250mls</td>
<td>Placebo</td>
<td>Double blind randomised placebo controlled</td>
<td>28</td>
<td>Vascular function - Flow mediated dilatation (FMD) and (placebo)</td>
<td>64 pts</td>
<td>Hypertensive</td>
<td>Atherosclerotic disease – IHD or stroke</td>
<td>Diet – no change</td>
<td>Oxygen consumption</td>
</tr>
<tr>
<td>6.4mmol</td>
<td>nitrate deplete 0.001mmol nitrate/d</td>
<td>6.4mmol nitrate Vs placebo</td>
<td></td>
<td>56.3 yrs.</td>
<td>BP&gt;130/85</td>
<td>Hypertensive</td>
<td>Age 18-85, eGFR&gt;50 mL/min, no CVD, LFTs</td>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td>(88)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HbA1c&gt;10%</td>
<td></td>
<td>Blood pressure</td>
</tr>
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</tr>
</tbody>
</table>

### Summary Effects of dietary nitrate supplementation in clinical populations

- **Placebo nitrate deplete 0.001mmol nitrate/d**
- **Study design**: Double blind randomised placebo controlled
- **Exercise**: Vascular function - Flow mediated dilatation (FMD) and (placebo)
- **No pts**: 64 pts (38F)
- **Age**: 56.3 yrs.
- **Disease**: Hypertensive
- **Exclusions**: Atherosclerotic disease – IHD or stroke, Age 18-85, eGFR>50 mL/min, no CVD, LFTs, HbA1c>10%
- **Aims**: Diet – no change
- **Findings**:
  - Significant increase in FMD in nitrate group after 6 weeks supplementation
  - No change in methaemoglobin
  - Significantly reduced PMA levels and trend towards reduction p selectin (marker of platelet activation)
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / days</th>
<th>No pts</th>
<th>Disease</th>
<th>Exclusions</th>
<th>Aims</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>placebo</td>
<td>Nitrate dose</td>
<td></td>
<td>pulse wave</td>
<td>57.6</td>
<td>CCF NYHAII or more, or EF&lt;30%</td>
<td>Washout – 2 weeks Medications – continue usual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nitrate deplete</td>
<td></td>
<td></td>
<td>velocity</td>
<td>yrs.</td>
<td>Malignancy in last 5 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRJ</td>
<td></td>
<td></td>
<td>BMI</td>
<td>26.8</td>
<td>Currently life-threatening condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(nitrate)</td>
<td></td>
<td>e.g. severe COPD, HIV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(nitrates)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Summary Effects of dietary nitrate supplementation in clinical populations**

- **Aims**: Findings
  - Significant decreased BP, subgroup analysis on treated hypertensives, significant decrease BP
  - Change in plasma nitrite inversely correlated with change in SBP
  - No evidence of tolerance over 4-week period
  - Peak decreases in BP at week 4

**Oxygen consumption**

**Others**

- Significantly improved
**Summary Effects of dietary nitrate supplementation in clinical populations**

<table>
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<tr>
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<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(89)</td>
<td>70mls BRJ mmol AM + PM 3.1g/L nitrate</td>
<td>Double blind randomised crossover</td>
<td>7 24 hr BP monitor</td>
<td>27 pts</td>
<td>Hypertensive</td>
<td>Smoker or ex-smoker &lt;6 months BMI&lt;18 or &gt;35 Systolic BP &lt;120 or &gt;160, diastolic &gt;100 4 or more antihypertensives, change within last month IHD or PVD Diabetes</td>
<td><strong>endothelial function</strong> as increase in peak FMD and reduced PWV, augmentation index • Significant decrease in arterial stiffness <strong>Plasma</strong> • Significantly increased plasma, salivary, urinary nitrate and nitrite • High plasma nitrate after low nitrate intervention ?artefact <strong>Blood pressure</strong> • No significant changes in BP at</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vs placebo low nitrate juice 0.3g/L nitrate</td>
<td></td>
<td>14 Urinary nitrate / nitrite</td>
<td>63.2 +/- 4.4 years</td>
<td>1-3 antihypertensive medications Age 50-70</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
## Summary Effects of dietary nitrate supplementation in clinical populations

<table>
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<tr>
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<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(90)</td>
<td>BRJ 250mls</td>
<td>7.5mmol</td>
<td>Double blind randomised placebo-controlled crossover</td>
<td>14 OD</td>
<td>FMD</td>
<td>27 (9F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vs BRJ</td>
<td>14 doses</td>
<td>24hr ABPM</td>
<td>67.2 +/- 4.9 years</td>
<td>BMI 30.8</td>
<td>Diabetes</td>
<td>Smokers alcohol per week &gt;21 units F / 28 units M</td>
<td>BP, endothelial function and insulin sensitivity in T2DM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>nitrate deplete BRJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet - Normal Washout – 4 weeks Medications – normal, had to be on one antihypertensive</td>
</tr>
</tbody>
</table>

**Oxygen consumption**

**Others**

- No changes in urinary sodium, potassium, creatine

**Plasma**

- Significantly increased plasma nitrate and nitrite after 2 weeks

**Blood pressure**

- No change in BP

**Oxygen consumption**

**Others**

- No change in macrovascular
<table>
<thead>
<tr>
<th>Author, year</th>
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<th>Nitrate dose</th>
<th>Study design</th>
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<th>disease</th>
<th>exclinations</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(62)</td>
<td>70mls BRJ</td>
<td>6.43mmol</td>
<td>Double blind randomised controlled crossover</td>
<td>Treadmill, 4</td>
<td>48 pts</td>
<td>63.3 +/- 7 years</td>
<td>Diabetes</td>
<td>CKD eGFR&lt;30 ml/min/1.73m2, uncontrolled HBP, BMI &lt;25 or &gt;35, MI or stroke</td>
<td>Type 2 DM, effect on oxygen cost of exercise and walking</td>
<td>Plasma</td>
</tr>
<tr>
<td>vs</td>
<td>placebo</td>
<td>nitrate deplete juice</td>
<td>Placebo</td>
<td>6MWT</td>
<td>(13F)</td>
<td>BMI</td>
<td>30.2</td>
<td>Smoker</td>
<td>Medications organic nitrates, nicorandil</td>
<td>No difference in baseline plasma nitrite ACE, ARB, metformin, insulin, statins, sulfonylurea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>submax</td>
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<td></td>
<td>Blood pressure</td>
<td>No significant change in systolic or diastolic BP</td>
</tr>
</tbody>
</table>

endothelial function measured by FMD
- No change in microvascular endothelial function
- No improvement in insulin sensitivity
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / days</th>
<th>No pts</th>
<th>Exclusions</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berry 2014 (79)</td>
<td>Beetroot juice 70ml X 2</td>
<td>Randomised single blind crossover</td>
<td>Cycle ergometer 1</td>
<td>15 pts (3F) COPD Baseline</td>
<td>Excluded if on nitroglycerine nitrate angina medications, PDE5 inhibitors, medications</td>
<td>Effect of dietary nitrate on submaximal exercise in COPD patients</td>
</tr>
</tbody>
</table>

**Summary Effects of dietary nitrate supplementation in clinical populations**

- **Oxygen consumption**
  - No significant difference in cost of walking, no change baseline or end exercise pulmonary VO₂

- **Others**
  - No improvement in 6MWT
  - ‘Small differences in antioxidants between BRJ and placebo unlikely to have a clinically meaningful affect’

- **Plasma**
  - Elevated plasma nitrate / nitrite

- **Blood pressure**
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts龄</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vs</td>
<td>Placebo prune juice</td>
<td>163ml</td>
<td>69.6 (+/- 8.5) years</td>
<td>VO2 max 13.9 +/- 3.5 ml/kg/min</td>
<td>that later stomach pH (PPIs, antacids)</td>
<td>Diet – low nitrate 48 hours prior to test</td>
<td>Washout – 7 days</td>
<td>Medications – normal medications</td>
</tr>
</tbody>
</table>

**Summary Effects of dietary nitrate supplementation in clinical populations**

- **Oxygen consumption**
- **Others**
  - Significant increased exercise time during submaximal constant work
  - No significant change in HR, oxygen consumption, arterial oxygen saturation, dyspnoea ratings,
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Nitrate dose</th>
<th>Study design</th>
<th>Exercise / days</th>
<th>No pts</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(86) Beetroot juice 140ml</td>
<td>12.9mmol + 200ml blackcurrant cordial</td>
<td>Double blind, randomised, placebo-controlled crossover trial</td>
<td>Incremental shuttle walk test ISWT 1</td>
<td>11 (6F)</td>
<td>COPD</td>
<td>Excluded vasodilators, organic nitrates</td>
<td>G</td>
<td>Diet – normal</td>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td>vs Placebo 140ml water + 200ml blackcurrant cordial</td>
<td>1</td>
<td>Baseline</td>
<td>69 +/- 7 years</td>
<td>Maximal</td>
<td>%FEV1 predicted 43.4</td>
<td>One patient on PPI, no benefit</td>
<td></td>
<td>Washout – 7 days</td>
<td>Blood pressure</td>
<td></td>
</tr>
<tr>
<td>27.3</td>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td>PMH Pulmonary hypertension, OSA, angina, MSK conditions</td>
<td></td>
<td>Medications – normal medications</td>
<td>Oxygen consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Others</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Significant increase in serum nitrate and nitrite</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• Significant lowering of resting systolic and diastolic BP</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>• Significant Increase in ISWT (likely to be clinically meaningful)</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• No change in %SpO2, HR or dyspnoea, but</td>
<td></td>
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</tr>
<tr>
<td>Author, year</td>
<td>Type supplement</td>
<td>Study design</td>
<td>Exercise / days</td>
<td>No pts</td>
<td>disease</td>
<td>exclusions</td>
<td>Aims</td>
<td>findings</td>
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</tr>
</tbody>
</table>
| (80) BRJ 70ml BD 4.8mmol | Double blind randomised placebo-controlled crossover | 3 | Endurance shuttle walk test | 19 | COPD | Acute exacerbation within 1 month Home oxygen IHD CCF If had >20mmHg systolic BP fall to test dose BRJ, MSK disease limiting exercise tolerance Medications Beta blocker Oral steroids | • Effect dietary nitrate supplementation on exercise tolerance in pts with stable moderate COPD
| Vs Placebo nitrate deplete BRJ | BD | 5 doses | 67 +/- 7.9 years | (14F) | moderate stable %FEV1 predicted 62.0 BMI | Diet – no beetroot Washout 4 days Medications - unchanged | Plasma
<p>| Plasma | Heterogenous response to dietary nitrate Blood pressure | Significant decrease resting systolic BP and increased HR Oxygen consumption Others | • No significant increase in submaximal exercise endurance |</p>
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Nitrate dose</th>
<th>Exercise / days</th>
<th>No pts</th>
<th>disease</th>
<th>Exclusions</th>
<th>Aims</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(81)</td>
<td>70mls BRJ</td>
<td>Double blind</td>
<td>6.77mmol AM</td>
<td>Cycle ergometer and 6MWT</td>
<td>14 pts</td>
<td>COPD</td>
<td>Mild – moderate</td>
<td>Recruited 30-80% predicted FEV1 and age 40-75</td>
<td>• Does dietary nitrate supplereffect on oxygen cost submax exercise and walking on CPD pts Diet – nitrate low 24 hours Washout – 7 days Medications – phosphodiesterase inhibitors held</td>
</tr>
<tr>
<td></td>
<td>Nitrate dose</td>
<td>randomised</td>
<td>+ PM</td>
<td>2.5 submax</td>
<td></td>
<td>%FEV1, predicted 57</td>
<td>Excluded CKD efGR&lt;30 ml/min/1.73m2, uncontrolled hypertension sys&gt;160, diastolic &gt;100, smokers, on regular organic nitrate or nicorandil</td>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vs placebo</td>
<td>crossover</td>
<td>Vs</td>
<td>64.7 +/- 7.7yrs submax</td>
<td></td>
<td>BMI</td>
<td>29</td>
<td>• Non-significant increase IESWT 11%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>nitrate deplete</td>
<td></td>
<td>juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Non-significant improvement in time to fatigue 6%</td>
<td></td>
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<tr>
<td></td>
<td>juice</td>
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<td></td>
<td><strong>Blood pressure</strong></td>
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<td></td>
<td></td>
<td></td>
<td>• No significant change in systolic or diastolic BP</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Oxygen consumption</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• No effect on baseline or end</td>
<td></td>
</tr>
<tr>
<td>Author, year</td>
<td>Type supplement</td>
<td>Study design</td>
<td>Exercise / max vs submax</td>
<td>No pts</td>
<td>Age</td>
<td>disease</td>
<td>exclusions</td>
<td>Aims</td>
<td>findings</td>
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<td>---------</td>
</tr>
<tr>
<td>Borlaug et al., 2015</td>
<td>Sodium nitrite iv 50 mcg/kg/min</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vs placebo N saline</td>
<td>Double blind randomised placebo controlled parallel group</td>
<td>1 dose</td>
<td>Submax</td>
<td>28 pts, 8F placebo, 9F nitrite</td>
<td>Supine 20W workload for 5 min</td>
<td>70 +/- 8 yrs. BMI 33.4 HBP 11 Nitrite 69 +/- 6 yrs. BMI 32.0 HBP 12</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heart failure with preserved EF (HFpEF)</td>
<td>EF&gt;50%, increase left heart filling pressure at rest &gt;15mmHg and / or exercise &gt;25mmHg</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significant valvular heart disease, cor pulmonale, significant pulmonary disease, congenital heart disease, g6PD deficiency, MI within 60days, unstable angina, left to right shunt, hypertrophic / infiltrative cardiomyopathy, primary renal or hepatic disease, high output cardiac failure, constrictive pericarditis</td>
<td>Measure pulmonary capillary wedge pressure at rest and during exercise</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No washout (parallel group)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Diet – no mention Medications – organic nitrate or phosphodiesterase 5 inhibitors excluded</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Blood pressure</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly reduced exercise VO2</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly increased plasma nitrite, greater than expected decay during exercise consistent with active consumption</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly reduced VO2, increased cardiac output and stroke volume</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Others**
- No significant change in 6mWT
- Measure pulmonary capillary wedge pressure at rest and during exercise
- No washout (parallel group)
- Diet – no mention Mediations – organic nitrate or phosphodiesterase 5 inhibitors excluded
- Significantly reduced exercise VO2, increased cardiac output and stroke volume
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts Age</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nitrate dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Summary Effects of dietary nitrate supplementation in clinical populations**

- Significant reduction in central resting pressures
- No change in exercise HR or systolic BP
- Non-significant reduced mean BP during exercise

**Oxygen consumption**

- Significantly reduced VO2

**Others**

- Significant reduction in resting and exercise PCWP
- Exercise PCWP reduction
- Significantly increased cardiac output
### Summary Effects of dietary nitrate supplementation in clinical populations

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Nitrate dose</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>disease</th>
<th>Exclusions</th>
<th>Aims</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(82)</td>
<td>BRJ 140ml</td>
<td>Double blind placebo controlled randomised crossover</td>
<td>1 dose</td>
<td>6MWT</td>
<td>9 pts (4F)</td>
<td>Heart failure</td>
<td>Major organ system dysfunction</td>
<td>Heart failure</td>
<td>Output and stroke volume</td>
</tr>
<tr>
<td></td>
<td>11.2mmol nitrate</td>
<td></td>
<td>Submax and maximal</td>
<td>Muscle function isokinetic dynamometry</td>
<td>57 +/-10 years</td>
<td>BMI 29.1</td>
<td>Major organ system dysfunction</td>
<td>Washout 1-2 weeks</td>
<td>Increased plasma nitrate, Plasma nitrite non-significantly increased</td>
</tr>
<tr>
<td></td>
<td>Vs placebo</td>
<td></td>
<td></td>
<td>Submax and maximal</td>
<td></td>
<td></td>
<td>Medications - All patients were on beta blockers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrate deplete</td>
<td></td>
<td></td>
<td>Muscular function</td>
<td></td>
<td></td>
<td>Medications - All patients were on beta blockers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beetroot juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medications - All patients were on beta blockers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Decreased exercise Right Atrial Pressure and PA pressure
- Reduced exercise SVR >2x resting, greater effect of nitrite on stress PCWP

### Plasma

- Significantly increased plasma nitrate, Plasma nitrite non-significantly increased
- Increase in NO breath levels =
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / days</th>
<th>Nitrate dose</th>
<th>No pts</th>
<th>exclusions</th>
<th>disease</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>max vs submax</td>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td>increased NO bioavailability</td>
</tr>
</tbody>
</table>

**Blood pressure**
- No change blood pressure or heart rate

**Oxygen consumption**

**Others**
- No significant change in muscle function
- No significant change in 6MWT (not powered)
- Significantly improved muscle contractile function
  - increased peak torque and power therefore calculated
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts</th>
<th>Age</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(91)</td>
<td>Beetroot juice</td>
<td>Double blind randomised crossover</td>
<td>Cycle ergometer</td>
<td>7</td>
<td></td>
<td></td>
<td>Heart failure with preserved EF (HFP EF)</td>
<td>Excluded if significant ischemic or valvular heart disease, pulmonary disease, anaemia, other disorder that could explain patient symptoms</td>
<td>• Does dietary nitrate improve exercise tolerance in pts with HFP EF?</td>
</tr>
<tr>
<td></td>
<td>70ml 6.1mmol nitrate</td>
<td></td>
<td>Submax</td>
<td>69 (+/-7) years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet – no mention Washout – 3-7 days Medications –including ACE / ARB, diuretics, Beta blockers, Calcium channel blockers</td>
</tr>
<tr>
<td></td>
<td>Vs Placebo</td>
<td></td>
<td></td>
<td></td>
<td>BMI</td>
<td>32.9</td>
<td></td>
<td></td>
<td>• Significantly increased plasma nitrate and nitrite after one and 7 doses, variable response</td>
</tr>
<tr>
<td></td>
<td>nitrate deplete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Submaximal aerobic endurance increased 24% after 7 days dosing, no change with 1 dose</td>
</tr>
<tr>
<td></td>
<td>beetroot juice</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Blood pressure</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Medications – nitroglycerine, nitrate preparations for angina, phosphodiesterase type 5 inhibitors, medications that alter stomach pH (antacids, PPIs, H2 receptor antagonists)</td>
<td></td>
<td>• Significantly reduced resting systolic BP after one dose</td>
</tr>
<tr>
<td>Author, year</td>
<td>Type supplement</td>
<td>Study design</td>
<td>Exercise / max vs submax</td>
<td>No pts</td>
<td>Age</td>
<td>disease</td>
<td>exclusions</td>
<td>Aims</td>
<td>findings</td>
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</tr>
<tr>
<td>hirai (83)</td>
<td>Beetroot juice</td>
<td>Double blind randomised crossover</td>
<td>9</td>
<td>Constant load Cycle ergometer</td>
<td>10 pts</td>
<td>63 (+/5) years</td>
<td><strong>Heart failure with reduced EF (HFrEF)</strong></td>
<td>Excluded unstable angina, significant arrhythmia</td>
<td>• Does dietary nitrate supple improve cardiorespiratory function an exercise tolerance in pts with HFrEF</td>
</tr>
<tr>
<td></td>
<td>70ml 6.45mmol nitrate</td>
<td>morning and evening</td>
<td>Submax and max</td>
<td>BMI</td>
<td>31.0</td>
<td>VO2 peak 15.3 +/-0.7 ml/kg/min</td>
<td>Medications that directly potentiate NO mediated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Summary Effects of dietary nitrate supplementation in clinical populations**

  - **Oxygen consumption**
  - **Others**
    - Submaximal aerobic endurance increased 24% after 7 days dosing, no change with 1 dose

- **Blood pressure**
  - No significant change in resting or maxima BP

- **Plasma**
  - Significantly
  - Elevated plasma nitrite

- **Significantly reduced resting and unloaded BP after one week of BRJ**
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type supplement</th>
<th>Study design</th>
<th>Exercise / max vs submax</th>
<th>No pts Age</th>
<th>disease</th>
<th>exclusions</th>
<th>Aims</th>
<th>findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nitrate dose</td>
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<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>AT 9.4 +/- 0.7 ml/kg/min</td>
<td>effects e.g. ISDN, phosphodiesterase type 5 inhibitors</td>
<td>Diet – regular dietary habits Washout – 5 days Medications – took usual meds</td>
<td>Oxygen consumption</td>
<td></td>
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<tr>
<td></td>
<td>nitrate deplete</td>
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</tr>
<tr>
<td></td>
<td>beetroot juice</td>
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</tr>
</tbody>
</table>

**Oxygen consumption**
- No significant difference in pulmonary VO$_2$ kinetics
- Did not reduce o$_2$ cost of exercise

**Others**
- No significant difference in time to exercise intolerance
- No significant difference in central haemodynamics (heart rate, stroke volume and cardiac output), blood lactate
### Appendix 5 Plasma nitrate and nitrite levels in clinical populations after dietary nitrate supplementation

<table>
<thead>
<tr>
<th>Study</th>
<th>Nitrate dose mmol</th>
<th>Plasma nitrate (micromole/L) NO₂</th>
<th>Plasma nitrate (micromole/L) NO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline/placebo</td>
<td>Post-intervention</td>
</tr>
<tr>
<td>(89)</td>
<td>70mls of 3.1g/L 7 days BD</td>
<td>5.8 +/- 2.9</td>
<td>123.4 +/- 53.3</td>
</tr>
<tr>
<td>(90)</td>
<td>7.5 14 days OD</td>
<td>232 (200,265) nmol</td>
<td>390 (312,537) nmol</td>
</tr>
<tr>
<td>(62)</td>
<td>6.43 4 days OD</td>
<td>680 +/- 256 nmol</td>
<td>1065 +/- 607 nmol</td>
</tr>
<tr>
<td>(80)</td>
<td>4.8mmol twice a day for 3 days (total 5 doses)</td>
<td>+612 +/- 603 nmol</td>
<td>+452 +/- 167</td>
</tr>
<tr>
<td>(81)</td>
<td>6.8mmol twice a day for 3 days (total 5 doses)</td>
<td>48 +/- 85</td>
<td>215 +/- 84</td>
</tr>
<tr>
<td>(82)</td>
<td>11.2mmol one dose</td>
<td>0.48 +/- 0.09 PB, 0.25 NB</td>
<td>0.50 +/- 0.06 NSC</td>
</tr>
<tr>
<td>(91)</td>
<td>One week</td>
<td>0.34 +/- 0.26</td>
<td>0.78 +/- 0.52</td>
</tr>
<tr>
<td>(79)</td>
<td>One dose 7.58mmol</td>
<td>+379%</td>
<td>+938%</td>
</tr>
<tr>
<td>(86)</td>
<td>One dose 12.9mmol</td>
<td>96 +/- 91nmol, 139 +/- 86</td>
<td>751 +/- 632</td>
</tr>
<tr>
<td>Hirai (83)</td>
<td>6.45mmol twice a day for 9 days</td>
<td>+184.1 nmol</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 6 Blood pressure changes in clinical populations after dietary nitrate supplementation

<table>
<thead>
<tr>
<th>study</th>
<th>Disease</th>
<th>Nitrate dose (mmol)/duration</th>
<th>medications</th>
<th>Sys BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rest</td>
<td>exercise</td>
</tr>
<tr>
<td>Kenjale et al (63)</td>
<td>PAD 8 pts</td>
<td>9.1 X1 dose</td>
<td>PPIs excluded</td>
<td>NSC</td>
<td>SR 2 min recovery p≤0.05</td>
</tr>
<tr>
<td>Eggebeen et al (91)</td>
<td>Heart Failure 20 pts</td>
<td>6.1mmol x7 days</td>
<td>Excluded if on Nitrate prep including nitro-glycerine, phosphodiesterase inhibitors, medications that later stomach pH</td>
<td>SR 14mmHg, p&lt;0.001</td>
<td>Trend to reduction p=0.054</td>
</tr>
<tr>
<td>Kapil et al (88)</td>
<td>Hypertension 64 pts</td>
<td>6.4 x28 days</td>
<td>Continue usual medications and diet</td>
<td>SR 7.7mmHg p&lt;0.001</td>
<td>X</td>
</tr>
<tr>
<td>Berry et al (79)</td>
<td>COPD 15 pts</td>
<td>7.58 x1</td>
<td>Excluded if on nitro-glycerine nitrate angina medications, PDE5 inhibitors, medications that alter stomach pH (PPIs, antacids)</td>
<td>SR 8mmHg p= 0.019</td>
<td></td>
</tr>
<tr>
<td>Kerley et al (86)</td>
<td>COPD 11 pts</td>
<td>12.9 x</td>
<td>Excluded vasodilators, organic nitrates</td>
<td>SR 12mmHg p=0.03</td>
<td>X</td>
</tr>
<tr>
<td>Study</td>
<td>Disease</td>
<td>Number of patients</td>
<td>Nitrate dose (mmol) /duration</td>
<td>Medications</td>
<td>Sys BP</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------</td>
<td>--------------------</td>
<td>------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Leong et al (80)</td>
<td>COPD</td>
<td>19 pts</td>
<td>4.8mmol twice a day X5 doses</td>
<td>Excluded beta blockers and oral steroids excluded if had &gt;20mmHg drop with test dose</td>
<td>SR 10mmHg p= 0.01</td>
</tr>
<tr>
<td>Velmurugan et al (87)</td>
<td>Hypercholesterolaemia</td>
<td>65 pts</td>
<td>6.0 X 6 weeks</td>
<td>14 pts on antihypertensives</td>
<td>SR p=0.004 not intergroup</td>
</tr>
</tbody>
</table>

**No change in blood pressure**

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease</th>
<th>Number of patients</th>
<th>Nitrate dose (mmol) /duration</th>
<th>Medications</th>
<th>Sys BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilchrist et al (90)</td>
<td>Diabetes</td>
<td>14 pts</td>
<td>75 x 14 days</td>
<td>Continue usual medications, and diet All pts on one antihypertensive</td>
<td>NSC</td>
<td>NSC</td>
</tr>
<tr>
<td>Bondonno et al (89)</td>
<td>Hypertension</td>
<td>27 pts</td>
<td>0.21g twice a day x7 days</td>
<td>Continue usual medications Low nitrate diet</td>
<td>NSC</td>
<td>NSC</td>
</tr>
<tr>
<td>Shepherd (62)</td>
<td>Diabetes</td>
<td>48 pts</td>
<td>6.4 x 4 doses</td>
<td>Excluded Organic nitrates and nicorandil, phosphodiesterase inhibitors withheld</td>
<td>NSC</td>
<td>X</td>
</tr>
<tr>
<td>Shepherd et al (81)</td>
<td>COPD</td>
<td>14 pts</td>
<td>6.77mmol x5 doses</td>
<td>Held phosphodiesterase inhibitors</td>
<td>NSC</td>
<td>NSC</td>
</tr>
<tr>
<td>Ref.</td>
<td>Condition</td>
<td>Dose</td>
<td>Details</td>
<td>Result 1</td>
<td>Result 2</td>
<td>Result 3</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------</td>
<td>-------------</td>
<td>------------------------------------------------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Coggan et al (82)</td>
<td>Heart failure</td>
<td>11.2mmol</td>
<td>All patients on beta blockers Excluded if on PPIs, antacids and xanthine oxidase inhibitors</td>
<td>NSC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirai (83)</td>
<td>Heart Failure</td>
<td>6.45mmol</td>
<td>twice a day x 9 days</td>
<td>NSC</td>
<td>NSC</td>
<td>NSC</td>
</tr>
</tbody>
</table>

SR = significant reduction, NSC = no significant change,
Appendix 7 CPX report variables

AT (ml/kg/min)

- **Anaerobic / lactate / Gas Exchange Threshold**
  - **Definition** – Oxygen uptake at which anaerobic ATP synthesis significantly supplements aerobic synthesis. (Point at which line jumps on VCO2 vs Vo2 graph ‘V slope’, excess CO2 production). AT (usually as an absolute value (mls/min) is measured as the inflection point / the change of slope in the VO2-VCO2 curve and represents excess CO2 production caused by buffering of acid metabolites from anaerobic metabolism. It is also the point at which Ve/VCO2 stays the same while Ve/VO2 rises sharply.
  - **Significance** – Objective marker of cardiorespiratory fitness, exercise capacity / physiological reserve. No volitional component, not possible to alter by feigning exhaustion or trying harder.
  - **This represents the point in exercise when anaerobic metabolism starts to play a significant role in total metabolism.** It represents a level of metabolic beyond which exercise is not sustainable due to the accumulation of acid metabolites faster than they can be cleared.
    - Below AT exercise is sustainable.
    - Above AT exercise
      - During ‘Heavy’ exercise, plasma lactate and acid elevated but may decrease or stabilise as exercise continues.
      - During severe exercise, plasma lactate and acid elevated and continue to increase which is not sustainable.
  - A low anaerobic threshold predicts postoperative cardiopulmonary morbidity. The thresholds vary for different surgical procedures, possibly due to the differing oxygen load caused by complications. Low AT has also been shown to correlate with cardiac failure, with an AT of <11mls/Kg/min representing NYHA class II failure.
  - AT is a predictor of short and long-term outcomes after surgery and a predictor of 30-day outcome postoperatively.

Peak VO2 (ml/min)

- **Definition** - Highest value for oxygen uptake attained during test,
- **Reproducible but significant volitional component** - subject can terminate test early
- **Related to clinical outcome**
- **May be equal to VO2 max but likely to be less,** (VO2 max is maximal oxygen uptake, point where VO2 plateaus despite increase in work rate, dependant on WR slope, and has no volitional component. A test is maximal (and therefore peak VO2 is VO2 max if RER>1.15 or the heart rate attains the age predicted maximum): A maximal test refers to one where the effort has been sufficient to produce significant anaerobic metabolism. A test can be maximal and still not achieve VO2max.
• Significance - prognostic marker of postoperative morbidity and is a predictor of short and long-term outcomes post-surgery

**VO₂ (ml/kg/min)**

• Definition – Total body oxygen consumption measured at the mouth, indexed to body weight.
• Significance – at steady state is equal to the cellular oxygen uptake as little facility in body to store oxygen

**Measured Peak O₂ pulse (mls/bt)**

• Definition - Amount of oxygen delivered to tissues by each heart beat
• Dependant on stroke volume and arterio-venous oxygen difference
• Significance – Can be used as an index of Stroke volume especially at peak exercise, as the A-V DO2 is predictable at 75% at this stage in exercise. Therefore, decreased in patients with severe LV dysfunction or valvular heart disease, often increased in beta blocked patients.

**VO₂ at AT (ml/min)**

• Definition - Oxygen uptake at Anaerobic Threshold expressed as an absolute value. Usually not used in clinical literature, as makes sense only when either indexed or expressed as a proportion of something.

**AT/Predicted VO₂ max (%)**

• Definition – Oxygen uptake at Anaerobic threshold expressed as a proportion of predicted peak VO2
• Normal range - 40-60%
• Significance – In normal exercise physiology, the AT is reached between 40-60% of peak predicted VO2. This implies that normally anywhere from 1/3 to 2/3 of exercise metabolism can be aerobic (a wide range). Anything lower suggests low cardiorespiratory fitness.

**VeVCO₂ at AT**

• Definition – Ventilation per unit minute volume at the AT. A measure of ventilation / perfusion in the lungs matching at its best during exercise. Beyond the AT, particularly beyond the respiratory compensation point, the minute ventilation may rise disproportionate to the VCO2 to keep pH constant, in response to the overriding of buffering capacity.
• Normal range - <35. This is a unitless value (mls/mls)
VeVCO₂ slope

- Definition - ventilatory equivalent for CO₂, i.e. ratio of ventilation to carbon dioxide output, volume of gas breathed divided by CO₂ produced across the entire exercise period (excluding the recovery phase). It does not represent an instantaneous value, but one across the period of exercise.
- Normal range - 25-35
- Significance – If lower represents excellent V/Q matching, as in athletes, if higher increased dead space or shunt caused by cardiac or respiratory or dual pathology. Athletes/ anxious individuals may initially hyperventilate but drops to normal once loaded pedalling starts

Peak Ve (L/min)

- Definition – minute ventilation at peak exercise.
- Has no ‘normal’ value but is a key number to calculate the ‘breathing reserve’: [Maximum voluntary ventilation (MVV) - peak Ve/ MVV] *100
- Significance – In normal subjects, exercise limitation is due to exhaustion of cardiac reserve: at termination, most subjects should have a breathing reserve at or above 30%. A breathing reserve <30% at termination, particularly if accompanied by a high heart rate reserve suggests a ventilatory cause for exercise limitation.

ΔVO₂/ΔWR (ml/min/watt)

- Relationship between oxygen uptake and WR
- Normally 10 ml/min/watt
- Reduced in any condition that reduces blood flow to periphery
- Not reduced in obesity so can determine if a reduction in AT is due to obesity or poor LV function

Max load achieved (watts)

- Definition - Amount of work achieved in watts
- Significance – represents peak work achieved. Will correlate well with peak VO₂ if the ΔVO₂/ΔWR relationship is normal. Can be used as a surrogate for peak VO₂ in prehab programmes.
- Normal range – volitional and training/ fitness dependant
Patient information leaflet

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer

Professor Timothy Rockall, Professor of Surgery,
Dr Mike Scott, Consultant Anaesthetist
Miss Vanessa Brown, Research Fellow, Principal Investigator

“Does drinking beetroot juice improve exercise test results and recovery after surgery in people with colorectal cancer?”

Invitation to participate
You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. This information sheet is designed to help you decide whether you would like to participate in this study. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?
Drinking extra nitrates in the form of beetroot juice has been shown to improve exercise performance in professional athletes. Cardiopulmonary exercise testing (CPX) measures the function of your heart and lungs in response to exercise (see separate information leaflet ‘Your Cardio Pulmonary Exercise test’). Studies have shown that the better your CPX result, the less likely you are to have complications after a big operation.
The aim of this study is to see if dietary nitrate supplementation improves performance in CPX and recovery after surgery.
The study will last up to approximately 28 days in total.

Why have I been chosen?
You have been invited to take part in the study because you have a diagnosis of colorectal cancer, are awaiting surgery and fit the required criteria. Approximately 80 people will be observed and tested in this study.

1. Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC 15/LO/0945, R&D 15/0099, NCT02318356)
Version 2.2 14/3/2016
Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
The study will last up to approximately 28 days. You will be asked to give up certain foods which are high in nitrates for the duration of the study (see attached list ‘Nitrates rich foods’) and not chew chewing gum or use mouthwash as these affect nitrate absorption into the body.

You will be asked to perform two CPX tests, one week apart.
- From 10 days before your first CPX you will be asked to give up foods which are high in nitrates (see attached list ‘Nitrates rich foods’) and not chew chewing gum or use mouthwash. You should continue this until the end of the study.
- For the 24 hours before your first CPX you will be asked to record all food and drink you eat. You will then be asked to replicate this on the day before your second CPX
- In between the two tests you will be asked to drink a 70ml shot of either beetroot juice or placebo (beetroot juice with nitrates removed) a day.

After the second CPX test you will continue to drink your assigned drink at 10.30am every day. On the day of surgery you should drink your assigned shot with the morning dose of preload (mixed with 330mls of water instead of 400mls) and drink it at either 06am (morning list) or 10.30am (afternoon list) as per the “Enhanced Recovery Programme Bowel Surgery” leaflet.

After the surgery you should continue drinking your assigned drink along with your first nutritional drink every morning until discharge. Your grip strength will be measured everyday in the morning and evening and we will ask you to record your weight on day 14 and 28 post operatively. Other information from your medical notes will be captured approximately 90 days after your operation.

Please see page 5 for a detailed flow chart of what will happen to you during the study.

You will not be expected to need to visit the GP more often during the study.

This study is a randomised trial. Because we do not know if nitrates will improve things, patients will be put into groups and then compared. The groups are randomly

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC 15/LC/0945, R&D 15ONCN0009, NCT02319356)
Version 2.2 14/3/2016
selected by a computer by chance. Patients in one group will have normal beetroot juice containing nitrates, in the other group patients will receive placebo beetroot juice with the nitrates removed. A placebo is a dummy treatment which looks like the real thing but is not. It contains no active ingredient. You will have a one in two chance of getting the active beetroot or the placebo. This is a blind trial. Neither you or your doctors will know which group you are in (although, if your doctor needs to find out he/she can do so).

What are my responsibilities?

- Refrain from eating foods on the attached list ‘Nitrate rich foods’ for the duration of the study
- Do not chew gum or use mouthwash for the duration of the study
- Drink your 70ml shots of your assigned drink a day from days 12 onwards at the assigned time
- Keep a food diary for the 24 hours before your first CPX and replicate this diet on the day before your second CPX
- If female and appropriate – agree to use effective contraception during the course of the study

You can drive and take part in sport as normal. You should continue to take your regular medication unless we specifically advise you otherwise. You should not donate blood in the study period as this may change the results of your exercise test.

What is the drug or procedure that is being tested?

We are testing if the nitrates in beetroot juice improve exercise performance. Once you have completed your first CPX you will be asked to drink your 70ml shot of your assigned drink at 2.5 hours before your appointment time each day (e.g. if your appointment time is 2.45pm you should have your shot at 12.15pm everyday).

What are the side effects of any treatment received when taking part?

Beetroot juice may turn your urine pink and your stools red. If you become concerned you can contact one of us via the contact details given at the end of this document. In the event of an emergency please call 999 or go to your local A&E.

What are the possible disadvantages and risks of taking part?

You will not be able to eat foods on the attached list ‘Nitrate rich foods’ for the duration of the study. This is inconvenient but should not affect your recovery after surgery.

If we find a condition of which you were unaware this will allow us to refer you to the appropriate specialist for further treatment.

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with colorectal cancer

(REC15/LO/0945, R+D 150NCN0009, NCT02319356)

Version 2.2 14/3/2016
CPX - As with all medical tests there is the chance of unwanted side effects or complications. The risk of these with CPX are the same as for moderate exercise. The number of patients that develop problems during the test is low (1 in 1000). The complications that may occur during the test include abnormal blood pressure, fainting, irregular, fast or slow heart rhythms. In exceptionally rare instances there can be serious complications such as a heart attack or stroke. Please see the separate information leaflet ‘Your Cardio Pulmonary Exercise test’ for more details.

Note for female patients
High levels of nitrates are thought to be harmful to unborn children therefore it is possible that if the treatment is given to a pregnant woman it will harm the unborn child. Pregnant women must not therefore take part in this study, neither should women who plan to become pregnant during the study. Women who could be pregnant may be asked to have a pregnancy test before taking part to exclude the possibility of pregnancy. Women who could become pregnant must use an effective contraceptive during the course of this study. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.
All women of child-bearing age will have a pregnancy test as part of their routine care.

What are the possible benefits of taking part?
We hope that this study will go on to produce further studies which may in time show a definite relationship between drinking beetroot juice and faster recovery after surgery.
The study preparation is not a ‘drug’ in its truest sense but more a food supplement which has minimal side effects.
A CPX test stresses your heart and lungs in a systematic controlled fashion. If the test shows you are very unfit it will allow us to refer you to the appropriate specialist for further treatment of the medical condition. Or it may allow us to offer you alternatives that you are more able to tolerate.

What if new information becomes available?
Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.
Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with colorectal cancer
(REC 15/LO/0945, R&D 1501NO0009, NCT02313556)
Version 2.2 14/3/2016
Day 1
Please do not
- Eat nitrate rich foods (see attached list)
- Chew gum
- Use mouthwash

until the end of the trial

Day 10
24 hours before 1st exercise test
- Record all food / drink you eat in attached food diary
- Avoid alcohol and strenuous exercise

Day 11 - 1st CPX (Exercise test)
- Do not eat any food after ________ (3 hours before test)
- Arrive for CPX in rested and hydrated state

This visit will last approximately an hour

Day 12-16
- Drink your assigned 70ml shot at ________ (2.5 hours before the time of your next CPX)
- Please continue NOT to eat nitrate rich foods, chewing gum and mouthwash

Day 17
24 hours before 2nd exercise test
- Eat / drink the same food you did before first test, as recorded in your food diary
- Avoid alcohol and strenuous exercise

5 Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with colorectal cancer
(REC 15/LDO/0945, R&D 15/09/20159, NCT02319356)
Version 2.2 14/3/2015
Day 18 - 2nd CPX (Exercise test)

- Do not eat after ________ (3 hours before test)
- Arrive for CPX in rested and hydrated state

This visit will last approximately an hour

Day 19 - to day before surgery

Drink your assigned juice shot at 10:30am every day

Day of Surgery

Drink your assigned juice shot along with your morning dose of preload (mix one sachet with 330/mls water not 400/mls)

- 6am (morning list)
- 10:30am (afternoon list)

After surgery

Drink your assigned 70ml shot every morning with your first drink
Remain on your nitrate free diet

Once you have been discharged from hospital the trial is over and you can resume a normal diet

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer

(REC 15/LO/0945, R+D IS0NCN0009, NCT02319356)
Version 2.2 14/3/2016
What happens when the research study stops?
The juice will not be available after the research finishes.

What if something goes wrong?
It is very unlikely that you will come to any harm during this study. However if you do come to harm there are no special compensation arrangements.
If you wish to complain or have any concerns about your treatment by members of the team or about the research itself the Patient Advice and Liaison Service (PALS) are available to provide independent help, advice and support. They can be found at the far left corner as you enter the main reception area. They can also be contacted by -

  Telephone: 01483 402757
  Email: rsc-tr.pals@nhs.net
  In person: opening hours 9am-4pm Monday to Friday

Who has reviewed the study?
This study has been reviewed and received a favourable opinion by South East Coast-Surrey Research Ethics Committee.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Data will be stored in accordance with the data protection act 1998. Data will be stored on a password protected computer or encrypted USB storage device which will be securely retained in a locked office in a separate building which requires swipe card access. Data will only be accessed by members of the direct care team and will be anonymised by assigning a unique study number. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized from it.
Your own GP will be notified of your participation in the trial.

What will happen to the results of the research study?
The results of the research may be published in a medical journal, but you will not be identified by name in any publications. Once the study has been completed and analysed we can send you a summary of the results if you would like. Please let us know by indicating this on the consent form.

Who is organizing the research?
This study is being organized and coordinated by the Royal Surrey County Hospital Guildford. Your doctors will not be paid for including you in this study.

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with colorectal cancer
(REC 15/LO/0945, R+D 15OCTR00009, NCT021319356)
Version 2.2 14/3/2016
Will I get travel expenses?
Parking charges and travelling expenses incurred in addition to your routine care can be reimbursed on production of receipts.

Contact for Further Information
If you have any questions concerning the study please do not hesitate to contact us:

Miss Vanessa Brown, MBBS, MRCS
Research Fellow
Department of Surgery
Royal Surrey County Hospital
Egerton Rd
Guildford
GU27XX

Telephone: 01483 406 729
Email: rsc-tr.ColoRectalCNS@nhs.net

Thank you for taking the time to read this information leaflet

You will be contacted shortly by the research team by telephone, during the call you will have the opportunity to raise any concerns or questions and if you decide to take part in the study, you will be asked to give verbal consent.
You will be requested to sign a written consent at your CPX appointment.

You will be given a copy of the information sheet and a signed consent form to keep

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC 15/LO/0945, R+D 15ONCN0009, NCT02319356)
Version 2.2 14/3/2016
Nitrate rich foods

Please find below the list of foods you should not eat for the duration of the study

- Celery
- Celeriac
- Chervil
- Chinese cabbage
- Cress
- Endive
- Fennel
- Greens including Collard, Kale, Musturd and Turnip
- Kohlrabi
- Leek
- Lettuce
- Parsley
- Rocket (Rocula)
- Red beetroot
- Spinach

Please also do not chew chewing gum or use mouthwash for the duration of the study

Vegetables you can eat
(This list is not exhaustive)

Salads
Baby Kale leaves
Pea shoots
Avocado
Cucumber
Tomatoes

Others
Beans
Broccoli / cauliflower
Brussel sprouts
Carrots
Peppers
All types of fruit

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC15/LO/0945, R+D 15ONCN0009, NCT02319356)
Version 2.2 14/3/2016
Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer

(REC 15/LO/0945, R+D 15ONCN0009, NCT02319356)

Professor Timothy Rockall, Professor of Surgery
Dr Mike Scott, Consultant Anaesthetist
Miss Vanessa Brown, Research Fellow

Background
Major surgery generates a systemic inflammatory response that places extra metabolic demands on the body and increases oxygen demand and consumption postoperatively.

Dietary nitrate supplementation has been shown to enhance exercise tolerance and performance in professional athletes and physically active males. In patients with COPD a single dose of beetroot juice reduced oxygen consumption and extended time to exhaustion during submaximal exercise. In patients with peripheral arterial disease dietary nitrate supplementation increased claudication time and walking time.

The majority of interventions in enhanced recovery have focused on improving oxygen delivery, for example by strict management of fluid balance and decreasing oxygen demand by minimising the stress response for example by decreasing the fasting period.

Nitrates however work to improve oxygen utilisation by tissues. The mechanism of action by which this occurs is unclear but several theories have been postulated including improving mitochondrial efficiency and acting as a vasodilator to improve blood flow to contracting muscle.

Cardiopulmonary exercise testing (CPX) is an established, non-invasive and safe method of assessing patients’ cardiopulmonary reserve prior to surgery. It has been shown to predict complications and length of stay in patients undergoing major elective surgery.

The anaerobic threshold (AT) is the oxygen uptake (VO2) at which anaerobic ATP synthesis supplements aerobic ATP synthesis; is calculated during CPX. The AT has no volitional component and so is not possible to alter by trying harder or pretending to be exhausted. The AT can predict short and long term outcomes after surgery and postoperative complications. Patients with colorectal cancer have a lower AT, which increases significantly post resection.

Peak VO2 is the highest value for oxygen uptake attained during CPX. It is also a prognostic marker of postoperative morbidity. In physically active males, supplementation with beetroot juice (BRJ) led to a 12% reduction in VO2 during walking, a 7% reduction in the steady-state VO2 during running, a 19% reduction in the pulmonary VO2 response and a 16% improvement in the time to task failure.
What is the aim of the study?
This study aims to see if outcomes after laparoscopic colorectal surgery can be improved by determining if nitrato loading with BRJ improves the AT and Peak VO2 in patients with colorectal cancer and decreases the rate of post-operative complications. It will allow refinement of the enhanced recovery programme.

Who is eligible to take part in the study?
Eligible patients are those who are
- aged 18-99 years old
- on the waiting list for laparoscopic surgery for colorectal cancer
Excluded are patients who
- have a contraindication to cardiopulmonary exercise testing: eg unstable cardiac disease, lower limb dysfunction, inability to perform CPX
- are not able to give informed consent
- are allergic to Beetroot
- are pregnant or planning to become pregnant

Once recruited patients who during CPX have an ischaemic ECG or are unable to reach AT or who have emergency surgery will be excluded.

What is my role?
We do not expect patients to require any extra support from you. As you may be aware, beetroot juice causes ‘beeturia’ and red stools which the patients will be warned about.

When will patients enter the study?
Patients who are undergoing elective laparoscopic colorectal resections and fulfill the inclusion and exclusion criteria will be invited to participate in the study.

Patients will be approached in the outpatient clinic by the chief investigator or clinical supervisor about the possibility of participation in the study. The principles behind the study will be explained. Patients will be informed that they will be randomized into one of two groups to receive either beetroot juice or placebo. The cardiopulmonary exercise test will be explained in detail. They will be provided with an information sheet and the opportunity to ask any questions they may have at that time.

Should they show interest in participating in the study they will be given a phone number and e-mail address to contact the chief investigator regarding any further questions. All patients will be assessed at a preoperative clinic and if any concerns are identified they will be reviewed by a consultant anaesthetist.

What is the study comparing?
The study is comparing whether dietary nitrato supplementation with beetroot juice improves performance in CPX compared with placebo.

Does dietary nitrato supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC 15/LO/0945, R+D 15NCIC100009, NCT02313356)
Version 2.1 29/7/2015
Patients will be given a list of foods that are rich in nitrates and asked to abstain from them for 10 days before the initial CPX and during the trial (up to approximately 28 days). They will also be asked to abstain from using antibacterial mouthwash and chewing gum. They will be asked to record their dietary intake for 24 hours preceding initial the initial CPX and to replicate this prior to the second CPX.

Alcohol and strenuous exercise should be avoided for 24hrs preceding each CPX and patients will be asked to arrive for each CPX in a rested and fully hydrated state, more than 3 hours post-prandial. Patients will undergo the initial CPX and will then be randomized to receive either beetroot juice (BRJ, containing NO\textsubscript{3}−, 6.5 mmol/day, administered as 70ml ‘Beet it Concentrated beetroot Juice Shots’, James White drinks, Ipswich UK) or ‘placebo’ (PL, NO\textsubscript{3}−-depleted BRJ) for 7 days. A second CPX will then be performed. Patients will be advised to drink their 70ml shot at 2.5 hours before their appointment time every day for the 7 days between CPXs.

Patients will be advised to drink their 70ml shot at 2.5 hours before their appointment time every day for the 7 days between CPXs. After their second CPX patients will be advised to continue drinking their assigned 70ml shot at 10.30am until the day of their surgery. On the day of surgery they will be advised to mix their shot with their morning dose of preload and drink at 6am (morning list) or 10.30am (afternoon list). They will then be asked to drink their assigned 70ml shot along with their first nutritional drink every morning until discharge.

They will receive verbal and written warnings about beeturia and red stools.

What will be measured during the study?
After they have signed their consent form the patients’ weight (kg), height (cm), resting blood pressure (BP) and heart rate (HR) will be measured. ASA and performance status will be assessed and recorded. At this stage the CPX process will be explained again to the patient. Saddle and handle bar height and configuration recorded and reproduced in second test.

CPX will be performed according to a standard ramped incremental exercise protocol on a bike following the American society of chest physicians recommendations. The study will be supervised by a medically qualified doctor and terminated once the attending physician is certain AT has been surpassed.

Early termination and therefore exclusion will occur if ischemic changes appear on the ECG, at patient request or intolerance. Standard measurements including AT and VO\textsubscript{2}Max will be calculated.

Patients will follow the standard post-operative enhanced recovery care pathway for all colorectal patients. Daily measurements of the patients’ temperature, blood pressure, heart rate and saturations will be recorded. The time until the patient is able to tolerate a normal diet and when they first open their bowels and pass flatus will be recorded. Grip strength will be measured with dynamometer on day of surgery and every morning and evening until discharge by the Colorectal Nurse specialists. The time in hours to when the patient is deemed medically fit for discharge and when the patient actually leaves the hospital will be recorded.

Patients will be asked to record their weight on day 14 and 28 post operatively. Morbidity / post operative complications will be recorded using the Clavien Dindo and POMs scoring systems.

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC 15/LO/0945, R+D 150CN0009, NCT02193556) Version 2.1 29/7/2015
Will I know which of my patients are taking part in the study?
Yes, you will always be informed. Should we suspect, at any point during the trial, that there might be another medical condition of which you should be aware, we will notify you as soon as possible.

Who is organizing and funding the research?
This study is being organised and co-ordinated by the Royal Surrey County Hospital Guildford.

Who has reviewed the study?
The study has been reviewed and received a favourable opinion by South East Coast-Surrey Research Ethics Committee.

Who do I contact for further information?
If you have any questions concerning the study please do not hesitate to contact us:

Miss Vanessa Brown, MBBS, MRCS
Research Fellow
Department of Surgery
Royal Surrey County Hospital
Egeron Rd
Guildford
GU27XX
01483 406729
rsc-tr.ColorectalCNS@nhs.net

Thank you for reading this

References

Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and post-operative recovery in patients with Colorectal cancer
(REC 15/LO/0945, R+D 150NCN00009, NCT02319356)
Version 2.1 29/7/2015
Food diary

Day before test

<table>
<thead>
<tr>
<th>Time consumed and amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eg Ham sandwich, ready salted crisps, apple, can of coke</td>
</tr>
</tbody>
</table>

Day of test

<table>
<thead>
<tr>
<th>Breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snacks</td>
</tr>
<tr>
<td>Lunch</td>
</tr>
</tbody>
</table>

NB – please do not eat for 3 hours before the test
## Appendix 11 Patients without cancer on histology

<table>
<thead>
<tr>
<th>Reason for resection</th>
<th>histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion in transverse colon suggestive of cancer, biopsy high grade dysplasia with progression</td>
<td>incidental appendiceal tumour LAMN</td>
</tr>
<tr>
<td>PET hot polyp on surveillance for SCC tongue, did not lift on endoscopy</td>
<td>benign</td>
</tr>
<tr>
<td>Incidental on MRI for back pain, not amenable to EMR on endoscopy, large, 15 cm, extends over 4/5 cm</td>
<td>Tubulovillous adenoma with low grade dysplasia</td>
</tr>
<tr>
<td>Polyp in segment of diverticular disease, biopsy high grade dysplasia</td>
<td>Benign, focal high-grade dysplasia</td>
</tr>
<tr>
<td>Recurrent polyp, biopsy high grade dysplasia, would not lift</td>
<td>Tubulovillous adenoma with high grade dysplasia</td>
</tr>
<tr>
<td>Rectosigmoid polyp, failed to lift</td>
<td>Tubulovillous adenoma with focal high-grade dysplasia</td>
</tr>
</tbody>
</table>

## Appendix 12 Laboratory values

<table>
<thead>
<tr>
<th></th>
<th>overall</th>
<th>Nitrates</th>
<th>Placebo</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>95% Cl</td>
<td>n</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>72</td>
<td>38</td>
<td>126</td>
<td>34</td>
</tr>
<tr>
<td>Albumin</td>
<td>65</td>
<td>34</td>
<td>42</td>
<td>31</td>
</tr>
</tbody>
</table>

## Appendix 13 Ramp data

<table>
<thead>
<tr>
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<th>Nitrates</th>
<th>Placebo</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>95% Cl</td>
<td>n</td>
</tr>
<tr>
<td>Ramp</td>
<td>67</td>
<td>35</td>
<td>16.7</td>
<td>32</td>
</tr>
</tbody>
</table>
### Appendix 14 Summary CPX results

<table>
<thead>
<tr>
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<th>Nitrates</th>
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<th>Baseline</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>95% CI</td>
<td>paired</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>change</td>
<td>95% CI</td>
<td>t test</td>
<td></td>
</tr>
<tr>
<td>AT (ml/kg/min)</td>
<td>65</td>
<td>34</td>
<td>12.3</td>
<td>11.33-13.34</td>
<td>0.70</td>
</tr>
<tr>
<td>VO₂ at AT (ml/min)</td>
<td>65</td>
<td>34</td>
<td>972.6</td>
<td>886.3-1059.0</td>
<td>56.2</td>
</tr>
<tr>
<td>Peak VO₂ (ml/min)</td>
<td>58</td>
<td>32</td>
<td>1584</td>
<td>1424-1744</td>
<td>-20.6</td>
</tr>
<tr>
<td>VeVCO₂ slope</td>
<td>57</td>
<td>31</td>
<td>29.0</td>
<td>27.2-30.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Peak VE (L/min)</td>
<td>58</td>
<td>32</td>
<td>72.3</td>
<td>64.6-80.1</td>
<td>-0.38</td>
</tr>
<tr>
<td>VO₂ / WR (ml/min/watt)</td>
<td>57</td>
<td>31</td>
<td>9.14</td>
<td>8.6-9.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Max load achieved (Watts)</td>
<td>58</td>
<td>32</td>
<td>128.6</td>
<td>110.5-146.7</td>
<td>3.2</td>
</tr>
<tr>
<td>VeVCO₂ at AT</td>
<td>65</td>
<td>34</td>
<td>32.1</td>
<td>30.6-33.6</td>
<td>0.20</td>
</tr>
<tr>
<td>VO₂ (ml/kg/min)</td>
<td>58</td>
<td>32</td>
<td>20.2</td>
<td>18.6-22.2</td>
<td>-0.31</td>
</tr>
<tr>
<td>Peak O₂ pulse (mls/bt)</td>
<td>58</td>
<td>32</td>
<td>10.6</td>
<td>9.5-11.7</td>
<td>-0.18</td>
</tr>
<tr>
<td>VO₂ (peak / m²)</td>
<td>58</td>
<td>32</td>
<td>824.3</td>
<td>748.7-899.8</td>
<td>-10.5</td>
</tr>
<tr>
<td>Watts</td>
<td>58</td>
<td>32</td>
<td>128.6</td>
<td>110.5-1467</td>
<td>3.2</td>
</tr>
</tbody>
</table>
## Appendix 15 Summary Spirometry results

<table>
<thead>
<tr>
<th></th>
<th>overall</th>
<th>Nitrates</th>
<th>Placebo</th>
<th>Baseline</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>95% CI</td>
<td>change</td>
<td>95% CI</td>
</tr>
<tr>
<td>FEV1</td>
<td>71</td>
<td>37</td>
<td>2.85</td>
<td>2.6-3.11</td>
<td>0.04</td>
</tr>
<tr>
<td>FVC</td>
<td>71</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC%</td>
<td>71</td>
<td>37</td>
<td>79.6</td>
<td>77.4-81.8</td>
<td>0.3</td>
</tr>
<tr>
<td>PEFR</td>
<td>71</td>
<td>37</td>
<td>7.9</td>
<td>7.2-8.7</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Appendix 16 Summary blood pressure results

<table>
<thead>
<tr>
<th></th>
<th>overall</th>
<th>Nitrates</th>
<th></th>
<th>Placebo</th>
<th></th>
<th>Baseline</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>95% CI</td>
<td>change</td>
<td>95% CI</td>
<td>Paired t test</td>
<td>n</td>
</tr>
<tr>
<td>Resting systolic</td>
<td>71</td>
<td>38</td>
<td>135.4</td>
<td>2.0</td>
<td>-2.3-6.3</td>
<td>0.344</td>
<td>33</td>
</tr>
<tr>
<td>Resting diastolic</td>
<td>71</td>
<td>38</td>
<td>78.3</td>
<td>-1.2</td>
<td>-4.2-1.9</td>
<td>0.447</td>
<td>33</td>
</tr>
<tr>
<td>Maximal systolic</td>
<td>71</td>
<td>38</td>
<td>179.9</td>
<td>-2.6</td>
<td>-0.3-9.7</td>
<td>0.427</td>
<td>33</td>
</tr>
<tr>
<td>Maximal diastolic</td>
<td>71</td>
<td>38</td>
<td>83.9</td>
<td>-3.2</td>
<td>-10.5-4.0</td>
<td>0.376</td>
<td>33</td>
</tr>
<tr>
<td>Hypertensives excluded</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting systolic</td>
<td>49</td>
<td>27</td>
<td>132.8</td>
<td>2.5</td>
<td>-2.9-7.9</td>
<td>0.344</td>
<td>22</td>
</tr>
<tr>
<td>Resting diastolic</td>
<td>49</td>
<td>27</td>
<td>77.8</td>
<td>-0.9</td>
<td>-4.5-2.8</td>
<td>0.620</td>
<td>22</td>
</tr>
<tr>
<td>Maximal systolic</td>
<td>49</td>
<td>27</td>
<td>174.6</td>
<td>-1.7</td>
<td>-9.8-6.3</td>
<td>0.661</td>
<td>22</td>
</tr>
<tr>
<td>Maximal diastolic</td>
<td>49</td>
<td>27</td>
<td>83.9</td>
<td>-3.7</td>
<td>-13.3-5.8</td>
<td>0.428</td>
<td>22</td>
</tr>
</tbody>
</table>
## Appendix 17 Clavien Dindo scoring system

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I</strong></td>
<td>Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgesics, diuretics, electrolytes, and physiotherapy. E.g.</td>
</tr>
<tr>
<td>II</td>
<td>Requiring pharmacological treatment with drugs other than such allowed for grade I complications. E.g.</td>
</tr>
<tr>
<td>III</td>
<td>Requiring surgical, endoscopic or radiological intervention.</td>
</tr>
<tr>
<td>IIIa</td>
<td>Grade IIIa Intervention not under general anaesthesia.</td>
</tr>
<tr>
<td>IIIb</td>
<td>Grade IIIb Intervention under general anaesthesia.</td>
</tr>
<tr>
<td>IV</td>
<td>Life-threatening complication (including CNS)</td>
</tr>
<tr>
<td>IVa</td>
<td>Heart failure leading to low-output syndrome</td>
</tr>
<tr>
<td></td>
<td>Lung failure requiring intubation</td>
</tr>
</tbody>
</table>
complications) * requiring IC/ICU management  • Renal insufficiency requiring dialysis

• Grade IVa Single organ dysfunction (including dialysis)  
  • Grade IVb Multiorgan dysfunction  
  IVb  
  Same as for IVa but in combination with respiratory / renal failure / hemodynamic instability

V
Death of a patient

Suffix “d”  
Suffix “d”

If the patient suffers from a complication at the time of discharge (see examples in Table 2), the suffix “d” (for “disability”) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

• Cardiac insufficiency after myocardial infarction (IVa–d)
• Stroke with sensorimotor hemi syndrome (IVa–d)
• Residual renal insufficiency after sepsis with multiorgan dysfunction (IVb–d)

### Appendix 18 All inpatient complications classified by Clavien Dindo

<table>
<thead>
<tr>
<th>Class</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vomited once, no intervention,</td>
</tr>
<tr>
<td>1</td>
<td>Vomited once, no intervention,</td>
</tr>
<tr>
<td>2</td>
<td>Blood transfusion, anaemic (Haemoglobin 87) preoperatively</td>
</tr>
<tr>
<td>2</td>
<td>Ileus, nasogastric tube inserted</td>
</tr>
<tr>
<td>2</td>
<td>Pulmonary Embolism, had had previously, was on treatment</td>
</tr>
<tr>
<td>2</td>
<td>Blood transfusion, large PR bleed</td>
</tr>
<tr>
<td>2</td>
<td>Antibiotics UTI, Known Colo-vesicle fistula, home with catheter,</td>
</tr>
<tr>
<td>2</td>
<td>Ileus requiring nasogastric tube</td>
</tr>
<tr>
<td>2</td>
<td>Blood transfusion intraoperatively</td>
</tr>
</tbody>
</table>
Abdo pain and raised inflammatory markers, started on **antibiotics**, CT no leak / collection

Readmitted with ileus, resolved with gastrografin

2. **blood transfusion**, PR bleed, anaemic (haemoglobin 80) preoperatively

3. **Return to theatre**

Laparoscopic washout of haematoma, haematoma causing ileus, **blood transfusion**

Readmission with collection / **wound infection**

3. **Return to theatre**

Prolonged ileus requiring nasogastric tube, Acute renal failure secondary to ileostomy

Returned to theatre for early reversal

3. **Return to theatre**

TME + ileostomy, ileus, EUA for anastomotic leak, managed with endosponge. **Blood transfusion.**

3. **Return to theatre**

TME and ileostomy, Prolonged ileus, Gastrografin enema no anastomotic leak, Reversed day 11, anastomotic leak, LRTI

Returned to theatre day 16 and defunctioned

3. **Return to theatre**

TME, anastomotic leak, returned to theatre for washout and defunctioning stoma

Wound infection

5. **Death of patient**

Aspiration pneumonia secondary to ileus, Died on ITU
### Appendix 19 Complications after discharge not requiring readmission

<table>
<thead>
<tr>
<th>Complications after discharge not requiring readmission</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>UTI</td>
<td>22, 32, 48, 58</td>
</tr>
<tr>
<td>Wound infection</td>
<td>7, 45</td>
</tr>
</tbody>
</table>

### Appendix 20 Readmissions

<table>
<thead>
<tr>
<th>Readmissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>placebo Readmitted with collection (previous haematoma requiring laparoscopic washout) and wound infection</td>
</tr>
<tr>
<td>nitrates Readmitted with ileus</td>
</tr>
<tr>
<td>nitrates Readmitted with catheter problems</td>
</tr>
<tr>
<td>placebo Admitted day 25 with carbon monoxide poisoning</td>
</tr>
<tr>
<td>Readmitted from clinic day 15, CT pelvic collection, had laparoscopy and washout, classify as late anastomotic leak</td>
</tr>
<tr>
<td>nitrates Readmitted day 19-35 with ileus, resolved with GG</td>
</tr>
<tr>
<td>placebo Readmitted day 7, CT no leak, treated as catheter associated UTI</td>
</tr>
<tr>
<td>placebo Readmitted with faint, had blood transfusion</td>
</tr>
<tr>
<td>placebo Readmitted with constipation, had enema then vasovagal, home next day</td>
</tr>
<tr>
<td>nitrates Readmitted with catheter blocking and associated UTI, treated with antibiotics</td>
</tr>
</tbody>
</table>
9. Bibliography


60. Alexander J, Benford D. Nitrate in vegetables. The EFSA Journal [Internet]. 2008; 689: [1-79 pp.]


