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The oral bioavailability of nitrate from vegetables investigated in healthy volunteers

A.C. Lambers, J.M. Kortboyer, R.C. Schothorst,
A.J.A.M. Sips, R.F.M.J. Cleven, J. Meulenbelt

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RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

Abstract

The major source of human nitrate exposure comes from vegetables. Several studies were performed to estimate the total daily dietary nitrate intake based on the nitrate contents of food and drinking-water. However, only nitrate that is absorbed from the gastro-intestinal tract may contribute to the toxicity of nitrate in the body. At present no data are available on the bioavailability of nitrate from vegetables. Therefore the present study was performed to evaluate the oral bioavailability of nitrate from frequently consumed nitrate-rich vegetables.

In a crossover design 12 participants were administered 365 mg nitrate intravenously and 300 grams of spinach, lettuce and beetroot with respectively 564 mg, 1013 mg and 643 mg nitrate. Plasma samples were collected to calculate the bioavailability of nitrate from the vegetables.

With correction for the endogenous nitrate production the bioavailability for spinach was calculated on 98% ± 12%, for lettuce 113% ± 14% and for beetroot 106% ± 15%.

When the endogenous nitrate production was not taken into account, the bioavailability for spinach was calculated on 91% ± 10%, for lettuce 89% ± 13% and for beetroot 93% ± 12%. It was concluded, that the bioavailability of nitrate from spinach, lettuce and beetroot is very high.

Contents

Abbreviations

ADI	Acceptable Daily Intake
ALAT	alanine amino transferase
ARO	Laboratory for Residue Analysis
ASAT	aspartate amino transferase
AUC	area under the curve
b.p.m.	beats per minute
bw	body weight
cm	centimetres
CV	coefficient of variation
D	dose
ECG	electrocardiogram
ESR	erythrocyte sedimentation rate
exp9	times 10^9
F	bioavailability
f	female
g	gram
GCP	Good Clinical Practice
γGT	gamma glutamyl transpeptidase
GLP	Good Laboratory Practice
Hb	haemoglobin
HPIC	high performance ion chromatography
hr	hour
hrs	hours
HR	heart rate
Ht	haematocrite
iv	intravenous
kg	kilogram
l	litre
LAC	Laboratory of Inorganic Analytical Chemistry
LDH	lactate dehydrogenase
m	male
MD	Medical Degree
min	minutes
mg	milligram
mm	millimetres
mmHg	millimetres mercury pressure
mmol	millimole
µmol	micromole
$NaNO_3$	sodium nitrate

NO_2^-	nitrite-ion
NO_3^-	nitrate-ion
NVIC	National Poisons Control Centre
or	oral
RAA-axis	renin-angiotensin-aldosteron axis
RIVM	National Institute of Public Health and the Environment
SD	standard deviation
SOP	standard operating procedure
t_{max}	time to maximum concentration
$t_{1/2}$	elimination half-life
U/l	units per litre
UMC	University Medical Center Utrecht
UV	ultraviolet
VSS	volume of distribution in steady state
WHO	World Health Organisation
WOM	Medical Ethics Committee of the University Medical Center Utrecht
yrs	years
y/m/d	year/month/day

Samenvatting

Het voorkomen van nitraat en nitriet in voedingsmiddelen is een onderwerp waar autoriteiten zich al lange tijd mee bezig houden. Risicoschattingen hebben geleid tot een maximaal dagelijkse toelaatbare hoeveelheid (ADI) van 3,65 mg nitraat-ion per kg lichaamsgewicht. Deze ADI was op rattenstudies gebaseerd. Momenteel wordt er gewerkt aan een risicoschatting op basis van humane gegevens. Daarvoor is door het RIVM een serie kinetische onderzoeken met betrekking tot nitraat en nitriet uitgevoerd, waaronder een onderzoek naar de biobeschikbaarheid van nitraat uit groente.

De grootste bron van nitraatblootstelling is groente. Voor zover bekend is op dit moment de nitraatinname van het grootste deel van de Nederlandse bevolking onder de ADI van 3,65 mg/kg lichaamsgewicht. Echter door het eten van nitraatrijke groente kan de ADI gemakkelijk overschreden worden. Het nitraat dat door het maagdarmkanaal wordt geabsorbeerd zou kunnen bijdragen aan de toxiciteit van nitraat. Op dit moment zijn er geen gegevens beschikbaar over de biobeschikbaarheid van nitraat uit groente. In dit onderzoek is daarom de biobeschikbaarheid van nitraat uit drie regelmatig geconsumeerde nitraatrijke groenten geëvalueerd.

De studie opzet was een cross-over design met 4 testperiodes, waarin twaalf deelnemers (zes mannen en zes vrouwen) het volgende kregen toegediend: A 500 mg natriumnitraat (bevattende: 365 mg nitraat) intraveneus, B 300 gram spinazie (bevattende: 564 mg nitraat), C 300 gram verse kropsla (bevattende: 1013 mg nitraat) en D 300 gram gekookte rode bieten (bevattende: 643 mg nitraat). Tussen twee testperiodes zat een wash-out periode van tenminste een week. De groenten en het infuus werden in nuchtere toestand toegediend.

De biobeschikbaarheid van nitraat uit groente werd berekend door blootstelling aan nitraat na toediening van groenten te vergelijken met de blootstelling aan nitraat na toediening via een infuus. Tevens werd de biobeschikbaarheid van nitraat uit groente berekend met en zonder rekening te houden met de endogene (background) nitraatproductie. De blootstelling aan nitraat werd bepaald door nitraatconcentraties in plasma.

Er werd een terminale eliminatie halfwaardetijd van 6-7 uur gevonden voor nitraat uit plasma. De biobeschikbaarheid met correctie voor de endogene nitraatproductie was voor spinazie 98% ± 12%, voor sla 113% ± 14% en voor bieten 106% ± 15%. De biobeschikbaarheid zonder correctie voor de endogene nitraatproductie was voor spinazie 91% ± 10%, voor sla 89% ± 13% en voor bieten 93% ± 12%.

Er kan worden geconcludeerd, dat de biobeschikbaarheid van nitraat uit de onderzochte groenten zeer hoog dan wel bijna compleet is.

Summary

The occurrence of nitrate and nitrite in food has been subject of concern to health authorities for a long time. Risk assessments have led to the establishment of an Acceptable Daily Intake of nitrate of 3.65 mg nitrate ion per kg bw. This ADI was extrapolated from rat experiments. At the moment a risk assessment is formed based on human data. Therefore series kinetic studies were performed by the National Institute of Public Health and the Environment. One of these studies is research on the bioavailability of nitrate from vegetables.

Vegetables form the major source of human nitrate exposure. At present, the nitrate intake of the major part of the population in the Netherlands is below the Acceptable Daily Intake (ADI) of 3.65 mg nitrate ion /kg bw. However, the ADI may easily be exceeded by eating vegetables with a high nitrate content. Nitrate that is absorbed from the gastro-intestinal tract may contribute to the toxicity of nitrate. At present no data are available on the bioavailability of nitrate from vegetables. In the present study, therefore, the oral bioavailability of nitrate from three frequently consumed nitrate-rich vegetables was evaluated.

In a cross-over design with four test-periods 12 participants (six men and six women) were administered the following treatments: A intravenous 500 mg sodium nitrate (containing: 365 mg nitrate), B 300 gram spinach (containing: 564 mg nitrate), C 300 gram fresh lettuce (containing: 1013 mg nitrate) and D 300 gram cooked beetroot (containing: 643 mg nitrate). Between the different test-periods was a wash-out period of at least one week. The treatments were administered to volunteers in fasting state.

The bioavailability of nitrate from vegetables was calculated by comparing exposure to nitrate after vegetable administration and exposure to nitrate after intravenous nitrate administration. Thereby bioavailability of nitrate from vegetables was calculated with and without taking into account the endogenous nitrate production. The nitrate exposure was determined by nitrate concentrations in blood.

An elimination half-life of 6-7 hours was observed for nitrate from plasma. The bioavailability with correction for endogenous nitrate production was for spinach 98% ± 12%, for lettuce 113% ± 14% and for beetroot 106% ± 15%. The bioavailability without correction for endogenous nitrate production was for spinach 91% ± 10%, for lettuce 89% ± 13% and for beetroot 93% ± 12%. Thus the bioavailability of nitrate from spinach, lettuce and beetroot is very high.

1. Introduction

The occurrence of nitrate and nitrite in food has been subject of concern to health authorities for a long time. Risk assessments have led to the establishment of an Acceptable Daily Intake of nitrate of 3.65 mg nitrate ion/kg body weight (bw). The ADI allocated to sodium nitrate by the WHO was extrapolated from the no-adverse effect level as observed in chronic rat experiments. Nitrate however is generally considered to be of low toxicity. The toxicity of nitrate has been ascribed to the conversion of nitrate into nitrite by bacterial reduction in the oral cavity (1,2) and gastro-intestinal tract (3,4). At the National Institute of Public Health and the Environment a risk assessment will be performed based mainly on human data. Therefore several kinetic studies on nitrate and nitrite in healthy volunteers have been performed. This report describes the bioavailability of nitrate from certain vegetables.

1.1 Background

Nitrate (NO_3^-) that is absorbed from the gastro-intestinal tract is partly transported from the blood into the salivary gland and excreted via saliva. Within the oral cavity, part of the nitrate is subsequently converted to nitrite (NO_2^-) by bacterial reduction. The conversion of nitrate into nitrite in humans was investigated in several human volunteer studies with different doses and formulations (5,6,7). In general it was found that approximately 27% of an oral nitrate dose is excreted into the saliva and around 25% is subsequently reduced to nitrite in the oral cavity leading to approximately 7% conversion of a nitrate dose into nitrite. The nitrite that is formed in the saliva, may be swallowed and subsequently absorbed from the gastro-intestinal tract, and thus may lead to systemic nitrite toxicity. Besides the exogenous exposure to nitrate, endogenous synthesis of nitrate within the body is an important source contributing to the body burden of nitrate. Nitrate is formed, endogenously, as the stable end product of nitric oxide. Nitric oxide is formed in many different cell types in the body through a common biochemical pathway that involves the oxidation of L-arginine. Nitric oxide is a cellular messenger and seems to play a major role as a messenger in several cell functions (8). The estimated daily endogenous production of nitrate, via the L-arginine-nitric oxide-nitrate pathway, is approximately 60 mg/day. The endogenous nitrate production may increase many folds during inflammatory processes within the body (9). Nitrate that is formed endogenously may also contribute to the formation of nitrite via excretion into the saliva.

It has been estimated that oral nitrate reduction represents 80% of the individual nitrite exposure (5,10,11). The other 20% of human nitrite exposure is coming from foods to which nitrite was added as a preservative or for other reasons, i.e. meat-products. The most important acute effects of nitrite exposure are methaemoglobinemia, vasodilatation and gastro-intestinal disturbances.

Nitrite gave positive results in a battery of in vitro and in vivo mutagenicity test systems (12,13). However, various carcinogenicity studies with nitrite in the rat were negative (14,15).

Although nitrite itself is considered not carcinogenic, the nitrite converted in the oral cavity from nitrate may react, in the stomach, with nitrosatable agents in the food to form carcinogenic N-nitroso compounds. N-nitrosocompounds are associated with an increased risk for (gastric) cancer (16). So far, there is no convincing evidence for a causal association between nitrate intake and cancer risk (17).
Two rat experiments showed that nitrite induced hypertrophy of the zona glomerulosa of the adrenals (18,19). In rats this hypertrophy was reversible after withdrawal of the nitrite exposure. In a recent study this hypertrophy of the zona glomerulosa of the adrenals in rats turned out to be a physiological adaptation to the vasodilatation induced by nitrite The adrenal gland plays a role in the regulation of blood pressure via the renin-angiotensin-aldosteron axis (RAA-axis). The absence of nitrite-induced hypertrophy in rats given ramipril, an inhibitor of angiotensin converting enzyme, indicated that the RAA-axis was involved in the etiology of the hypertrophy of the adrenal zona glomerulosa. This suggested that hypertrophy of the adrenal zona glomerulosa can be considered to be a physiological adaptation to repeated episodes of hypotension caused by nitrite. (20).

The major source of human nitrate exposure comes from food and drinking water. The estimated average intake of nitrate in the Netherlands ranges from <163 mg to 243 mg nitrate ion per day. The average nitrate intake of the major part of the population in the Netherlands is below the ADI of 3.65 mg nitrate ion / kg bw (17). However, the ADI is easily exceeded, when vegetables with a high nitrate content are eaten.
Nitrates in food may be present naturally or as an additive introduced for various technological reasons. The latter is strictly limited by regulatory control and only minor contributes to the total nitrate exposure. Non-additives nitrate in food mainly stems from vegetables. Vegetables provide approximately 80% of the average daily dietary nitrate intake (23). There is a wide variation in the nitrate content of vegetables depending on the type of vegetable, its source, conditions of cultivation and storage. Greenhouse vegetables generally contain higher nitrate levels then outdoor grown vegetables. During winter, when the light-intensity and the temperature are low, the nitrate content of the vegetables is also higher (17).
The nitrate levels in vegetables tend to increase over the years in Western Countries. Inadequate agricultural practice, especially the use of chemical fertilisers and manure, is an important cause for the increasing nitrate concentrations in certain vegetables. In various European countries growers encounter problems because they cannot meet the standards set for nitrate based on the present ADI. Under extreme conditions concentrations of nitrates in certain types of vegetables may reach levels up to 10.000 mg per kg (21). In the Netherlands regulatory limits for the nitrate content of certain vegetables exist. It concerns vegetables which constitute a high nitrate content and which are frequently consumed. These vegetables are lettuce, spinach, endive and beetroot (see Appendix 2).
Dejonckheere et al measured the nitrate loss of a number of vegetables after normal culinary practice such as washing, peeling, cooking and stewing (22). Washing of leafy vegetables (lettuce, endive) with tap water reduced the nitrate concentration with 10-15% and for lettuce

the elimination of the thick midrib resulted in a decrease of the nitrate content of 30-35%. Stewing spinach and carrots resulted in higher nitrate concentrations due to the loss of water. The nitrate content is decreased with 40% by eliminating the stem and midrib of the spinach before stewing because the nitrate content in these plant parts is two to three times higher than in leaf tissue. During cooking nitrate is partitioned between the boiling liquid and the leaf tissues. Thus the nitrate concentration decreases more by using an abundant amount of boiling water and by careful leaking of the water after cooking.

In figure 1 the contribution of different food products to the total dietary nitrate intake is presented, in which loss due to preparation is taken into account. Within the group of the leafy vegetables the main contribution to the nitrate intake comes from endive (36%), lettuce (31%) and spinach (29%). As for the total nitrate intake potatoes, endive, lettuce and spinach each contribute approximately 9% to the daily nitrate intake (23).

During inadequate storage practices of foods nitrite may be formed from nitrate. Under normal circumstances the nitrite content of vegetables is negligible.

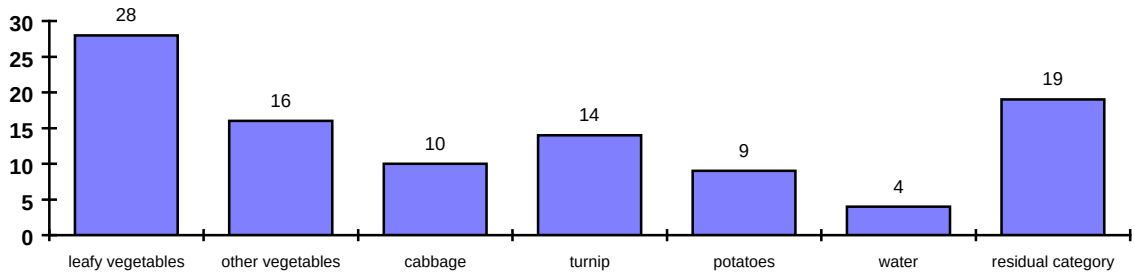

Figure 1. Contribution of food products to the total nitrate intake (%) accounted for nitrate loss due to culinary preparation

The ADI allocated to sodium nitrate by the WHO was extrapolated from the no-adverse effect level as observed in chronic rat experiments. Most animal toxicity studies were performed with aqueous oral nitrate solutions. Nitrate may be readily available from aqueous solutions. However, human nitrate exposure mostly occurs via intake of foods. The assessment of human nitrate exposure from dietary sources does not take into account that the gastro-intestinal absorption of nitrate may depend on the matrix in which the nitrate is present in the diet. Only nitrate that is indeed absorbed from the gastro-intestinal tract may contribute to the toxicity of nitrate. At present no data are available on the bioavailability of nitrate from food. A low bioavailability of nitrate from food may have implications for the risk analyses of nitrate exposure.

Since vegetables are the largest source of nitrate intake from food, in this study the bioavailability from vegetables was examined. Vegetables with regulatory limits (spinach, lettuce and beetroot) were chosen. From an earlier study it appeared that the bioavailability from these vegetables could be investigated without being confronted with procedural problems (24).

The normal daily consumption of vegetables is estimated to be between 100 and 200 gram, depending on the type of vegetable. However, to increase the oral intake of nitrate, in the

present study, 300 grams of vegetables were administered to the volunteers, in order to be able to investigate the bioavailability of nitrate properly. Furthermore, the vegetables were administered in fasting state in order to investigate the worst case scenario with respect to the bioavailability.

2. Materials and Methods

2.1 Study protocol

The present study was carried out conform the protocol entitled "The oral bioavailability of nitrate from vegetables investigated in healthy volunteers (project number 348801 006, project 235802, dated 28-05-1997, revised 15-07-1997)". The Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands, approved the study protocol.
The study was performed under the recommended principles of Good Clinical Practice for studies with medicine in the European Community.
The participants in the study were fully informed, both orally and in writing, about the purpose of the study, the study design and the possible risks involved. The volunteers signed a written statement of consent (Appendix 3).

2.2 Study population

Twelve volunteers (six men and six women) in the age of 21 to 28 years participated in the study (see Appendix 4). They were all non-smokers and did not use any medication except for an oral contraceptive. The participants were recruited by announcements on the bulletin boards in the building of the Utrecht University and through direct mailing to former participants or individuals that had notified us to be willing to participate in healthy volunteer studies.
One month to one week before the start of the study, 18 volunteers, willing to participate, were screened for enrolment by a pre-study medical examination. The medical examination consisted of a standard medical history questionnaire, a physical examination, 12-lead electrocardigraphy (using a Hewlett Packard cardiograph, type 4700 A) and non-invasive automated blood pressure measurement (using a Passport Monitor of Datascope®). Furthermore, blood and urine samples were collected for routine laboratory analyses (see section 2.5.4). Physicians other then the medical investigators involved in this study performed the pre-study medical examinations. The participants were finally selected for enrolment in the study on basis of the results of the medical screening and the selection criteria as summarised in Appendix 5.
If the routine blood and urine analyses revealed abnormal values, the clinical relevance was determined. If the results were considered to be of no clinical relevance the volunteer was included in the study. Otherwise, a new blood or urine sample was collected and analysed until normal results were obtained or the volunteer was excluded from participation in the study. Five of the volunteers who underwent the medical screening were excluded, one was reserve and twelve volunteers entered the study (see also section 3.3).
The General Practitioner was informed when his/her patient was going to participate in the study and was asked to inform the medical investigator when he/she disagreed on the decision to enrol the subject in the study.

After the study was completed the volunteers underwent a post-study medical examination. The volunteers were asked to report any change in their health status as compared to the pre-study medical screening. Furthermore, the physical examination and routine blood and urine investigations were repeated.

2.3 Products under study

2.3.1 Sodium nitrate

For intravenous administration of nitrate a sodium nitrate solution was sterilely prepared in one batch by the Pharmacy of the University Medical Center Utrecht. Sodium nitrate (CAS-no. 7631-99-4) was dissolved in distilled water with an isotonic concentration of 14 mg/ml $NaNO_3$ and delivered in vials of 100 ml. During the study the vials were stored in dark at room temperature.

2.3.2 Vegetables

The lettuce and the beetroot were purchased from a vegetable auction. The nitrate content of these vegetables was determined directly when they were brought in from vegetable auction by means of a quick nitrate analysis test (see section 2.5.1). The vegetables were rejected for the study in case the nitrate content was less then 1200 mg nitrate per kg product. The nitrate content in the batch of deep freeze spinach was already determined in a previous study and above 1200 mg per kg product (24).

The procedures for the culinary preparation of the vegetables were as much as possible copied from the home-situation. Extra care was taken to thoroughly homogenise the vegetable batch.

Spinach

One batch of sliced deep-freeze spinach in packages of 450 gram (lotnumber L 7143 BD 005, expiry date 05-1999), was kindly provided by IGLO-OLA BV, the Netherlands on July 1, 1997. The spinach was stored in the dark at -25 to -30 ^{o}C until usage.

Approximately 1.5 hours prior to their administration in the study packages of 450 gram of sliced deep-freeze spinach were taken out of the freezer and distributed over microwave boxes. Approximately 30 minutes prior to the start of the study the microwave boxes were each put in a microwave (Whirlpool) and heated for 20 minutes at 900 watt. The heated spinach was leaked and thoroughly mixed manually. Portions of 300 grams spinach were weighted (Sartorius Portable Balance isoCAL LC 2201s) and collected in plastic salad bowls and polypropylene boxes. Thereafter the spinach in the salad bowls was administered to the volunteers. Six polypropylene boxes with spinach were stored at -80^{o}C and analysed for the nitrate content.

Lettuce

Greenhouse lettuce, grown in the Netherlands, was obtained the day before it was used in the study from vegetable auction “Zon in Venlo” under auction number 84-1727 and delivered by

van Dijk BV, Heteren. The lettuce was kept in the dark and refrigerated at 3 - 6 ^{o}C until usage.

Preparation of the lettuce took place less than two hours prior to their administration to the volunteers. Crops of lettuce were cut in two. From each half the thick midrib was removed. The lettuce was then sliced into strips of approximately 2 cm. All lettuce was collected in a container and thoroughly mixed manually for several minutes. Portion of 300 gram lettuce were weighted and collected in plastic salad bowls (for the volunteers) and polypropylene boxes (for nitrate analyses). Thereafter, each portion of lettuce was washed with water, centrifuged and collected again in the salad bowls and polypropylene boxes. The lettuce in the salad bowls was administered to the volunteers. Eight polypropylene boxes with lettuce were stored at -80^{o}C and analysed for the nitrate content.

Beetroot

Raw beetroot, grown in the Netherlands, was obtained on 11 November 1997 and delivered by van Dijk BV, Heteren. The beetroot was kept in the dark and refrigerated at 3 - 6 ^{o}C until usage.

Beetroot was prepared less than two hours prior to administration to the volunteers. Approximately 6 kg of beetroot was cooked in a standard 40 litres-pan in the Central Kitchen of the University Medical Center Utrecht. Seven litres of boiling water were added. The beetroot was cooked for 35 minutes. After peeling the beetroot was sliced in particles of 5*5*5 mm with a slicing machine. A sample of the boiling water was frozen at -80^{o}C and analysed for the nitrate content. The beetroot was thoroughly mixed manually for several minutes. Portions of 300 grams beetroot were weighted and collected in plastic salad bowls and polypropylene boxes. Thereafter the beetroot in the salad bowls was administered to the volunteers. Eight polypropylene boxes with beetroot were stored at -80^{o}C and analysed for the nitrate content.

2.4 Study design

2.4.1 General

In an open, single dose study 12 healthy adult volunteers received each of the following treatments:

Treatment A = Single dose intravenous administration of 500 mg sodium nitrate
Treatment B = Single dose oral administration of 300 gram spinach (deep-freeze)
Treatment C = Single dose oral administration of 300 gram of raw lettuce
Treatment D = Single dose oral administration of 300 gram of cooked beetroot

The vegetables were administered in fasting state and fasting was maintained until 4 hours post dose. Clinical observation of the participants took place from approximately 1 hour before until 24 hours after administration of the vegetables. During this period blood samples were taken at timed intervals (see Appendix 6).

There was a washout period of at least one week between the treatments. The treatments were preceded by a 3-day nitrate and nitrite low diet (see Appendix 7).

2.4.2 Procedures

The volunteers arrived in fasting state at the Clinical Research Unit at approximately 08.00 hr. After handing over their food diary, a pregnancy test (TestPack Plus^{tm} hCG-urine, ABBOTT) was performed in female volunteers. The body weight of all volunteers was determined (Seca balance, Schinkel Medical Instruments) and the blood pressure and heart rate were recorded with a non-invasive automated blood pressure meter (Passport Monitor® of Datascope). An intravenous cannula was inserted in a forearm vein and a baseline blood sample was obtained.

At approximately 09.00 hr the volunteers received a single intravenous administration of 500 mg sodium nitrate infused over 30 minutes (study day A) or a single dose of 300 gram vegetables (beetroot, lettuce, spinach; study days B, C and D). The cannula for intravenous infusion was inserted in a forearm vein, in the opposite arm to which the cannula for blood sampling was inserted. The responsible physician made sure that the possibility of subcutaneous administration of the sodium nitrate was excluded. The sodium nitrate was administered using an IVAC^{R} 560 infusion pump (SOP NVIC/048). The volume that was infused was programmed into the pump. The first hour after the infusion had been started blood pressure and heart rate were checked every 10 minutes.

The vegetables were consumed with 100 ml distilled water. After intake of the vegetables the salad bowl was rinsed with 100 ml distilled water. This water was subsequently swallowed by the volunteer, simultaneously rinsing the mouth to remove remaining vegetable in the mouth.

The volunteers remained in bed in sitting position for 3 hours following intake of vegetables. The participants remained in the Clinical Research Unit for 24 hours following treatment. The volunteers were frequently asked about adverse effects during the day (see Appendix 6).

At scheduled time points, for 24 hours following the administration of the vegetables, blood samples of 5 ml were taken from a cannula inserted in a forearm vein. Blood sampling was performed without using tourniquet pressure. The blood samples were collected in heparinised tubes. The samples were immediately centrifuged for 10 minutes at 3000 r.p.m. and 4 ^{o}C. The supernatant (plasma) was transferred into polypropylene tubes and immediately frozen in liquid nitrogen. The plasma samples were subsequently stored at -80^{o}C until analysis for nitrate and nitrite content. The scheduled time points for blood sample collection are presented in Appendix 6. The total amount of blood taken from the participant during the investigation (including haematology and clinical chemistry tests) was approximately 465 ml.

2.4.3 Diet

Three days prior to a study day the volunteers consumed a nitrite and nitrate low diet. The dietary restrictions are presented in Appendix 7. The actual food intake was registered in a food diary.

The volunteers received a nitrate and nitrite low diet at fixed time points during the study days (see Appendix 6). This diet consisted of bread, butter, jam, tea, milk, apple and for dinner green peas, rice, meat ragout and custard. At 3 hours post dose (around 12.00 hr) 200 ml distilled water was administered to the volunteers. Thereafter distilled water was taken ad libitum.

2.5 Sample analysis

2.5.1 Quick nitrate analysis test

One day prior to the study day lettuce and beetroot were purchased from the vegetable auction and a quick nitrate analysis test was performed. At random 10 crops of lettuce and 10 beetroots were taken from the batch. A sample size of 10 gives a reasonable prediction of the true nitrate content of the vegetables within the batch.

Lettuce

The ten crops of lettuce were cut in two. One half of each crop was discarded. The thick midrib of the other half was removed. The ten lettuce crops were then weighted (Sartorius Portable Balance isoCAL LC 2201s) and distributed over 2 microwave boxes (in every box 5 half crops). The boxes were put in the microwave (Whirlpool) for 4 minutes at 900 watt and were weighted again. The contents of each box were subsequently put in a blender. After adding 200 ml distilled water to the blender the lettuce was blended for approximately 2 minutes (dilution factor = (200 + weight of the lettuce)/weight of the lettuce). A portion of 10 to 15 grams of the homogenated lettuce was put in water of 80 ^{o}C for 15-20 minutes. Thereafter, 100 ml distilled water was added and the lettuce was filtered over a filter (SNS filters 595 1/2, 150 mm). The jug was rinsed with 50 ml water which was also filtered and the filtrate was filled to 150 ml in total (dilution factor = (150 + weight homogenated portion)/ weight of the homogenated portion). The filtrate was analysed for the nitrate content.

Beetroot

The ten beetroots were cut in two. One half of each beetroot was discarded. The other half was cut into small pieces. The beetroot halves were then weighted per 5 halves (Sartorius Portable Balance isoCAL LC 2201s) and distributed over 2 containers. The contents of each container were put in a blender. After adding 350 ml distilled water to the blender the beetroot was blended for approximately 2 minutes (dilution factor = (350 + weight of the beetroot)/weight of the beetroot). A portion of 10 to 15 grams of the homogenated beetroot was put in water of 80 ^{o}C for 15 minutes. Thereafter, 100 ml distilled water was added and the beetroot was filtered over a filter (SNS filters 595 1/2, 150 mm). The jug was rinsed with 50 ml distilled water which was also filtered and filled to 150 ml in total (dilution factor = (150 + weight homogenated portion)/weight of the homogenated portion). The filtrate was analysed for the nitrate content.

The nitrate analyses of the filtrate from the beetroot and the lettuce was performed by the Laboratory of Inorganic Analytical Chemistry by means of HPIC. Because of the tight time schedule concessions were done to the accuracy of the results, i.e. with each series of samples

only 2 calibration-standards, enclosing the expected nitrate levels of the samples, were analysed. These adjustments were considered justifiable because the aim of the nitrate analysis test was only to provide an estimate of the nitrate level of the lettuce and the beetroot, to check whether the batch fulfilled the criterion of 1200 mg nitrate per kg vegetable. The lower limit of quantification of nitrate was 0.002 mmol/l.

Spinach

The spinach used came from the same batch as the batch used in the pilot study. At that time a “quick nitrate analysis” had already been performed and the procedure is described in a previous report (24).

2.5.2 Nitrate analyses of the vegetables

The nitrate content of the frozen vegetable portions were determined by the Laboratory for Residue Analysis by means of a previously established method based on High Performance Ion Chromatography and UV detection at 208 nm. Prior to the analyses, the vegetables were freeze-dried. All samples were analysed in triple (CV < 5%). Simultaneously with the study samples a nitrate added sample was frequently analysed (CV < 5%). Two samples from a prior study served as control samples for the nitrate analyses. The lower limit of quantification of nitrate in vegetables was 2 mg/kg.

2.5.3 Plasma nitrate and nitrite analyses

Concentrations of nitrate and nitrite in plasma were determined by the Laboratory of Residue Analysis by means of a previously established method based on High Performance Ion Chromatography and UV detection at 208 nm. The method is optimised to ensure a limit of quantification for plasma nitrate that is below baseline nitrate concentrations in the plasma. With each series of samples a duplo sample (CV < 10%), a blank sample and a spiked human plasma sample (CV < 10%) were analysed. Also recovery experiments were performed during the analytical sessions. All recovery experiments showed a recovery for nitrate and nitrite within 100 ± 10%. The lower limit of quantification was 0.2 mg/kg for both plasma nitrate and plasma nitrite.

2.5.4 Routine laboratory analyses of blood and urine specimen

During the pre- and post-study screening blood and urine samples were taken for routine laboratory investigations. The following parameters were determined: Blood analyses on Hb, Ht, leukocytes (+differentiation), sodium, potassium, urea, calcium, creatinin, bilirubin, ASAT, ALAT, LDH, γGT and ESR. The precision of the test-results was assured by interlaboratory surveillance procedures ("ring validation" method). Dipstick urine analyses on glucose, protein, blood and leukocytes were performed.

2.6 Data analysis

All kinetic parameters were first determined without any correction for baseline plasma nitrate concentration.

Kinetic analyses were performed on plasma nitrate concentrations with the kinetic computer programme Topfit 2.0 (25). Terminal elimination half-life ($t_{1/2}$), the area under the curve till 24 hours (AUC_{0-24}) and the volume of distribution (VSS) were calculated on the basis of non-compartemental analyses.

The terminal half-life was calculated from 4 hours onwards till 12 hours in all analyses.

The AUC_{0-24} was calculated from the data points, using the linear trapezoidal rule. The AUC_{0-24} was calculated in two different ways. First, the AUC_{0-24} was corrected for baseline (background) nitrate concentration. Second, the AUC_{0-24} was calculated without correction for the baseline nitrate concentration. It was assumed that the baseline plasma nitrate concentration reflects a steady state situation during the day. The correction was made by: nitrate concentration $_{\text{time x}}$ - baseline. Baseline was the average of the first two plasma nitrate samples taken on a study day before administration of $NaNO_3$-solution or vegetables. The corrected and the uncorrected nitrate concentrations were imported in Topfit and subsequently the AUC_{0-24} was calculated. If the measured nitrate plasma concentration at 21 and/or 24 hours after the administered dose (D) was below the baseline nitrate concentration (the difference was below zero), the AUC_{0-24} of that volunteer was excluded for calculation of the bioavailability.

The absolute bioavailability (F) of nitrate was then calculated by the formula:

$$F = \frac{AUC_{or}}{AUC_{iv}} \times \frac{D_{iv}}{D_{or}}$$

In this formula first the AUC_{0-24} with correction for baseline nitrate concentration were used. Also the bioavailability without correction for baseline nitrate concentration was calculated. Paired t-tests were performed to investigate, whether the bioavailability with and without correction for baseline nitrate concentration significantly differed within a vegetable.

2.7 Study sites

- The clinical part of the healthy volunteer study was performed at the Clinical Research Unit of the National Poisons Control Centre in Utrecht, the Netherlands.
- The routine laboratory analyses of the blood samples obtained from the volunteers during pre- and post-study screening were performed by the Department of Clinical Chemistry of the University Medical Center Utrecht (UMC), the Netherlands.
- The urine specimens obtained from the volunteers during the pre-study screening were analysed at the Clinical Research Unit of the National Poisons Control Centre in Utrecht, the Netherlands.

- The analyses of the plasma samples for nitrate and nitrite were performed by the Laboratory for Residue Analysis (ARO) of the National Institute of Public Health and the Environment (RIVM) in the Netherlands.
- The culinary preparation of the lettuce and the beetroot took place in the Central Kitchen of the University Medical Center Utrecht (UMC), the Netherlands.
- The spinach was prepared in the Clinical Research Unit of the National Poisons Control Centre in Utrecht, the Netherlands.
- The quick nitrate analyses of the vegetable batch were performed by the Laboratory for Inorganic Analytical Chemistry (LAC) of the National Institute of Public Health and the Environment (RIVM) in the Netherlands.
- The nitrate analyses of the frozen duplicate vegetable portions were performed by the Laboratory for Residue Analysis (ARO) of the National Institute of Public Health and the Environment (RIVM) in the Netherlands.

2.8 Archiving

All study documents, including this report will be kept on file in the GCP-archives of the Department of Medical Toxicology of the National Poisons Control Centre of the Netherlands for a period of at least 15 years.

3. Results

3.1 General

No major protocol violations occurred. In cases the study procedures were not in full compliance with the protocol this was registered. The deviations to the protocol are summarised in Appendix 8. The deviations to the protocol had no impact on the outcome of the study.

3.2 Nitrate analyses of the vegetables

In Appendix 9 the results of the vegetable nitrate analyses are given. Before the vegetables were administered to the volunteers the nitrate content was checked with a quick nitrate analysis test. One batch of spinach was used for both the pilot-study as this study. For the pilot-study a quick nitrate analysis test had already been performed and the nitrate content of the spinach was analysed on 2082 mg/kg. The first batch of beetroot was analysed on 482 and 379 mg/kg (average 431 mg/kg) nitrate. This batch was not used, because it was below the limit of 1000 mg/kg. The second batch was analysed on 1901 and 3234 mg/kg (average 2568 mg/kg) nitrate. This batch was used. Lettuce was analysed on 3842 and 3742 mg/kg (average 3792 mg/kg) nitrate with the quick nitrate analysis test.

During the study the nitrate content was measured at the Laboratory for Residue Analysis. The average nitrate content of spinach, lettuce and beetroot were respectively 1880, 3017 and 2144 mg/kg. As expected these nitrate values are somewhat lower than the nitrate values got with the quick nitrate analysis test, because the vegetables collected for analysis underwent culinary preparation.

3.3 Pre-study medical screening

Between 23 September 1997 and 30 September 1997 18 volunteers underwent a medical screening. Twelve persons entered and completed the study (see also section 2.2). The medical history and the physical examination of these volunteers did not reveal abnormalities, which were considered to have implications for their participation in the study. The routine laboratory test results of the blood showed no or minor abnormalities of no clinical relevance as judged by the physician. All dipstick urine analyses were negative, except for volunteer 4, who had a positive result for leukocytes. Eight days later a repeated test was negative for leukocytes. In Appendix 10 the results of the routine laboratory analyses and the dipstick urine test are tabulated.

One person entered the study as possible replacement, but did not receive any treatment, because all the other volunteers completed the study.

Five volunteers were excluded from the study. One person had a remained elevated plasma bilirubin concentration, when the test was repeated. Another had elevated activity of liver

enzymes (γGT, ASAT and ALAT). A third person was excluded because of an elevated ESR in combination with a positive urine test for leukocytes and blood. One person had a history of endometriosis and after an operation of 2 and a half years earlier the complaints had still not completely disappeared. The fifth person got dizzy, was heavily sweating, almost collapsed during blood sampling and the ECG showed ventricular extra systoles. This person recovered almost immediately.

3.4 Post-study medical screening

The post-study medical screenings were performed on 18, 19 and 20 November 1997. In general no changes in health condition were observed compared to the pre-study medical screening.

Volunteer 2 experienced eczema in both fossa cubiti, this was not due to the study. Volunteer 9 had at the left wrist a mild phlebitis visible at the site of the intravenous cannula, which developed after the third and fourth study period on 07-11-1997 and 13-11-1997. There was no erythema, but a mild swelling and pain on palpation on the vein. The volunteer was followed till 07-05-1998. The swelling could be palpated, but was not visible anymore and there was still improvement. According to the volunteer there was still no filling of the vein under tourniquet pressure. With this situation the medical dossier was closed.

The results of the routine laboratory tests and the vital signs are tabulated in Appendix 10. Some volunteers had a heart rate above 100 b.p.m. in most cases due to physical activity prior to the medical screening. Repeated measurements showed lower heart rates. Abnormalities seen in the results of the laboratory and urine tests were judged as of no clinical relevance by the physician, who did the medical screening. In some cases additional measurements were performed:

Volunteer 1 had an elevated bilirubin of 19 μmol/l. In addition direct bilirubin and alkaline phosphatase were determined. Because these parameters were in the normal range, the elevated bilirubin was judged as of no clinical relevance. The ESR was repeated for volunteer 2. The repeated ESR of 8 mm/hr was in the normal range. Volunteer 6 had an elevated bilirubin (25 μmol/l) level. Direct bilirubin was within the normal range (4 μmol/l). During pre-study screening this volunteer had also an elevated bilirubin (23 μmol/l) level, though it had no clinical implications. Volunteer 10 had an elevated ESR of 10 mm/hr. This was repeated and decreased to 6 mm/hr. This slight elevation was judged to be of no clinical relevance.

3.5 Study dates and treatments

The clinical part of the study was performed between 13 October and 13 November 1997. For the exact dates see Appendix 11.

The mean nitrate content for the spinach, lettuce (inclusive some wash-water) and beetroot after culinary preparation were respectively 1880, 3017 and 2144 mg/kg vegetable (see

Appendix 9). The weight of the administered spinach and beetroot portions was between 300.0 gram and 300.8 gram. The calculated nitrate contents of the vegetables were not corrected for these slight differences. The nitrate content of the lettuce is only known inclusive the wash-water that was cling to the lettuce. Six lettuce portions with wash-water were weighted and the weights varied from 321 g to 380 g and the average was 336 g. This average was taken to calculate the amount of nitrate for lettuce. The amounts of nitrate in the administered vegetable portions were for spinach 564 mg, for lettuce 1013 mg and for beetroot 643 mg.
All volunteers ate the spinach within 30 minutes. To eat the lettuce it took longer than 30 minutes for volunteers 1, 3, 4, 9 and 11, respectively 39, 43, 44, 46 and 38 minutes. The beetroot was eaten in respectively 32, 46, 36 and 41 minutes by volunteers 1, 3, 9 and 10. (See Appendix 12).

3.6 Adverse effects

Most of the volunteers did not experience any adverse effects. The volunteers who experienced adverse effects are summarised in Appendix 13. Two volunteers had adverse effects, which were possibly related to the treatment. After infusion of the sodium nitrate volunteer 3 had a headache several times that day, had been nauseous for 10 minutes after 3¾ hour and experienced head discomfort for 5 hours and 50 minutes 4 hours after infusion of the sodium nitrate. Volunteer 4 experienced a headache 5 hours after administration, which became worse after 10 hours.
After consuming the spinach volunteer 3 had a headache for 6 hours and 15 min starting 11½ hours after treatment. Volunteer 4 had a mild headache for 18 hours, which started 3 hours after administration. After 12 hours and 25 minutes volunteer 4 became nauseous and vomited three times.
After the consumption of the lettuce volunteer 3 experienced a mild headache for 10 hours starting after 3 hours. After 5 hours volunteer 3 became nauseous and vomited 7 times (the first time about 6 hours after lettuce intake). Volunteer 4 experienced a moderate headache after 3½ hours during 9 hours and 25 minutes.
After the intake of the beetroot volunteer 3 was nauseous for 1 hour starting after 9 hours. She had a headache after 10 hours, which lasted for 3 hours and vomited one time after 13 hours.

3.7 Plasma nitrate concentrations

In Appendices 14-17 individually plasma nitrate concentrations are tabulated. In these Appendices the maximum measured plasma concentrations are underlined. The kinetic parameters are tabulated in Appendix 18. Figure 2 shows mean plasma nitrate concentrations after infusion of sodium nitrate and intake of the vegetables.
After infusion of the sodium nitrate dose over 30 minutes the peak-plasma nitrate concentration was reached between 30 and 40 minutes. The terminal elimination half-life

determined after the intravenous administration was 6.57 ± 1.46 hours. The volume of distribution in steady state (VSS) was calculated to be between 18 and 32 l with an average of 24 l. When the VSS is expressed in l/kg body weight this is between 0.24 and 0.44 l/kg bw with an average of 0.32 l/kg bw.

Every volunteer ate his or her spinach within 15 minutes (see Appendices 12 and 18). The peak-plasma concentration was reached within two hours, except for volunteer 9, whose peak-plasma concentration was reached after 3 hours. The half-life was calculated on 6.05 ± 1.18 hours after the intake of spinach.

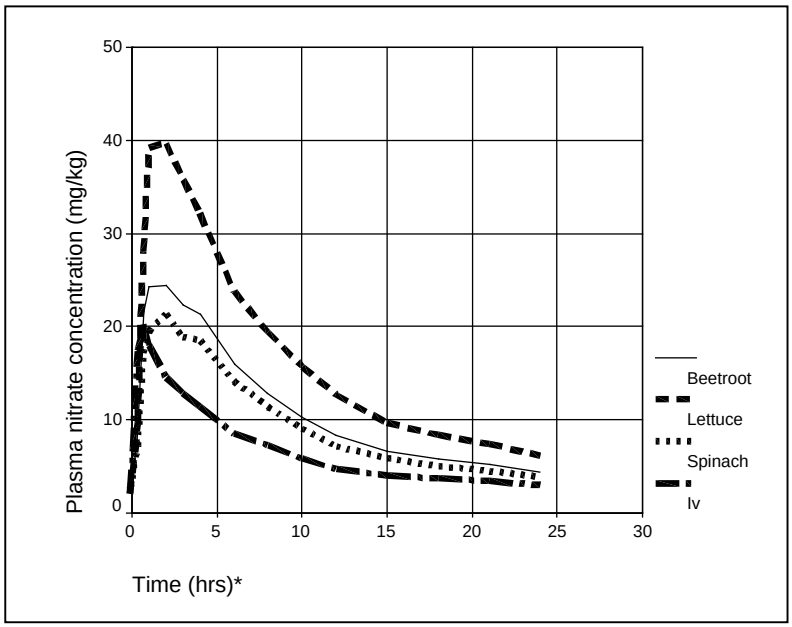

*Figure 2. Measured plasma nitrate concentrations after infusion of sodium nitrate and intake of the vegetables. * Time after dosing.*

The intake of lettuce took the volunteers 18 to 46 minutes. The peak-plasma nitrate concentration was reached within two hours, except for volunteer 9, whose peak-plasma concentration was reached after 2,5 hours. After the lettuce intake the half-life was calculated on 6.31 ± 1.32 hours.

The intake-time of beetroot was between 11 and 46 minutes. Within two hours the peak-plasma nitrate concentration was reached. The half-life was then calculated on 6.21 ± 1.13 hours.

To calculate the bioavailability of nitrate of the different vegetables, a correction was made for the different nitrate contents of the vegetables (see 2.6). Volunteer 6 was excluded for calculation of the bioavailability with correction for baseline nitrate, since after the 18th hour sample plasma nitrate concentrations became negative, when corrected for baseline nitrate. Volunteer 4 was excluded for calculation of the bioavailability for spinach, because after the 18th hour sample plasma nitrate concentrations became negative, when corrected for baseline nitrate. The bioavailability with correction for baseline was for spinach 98% ± 12%, for lettuce 113% ± 14% and for beetroot 106% ± 15%. The bioavailability without correction for baseline was for spinach 91% ± 10%, for lettuce 89% ± 13% and for beetroot 93% ± 12%.

For lettuce and beetroot the results of the bioavailability calculations with and without correction for baseline nitrate were significantly different (respectively $t = 6.010$, $p < 0.001$ and $t = 2.642$, $p = 0.025$). For spinach the results of the bioavailability with and without correction for baseline nitrate were not significantly different ($t = 1.539$, $p = 0.158$).

3.8 Plasma nitrite concentrations

In Appendices 19-22 individual plasma nitrite concentrations are tabulated. Before the infusion of 500 mg $NaNO_3$ the baseline of volunteer 1 was 0.34 mg/kg nitrite and of volunteer 3 was 0.31 mg/kg nitrite. Before the intake of beetroot the baseline of volunteer 2 was 0.22 mg/kg nitrite and of volunteer 6 was 0.23 mg/kg nitrite. All other baselines were < 0.2 mg/kg nitrite (the quantification limit).

Volunteer 2 had a plasma nitrite concentration above 0.3 mg/kg, that was higher than the baseline nitrite concentration, in 5 of the 11 blood samples between half an hour and 6 hours after the infusion of sodium nitrate. The other volunteers had no rise of the plasma nitrite concentration after the infusion of sodium nitrate.

Volunteer 8 had a plasma nitrite concentration of 0.74 mg/kg after 8 hours of the intake of spinach.

In general in most blood samples the plasma nitrite concentration was below the quantification limit. Incidentally the concentration was between 0.2 and 0.25 (see Appendices 19-22).

3.9 Blood pressure and heart rate

Blood pressure and heart rate were determined as a safety parameter the first hour during and after infusion of sodium nitrate. Volunteers 8 and 11 had a decrease in both systolic and diastolic blood pressure (see Appendix 23). Volunteer 9 had a decrease in diastolic blood pressure and volunteer 12 had a decrease in systolic blood pressure. A decrease in heart rate of about 10 (5-20) beats per minute comparing the first and second measurement was seen in all volunteers, except for one volunteer, who had an increase in heart rate of 5 beats per minute (Appendix 23).

4. Discussion

Bioavailability of nitrate from various vegetables appeared to be high. These results are in accordance with the results of a prior pilot study (24). In that study high plasma nitrate concentrations were found after vegetable intake.

It is however difficult to give exact values because of the endogenous nitrate production (baseline). In this study a correction for the baseline plasma nitrate concentration was performed, in which it was assumed that the baseline is constant during one day. However, not enough is known about the endogenous nitrate production whether or not this assumption is fully correct. Ignoring the nitrate baseline in calculating the bioavailability of nitrate from vegetables would not be correct, since it is known there is an endogenous production of nitrate. In this study therefore the bioavailability of nitrate from vegetables was calculated with and without a correction for the baseline plasma nitrate concentration to get an impression of the bioavailability. For nitrate baseline correction the average of two plasma nitrate concentrations before every administration of nitrate was taken. This choice was made because in spite of controlled test conditions (nitrate-low diet, fasting) some day-to-day variance in the baseline was found. This variance could not be fully explained by dietary variance, since only some minor deviations from this diet had been made and in general persons with a high baseline had also higher plasma nitrate concentrations 24 hours after nitrate administration compared to nitrate concentrations of the other volunteers. These facts together were reason to correct for the baseline as measured before every single administration and not correct for an average baseline of all the plasma concentrations before administration. The bioavailability was high (90-100%) either with or without correction for the endogenous nitrate production.

The vegetables in this study were administered under fasting conditions to investigate a worst case scenario. Normally the investigated vegetables are eaten together with other foods. It is known, that bioavailability of substances, like nitrate, can be affected by binding to other substances. So the bioavailability of nitrate from vegetables eaten during a meal, might be lower than the bioavailability measured during this study in a healthy population.

The elimination half-life of nitrate in this study was 6-7 hours, when it was calculated from the plasma nitrate concentration 4 to 12 hours after administration of nitrate. This elimination half-life was comparable with the half-life found in a study of Kortboyer et al. (7) who found a half-life of 6.5 hours after orally administrating a nitrate solution containing two times the ADI. The elimination half-life in this study was also comparable with the elimination half-life found in a study of Wagner et al. (9), who found a half-life of nitrate of 5 hours. In the Wagner study 12 healthy young adults had been orally administrated a nitrate solution containing 3.5 mmol (217 mg) ^{15}N-labelled nitrate. In our study 15 hours after administration of nitrate a change was observed in half-life from the 6-7 hours to 17.5 hours, when calculated from the nitrate concentrations 15 to 24 hours after administration of nitrate. We assume the calculated elimination half-life of 6-7 hours is the elimination half-life and is not a

matter of redistribution, since Wagner et al (9) observed 90% of the labelled nitrate appearing in the urine was excreted within 24 hours. This apparent change in elimination half-life of nitrate several hours after nitrate administration has not been reported before. It is possible, that the endogenous nitrate production influences the nitrate plasma concentration in this study from 15 hours after nitrate administration in such a way, that an elimination half-life cannot be calculated reliably from the nitrate plasma concentrations measured 15 hours and later after nitrate administration.

In this study the VSS was calculated on 24 l or 0.32 l/kg. In the study of Wagner et al. (9) the volume of distribution was calculated on 21.1 l or 0.30 l/kg. These are comparable data in spite of nitrate in the Wagner study was given as an oral dose and in our study nitrate was given intravenously.

All peak plasma nitrate concentrations occurred within 3 hours and most peaks appeared within 2 hours. A longer duration of the intake of the vegetables did not lead to a relevant later appearance of the peak plasma nitrate concentration. So the duration of the intake of the vegetables caused only a minor or no error in the calculation of the bioavailability of nitrate from the vegetables. Sometimes more than 1 peak was seen. A less plausible explanation for this phenomenon is the variance in the plasma nitrate analyses. A more plausible explanation is the difference in emptying of the stomach contents.

Plasma samples were analysed a year and a half after the experimental phase of the study. The samples were kept at -80 ^{o}C. Since in the pilot study the plasma nitrite concentrations were comparable with this study and the nitrate-ion is even more stable than the nitrite-ion, we assume no loss in nitrate and nitrite has been taken place.

The plasma nitrite concentrations were too low to expect any adverse effects. Two volunteers did however demonstrate adverse effects (headache, vomiting), that could be related to nitrite. But we expect that these adverse effects probably had other causes than the nitrate intake. The volunteer, who vomited after the lettuce intake, had some trouble with eating the amount of raw lettuce. This might have played a role in the nausea and vomiting. Also staying in an air-conditioned room for more than 24 hours could have played a role in the nausea and headache. Vomiting was no reason to exclude volunteers from statistics, because volunteers vomited 6 or more hours after vegetable intake. At those times nitrate from the vegetables had already been absorbed.

The AUC of nitrite could not be calculated because of low plasma nitrite concentrations (most concentrations were below the lower limit of quantification). It seems therefore that systemic nitrite is no matter of concern when vegetables containing high amounts of nitrate are eaten by healthy volunteers. Unfortunately no comparison of $AUC_{nitrate}$ versus $AUC_{nitrite}$ could be made, which could have provided information, whether metabolism is a fixed factor.

One volunteer had a nitrite concentration of 0.74 mg/kg, which was higher than the other nitrite concentrations of < 0.35 mg/kg. This concentration was found eight hours after intake of spinach. All other concentrations for that person on that day were < 0.2 mg/kg. So this solely high concentrations cannot be explained by the nitrate and/or nitrite intake from

spinach. Since the blood sample was also not haemolytic, the reason for the higher nitrite concentrations remains unknown.
It is known that nitrite has vasodilatated effects (26). In this study there was no rise in plasma nitrite concentration measured, consequently no effect on the blood pressure and heart rate were expected. This expectation is confirmed by higher heart rates before administration of sodium nitrate than after administration of sodium nitrate. During infusion of sodium nitrate the volunteers were in rest. It seems that being in rest influenced the heart rate more than the infusion of the sodium nitrate.
Overall it can be concluded that the bioavailability of nitrate from the vegetables is high.

5. Conclusions

The aim of this study was to investigate the bioavailability of frequently consumed vegetables and especially that of cooked spinach, raw lettuce and cooked beetroot. Twelve healthy volunteers received a single dose of 500 mg $NaNO_3$ and a single dose of 300 g spinach, lettuce and beetroot. All bioavailabilities of nitrate from the given vegetables were close to 100%. So it can be concluded that nitrate from spinach, lettuce and beetroot is very highly available.

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Statement of GCP Compliance

The undersigned hereby certify that the clinical phase of the study, as described herein, was performed in accordance with the recommended principles of Good Clinical Practice for studies with medicine in the European Community (CPMP/ICH/135/95 Final: 17-07-1996). This report provides a complete, correct and faithful record of the results obtained.

Name: J. Meulenbelt, MD, PhD (Head National Poisons Control Centre)
Laboratory: National Poisons Control Centre, RIVM
Date: 05-04-2000
Signature:

Declaration of quality control

Undersigned states herewith that the research presented in this report has been carried out according to the EC principles of Good Clinical Practice (GCP) and that this report reflects a complete, correct and reliable overview of the results obtained.

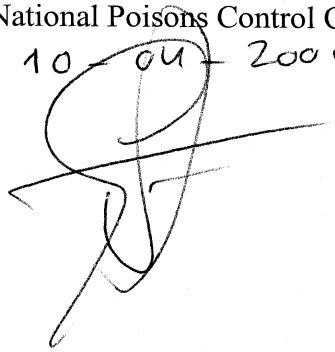
GCP inspections of the experiments and reports submitted to the management research team leader took place on:

Report publication date	Report inspection date
05-11-1997	13-10-1997
	20-10-1997
	29-10-1997
	05-11-1997

This report was inspected on:	14-01-2000
	17-01-2000
	25-01-2000
	31-01-2000
	04-04-2000

Report publication date:	31-01-2000
	04-04-2000

Substitute quality control officer:

Name	:	G.A. van Zoelen
Laboratory	:	National Poisons Control Centre, RIVM
Date	:	10-04-2000
Signature	:	

Appendix 1 Mailing list

1-5 Inspectorate for Health Protection, Commodities and Veterinary Public Health, dr ir M.W.J. Wolfs
6-10 Director of the Directorate of Health Policy, dr A.A. W. Kalis
11 Director-General of Public Health, prof dr H.J. Schneider
12 Inspectorate for Health Care, drs P.H. Vree
13 Medical Ethics Committee (METC), University Medical Center Utrecht
14 Depot of Dutch Publications and Dutch Bibliography
15 Directorate-General of the National Institute of Public Health and the Environment
16 Director Division Substances and Risks (Division 3/4), RIVM, dr ir G. de Mik
17 Laboratory for Residue Analysis, RIVM, dr R.W. Stephany
18 Laboratory of Inorganic Analytical Chemistry, RIVM, ir H.J. van de Wiel
19 Laboratory for Health Effects Research, RIVM dr A. Opperhuizen
20 Laboratory for Health Effects Research, RIVM, dr A.B.T.J. Boink
21 Centre for Substances and Risk Assessment, RIVM, dr G.J.A. Speijers
22 Laboratory of Exposure Assessment and Environmental Epidemiology, RIVM, dr ir E. Lebret
23 Laboratory of Exposure Assessment and Environmental Epidemiology, RIVM, dr ir M.J. Zeilmaker
24 National Poisons Control Centre, RIVM, dr Tj.T. Mensinga
25 National Poisons Control Centre, RIVM, drs I. de Vries
26 National Poisons Control Centre, RIVM, drs E. Spaans
27 National Poisons Control Centre, RIVM, Ing A.W.M. Hofstee
28 National Poisons Control Centre, RIVM, drs G.A. van Zoelen
29-35 Authors
36 Public Relations Department, RIVM
37 Bureau Report Registration, RIVM
38 Library, RIVM
39-54 Sales Department
55-60 Reserve

Appendix 2 Regulatory limits for the nitrate content of vegetables in The Netherlands

	November - April (mg/kg fresh product)	April - November (mg/kg fresh product)
Spinach	4500	3500
Lettuce	4500	3500
Endive	3500	2500

	July - April (mg/kg fresh product)	April - July (mg/kg fresh product)
Beetroot	3500	4000

Adapted from Nitrates and nitrites in foodstuff, Dr. L.J. Schuddeboom, Council of Europe Press, 1993 (27).

Appendix 3 Informed consent (in Dutch)

INFORMED CONSENT FORMULIER

Afd. IC-1 en Klinische Toxicologie van het Academisch Ziekenhuis Utrecht
Nationaal Vergiftigingen Informatie Centrum van het RIVM

Ondergetekende verklaart een exemplaar te hebben ontvangen van de "Informatie voor Deelnemers" betreffende het onderzoek getiteld:
“Studie naar de opname van nitraat uit verschillende groenten: een biobeschikbaarheidsonderzoek” (projectnummer 348801 006/p; project: 235802; datum 28-05-1997; revised 15-07-1997)”.

Ondergetekende heeft een mondelinge toelichting op dit informatieformulier gekregen en is in de gelegenheid geweest iedere gewenste vraag te stellen.

Ondergetekende verklaart dat hij/zij, na kennis te hebben genomen van bovengenoemde informatie, als vrijwilliger wil deelnemen aan bovengenoemd onderzoek.
Ondergetekende verklaart dat hij/zij naar eer en geweten alle vragen gesteld tijdens de medische keuring zal beantwoorden en geen informatie bewust zal achtergehouden.
Afgesproken is dat hij/zij zich gedurende de totale onderzoeksperiode zal houden aan de opzet van het onderzoek en aan de restricties zoals beschreven in de schriftelijke informatie voor deelnemers.

Voorts verklaart de vrijwilliger dat hij/zij in een periode van 30 dagen voorafgaand aan, en tijdens dit onderzoek, niet deelneemt aan enig ander vrijwilligersonderzoek.

Aan ondergetekende is medegedeeld dat bij vragen en problemen gedurende 24 uur per dag, 7 dagen per week, direkt kontakt kan worden opgenomen met de onderzoekers via het Academisch Ziekenhuis Utrecht, afdeling Intensive Care-I en Klinische Toxicologie, tel. 030-2507330 (tijdens kantooruren) en het Nationaal Vergiftigingen Informatie Centrum, tel. 030-2748888 (buiten kantooruren).

Voorts is ondergetekende medegedeeld dat hij/zij zonder opgave van redenen op elk moment deelname aan het onderzoek kan staken.

Ondergetekende gaat accoord met inzagerecht van persoonlijke (medische) gegevens door medewerkers van de afdeling IC-1 en Klinische Toxicologie en medewerkers van inspecterende instanties.

Naam : .....

Voornamen : .....

Geboortedatum : .....

Adres : .....

Postcode+Woonplaats : .....

Telefoonnummer : .....

Utrecht, ...(datum en jaar)

Handtekening vrijwilliger: Naam en handtekening onderzoeker:

....

Appendix 4 Demographic variables

Volunteer-number	Gender (m/f)	Age (yrs)	Height (cm)	Weight (kg)	Comment
101*	m	25	183	82	Excluded
102*	f	24	164	65	Excluded
103*	f	20	176	66	Excluded
104*	f	33	175	65	Excluded
105*	m	24	182	64.5	Excluded
106*	f	23	172	62	Reserve
1	m	24	196	88	
2	f	21	174	78	
3	f	24	180	62	
4	f	28	197	91	
5	m	27	174	64.5	
6	m	24	189	84	
7	m	21	187	79	
8	m	24	183	75	
9	f	22	172	61.5	
10	m	28	187	80	
11	f	24	184	79	
12	f	25	173	62	
Mean		24	183	75	
Men			186	78	
Women			180	72	
SD		2.39	8.67	10.5	
Men			7.27	8.1	
Women			9.53	12.3	

* These persons are excluded from the statistics

Appendix 5 Selection criteria

Selection criteria as applied for enrolment of the volunteers in the study on the oral bioavailability of nitrate from vegetables

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Inclusion criteria

- female/male
- age 18-35 years
- weight within 10 % of the normal range of the Metropolitan Height and Mass Tables
- willing to give Written Informed Consent
- healthy as judged by a physician from the medical history, physical examination, electrocardiography and routine laboratory blood and urine analyses
- good venous accessibility

Exclusion criteria

- difficulties with swallowing raw lettuce, heated deep-freeze spinach or cooked beetroot (i.e. without use of additional flavouring)
- smoking
- chronic drug treatment (especially antacids)
- drug treatment within one week before the start of the study, except for oral contraceptives. This to the judgement of the physician and/or medical investigator.
- use of narcotics
- more than 3 units of alcohol per day
- blood donation within 30 days before the start of the study
- participation in another drug study within 30 days before the experiment or during the study
- pregnant or lactating females
- mental illness
- any chronic illness
- family history positive for pernicious anaemia
- family history positive for G6PD-deficiency
- excessive pyrosis or history of gastritis or gastric or duodenal ulcer
- abnormal dietary habits as judged by a physician
- anaemia

Appendix 6 Flow chart of scheduled study procedures

Procedures during the day of the administration of sodium nitrate

Clock-time	Scheme-time	Scheduled Procedure
08.00 h	-1.00	Pregnancy test, Body weight,
		Blood pressure and heart rate
		Insertion of two intravenous cannulas
08.30 h	-0.50	Blood sampling
08.55 h	-0.08	Blood sampling, Adverse effects
09.00-09.30 h	0.00-0.50	***Intravenous infusion of sodium nitrate***
09.10 h	0.17	Blood sampling, Blood pressure, Adverse effects
09.20 h	0.33	Blood sampling, Blood pressure, Adverse effects
09.30 h	0.50	Blood sampling, Blood pressure, Adverse effects
09.40 h	0.67	Blood sampling, Blood pressure, Adverse effects
09.50 h	0.83	Blood sampling, Blood pressure, Adverse effects
10.00 h	1.00	Blood sampling, Blood pressure, Adverse effects
10.15 h	1.25	Blood sampling, Adverse effects
10.30 h	1.50	Blood sampling, Adverse effects
11.00 h	2.00	Blood sampling, Adverse effects
11.30 h	2.50	Blood sampling, Adverse effects
12.00 h	3.00	Blood sampling, Adverse effects
12.00 h	3.00	200 ml distilled water
13.00 h	4.00	Blood sampling, Adverse effects
13.00-13.30 h	4.00-4.50	Lunch
15.00 h	6.00	Blood sampling, Adverse effects
15.00-15.30 h	6.00-6.50	Tea
17.00 h	8.00	Blood sampling, Adverse effects
17.30-18.00 h	8.50-9.50	Diner
19.00 h	10.00	Blood sampling, Adverse effects
21.00 h	12.00	Blood sampling, Adverse effects
22.00 h	13.00-13.50	Snack
00.00 h	15.00	Blood sampling, Adverse effects
03.00 h	18.00	Blood sampling, Adverse effects
06.00 h	21.00	Blood sampling, Adverse effects
09.00 h	24.00	Blood sampling, Adverse effects
09.30 h	24.50	Breakfast and departure

Appendix 6 (continued) Flow chart of scheduled study procedures low chart

Procedures during the day of the administration of vegetables

Clock-time	Scheme-time	Activity
08.00 h	-1.00	Pregnancy test, Body weight,
		Blood pressure and heart rate
		Insertion of intravenous cannula
08.30 h	-0.50	Blood sampling
08.55 h	-0.08	Blood sampling, Adverse effects
09.00-09.30 h	0.00-0.50	***Administration of the vegetables***
09.20 h	0.33	Blood sampling, Adverse effects
09.40 h	0.67	Blood sampling, Adverse effects
10.00 h	1.00	Blood sampling, Adverse effects
10.20 h	1.33	Blood sampling, Adverse effects
10.40 h	1.67	Blood sampling, Adverse effects
11.00 h	2.00	Blood sampling, Adverse effects
11.20 h	2.33	Blood sampling, Adverse effects
11.40 h	2.67	Blood sampling, Adverse effects
12.00 h	3.00	Blood sampling, Adverse effects
12.00 h	3.00	200 ml distilled water
12.30 h	3.50	Blood sampling, Adverse effects
13.00 h	4.00	Blood sampling, Adverse effects
13.00-13.30 h	4.00-4.50	Lunch
14.00 h	5.00	Blood sampling, Adverse effects
15.00 h	6.00	Blood sampling, Adverse effects
15.00-15.30 h	6.00-6.50	Tea
17.00 h	8.00	Blood sampling, Adverse effects
17.30-18.30 h	8.50-9.50	Diner
19.00 h	10.00	Blood sampling, Adverse effects
21.00 h	12.00	Blood sampling, Adverse effects
22.00 h	13.00	Snack
00.00 h	15.00	Blood sampling, Adverse effects
03.00 h	18.00	Blood sampling, Adverse effects
06.00 h	21.00	Blood sampling, Adverse effects
09.00 h	24.00	Blood sampling, Adverse effects
09.30 h	24.50	Breakfast and departure

Appendix 7 Nitrate and nitrite-low diet

The study participants had to adhere to the following diet instructions for three days prior to every study day.

VEGETABLES

Vegetables provide a large part of daily nitrate. The nitrate-content of the vegetables can vary enormously. The diet instructions given here are based on mean nitrate concentrations in various vegetables. Because the nitrate content of the vegetables can vary enormously it is always important to eat different vegetables every day.

A Vegetables containing more than 1000 mg nitrate per kg product. These vegetables are **forbidden**

endive
celery
Chinese cabbage
chervil
turnip cabbage
paksoi
purslane
turnip tops
radish
black radish
beetroot (red)
lettuce
beetroot
spinach
cabbage
fennel
watercress

B Vegetables containing between 500 and 1000 mg nitrate per kg product. **Limited use** of these vegetables is permitted. The day before the actual study day these vegetables should not be eaten.

cauliflower
curly kale
paprika
leek
sliced beans

C Vegetables containing less than 500 mg nitrate per kg product. These vegetables can be eaten **without restriction**.

artichokes
asparagus
aubergine
broccoli
courgette
green peas
peas
cucumber
turnip rooted celery
maize
mushrooms
padded peas
rhubarb
tauge
tomato
savoy cabbage
butter beans
sprouts
broad beans
onions
carrots
white cabbage
Belgian endive
sauer kraut
red cabbage

Appendix 7 (continued) Nitrate and nitrite-low diet

POTATOES

Limited use of potatoes is permitted. This also accounts for other potato-products such as chips and French-fries.
The day before the actual study day these products should not be eaten.

RICE AND PASTE
These products can be eaten **without restriction**.

BREAD
Bread and bread-products can be eaten **without restriction**.

MEAT AND FISH
Fish and fresh meat can be eaten **without restriction**.

MEAT-PRODUCTS/MEAT-DELICACIES
In these products nitrate and nitrite are used as preservatives. Therefore, only limited use of these products is permitted. The day before the actual study day the following products should not be eaten.

bacon
ham
smoked beef
smoked sausage

MILK, EGGS AND DAIRY-PRODUCTS
These products can be eaten **without restriction**.

FRUIT
Fruit can be eaten **without restriction**.

DRINKS
The use of potable water from a private well is **forbidden**.
Consumption of vegetable juices is **forbidden**.
All other drinks can be consumed **without restriction**.

For the duration of the experiment alcohol consumption, with a maximum of three glasses a day, is permitted.
Alcohol consumption is not allowed on the day before the actual study day.

Appendix 8 Deviations from protocol

- The quick nitrate analysis of the beetroot and the lettuce that was done prior to the study session was not performed with the Bioquant® Nitrat Kompakt Method of Merck. Instead, the vegetables were analysed by the Laboratory of Inorganic Analytical Chemistry by means of High Performance Ion Chromatography. The Laboratory of Inorganic Analytical Chemistry performs nitrate analyses on a regular base and was able to schedule the nitrate analyses of the present study in their program. In addition, this method is more precise then the Bioquant® Nitrat Kompakt Method of Merck.

- A lower limit for nitrate in beetroot of 1000 mg/kg, in stead of the protocolised lower limit of 1200 mg/kg nitrate in beetroot, was used acting on information received from the pilot-study.

- The body mass of volunteers 2 and 4 were outside 10% of the normal range of the Metropolitan Height and Mass Tables. Volunteer 2 was 2.6 kg overweight. Volunteer 4 was approximately 2.5 kg overweight. However, she fell outside the range of the Metropolitan Height and Mass Tables, because of her extreme length for a woman (197 cm) and the weight range for women of that length had to be estimated.

- The last blood samples were taken 12, 15, 18, 21 and 24 hours after infusion of sodium nitrate, in stead of 12, 16, 20, 24 and 32 hours as described in the protocol.

- In general the blood samples were taken within 5 minutes within the scheme time after administration of the treatment. Several times the first two blood samples (to determine the baseline nitrate level) were taken somewhat earlier. The 24 hrs(after administration) blood samples the next morning was taken somewhat earlier or later several times. For most volunteers only incidentally blood samples were taken with a time difference of more than 5 minutes. Exceptions were volunteer 2 and volunteer 9. For volunteer 2 the 10 hrs, 12 hrs and 21 hrs samples after intake of beetroot were taken approximately half an hour late due to difficult blood sampling. For volunteer 9 the 2.67 hrs and 3 hrs samples were taken respectively 6 and 7 minutes late and the 4, 5 and 6 hrs samples were taken 30-45 minutes late due to difficult blood sampling after the intake of beetroot.

Appendix 8 (continued) Deviations from protocol

- Diet: volunteer 2 ate the day before day D nasi containing leek, but this did not affect the nitrate baseline, which was 1.68 mg/kg. Volunteer 6 ate the day before day A about 50 grams potatoes, this may have affected the nitrate baseline, which was 4.38 mg/kg. Volunteer 8 ate the day before day B a plate of potatoes and sauerkraut. This may have slightly affected the baseline which was 2.40 mg/kg. The other baselines of this person were ≤ 1.60 mg/kg. Volunteer 12 ate the day before day C one roll with salami and about 100 grams shrimp crackers (with potato-flour). Also the day before day B volunteer 12 ate shrimp crackers. This may have affected the nitrate baseline which were 4.12 mg/kg on day B and 4.03 mg/kg on day C.

- Adjustments were made to the lunch, tea, diner and snack that were consumed during the study session. Volunteers 5, 6, 7 and 8 had one slice of bread with (butter) and jam extra for lunch. Volunteers 4 and 9 ate no butter at lunch and tea. Volunteer 1 peeled the apple (that was served as snack) day A. On day C volunteer 3 had for tea one slice of bread and vomited a short while thereafter. Probably the lunch came out, too. Because of ongoing nausea volunteer 3 also did not have diner and snack in the evening. On day D volunteer 3 ate one slice of bread with butter and jam less during tea. Because of nausea she did not have diner. She ate the snack, but vomited after 10 minutes. Volunteer 4 ate one butter-tube less than prescribed during lunch and tea. On day B she did not eat the snack due to nausea. Volunteer 5 ate one butter-tube less for tea and had no tea during the evening snack except on day D. Volunteer 6 ate one butter-tube less during tea. Volunteer 7 had one slice of bread with jam extra for lunch and tea. Volunteer 8 had one slice of bread extra for lunch and tea and ate on day D one butter-tube less during lunch. Volunteer 10 had one butter-tube less for lunch and tea. Volunteer 11 ate one butter-tube and one jam-tube less during lunch and tea. On day D no distilled water was administered. Volunteer 12 had one jam less for lunch and tea.

Appendix 9 Nitrate content of the vegetables

Vegetable	Nitrate content of the unprepared vegetables prior to the study (mg/kg)	average	Nitrate content of the prepared vegetables during the study (mg/kg)	average
Beetroot	1901 3234	2568 (N = 2)	2126 2127 2171 2168 2194 2174 2133 2061	2144 (N= 8)
Boiling water			258 mg/l 298 mg/l	278 mg/l (N = 2)
Lettuce[1]	3842 3742	3792 (N = 2)	3059 3022 3055 3003 3047 2976 3022 2952	3017 (N = 8)
Spinach	1942[2] 2222[2]	2082[2] (N = 2)	1868 1901 1888 1865 1873 1882	1880 (N = 6)
Leaked water	370 mg/l			

1. The nitrate content of the lettuce during the study was analysed inclusive some wash-water
2. The spinach used is this study came from the same batch as the spinach used in the pilot study (24)

Appendix 10 Results of pre- and postmedical screening

Volunteer	1		2		3		
	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	
Date	23-09-97	19-11-97	24-09-97	18-11-97	23-09-97	18-11-97	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	142/74	140/75	126/77	125/85	121/65	109/67	(mmHg)
Heart rate	65	85	73	62	63	84	(b.p.m.)
Standing:							
Blood pressure	142/84	128/92	133/77	135/87	122/74	115/72	(mmHg)
Heart rate	68	86	81	84	73	101	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	10.3	9.8	7.8	8.2	8.6	8.2	M: 8.6-10.7 mmol/l F: 7.4-9.6 mmol/l
Haematocrite	0.46	0.45	0.38	0.38	0.41	0.38	M: 0.41-0.55 l/l F: 0.36-0.46 l/l
Leukocytes	5.1	6.8	7.1	8.3	6.4	6.9	$4.0\text{-}10.0*10^9$/l
Eosinophiles	2	2	2	2	4	3	< 5 %
Basophiles	0	2	1	0	1	1	< 2 %
Neutrophiles	56	53	61	61	53	50	40-72 %
Lymphocytes	33	34	28	31	34	40	20-45 %
Monocytes	9	9	8	6	8	6	3-10 %
ESR	2	2	9	20*	8	7	M: 1-5 mm/hr F: 2-12 mm/hr
Sodium	142	141	139	137	140	140	136-146 mmol/l
Potassium	4.1	4	3.9	4.1	3.8	3.8	3.8-5.0 mmol/l
Calcium	2.29	2.16*	2.16*	2.18*	2.24	2.15*	2.20-2.60 mmol/l
Urea	5.7	6.6	3.7	3.1	4.5	3.5	3.0-7.5 mmol/l
Creatinin	81	85	73	66	66	67	50-120 µmol/l
Bilirubin	17	19*	8	14	20*	29*	< 17 µmol/l
LDH	428	537	442	562	568	500	300-620 U/l
γGT	43	36	18	24	23	19	M: 15-70 U/l F: 15-45 U/l
ASAT	36	30	27	23	27	29	15-45 U/l
ALAT	40	39	30	29	29	29	10-50 U/l
URINARY ANALYSES							
Proteins/albumin	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Glucose	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Leukoscreen	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Blood	neg.	neg.	neg.	neg.	neg.	neg.	Negative

* = Out of normal range
neg. = negative
pos. = positive

Volunteer	4		5		6		
	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	
Date	24-09-97	19-11-97	23-09-97	19-11-97	30-09-97	18-11-97	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	114/69	114/69	122/67	126/70	119/67	120/68	(mmHg)
Heart rate	67	84	70	61	55	73	(b.p.m.)
Standing:							
Blood pressure	113/77	122/78	127/62	122/85	121/76	123/72	(mmHg)
Heart rate	105	116	84	69	67	96	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	8.1	8	8.6	7.9*	9.6	8.9	M: 8.6-10.7 mmol/l F: 7.4-9.6 mmol/l
Haematocrite	0.4	0.38	0.41	0.38*	0.47	0.43	M: 0.41-0.55 l/l F: 0.36-0.46 l/l
Leukocytes	4.4	7.5	3.9*	4.1	6.6	6.6	$4.0\text{-}10.0*10^9$/l
Eosinophiles	2	2	8*	2	2	1	< 5 %
Basophiles	1	2	0	0	1	1	< 2 %
Neutrophiles	57	49	40	42	65	66	40-72 %
Lymphocytes	32	35	39	45	27	26	20-45 %
Monocytes	8	12*	13*	11	5	6	3-10 %
ESR	10	9	7*	7*	6*	5	M: 1-5 mm/hr F: 2-12 mm/hr
Sodium	140	139	142	140	142	141	136-146 mmol/l
Potassium	4.8	4.2	4.3	4.1	4.4	4	3.8-5.0 mmol/l
Calcium	2.19*	2.12*	2.32	2.20	2.11*	2.21	2.20-2.60 mmol/l
Urea	3	4.1	4.2	6.1	4.1	3.8	3.0-7.5 mmol/l
Creatinin	68	61	84	90	90	88	50-120 µmol/l
Bilirubin	8	11	12	13	23*	25*	< 17 µmol/l
LDH	408	496	406	441	440	361	300-620 U/l
γGT	22	23	26	23	25	21	M: 15-70 U/l F: 15-45 U/l
ASAT	23	26	45	25	44	20	15-45 U/l
ALAT	14	18	35	28	31	25	10-50 U/l
URINARY ANALYSES							
Proteins/albumin	neg.	weak pos.*	neg.	neg.	neg.	neg.	Negative
Glucose	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Leukoscreen	pos.*	neg.	neg.	neg.	neg.	neg.	Negative
Blood	neg.	neg.	neg.	neg.	neg.	neg.	Negative

* = Out of normal range
neg. = negative
pos. = positive

Volunteer	**7**		**8**		**9**		
	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	
Date	23-09-97	20-11-97	24-09-97	18-11-97	24-09-97	20-11-97	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	116/62	120/72	103/58	110/64	118/75	112/68	(mmHg)
Heart rate	54	71	48	53	65	48	(b.p.m.)
Standing:							
Blood pressure	111/61	111/73	119/57	111/67	131/78	115/77	(mmHg)
Heart rate	60	105	52	72	81	62	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	9.4	9.3	8.7	8.9	9.5	8.9	M: 8.6-10.7 mmol/l F: 7.4-9.6 mmol/l
Haematocrite	0.46	0.45	0.4*	0.45	0.44	0.42	M: 0.41-0.55 l/l F: 0.36-0.46 l/l
Leukocytes	6.7	5.2	4.2	5.3	10.2*	7.6	$4.0\text{-}10.0*10^9$/l
Eosinophiles	1	2	3	3	0	4	< 5 %
Basophiles	0	1	0	0	0	1	< 2 %
Neutrophiles	66	47	41	44	72	47	40-72 %
Lymphocytes	28	41	49*	45	22	41	20-45 %
Monocytes	5	9	7	8	6	7	3-10 %
ESR	2	6*	2	2	6	8	M: 1-5 mm/hr F: 2-12 mm/hr
Sodium	141	140	141	142	141	139	136-146 mmol/l
Potassium	4.0	4.1	3.7*	4	4.4	4	3.8-5.0 mmol/l
Calcium	2.26	2.23	2.14*	2.24	2.26	2.11	2.20-2.60 mmol/l
Urea	4.9	4.4	4.3	4.1	3	3.4	3.0-7.5 mmol/l
Creatinin	76	77	78	92	76	73	50-120 µmol/l
Bilirubin	10	15	9	15	8	12	< 17 µmol/l
LDH	433	422	450	427	529	482	300-620 U/l
γGT	23	22	20	21	36	29	M: 15-70 U/l F: 15-45 U/l
ASAT	32	29	27	33	35	22	15-45 U/l
ALAT	36	33	23	23	27	17	10-50 U/l
URINARY ANALYSES							
Proteins/albumin	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Glucose	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Leukoscreen	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Blood	neg.	neg.	neg.	neg.	neg.	neg.	Negative

* = Out of normal range
neg. = negative
pos. = positive

Volunteer	**10**		**11**		**12**		
	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	Prestudy Screening	Poststudy Screening	
Date	30-09-97	19-11-97	25-09-97	18-11-97	25-09-97	20-11-97	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	125/70	114/57	120/60	125/63	120/63	116/56	(mmHg)
Heart rate	48	41	62	68	54	59	(b.p.m.)
Standing:							
Blood pressure	129/88	113/83	123/81	118/77	132/67	111/64	(mmHg)
Heart rate	51	70	84	103	61	67	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	9	8.7	7.8	7.8	8.2	7.5	M: 8.6-10.7 mmol/l F: 7.4-9.6 mmol/l
Haematocrite	0.43	0.4	0.38	0.34*	0.38	0.36	M: 0.41-0.55 l/l F: 0.36-0.46 l/l
Leukocytes	6.9	6.3	6.2	7.4	7.8	6.5	$4.0\text{-}10.0*10^9$/l
Eosinophiles	2	3	6*	5	0	2	< 5 %
Basophiles	1	1	0	0	0	0	< 2 %
Neutrophiles	64	51	58	57	52	50	40-72 %
Lymphocytes	29	36	30	32	46*	40	20-45 %
Monocytes	4	9	6	6	2*	8	3-10 %
ESR	6*	10*	8	12	2	4	M: 1-5 mm/hr F: 2-12 mm/hr
Sodium	142	140	141	142	142	140	136-146 mmol/l
Potassium	4.2	4.2	4	4	3.9	4.3	3.8-5.0 mmol/l
Calcium	2.38	2.2	2.22	2.22	2.21	2.09	2.20-2.60 mmol/l
Urea	5.5	6.5	3	3.6	3.1	3.1	3.0-7.5 mmol/l
Creatinin	94	90	75	80	74	76	50-120 µmol/l
Bilirubin	8	15	7	11	10	13	< 17 µmol/l
LDH	361	554	370	428	487	503	300-620 U/l
γGT	27	25	15	19	17	13*	M: 15-70 U/l F: 15-45 U/l
ASAT	24	31	20	21	22	23	15-45 U/l
ALAT	23	28	25	19	14	20	10-50 U/l
URINARY ANALYSES							
Proteins/albumin	neg.	neg.	neg.	weak pos.*	neg.	neg.	Negative
Glucose	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Leukoscreen	neg.	neg.	neg.	neg.	neg.	neg.	Negative
Blood	neg.	neg.	neg.	neg.	neg.	neg.	Negative

* = Out of normal range
neg. = negative
pos. = positive

Appendix 11 Study dates and treatments

A = Single dose intravenous administration of 500 mg sodium nitrate (365 mg nitrate)

B = Single dose oral administration of 300 gram spinach (deep-freeze) (564 mg nitrate)

C = Single dose oral administration of 300 gram of raw lettuce (1013 mg nitrate)

D = Single dose oral administration of 300 gram of cooked beetroot (643 mg nitrate)

Treatment				
	A	**B**	**C**	**D**
	Date	**Date**	**Date**	**Date**
Volunteer-number				
1	13-10-1997	27-10-1997	05-11-1997	13-11-1997
2	13-10-1997	27-10-1997	05-11-1997	12-11-1997
3	15-10-1997	27-10-1997	05-11-1997	12-11-1997
4	15-10-1997	27-10-1997	05-11-1997	12-11-1997
5	16-10-1997	29-10-1997	05-11-1997	12-11-1997
6	16-10-1997	29-10-1997	05-11-1997	12-11-1997
7	20-10-1997	29-10-1997	06-11-1997	13-11-1997
8	20-10-1997	29-10-1997	06-11-1997	12-11-1997
9	22-10-1997	30-10-1997	06-11-1997	13-11-1997
10	22-10-1997	30-10-1997	06-11-1997	13-11-1997
11	23-10-1997	30-10-1997	06-11-1997	13-11-1997
12	23-10-1997	30-10-1997	06-11-1997	13-11-1997

Appendix 12 Infusion and intake times of treatment

Treatment A = Single dose intravenous administration of 500 mg sodium nitrate

Treatment B = Single dose oral administration of 300 gram spinach (deep-freeze)

Treatment C = Single dose oral administration of 300 gram of raw lettuce

Treatment D = Single dose oral administration of 300 gram of cooked beetroot

Infusion and intakes times of the different treatments (in minutes)				
	Treatment			
Volunteer number	**A**	**B**	**C**	**D**
1	30	13	39	32
2	30	10	19	14
3	30	11	43	46
4	30	9	44	16
5	30	6	27	22
6	30	8	19	14
7	30	8	19	19
8	30	9	18	11
9	30	14	46	36
10	30	12	15	41
11	30	13	38	16
12	30	6	28	15
Average (± S.D.)	30 (± 0)	10 (± 3)	30 (± 12)	24 (± 12)

Appendix 13 Table of adverse effects

Adverse effects after infusion of 500 mg sodium nitrate

Volunteer number	Adverse effect	Time started after treatment (hrs.min)	Duration (hrs.min)	Intensity (mild/mode-rate/severe)	Related to test-product
3	Headache	1.30	2.40	mild	Possible
	Nausea	3.45	0.10	mild	Possible
	Head discomfort[2]	4.10	5.50	mild	Possible
	Headache	10.00	2.00	moderate	Possible
	Headache	15.00	3.00	mild	Possible
4	Headache	5.00	5.10	mild	Possible
	Headache[1]	10.10	7.45	severe	Possible
5	Throat ache	18.00	0.10	mild	no
9	Throat ache	predose	continuing	mild	no
10	Nose congestion	predose	continuing	mild	no
12	Light dizziness	predose	0.05	mild	no

1. 500 mg paracetamol administered 13.25 hrs.min after the start of the intravenous infusion of the sodium nitrate
2. Between two periods of headache, this person had an uncomfortable feeling in the head, but no headache.

Adverse effects after administration of 300 grams spinach

Volunteer number	Adverse effect	Time started after treatment (hrs.min)	Duration (hrs.min)	Intensity (mild/mode-rate/severe)	Related to test-product
3	Headache	11.30	6.15	mild	Possible
4	Headache	3.00	18.00	mild	Possible
	Nausea	12.25	2.15	moderate	Possible
	Vomiting	13.40	0.05	moderate	Possible
	Vomiting	14.10	0.05	moderate	Possible
	Vomiting	14.35	0.05	moderate	Possible
11	Light headache	predose	4.00	mild	no

Adverse effects after administration of 300 grams lettuce

Volunteer number	Adverse effect	Time started after treatment (hrs.min)	Duration (hrs.min)	Intensity (mild/mode-rate/severe)	Related to test-product
3	Headache	3.00	10.10	mild	Possible
	Nausea	5.00	9.40	mild	Possible
	Vomiting	6.25	0.05	mild	Possible
	Vomiting	8.55	0.05	mild	Possible
	Vomiting	9.20	0.05	mild	Possible
	Vomiting	10.05	0.05	mild	Possible
	Vomiting	10.35	0.05	mild	Possible
	Vomiting	11.20	0.05	mild	Possible
	Vomiting	14.35	0.05	mild	Possible
4	Headache*	3.30	9.45	moderate	Possible

* 500 mg paracetamol/coffein administered 9.30 hrs.min after the administration of the lettuce

Adverse effects after administration of 300 grams beetroot

Volunteer number	Adverse effect	Time started after treatment (hrs.min)	Duration (hrs.min)	Intensity (mild/mode-rate/severe)	Related to test-product
3	Nausea	8.55	1.05	mild	Possible
	Headache	10.00	3.05	mild	Possible
	Vomiting	13.05	0.02	mild	Possible

Appendix 14 Plasma Nitrate concentrations after infusion of 500 mg $NaNO_3$ over 30 minutes (365 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-0.58	1.56	-0.92	1.11	-0.45	1.94	-0.83	1.97	-0.83	4.32	-0.83	4.45
8.55	-0.08	-0.23	1.52	-0.22	1.10	-0.17	1.84	-0.17	1.80	-0.17	4.02	-0.20	4.30
9.10	0.17	0.17	9.46	0.17	8.21	0.17	9.61	0.17	9.55	0.17	13.89	0.17	13.62
9.20	0.33	0.33	16.33	0.33	14.02	0.33	19.41	0.25	15.84	0.33	22.56	0.33	19.44
9.30	0.50	0.52	20.82	0.52	20.40	0.52	27.21	0.52	19.50	0.52	23.67	0.52	23.99
9.40	0.67	0.67	17.03	0.67	18.27	0.67	22.99	0.67	18.06	0.67	23.28	0.67	21.27
9.50	0.83	0.83	16.01	0.83	17.02	0.83	20.92	0.83	16.27	0.83	21.17	0.75	19.28
10.00	1.00	1.00	14.93	1.00	16.60	1.00	20.25	1.00	15.32	1.00	20.66	1.00	18.49
10.15	1.25	1.25	14.36	1.25	15.75	1.25	19.15	1.25	14.54	1.25	19.58	1.25	16.73
10.30	1.50	1.50	14.13	1.50	14.70	1.50	17.13	1.50	13.38	1.50	19.06	1.50	16.97
11.00	2.00	2.00	12.49	2.00	13.93	2.00	16.13	2.00	12.36	2.00	18.11	2.00	16.04
11.30	2.50	2.52	11.43	2.50	13.48	2.50	14.42	2.50	12.01	2.50	16.31	2.50	15.19
12.00	3.00	2.98	10.74	3.00	12.47	3.00	14.24	3.00	11.11	3.00	16.16	3.03	14.45
13.00	4.00	4.00	9.76	4.00	11.47	4.00	11.97	4.00	9.92	4.00	14.32	4.00	12.91
15.00	6.00	6.00	7.96	6.00	7.91	6.13	8.55	6.00	7.46	6.00	10.38	5.98	10.26
17.00	8.00	8.00	6.70	8.03	6.55	8.00	6.37	8.00	5.94	8.00	9.68	7.98	8.81
19.00	10.00	10.00	5.73	10.00	5.41	10.03	4.95	10.03	4.85	10.00	8.34	9.97	7.30
21.00	12.00	11.97	5.05	12.02	3.94	12.02	3.61	12.02	3.91	12.00	7.58	11.97	6.33
0.00	15.00	15.00	4.34	15.00	3.22	15.00	3.13	15.00	3.48	14.98	7.28	14.98	5.32
3.00	18.00	18.00	3.81	18.00	3.08	17.98	2.74	17.97	3.04	17.98	7.70	18.00	4.58
6.00	21.00	21.00	3.54	21.00	2.80	21.00	2.57	21.00	2.70	21.00	7.58	21.00	4.32
9.00	24.00	24.00	3.14	23.90	2.43	24.75	2.22	24.75	2.40	24.00	6.92	23.98	3.70

The underlined concentrations are the maximum measured concentrations

Appendix 14 (continued) Plasma Nitrate concentrations after infusion of 500 mg $NaNO_3$ over 30 minutes (365 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-0.63	2.81	-0.95	1.52	-0.92	1.09	-0.75	2.56	-0.42	1.97	-0.70	2.51
8.55	-0.08	-0.15	2.76	-0.18	1.28	-0.17	0.93	-0.20	2.25	-0.17	1.64	-0.17	1.87
9.10	0.17	0.17	9.09	0.17	11.01	0.17	13.84	0.17	10.73	0.17	8.46	0.17	13.80
9.20	0.33	0.33	15.48	0.33	17.62	0.33	23.43	0.33	16.00	0.33	14.00	0.33	14.56
9.30	0.50	0.52	17.15	0.52	22.58	0.52	30.81	0.52	18.48	0.52	20.85	0.53	21.85
9.40	0.67	0.67	17.71	0.67	17.78	0.67	25.87	0.67	20.86	0.67	20.09	0.67	19.86
9.50	0.83	0.83	16.05	0.83	17.00	0.83	24.23	0.83	20.83	0.83	16.35	0.83	22.75
10.00	1.00	1.00	15.89	1.00	15.86	1.00	21.96	1.00	18.05	1.00	16.19	1.00	21.75
10.15	1.25	1.25	15.04	1.25	15.33	1.25	20.84	1.25	17.06	1.25	15.48	1.25	18.34
10.30	1.50	1.52	14.45	1.50	14.73	1.50	19.46	1.50	15.00	1.50	14.22	1.50	16.70
11.00	2.00	2.00	13.24	2.00	13.45	2.00	18.00	2.00	12.81	2.00	13.02	2.00	15.09
11.30	2.50	2.50	12.10	2.52	12.65	2.50	16.48	2.50	12.32	2.50	11.94	2.50	14.54
12.00	3.00	3.00	11.15	3.00	11.80	3.00	15.17	3.00	12.96	3.00	11.39	3.00	13.38
13.00	4.00	4.00	9.81	4.00	11.09	4.00	13.35	3.98	12.13	4.00	9.52	3.98	11.70
15.00	6.00	6.02	7.45	6.00	7.97	6.00	9.20	6.00	9.22	6.08	6.55	6.03	9.34
17.00	8.00	7.97	6.16	7.97	6.88	8.00	7.58	7.98	8.04	8.03	6.43	8.00	8.63
19.00	10.00	9.98	5.25	9.95	5.32	9.97	5.51	9.97	6.91	10.03	4.56	9.98	5.75
21.00	12.00	12.00	4.05	11.97	4.16	12.00	3.99	11.97	5.73	12.05	3.28	12.03	5.22
0.00	15.00	15.00	3.75	15.00	3.45	14.98	2.80	14.97	4.07	15.00	2.86	15.00	4.42
3.00	18.00	17.98	3.50	17.98	2.88	18.03	2.67	18.03	3.97	18.08	2.40	17.95	3.96
6.00	21.00	21.17	3.21	21.17	2.61	21.17	2.30	21.25	3.52	21.92	2.12	21.75	3.81
9.00	24.00	24.00	2.80	23.95	2.26	24.00	2.10	23.83	3.22	23.97	1.91	23.92	3.25

The underlined concentrations are the maximum measured concentrations

Appendix 15 Plasma Nitrate concentrations after intake of 300 g spinach (564 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-0.30	1.39	-1.20	1.82	-1.00	2.39	-0.83	2.93	-1.20	2.77	-0.88	2.09
8.55	-0.08	-0.17	1.27	-0.33	1.39	-0.30	2.20	-0.40	3.22	-0.18	3.46	-0.23	2.41
9.20	0.33	0.33	10.99	0.33	5.78	0.33	6.06	0.33	4.38	0.33	8.34	0.33	11.79
9.40	0.67	0.67	17.08	0.67	13.51	0.67	17.34	0.67	9.81	0.67	15.98	0.67	22.27
10.00	1.00	1.00	16.50	1.00	15.39	1.00	16.70	1.00	15.19	1.00	20.57	1.00	22.50
10.20	1.33	1.33	17.77	1.33	18.80	1.33	22.60	1.33	15.97	1.33	22.01	1.33	21.90
10.40	1.67	1.68	17.21	1.67	21.46	1.67	18.67	1.67	20.36	1.67	24.70	1.67	23.99
11.00	2.00	2.02	17.80	2.00	20.45	2.00	20.71	2.00	19.37	2.00	22.48	2.00	20.67
11.20	2.33	2.35	15.54	2.33	18.49	2.33	20.89	2.33	17.78	2.33	21.96	2.33	19.31
11.40	2.67	2.67	14.95	2.67	18.28	2.67	18.68	2.67	17.40	2.67	21.26	2.67	17.19
12.00	3.00	3.00	15.73	3.00	19.65	3.00	18.57	3.00	14.31	3.00	18.35	3.00	16.74
12.30	3.50	3.42	13.61	3.50	17.38	3.50	19.61	3.50	15.27	3.50	17.66	3.50	16.72
13.00	4.00	4.00	14.73	4.00	17.73	4.00	16.71	4.00	16.96	4.00	21.29	4.00	18.14
14.00	5.00	5.00	12.72	5.05	13.95	5.00	16.09	5.00	16.17	5.00	19.58	5.00	16.15
15.00	6.00	6.00	10.49	6.00	12.70	6.00	12.91	6.00	14.57	6.00	15.28	6.00	16.02
17.00	8.00	8.00	9.79	8.00	9.97	8.00	9.91	7.92	8.97	8.00	13.10	8.00	13.54
19.00	10.00	10.00	7.76	9.98	7.49	10.00	6.30	10.00	8.99	10.00	10.63	10.00	10.79
21.00	12.00	12.00	6.55	11.98	5.87	11.98	5.65	12.00	6.52	12.02	8.44	12.02	9.89
0.00	15.00	15.00	6.27	15.00	4.83	15.00	4.83	15.00	4.46	15.00	7.33	15.00	7.48
3.00	18.00	18.03	4.56	18.00	4.02	18.00	3.95	18.00	3.25	18.03	6.26	18.03	6.73
6.00	21.00	21.00	4.56	21.00	3.23	21.00	3.55	21.00	2.89	21.03	6.17	21.03	6.04
9.00	24.00	23.83	4.23	23.82	2.94	23.83	2.55	23.78	2.09	23.83	5.29	23.83	4.61

The underlined concentrations are the maximum measured concentrations

Appendix 15 (continued) Plasma Nitrate concentrations after intake of 300 g spinach
(564 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-0.75	2.60	-0.92	2.37	-1.18	2.27	-1.08	2.20	-0.72	1.61	-0.92	4.05
8.55	-0.08	-0.27	3.04	-0.30	2.43	-0.15	3.34	-0.18	3.18	-0.22	1.68	-0.23	4.19
9.20	0.33	0.33	5.09	0.33	6.65	0.33	6.21	0.33	9.90	0.33	5.02	0.33	6.85
9.40	0.67	0.67	15.81	0.67	16.34	0.67	20.40	0.67	20.23	0.67	17.14	0.67	19.74
10.00	1.00	1.00	18.63	1.00	20.41	1.00	25.12	1.00	21.27	1.00	20.49	1.00	21.56
10.20	1.33	1.33	18.55	1.33	21.73	1.33	25.65	1.33	21.20	1.33	21.58	1.33	25.64
10.40	1.67	1.67	19.23	1.67	22.08	1.67	24.51	1.67	21.40	1.67	21.73	1.67	26.08
11.00	2.00	2.00	18.38	2.00	21.80	2.00	25.84	2.00	20.77	2.00	21.93	2.00	26.16
11.20	2.33	2.33	17.45	2.33	21.43	2.33	24.25	2.33	20.24	2.33	21.56	2.33	25.70
11.40	2.67	2.67	16.39	2.67	20.15	2.67	23.63	2.67	19.45	2.67	20.37	2.67	25.12
12.00	3.00	3.00	15.30	3.00	19.95	3.00	26.09	3.00	19.35	3.00	18.82	3.00	23.85
12.30	3.50	3.50	16.89	3.50	19.55	3.52	23.57	3.50	18.88	3.50	18.69	3.50	23.02
13.00	4.00	4.00	16.01	4.00	18.65	3.98	23.25	4.00	18.42	4.00	17.73	3.97	22.34
14.00	5.00	5.00	12.82	5.00	16.24	5.00	19.56	5.00	16.11	5.00	14.90	5.00	19.61
15.00	6.00	6.00	12.49	6.02	13.80	6.00	17.09	6.00	14.66	6.00	12.58	6.00	17.39
17.00	8.00	8.00	9.22	8.00	11.29	8.00	13.67	8.00	12.56	8.00	9.88	8.07	14.56
19.00	10.00	10.00	7.59	10.08	8.85	10.00	10.99	10.00	10.07	10.00	8.13	10.00	11.33
21.00	12.00	12.02	5.67	12.02	6.57	12.00	7.86	12.00	8.35	12.02	5.84	12.03	8.77
0.00	15.00	15.00	5.18	15.00	?	15.00	5.93	15.00	7.33	15.05	4.47	15.08	6.82
3.00	18.00	18.03	4.77	18.03	5.11	18.17	6.03	18.15	5.74	18.17	3.82	18.25	5.90
6.00	21.00	21.03	4.51	21.03	4.49	21.00	4.44	21.00	5.09	21.00	3.80	21.00	5.42
9.00	24.00	23.80	3.72	23.78	4.14	23.83	4.09	23.83	4.28	23.82	3.41	23.83	5.12

The underlined concentrations are the maximum measured concentrations

Appendix 16 Plasma Nitrate concentrations after intake of 300 g lettuce (1013 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-1.20	1.34	-1.22	1.57	-1.03	1.36	-1.03	2.00	-1.35	1.35	-1.27	1.77
8.55	-0.08	-0.28	1.95	-0.33	1.93	-0.37	1.42	-0.38	2.65	-0.42	1.39	-0.37	1.78
9.20	0.33	0.33	7.15	0.35	4.44	0.33	1.68	0.33	2.12	0.33	10.00	0.42	25.47
9.40	0.67	0.67	29.04	0.67	27.37	0.67	11.57	0.67	7.44	0.67	33.19	0.75	38.71
10.00	1.00	1.00	38.85	1.00	40.98	1.00	29.20	1.00	31.08	1.00	39.05	1.08	40.85
10.20	1.33	1.33	36.46	1.33	42.62	1.33	38.47	1.33	34.74	1.37	43.85	1.42	38.45
10.40	1.67	1.67	34.51	1.67	42.46	1.67	41.55	1.67	32.36	1.67	42.39	1.75	38.33
11.00	2.00	2.00	32.17	2.00	41.81	2.00	40.83	2.00	32.26	2.00	41.34	2.08	35.75
11.20	2.33	2.33	32.58	2.33	39.79	2.33	39.33	2.33	30.26	2.33	38.38	2.42	33.77
11.40	2.67	2.68	30.43	2.67	39.85	2.67	37.10	2.72	29.02	2.68	37.35	2.75	34.29
12.00	3.00	3.00	29.24	3.00	37.20	3.00	36.41	3.00	29.09	3.00	36.86	3.08	32.83
12.30	3.50	3.50	27.52	3.50	32.94	3.50	33.89	3.50	28.72	3.50	34.01	3.58	31.02
13.00	4.00	4.00	25.66	3.98	33.16	4.00	33.07	4.00	28.07	4.00	33.46	4.07	29.45
14.00	5.00	5.00	23.48	5.00	26.33	5.00	28.26	5.03	24.21	5.00	25.90	5.08	26.47
15.00	6.00	6.00	21.02	6.03	22.81	6.00	24.72	6.00	22.23	6.00	22.72	6.08	24.70
17.00	8.00	8.00	19.12	8.03	18.22	8.08	18.89	8.00	16.92	8.00	18.77	8.08	21.11
19.00	10.00	10.00	16.09	10.00	15.02	10.00	13.84	10.00	14.07	10.00	15.33	10.08	16.86
21.00	12.00	12.00	13.64	12.00	11.46	12.00	9.83	12.00	10.90	12.00	12.50	12.08	14.55
0.00	15.00	15.00	11.01	15.02	8.66	14.98	6.08	14.98	8.62	15.00	10.22	15.08	11.11
3.00	18.00	18.00	9.73	18.02	7.52	18.00	4.49	17.98	8.09	17.98	9.13	18.08	10.04
6.00	21.00	21.00	8.10	21.02	6.69	21.00	3.74	21.03	6.36	21.02	8.37	21.12	8.48
9.00	24.00	23.83	6.44	23.83	5.44	23.83	3.65	23.88	5.45	23.92	7.11	24.00	7.29

The underlined concentrations are the maximum measured concentrations

Appendix 16 (continued) Plasma Nitrate concentrations after intake of 300 g lettuce

(1013 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-1.05	1.47	-1.03	1.51	-1.07	1.19	-1.05	2.42	-0.97	1.34	-0.93	3.55
8.55	-0.08	-0.30	1.70	-0.35	1.70	-0.37	1.33	-0.38	2.45	-0.37	1.46	-0.37	4.52
9.20	0.33	0.33	13.53	0.33	15.37	0.33	5.25	0.33	21.49	0.37	3.77	0.33	6.67
9.40	0.67	0.67	32.39	0.67	37.62	0.67	27.03	0.68	42.27	0.70	21.10	0.68	29.09
10.00	1.00	1.02	35.23	1.00	43.29	1.00	48.59	1.00	48.71	1.00	34.27	1.00	40.48
10.20	1.33	1.33	32.71	1.33	41.77	1.33	50.35	1.33	45.84	1.33	38.46	1.33	46.64
10.40	1.67	1.67	32.98	1.67	43.68	1.67	54.18	1.67	42.14	1.67	39.81	1.67	46.95
11.00	2.00	2.00	32.98	2.00	39.21	2.00	48.82	2.00	42.09	2.00	42.22	2.00	47.17
11.20	2.33	2.33	29.09	2.33	37.02	2.33	57.54	2.33	41.41	2.33	39.02	2.33	44.90
11.40	2.67	2.67	27.72	2.67	37.51	2.67	48.15	2.68	41.07	2.67	35.57	2.67	45.64
12.00	3.00	3.00	27.77	3.00	37.12	3.00	42.92	3.00	39.35	3.00	34.09	3.02	46.64
12.30	3.50	3.50	27.53	3.50	33.27	3.50	43.09	3.50	38.50	3.50	32.63	3.52	43.16
13.00	4.00	4.02	25.02	4.00	31.38	4.00	40.53	4.00	37.05	4.00	31.91	4.00	37.03
14.00	5.00	5.03	20.90	5.00	27.44	5.00	33.50	5.00	31.17	5.00	23.70	5.00	33.11
15.00	6.00	6.00	18.00	6.00	24.23	6.00	27.78	6.00	31.34	6.30	18.58	6.05	27.18
17.00	8.00	8.00	14.54	8.00	20.77	8.00	23.26	8.00	24.10	8.00	15.14	8.00	22.54
19.00	10.00	10.00	11.43	10.00	17.60	10.00	17.43	10.00	20.93	10.00	12.20	10.00	17.79
21.00	12.00	12.00	9.07	12.00	13.06	12.00	15.25	12.00	17.76	12.00	8.89	12.00	15.15
0.00	15.00	15.05	7.97	15.02	9.89	15.00	10.62	15.00	14.48	15.02	6.60	15.10	10.99
3.00	18.00	18.00	7.02	18.00	8.49	18.00	8.58	18.00	12.38	18.03	4.96	18.05	9.74
6.00	21.00	21.00	5.75	21.00	7.32	21.00	8.45	21.00	11.73	21.02	5.13	21.03	8.72
9.00	24.00	23.83	4.69	23.87	5.94	23.87	6.54	23.83	9.61	23.83	5.08	23.88	6.92

The underlined concentrations are the maximum measured concentrations

Appendix 17 Plasma Nitrate concentrations after intake of 300 g beetroot (643 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-1.02	2.46	-0.98	1.92	-0.78	1.75	-0.75	4.35	-1.18	2.14	-1.20	2.34
8.55	-0.08	-0.30	2.73	-0.50	1.44	-0.38	1.42	-0.37	2.72	-0.40	0.93	-0.42	2.69
9.20	0.33	0.33	5.27	0.33	8.11	0.33	1.60	0.33	6.05	0.33	9.53	0.33	14.46
9.40	0.67	0.67	20.51	0.67	24.34	0.68	4.66	0.67	19.44	0.67	27.21	0.67	24.71
10.00	1.00	1.00	23.27	1.00	24.57	1.00	16.97	1.00	19.01	1.00	22.76	1.00	24.92
10.20	1.33	1.33	22.08	1.35	24.05	1.33	17.66	1.33	20.46	1.33	22.28	1.33	22.07
10.40	1.67	1.67	23.60	1.67	27.36	1.67	18.26	1.67	23.90	1.67	30.02	1.67	20.39
11.00	2.00	2.00	22.99	2.02	24.08	2.00	25.02	2.00	19.96	2.00	24.38	2.00	26.11
11.20	2.33	2.33	21.55	2.33	23.92	2.33	18.42	2.33	20.93	2.33	21.55	2.33	20.04
11.40	2.67	2.67	20.96	2.67	23.04	2.67	21.22	2.67	21.68	2.67	28.75	2.67	18.22
12.00	3.00	3.00	20.66	3.05	20.77	3.05	25.04	3.00	16.79	3.00	19.61	3.00	25.68
12.30	3.50	3.50	19.19	3.50	21.06	3.53	21.79	3.50	18.38	3.50	23.04	3.50	18.64
13.00	4.00	4.00	18.48	4.00	21.12	4.00	23.56	4.00	18.88	4.00	22.93	4.00	19.67
14.00	5.00	5.00	15.70	5.00	17.98	5.00	19.26	5.00	15.69	5.00	18.75	5.05	18.16
15.00	6.00	6.00	14.31	6.00	15.03	6.03	17.83	6.00	14.14	6.00	15.78	6.00	15.69
17.00	8.00	8.00	11.66	8.00	11.66	8.00	13.81	8.00	10.46	8.00	11.91	8.00	13.43
19.00	10.00	10.00	10.34	10.60	9.04	10.00	10.37	10.13	8.30	10.08	9.27	10.05	11.33
21.00	12.00	12.00	8.64	12.43	6.83	12.08	8.87	12.05	0.39	12.00	8.02	12.00	10.21
0.00	15.00	15.00	6.83	15.00	5.22	15.00	6.06	14.97	6.37	15.00	6.17	14.98	8.07
3.00	18.00	18.03	6.55	18.00	4.71	18.02	4.89	17.98	5.50	17.97	5.87	17.97	8.03
6.00	21.00	21.00	5.68	21.53	4.14	21.00	4.25	20.98	5.09	20.95	5.18	20.95	6.57
9.00	24.00	24.00	4.82	24.08	4.52	24.07	3.29	24.05	4.19	24.00	3.87	23.97	5.64

The underlined concentrations are the maximum measured concentrations

Appendix 17 (continued) Plasma Nitrate concentrations after intake of 300 g beetroot (643 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)	Scheme time (hr)	Nitrate (mg/kg)
8.30	-0.50	-1.00	1.72	-1.20	1.27	-0.97	1.27	-0.98	5.48	-0.97	1.94	-0.77	2.61
8.55	-0.08	-0.35	2.27	-0.43	1.23	-0.38	1.23	-0.40	5.14	-0.43	1.82	-0.45	1.88
9.20	0.33	0.33	7.76	0.33	14.07	0.33	5.65	0.33	14.62	0.33	4.58	0.33	2.40
9.40	0.67	0.67	19.66	0.67	20.50	0.67	23.41	0.67	23.39	0.67	22.83	0.67	28.27
10.00	1.00	1.00	20.91	1.00	21.26	1.03	30.95	1.00	32.03	1.00	25.89	1.00	28.50
10.20	1.33	1.33	21.49	1.33	28.63	1.33	29.07	1.33	30.17	1.33	26.06	1.33	28.89
10.40	1.67	1.67	21.66	1.67	22.28	1.67	29.69	1.67	28.02	1.67	24.13	1.67	28.72
11.00	2.00	2.00	20.86	2.00	18.90	2.00	29.59	2.00	28.03	2.00	24.08	2.00	29.28
11.20	2.33	2.33	19.48	2.33	25.40	2.42	28.57	2.35	26.35	2.33	23.41	2.33	29.21
11.40	2.67	2.67	19.72	2.67	20.31	2.77	28.64	2.67	26.30	2.67	22.52	2.72	27.69
12.00	3.00	3.00	18.39	3.00	18.96	3.12	28.45	3.00	24.86	3.00	21.45	3.00	27.65
12.30	3.50	3.50	18.72	3.50	21.72	3.58	28.09	3.50	24.65	3.50	21.21	3.50	26.22
13.00	4.00	4.00	17.20	3.98	18.65	4.50	24.53	4.03	22.80	4.03	20.80	4.00	27.02
14.00	5.00	5.00	13.70	5.00	15.94	5.58	19.33	5.00	20.36	5.00	16.77	5.00	22.48
15.00	6.00	6.00	12.98	6.00	14.46	6.22	18.00	6.02	18.93	6.00	14.32	6.05	19.99
17.00	8.00	8.00	11.26	8.00	11.32	8.00	14.21	8.00	15.57	8.42	11.93	8.03	17.16
19.00	10.00	10.00	9.27	10.00	8.83	10.00	11.48	10.00	12.88	10.02	9.88	10.00	12.77
21.00	12.00	12.02	6.96	11.97	7.57	11.98	7.70	12.00	11.11	12.00	6.63	11.97	10.69
0.00	15.00	14.97	5.89	14.98	5.74	14.98	5.90	14.95	9.07	15.08	5.29	14.92	8.22
3.00	18.00	18.00	5.39	17.95	4.66	17.97	4.72	17.93	7.98	17.92	4.50	18.00	6.27
6.00	21.00	20.98	4.65	20.95	4.86	20.98	14.82	21.05	7.18	21.07	4.34	21.10	5.86
9.00	24.00	24.00	3.97	23.95	3.33	24.00	3.37	24.00	5.84	24.00	3.68	23.98	5.57

The underlined concentrations are the maximum measured concentrations

Appendix 18 Kinetic parameters derived from the plasma nitrate concentrations

$NaNO_3$-solution (365 mg, intravenous)

Volunteer number	t-1/2 (hrs)	Corr. coef. (t-1/2)	AUC_{0-24} (mg*hr/kg)	Baseline nitrate (mg/kg)	Baseline (B) corrected AUC_{0-24} (mg*hr/kg)	VSS[1] (l)	VSS[1] (l/kg)
1	8.39	0.993	153.214	1.54	116.254	32	0,36
2	5.52	0.984	146.541	1.10	120.102	26	0,34
3	4.69	0.998	155.787	1.89	109.010	18	0,29
4	6.07	0.995	140.452	1.88	93.841	26	0,29
5	9.30	0.929	238.725	4.17	138.645	28	0,44
6	7.82	0.993	193.287	4.38	89.209[2]	19	0,24
7	6.53	0.992	144.497	2.78	77.693	26	0,32
8	5.83	0.990	146.394	1.40	112.864	24	0,32
9	4.73	0.993	166.481	1.01	142.241	18	0,29
10	7.73	0.985	170.287	2.40	113.013	24	0,30
11	5.57	0.956	129.984	1.81	86.749	21	0,27
12	6.62	0.954	175.241	2.19	122.857	24	0,38
Mean	6.57	0.980	163.408	2.21	112.115	24	0,32

300 g cooked spinach (564 mg, oral)

Volunteer number	t-1/2 (hrs)	Corr. coef. (t-1/2)	AUC_{0-24} (mg*hr/kg)	Bioavailability (F)	Baseline nitrate (mg/kg)	B. corrected AUC_{0-24} (mg*hr/kg)	Corrected Bioavailability (F)
1	7.19	0.972	198.983	0.84	1.33	167.289	0.93
2	5.18	0.993	197.250	0.87	1.61	158.974	0.86
3	4.63	0.975	199.971	0.83	2.30	145.282	0.86
4	5.63	0.939	191.325	0.88	3.08	119.850[2]	
5	6.07	0.984	262.772	0.71	3.11	188.522	0.88
6	8.92	0.980	262.951	0.88	2.25	209.334[2]	
7	5.60	0.989	198.012	0.89	2.82	130.896	1.09
8	5.51	0.996	228.221	1.01	2.40	171.150	0.98
9	5.38	0.994	267.976	1.04	2.80	201.203	0.92
10	7.18	0.997	247.770	0.94	2.69	183.667	1.05
11	5.24	0.993	205.971	1.03	1.64	166.788	1.24
12	6.11	0.996	278.971	1.03	4.12	180.792	0.95
Mean	6.05	0.984	228.348	0.91	2.51	169.456	0.98

300 g raw lettuce (1013 mg, oral)

Volunteer number	t-1/2 (hrs)	Corr. coef. (t-1/2)	AUC_{0-24} (mg*hr/kg)	Bioavailability (F)	Baseline nitrate (mg/kg)	B. corrected AUC_{0-24} (mg*hr/kg)	Corrected Bioavailability (F)
1	9.05	0.993	380.345	0.89	1.64	341.105	1.06
2	5.56	0.987	381.635	0.94	1.75	339.933	1.02
3	4.65	0.997	340.580	0.79	1.39	307.456	1.02
4	5.96	0.997	337.877	0.87	2.32	283.313	1.09
5	6.04	0.974	405.459	0.61	1.37	372.688	0.97
6	7.84	0.996	422.802	0.79	1.78	380.158[2]	
7	5.61	0.994	314.347	0.78	1.58	276.610	1.28
8	6.76	0.987	412.778	1.02	1.60	374.431	1.20
9	5.73	0.979	469.806	1.02	1.26	439.729	1.11
10	7.71	0.981	513.082	1.09	2.43	455.017	1.45
11	4.66	0.976	325.197	0.90	1.40	291.835	1.21
12	6.13	0.989	462.753	0.95	4.03	366.370	1.07
Mean	6.31	0.988	397.222	0.89	1.88	349.862	1.13

300 g cooked beetroot (643 mg, oral)

Volunteer number	t-1/2 (hrs)	Corr. coef. (t-1/2)	AUC_{0-24} (mg*hr/kg)	Bioavailability (F)	Baseline nitrate (mg/kg)	B. corrected AUC_{0-24} (mg*hr/kg)	Corrected Bioavailability (F)
1	7.64	0.984	253.096	0.94	2.60	190.787	0.93
2	5.38	0.993	245.859	0.95	1.68	205.405	0.97
3	5.73	0.989	248.026	0.90	1.58	209.805	1.09
4	5.36	0.996	229.887	0.93	3.53	144.862	0.88
5	5.28	0.980	261.729	0.62	1.53	224.920	0.92
6	8.27	0.985	285.251	0.84	2.52	224.939[2]	
7	6.82	0.974	226.150	0.89	2.00	178.263	1.30
8	6.08	0.994	237.360	0.92	1.25	207.423	1.04
9	4.80	0.987	285.738	0.97	1.25	255.738	1.02
10	7.65	0.997	325.221	1.08	5.31	197.781	0.99
11	5.36	0.972	240.773	1.05	1.88	195.653	1.28
12	6.11	0.990	321.333	1.04	2.24	267.517	1.24
Mean	6.21	0.987	263.369	0.93	2.28	207.105	1.06

1. VSS is calculated with correction of baseline.

2. These data are not included in the mean corrected AUC_{0-24}, since for UTN 6 (intravenous) and UTN 4 (spinach) only the AUC_{0-18} could be presented. Therefore the bioavailabilities were not calculated for these persons.

Appendix 19 Plasma Nitrite concentrations after infusion of 500 mg $NaNO_3$ over 30 minutes (365 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-0.58	0.34	-0.92	<0.2	-0.45	0.31	-0.83	<0.2	-0.83	<0.2	-0.83	<0.2
8.55	-0.08	-0.23	<0.2	-0.22	<0.2	-0.17	<0.2	-0.17	<0.2	-0.17	<0.2	-0.20	<0.2
9.10	0.17	0.17	<0.2	0.17	<0.2	0.17	<0.2	0.17	<0.2	0.17	<0.2	0.17	<0.2
9.20	0.33	0.33	0.32	0.33	<0.2	0.33	<0.2	0.25	<0.2	0.33	<0.2	0.33	<0.2
9.30	0.50	0.52	<0.2	0.52	0.34	0.52	0.31	0.52	<0.2	0.52	<0.2	0.52	<0.2
9.40	0.67	0.67	0.34	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2
9.50	0.83	0.83	<0.2	0.83	0.34	0.83	0.27	0.83	<0.2	0.83	<0.2	0.75	<0.2
10.00	1.00	1.00	<0.2	1.00	<0.2	1.00	0.31	1.00	<0.2	1.00	<0.2	1.00	<0.2
10.15	1.25	1.25	<0.2	1.25	0.33	1.25	<0.2	1.25	<0.2	1.25	<0.2	1.25	<0.2
10.30	1.50	1.50	<0.2	1.50	<0.2	1.50	0.30	1.50	<0.2	1.50	<0.2	1.50	<0.2
11.00	2.00	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2
11.30	2.50	2.52	0.34	2.50	0.31	2.50	0.29	2.50	<0.2	2.50	<0.2	2.50	<0.2
12.00	3.00	2.98	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.03	<0.2
13.00	4.00	4.00	<0.2	4.00	0.21	4.00	0.30	4.00	<0.2	4.00	<0.2	4.00	<0.2
15.00	6.00	6.00	0.33	6.00	0.36	6.13	<0.2	6.00	<0.2	6.00	<0.2	5.98	<0.2
17.00	8.00	8.00	<0.2	8.03	<0.2	8.00	<0.2	8.00	<0.2	8.00	<0.2	7.98	<0.2
19.00	10.00	10.00	<0.2	10.00	<0.2	10.03	0.30	10.03	<0.2	10.00	<0.2	9.97	<0.2
21.00	12.00	11.97	<0.2	12.02	<0.2	12.02	<0.2	12.02	<0.2	12.00	<0.2	11.97	<0.2
0.00	15.00	15.00	<0.2	15.00	<0.2	15.00	<0.2	15.00	<0.2	14.98	<0.2	14.98	<0.2
3.00	18.00	18.00	<0.2	18.00	<0.2	17.98	<0.2	17.97	<0.2	17.98	<0.2	18.00	<0.2
6.00	21.00	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.00	<0.2
9.00	24.00	24.00	<0.2	23.90	<0.2	24.75	0.28	24.75	<0.2	24.00	<0.2	23.98	<0.2

Appendix 19 (continued) Plasma Nitrite concentrations after infusion of 500 mg $NaNO_3$ over 30 minutes (365 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-0.63	<0.2	-0.95	<0.2	-0.92	<0.2	-0.75	<0.2	-0.42	<0.2	-0.70	<0.2
8.55	-0.08	-0.15	<0.2	-0.18	<0.2	-0.17	<0.2	-0.20	<0.2	-0.17	<0.2	-0.17	<0.2
9.10	0.17	0.17	<0.2	0.17	<0.2	0.17	<0.2	0.17	<0.2	0.17	<0.2	0.17	<0.2
9.20	0.33	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2
9.30	0.50	0.52	<0.2	0.52	<0.2	0.52	<0.2	0.52	<0.2	0.52	<0.2	0.53	<0.2
9.40	0.67	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2
9.50	0.83	0.83	<0.2	0.83	<0.2	0.83	<0.2	0.83	<0.2	0.83	<0.2	0.83	<0.2
10.00	1.00	1.00	<0.2	1.00	0.20	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2
10.15	1.25	1.25	<0.2	1.25	<0.2	1.25	<0.2	1.25	<0.2	1.25	<0.2	1.25	<0.2
10.30	1.50	1.52	<0.2	1.50	<0.2	1.50	<0.2	1.50	<0.2	1.50	<0.2	1.50	<0.2
11.00	2.00	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2
11.30	2.50	2.50	<0.2	2.52	<0.2	2.50	<0.2	2.50	<0.2	2.50	<0.2	2.50	<0.2
12.00	3.00	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2
13.00	4.00	4.00	0.21	4.00	<0.2	4.00	<0.2	3.98	<0.2	4.00	<0.2	3.98	<0.2
15.00	6.00	6.02	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.08	<0.2	6.03	<0.2
17.00	8.00	7.97	<0.2	7.97	<0.2	8.00	<0.2	7.98	<0.2	8.03	<0.2	8.00	<0.2
19.00	10.00	9.98	<0.2	9.95	<0.2	9.97	<0.2	9.97	<0.2	10.03	<0.2	9.98	<0.2
21.00	12.00	12.00	<0.2	11.97	<0.2	12.00	<0.2	11.97	<0.2	12.05	<0.2	12.03	<0.2
0.00	15.00	15.00	<0.2	15.00	<0.2	14.98	<0.2	14.97	<0.2	15.00	<0.2	15.00	<0.2
3.00	18.00	17.98	<0.2	17.98	<0.2	18.03	<0.2	18.03	<0.2	18.08	<0.2	17.95	<0.2
6.00	21.00	21.17	<0.2	21.17	<0.2	21.17	<0.2	21.25	<0.2	21.92	<0.2	21.75	<0.2
9.00	24.00	24.00	<0.2	23.95	<0.2	24.00	<0.2	23.83	<0.2	23.97	<0.2	23.92	<0.2

Appendix 20 Plasma Nitrite concentrations after intake of 300 g spinach (564 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-0.30	<0.2	-1.20	<0.2	-1.00	<0.2	-0.83	<0.2	-1.20	<0.2	-0.88	<0.2
8.55	-0.08	-0.17	<0.2	-0.33	<0.2	-0.30	<0.2	-0.40	<0.2	-0.18	<0.2	-0.23	<0.2
9.20	0.33	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2
9.40	0.67	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2
10.00	1.00	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2
10.20	1.33	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2
10.40	1.67	1.68	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2
11.00	2.00	2.02	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2
11.20	2.33	2.35	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2
11.40	2.67	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2
12.00	3.00	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2
12.30	3.50	3.42	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2	3.50	-
13.00	4.00	4.00	<0.2	4.00	<0.2	4.00	<0.2	4.00	<0.2	4.00	<0.2	4.00	<0.2
14.00	5.00	5.00	<0.2	5.05	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2
15.00	6.00	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2
17.00	8.00	8.00	<0.2	8.00	<0.2	8.00	<0.2	7.92	<0.2	8.00	<0.2	8.00	<0.2
19.00	10.00	10.00	<0.2	9.98	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2
21.00	12.00	12.00	<0.2	11.98	<0.2	11.98	<0.2	12.00	<0.2	12.02	<0.2	12.02	<0.2
0.00	15.00	15.00	<0.2	15.00	<0.2	15.00	<0.2	15.00	<0.2	15.00	<0.2	15.00	<0.2
3.00	18.00	18.03	<0.2	18.00	<0.2	18.00	<0.2	18.00	<0.2	18.03	<0.2	18.03	<0.2
6.00	21.00	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.03	<0.2	21.03	<0.2
9.00	24.00	23.83	<0.2	23.82	<0.2	23.83	<0.2	23.78	-	23.83	-	23.83	<0.2

- No nitrite found in the plasma

Appendix 20 (continued) Plasma Nitrite concentrations after intake of 300 g spinach
(564 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-0.75	<0.2	-0.92	<0.2	-1.18	<0.2	-1.08	<0.2	-0.72	<0.2	-0.92	<0.2
8.55	-0.08	-0.27	-	-0.30	<0.2	-0.15	<0.2	-0.18	-	-0.22	<0.2	-0.23	<0.2
9.20	0.33	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2
9.40	0.67	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2
10.00	1.00	1.00	<0.2	1.00	-	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2
10.20	1.33	1.33	<0.2	1.33	-	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2
10.40	1.67	1.67	-	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2
11.00	2.00	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2
11.20	2.33	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2
11.40	2.67	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2
12.00	3.00	3.00	<0.2	3.00	-	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2
12.30	3.50	3.50	<0.2	3.50	<0.2	3.52	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2
13.00	4.00	4.00	-	4.00	<0.2	3.98	<0.2	4.00	-	4.00	<0.2	3.97	<0.2
14.00	5.00	5.00	<0.2	5.00	-	5.00	0.21	5.00	-	5.00	<0.2	5.00	<0.2
15.00	6.00	6.00	<0.2	6.02	-	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2
17.00	8.00	8.00	<0.2	8.00	0.74	8.00	<0.2	8.00	-	8.00	<0.2	8.07	<0.2
19.00	10.00	10.00	<0.2	10.08	<0.2	10.00	-	10.00	<0.2	10.00	<0.2	10.00	<0.2
21.00	12.00	12.02	<0.2	12.02	<0.2	12.00	<0.2	12.00	<0.2	12.02	<0.2	12.03	<0.2
0.00	15.00	15.00	<0.2	15.00	?	15.00	<0.2	15.00	<0.2	15.05	<0.2	15.08	-
3.00	18.00	18.03	<0.2	18.03	-	18.17	<0.2	18.15	<0.2	18.17	<0.2	18.25	0.24
6.00	21.00	21.03	<0.2	21.03	<0.2	21.00	<0.2	21.00	-	21.00	<0.2	21.00	<0.2
9.00	24.00	23.80	-	23.78	-	23.83	<0.2	23.83	-	23.82	<0.2	23.83	<0.2

- No nitrite found in the plasma

Appendix 21 Plasma Nitrite concentrations after intake of 300 g lettuce (1013 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-1.20	<0.2	-1.22	<0.2	-1.03	<0.2	-1.03	<0.2	-1.35	<0.2	-1.27	<0.2
8.55	-0.08	-0.28	-	-0.33	<0.2	-0.37	-	-0.38	<0.2	-0.42	<0.2	-0.37	<0.2
9.20	0.33	0.33	<0.2	0.35	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.42	<0.2
9.40	0.67	0.67	-	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.75	<0.2
10.00	1.00	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.08	<0.2
10.20	1.33	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.37	<0.2	1.42	<0.2
10.40	1.67	1.67	<0.2	1.67	<0.2	1.67	-	1.67	-	1.67	<0.2	1.75	<0.2
11.00	2.00	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.08	<0.2
11.20	2.33	2.33	-	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.42	-
11.40	2.67	2.68	<0.2	2.67	-	2.67	<0.2	2.72	<0.2	2.68	<0.2	2.75	<0.2
12.00	3.00	3.00	-	3.00	<0.2	3.00	<0.2	3.00	-	3.00	<0.2	3.08	<0.2
12.30	3.50	3.50	-	3.50	<0.2	3.50	-	3.50	<0.2	3.50	<0.2	3.58	<0.2
13.00	4.00	4.00	-	3.98	<0.2	4.00	<0.2	4.00	<0.2	4.00	-	4.07	<0.2
14.00	5.00	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.03	<0.2	5.00	<0.2	5.08	<0.2
15.00	6.00	6.00	<0.2	6.03	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.08	<0.2
17.00	8.00	8.00	<0.2	8.03	<0.2	8.08	<0.2	8.00	<0.2	8.00	-	8.08	<0.2
19.00	10.00	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.08	-
21.00	12.00	12.00	-	12.00	<0.2	12.00	<0.2	12.00	<0.2	12.00	-	12.08	<0.2
0.00	15.00	15.00	<0.2	15.02	<0.2	14.98	<0.2	14.98	<0.2	15.00	<0.2	15.08	<0.2
3.00	18.00	18.00	<0.2	18.02	<0.2	18.00	<0.2	17.98	<0.2	17.98	<0.2	18.08	-
6.00	21.00	21.00	<0.2	21.02	<0.2	21.00	<0.2	21.03	<0.2	21.02	<0.2	21.12	-
9.00	24.00	23.83	<0.2	23.83	-	23.83	-	23.88	<0.2	23.92	<0.2	24.00	-

- No nitrite found in the plasma

Appendix 21 (continued) Plasma Nitrite concentrations after intake of 300 g lettuce
(1013 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-1.05	<0.2	-1.03	<0.2	-1.07	<0.2	-1.05	<0.2	-0.97	<0.2	-0.93	<0.2
8.55	-0.08	-0.30	<0.2	-0.35	<0.2	-0.37	<0.2	-0.38	<0.2	-0.37	-	-0.37	<0.2
9.20	0.33	0.33	<0.2	0.33	<0.2	0.33	-	0.33	<0.2	0.37	<0.2	0.33	-
9.40	0.67	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.68	<0.2	0.70	<0.2	0.68	<0.2
10.00	1.00	1.02	-	1.00	-	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2
10.20	1.33	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2	1.33	<0.2
10.40	1.67	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2
11.00	2.00	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2
11.20	2.33	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	0.22
11.40	2.67	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.68	<0.2	2.67	<0.2	2.67	<0.2
12.00	3.00	3.00	<0.2	3.00	<0.2	3.00	<0.2	3.00	-	3.00	<0.2	3.02	<0.2
12.30	3.50	3.50	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2	3.52	<0.2
13.00	4.00	4.02	<0.2	4.00	<0.2	4.00	<0.2	4.00	<0.2	4.00	<0.2	4.00	<0.2
14.00	5.00	5.03	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2
15.00	6.00	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2	6.30	<0.2	6.05	<0.2
17.00	8.00	8.00	<0.2	8.00	<0.2	8.00	<0.2	8.00	<0.2	8.00	<0.2	8.00	<0.2
19.00	10.00	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2
21.00	12.00	12.00	<0.2	12.00	<0.2	12.00	<0.2	12.00	<0.2	12.00	<0.2	12.00	<0.2
0.00	15.00	15.05	<0.2	15.02	<0.2	15.00	<0.2	15.00	<0.2	15.02	<0.2	15.10	<0.2
3.00	18.00	18.00	<0.2	18.00	<0.2	18.00	<0.2	18.00	<0.2	18.03	<0.2	18.05	<0.2
6.00	21.00	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.00	<0.2	21.02	<0.2	21.03	<0.2
9.00	24.00	23.83	<0.2	23.87	<0.2	23.87	<0.2	23.83	<0.2	23.83	<0.2	23.88	<0.2

- No nitrite found in the plasma

Appendix 22 Plasma Nitrite concentrations after intake of 300 g beetroot (643 mg nitrate)

Volunteernumber		1		2		3		4		5		6	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-1.02	<0.2	-0.98	0.22	-0.78	<0.2	-0.75	<0.2	-1.18	<0.2	-1.20	0.23
8.55	-0.08	-0.30	<0.2	-0.50	<0.2	-0.38	<0.2	-0.37	<0.2	-0.40	<0.2	-0.42	<0.2
9.20	0.33	0.33	-	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2
9.40	0.67	0.67	-	0.67	<0.2	0.68	<0.2	0.67	<0.2	0.67	<0.2	0.67	0.22
10.00	1.00	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2	1.00	0.23	1.00	<0.2
10.20	1.33	1.33	-	1.35	0.23	1.33	0.22	1.33	<0.2	1.33	<0.2	1.33	0.21
10.40	1.67	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2
11.00	2.00	2.00	<0.2	2.02	<0.2	2.00	<0.2	2.00	<0.2	2.00	0.23	2.00	<0.2
11.20	2.33	2.33	-	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2	2.33	<0.2
11.40	2.67	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2	2.67	<0.2
12.00	3.00	3.00	<0.2	3.05	<0.2	3.05	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2
12.30	3.50	3.50	<0.2	3.50	0.23	3.53	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2
13.00	4.00	4.00	<0.2	4.00	<0.2	4.00	<0.2	4.00	-	4.00	<0.2	4.00	<0.2
14.00	5.00	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2	5.05	-
15.00	6.00	6.00	<0.2	6.00	<0.2	6.03	<0.2	6.00	<0.2	6.00	<0.2	6.00	<0.2
17.00	8.00	8.00	<0.2	8.00	<0.2	8.00	<0.2	8.00	<0.2	8.00	<0.2	8.00	-
19.00	10.00	10.00	<0.2	10.60	<0.2	10.00	<0.2	10.13	<0.2	10.08	<0.2	10.05	<0.2
21.00	12.00	12.00	<0.2	12.43	-	12.08	<0.2	12.05	<0.2	12.00	<0.2	12.00	<0.2
0.00	15.00	15.00	<0.2	15.00	<0.2	15.00	<0.2	14.97	-	15.00	<0.2	14.98	<0.2
3.00	18.00	18.03	<0.2	18.00	<0.2	18.02	<0.2	17.98	<0.2	17.97	-	17.97	-
6.00	21.00	21.00	<0.2	21.53	<0.2	21.00	-	20.98	<0.2	20.95	<0.2	20.95	<0.2
9.00	24.00	24.00	-	24.08	<0.2	24.07	<0.2	24.05	<0.2	24.00	<0.2	23.97	-

- No nitrite found in the plasma

Appendix 22 (continued) Plasma Nitrite concentrations after intake of 300 g beetroot
(643 mg nitrate)

Volunteernumber		7		8		9		10		11		12	
Scheduled time (hr.min)	Scheme time (hr)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)	Scheme time (hr)	Nitrite (mg/kg)
8.30	-0.50	-1.00	<0.2	-1.20	<0.2	-0.97	-	-0.98	<0.2	-0.97	<0.2	-0.77	<0.2
8.55	-0.08	-0.35	-	-0.43	-	-0.38	<0.2	-0.40	<0.2	-0.43	-	-0.45	<0.2
9.20	0.33	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	<0.2	0.33	-
9.40	0.67	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2	0.67	<0.2
10.00	1.00	1.00	<0.2	1.00	<0.2	1.03	<0.2	1.00	<0.2	1.00	<0.2	1.00	<0.2
10.20	1.33	1.33	<0.2	1.33	<0.2	1.33	-	1.33	<0.2	1.33	<0.2	1.33	<0.2
10.40	1.67	1.67	<0.2	1.67	0.21	1.67	<0.2	1.67	<0.2	1.67	<0.2	1.67	<0.2
11.00	2.00	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2	2.00	<0.2
11.20	2.33	2.33	<0.2	2.33	<0.2	2.42	<0.2	2.35	<0.2	2.33	<0.2	2.33	<0.2
11.40	2.67	2.67	<0.2	2.67	0.22	2.77	<0.2	2.67	<0.2	2.67	<0.2	2.72	<0.2
12.00	3.00	3.00	-	3.00	0.20	3.12	<0.2	3.00	<0.2	3.00	<0.2	3.00	<0.2
12.30	3.50	3.50	<0.2	3.50	<0.2	3.58	<0.2	3.50	<0.2	3.50	<0.2	3.50	<0.2
13.00	4.00	4.00	<0.2	3.98	<0.2	4.50	<0.2	4.03	<0.2	4.03	<0.2	4.00	<0.2
14.00	5.00	5.00	<0.2	5.00	<0.2	5.58	<0.2	5.00	<0.2	5.00	<0.2	5.00	<0.2
15.00	6.00	6.00	<0.2	6.00	<0.2	6.22	<0.2	6.02	<0.2	6.00	<0.2	6.05	<0.2
17.00	8.00	8.00	<0.2	8.00	-	8.00	<0.2	8.00	<0.2	8.42	<0.2	8.03	<0.2
19.00	10.00	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.00	<0.2	10.02	<0.2	10.00	<0.2
21.00	12.00	12.02	<0.2	11.97	<0.2	11.98	<0.2	12.00	<0.2	12.00	<0.2	11.97	<0.2
0.00	15.00	14.97	-	14.98	<0.2	14.98	<0.2	14.95	<0.2	15.08	<0.2	14.92	<0.2
3.00	18.00	18.00	<0.2	17.95	<0.2	17.97	<0.2	17.93	<0.2	17.92	<0.2	18.00	<0.2
6.00	21.00	20.98	<0.2	20.95	<0.2	20.98	<0.2	21.05	<0.2	21.07	<0.2	21.10	<0.2
9.00	24.00	24.00	<0.2	23.95	<0.2	24.00	<0.2	24.00	<0.2	24.00	<0.2	23.98	<0.2

- No nitrite found in the plasma

Appendix 23 Blood pressure and heart rate during the first hour of sodium nitrate infusion

Volunteer number		1			2			3			4		
Clock Time (hrs)	Scheme Time (hrs)	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.
8.30	-0.50	-0.45	139/78	77	-1.10	127/71	68	-0.87	107/65	71	-0.85	120/65	67
9.10	0.17	0.25	141/80	67	0.32	125/83	56	0.27	111/69	64	0.32	117/73	66
9.20	0.33	0.42	135/79	68	0.48	120/70	57	0.42	104/63	62	0.48	122/69	63
9.30	0.50	0.60	137/79	65	0.67	122/78	54	0.60	105/67	65	0.67	135/73	68
9.40	0.67	0.75	128/76	64	0.82	124/62	54	0.75	105/61	59	0.82	119/71	61
9.50	0.83	0.93	134/81	71	0.98	128/66	59	0.92	114/64	70	0.98	116/69	63
10.00	1.00	1.08	142/77	69	1.17	121/69	62	1.08	103/62	63	1.15	117/68	60

Volunteer number		5			6			7			8		
Clock Time (hrs)	Scheme Time (hrs)	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.
8.30	-0.50	-0.93	120/68	64	-0.88	128/73	63	-0.43	121/67	65	-0.93	121/73	71
9.10	0.17	0.27	126/71	54	0.32	117/69	58	0.27	129/66	57	0.32	111/58	53
9.20	0.33	0.45	120/66	57	0.50	118/74	49	0.42	119/64	61	0.48	114/57	49
9.30	0.50	0.62	122/79	54	0.67	124/64	51	0.60	112/67	55	0.67	110/69	53
9.40	0.67	0.75	120/69	60	0.82	124/64	51	0.77	119/70	59	0.82	110/60	55
9.50	0.83	0.92	101/60	63	1.00	123/63	52	0.92	116/63	58	0.98	117/64	59
10.00	1.00	1.10	126/70	60	1.15	119/72	55	1.08	115/69	56	1.15	118/68	61

Volunteer number		9			10			11			12		
Clock Time (hrs)	Scheme Time (hrs)	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.	Scheme Time (hrs)	Blood pressure mmHg systolic/diastolic	Heart rate b.p.m.
8.30	-0.50	-1.02	124/88	79	-0.78	129/64	45	-1.00	135/87	76	-0.62	120/60	74
9.10	0.17	0.27	115/77	59	0.33	124/72	49	0.27	117/64	64	0.33	104/65	59
9.20	0.33	0.42	124/67	55	0.48	121/70	45	0.43	129/67	76	0.50	109/65	55
9.30	0.50	0.60	117/71	59	0.67	123/65	48	0.62	119/62	75	0.68	104/63	48
9.40	0.67	0.77	118/69	57	0.83	127/67	43	0.75	114/63	63	0.82	108/69	58
9.50	0.83	0.92	115/79	58	1.00	120/58	43	0.93	105/69	74	1.00	101/60	52
10.00	1.00	1.10	118/72	64	1.15	129/64	42	1.10	120/65	61	1.15	109/54	54

b.p.m. = beats per minute