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The effect of nitrate on the thyroid gland function in healthy volunteers in a 4-week oral toxicity study

A.C. Lambers, H.P.F. Koppeschaar, J.W. van Isselt, W. Slob, R.C. Schothorst, Tj.T. Mensinga, 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

Increased nitrate intake might affect the thyroid gland function in humans, as has been observed in animals. The reason is that the nitrate-ion (NO_3^-) inhibits the iodide (I^-) transport into the thyroid gland because it shares the same transport mechanism. This inhibition could lead to a decrease in thyroid hormone (T_4, T_3) secretion, followed by an increase in the thyroid-stimulating hormone (TSH). In the end thyroid gland enlargement (goitre) could occur. Since there is only weak epidemiological evidence that thyroid gland enlargement may occur in humans, our aim was to investigate the effect of nitrate on the human thyroid gland function by means of a four-week nitrate exposure study. Once a day for 28 days ten volunteers received an oral solution of 15 mg sodium nitrate per kg body weight (three times the allowed daily intake, ADI) in 200 ml distilled water (nitrate group), and ten volunteers received 200 ml distilled water (control group). Both groups followed an iodine-restricted and low-nitrate diet; this was checked by measuring urinary iodide and plasma nitrate concentration. Before and after the 28-day exposure period the percentage (%) radioiodine (^{131}I) uptake (RAIU) was measured 5 hours and 24 hours after ^{131}I-capsule intake to investigate the competition of nitrate in the iodide transport. Before (nitrate) exposure and 2, 3 and 4 weeks after the start of the exposure period blood samples were taken to measure the hormones T_4, T_3, rT_3, TSH and IGF I to investigate the thyroid gland function. Nitrate was found to have no effect on the hormone concentrations during the four-week nitrate exposure of three times the ADI of nitrate. Within the nitrate group the 24-hr RAIU had increased 1.5 times (from 20 % to 29 %) after 28 days of nitrate exposure compared to the 24-hr RAIU before exposure, while a decrease in 24-hr RAIU in the nitrate group had been expected. So we can conclude that an exposure of three times the ADI of nitrate will not cause changes in thyroid gland function in a healthy population.

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Abbreviations

^{131}I	radioiodine
ADI	Acceptable Daily Intake
ALAT	alanine amino transferase
ARO	Laboratory for Residue Analysis
ASAT	aspartate amino transferase
AZU	University Hospital Utrecht (Academisch Ziekenhuis Utrecht)
BKG	background
BMI	body mass index
b.p.m.	beats per minute
bw	body weight
Ce^{3+}	$Cerium^{3+}$
Ce^{4+}	$Cerium^{4+}$
cm	centimetres
cpm	counts per minute
C-term	carboxyl terminal
CV	coefficient of variation
DCF	decay correction factor
ECG	electrocardiogram
ESR	erythrocyte sedimentation rate
exp9	times 10^9
F	female
FAO	Food and Agricultural Organisation
g	gram
GCP	Good Clinical Practice
γGT	gamma glutamyl transpeptidase
Hb	haemoglobin
HPIC	high performance ion chromatography
hr	hour
hrs	hours
HR	heart rate
Ht	haematocrite
I	iodine
I^-	iodide
IC-1	Intensive Care Unit 1
IGF I	insulin-like growth factor I
IRMA	Immunoradiometric assay
isofr	iso-fraction
kg	kilogram
l	litre
LAC	Laboratory of Inorganic Analytical Chemistry

LDH	lactate dehydrogenase
M	male
MBq	MegaBecquerel
MD	Medical Doctor
metHb	methaemoglobin
min	minutes
mg	milligram
ml	millilitres
mm	millimetres
mmHg	millimetres mercury pressure
mmol	millimole
µmol	micromole
$NaNO_3$	sodium nitrate
nmol	nanomole
NO_2^-	nitrite-ion
NO_3^-	nitrate-ion
N-term	amino terminal
NVIC	National Poisons Control Centre
QA	quality assurance
ra	retained activity
RAIU	radioactive iodine uptake
RIVM	National Institute of Public Health and the Environment
rT_3	reverse triiodothyronine
RT_3U	resin triiodothyronine uptake
SD	standard deviation
SOP	standard operating procedure
T_3	triiodothyronine
T_4	thyroxine
TSH	thyrotropin (thyroid stimulating hormone)
U/l	units per litre
UMC	University Medical Center Utrecht
USA	United States of America
UTN	unique trial number
UV	ultraviolet
WHO	World Health Organisation
WOM	Medical Ethics Committee of the University Medical Center Utrecht
yrs	years
y/m/d	year/month/day

Samenvatting

De mogelijkheid bestaat, dat nitraat de schildklierfunctie in de mens remt, zoals dit in proefdieronderzoek is gevonden. Het mechanisme hierachter is dat het nitraation (NO_3^-) opname van jodide (I^-) in schildkliercellen competitief remt door gebruik te maken van hetzelfde transportmechanisme. Een lagere schildklier jodide opname kan leiden tot een verlaagde schildklierhormoon secretie (T_4, T_3), gevolgd door een verhoogde productie van TSH (thyroid stimulerend hormoon). Hierdoor kan een schildklier vergroting (krop) ontstaan. Onze doelstelling was daarom om het effect van nitraat op de menselijke schildklierfunctie te onderzoeken in een vier weken nitraat blootstellingstudie. Gedurende 28 dagen kregen tien vrijwilligers dagelijks een oplossing met 15 mg natriumnitraat per kg lichaamsgewicht (driemaal de huidige maximaal toelaatbare dagelijkse hoeveelheid, ADI) in 200 ml gedestilleerd water oraal toegediend (nitraatgroep) en kregen tien vrijwilligers dagelijks 200 ml gedestilleerd water oraal toegediend (controlegroep). Beide groepen volgden een jodium-beperkt en nitraatarm dieet, wat gecontroleerd werd aan de hand van 24-uurs jodide in urine en plasma nitraat-concentraties. Voor en na 28 dagen blootstelling werd het percentage (%) radiojodium (^{131}I) opname (RAIU) gemeten op 5 uur en 24 uur na de inname van de ^{131}I-capsule om het competitief effect van nitraat op de schildklier jodide opname te meten. Voor (nitraat) blootstelling en twee, drie en vier weken na de start aan de blootstellingperiode werden bloedmonsters afgenomen voor de hormoonconcentraties van TSH, T_4, T_3, rT_3 en IGF I om de schildklierfunctie te bepalen. Er werd geen effect van nitraat op de hormoonconcentraties gevonden gedurende de vier weken blootstellingperiode aan driemaal de huidige ADI van nitraat. Na vier weken nitraat blootstelling was de 24-uurs RAIU 1,5 maal de 24-uurs RAIU gemeten voor nitraat blootstelling, van 20 % naar 29 %. Echter, een afname in 24-uurs RAIU in de nitraatgroep werd verwacht. De conclusie van dit onderzoek is daarom dat een blootstelling aan driemaal de huidige ADI van nitraat geen veranderingen in schildklierfunctie te weeg zal brengen in een gezonde populatie.

1. Introduction

In 1974, based on the literature that was available then, the Joint FAO/WHO Expert Committee on Food Additives established an Acceptable Daily Intake (ADI) of nitrate of 3.65 mg nitrate-ion (5 mg sodium nitrate) per kg body weight (1). Since this evaluation additional data have become available and several comprehensive reviews have been published (2,3,4,5,6). Most reviews resulted in a provisional advice to maintain the ADI at its current level but still mentioned the need for further research.

At the request of the Inspectorate for Health Protection, Commodities and Veterinary Public Health the National Poisons Control Centre performed several kinetic studies on nitrate and nitrite to study the validity of the current ADI (8, 9, 10, 11).

Nitrate is thought to be not toxic, although in several animal studies an effect of nitrate on the thyroid gland is found. The iodine uptake is affected (12, 13, 14), the production of thyroid hormones (T_4/T_3) is depressed (15, 16, 17, 18) and the production of TSH is stimulated. In the end goitre (increasing thyroid weight) may occur (19, 20). Besides decreased IGF I concentrations have been found (15, 16, 17). For humans only some weak epidemiological evidence exists (21, 22, 23).

The National Poisons Control Centre therefore performed an experimental study to evaluate the effect of nitrate on the thyroid gland in healthy volunteers. The hypothesis was that the iodine uptake would be depressed by nitrate by competitive inhibition and therefore thyroid hormone concentrations (T_4/T_3) would decrease and TSH (influenced by T_4 and T_3) concentration would increase. A decrease in IGF I concentration (influenced by T_4) was expected.

2. Materials and methods

2.1 Study protocol

The present study was carried out in accordance with the protocol entitled "The effect of nitrate on the thyroid gland function in healthy volunteers: 4 week oral toxicity study (project number 348801 007, project 235802, dated 01-09-1998)". The study protocol was approved by the Medical Ethics Committee of the University Hospital in Utrecht, the Netherlands. No major protocol violations occurred. The deviations from the protocol are summarised in Appendix 2. These deviations had no impact on the outcome of the study.
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

Six men and fourteen women in the age of 20 to 30 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 buildings 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, 30 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. 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. Ten of the volunteers who underwent the medical screening were excluded (see 3.1). Three persons were standby and twenty volunteers entered the study.
General practitioners were informed that their patients were going to participate in the study and were asked to inform the medical investigator in case they disagreed on the decision to enrol a particular subject in the study.

After the study was completed the volunteers underwent a post-study medical examination. The volunteers were asked to report any changes 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

One batch (Charge: 2G002_99B19, expiry date: 02-2000) of sodium nitrate solution (60 mg/ml) was prepared by the Central Pharmacy of the AZU/UMC. Bottles of 100 ml were kept in a dark place at room temperature. The laboratory for Residue Analysis has analysed 5 of these bottles after the study and found concentrations of 60.6 mg/ml, 59.6 mg/ml, 61.2 mg/ml, 60.1 mg/ml and 59.6 mg/ml.

The doses depended on the body weight of the volunteers and are shown in Appendix 6. Before the volunteers arrived the amounts of sodium nitrate solution were weighed in a mug on a Sartorius Portable Balance. The mug was then filled up to about 200 ml with distilled water and the distilled water and sodium nitrate were mixed. It was assumed that 1 ml sodium nitrate solution weighed 1 g. The intake of the sodium nitrate solution was orally.

2.4 Study design

2.4.1 General

The study was designed as a 4-week open nitrate exposure study. Of the 20 healthy adult volunteers that participated in this study, 10 received a sodium nitrate dose of 3 times the ADI (15 mg $NaNO_3$ per kg body weight) dissolved in 200 ml distilled water once daily (nitrate group) and 10 volunteers received 200 ml distilled water once daily (control group).

All volunteers followed dietary restrictions (iodide-restricted and nitrate low) for two weeks prior to and for the whole duration of the study. To check if the volunteers had kept to the diet, nitrate in plasma and iodide in 24-hr urine was determined.

2.4.2 Procedures

The clinical part of the study included a continuous period of 30 days (0 - 29). Each day the volunteers visited the Clinical Research Unit between 8.00 a.m. and 10.00 a.m. Daily the volunteers were asked about adverse effects and the dietary records were quickly checked for completeness. On day 1 the administration of nitrate or distilled water was started. Once daily, the nitrate group received an oral sodium nitrate dose of 15 mg per kg body weight (in 200 ml distilled water) and the control group received 200 ml distilled water. On days 0/1 and 28/29 the iodine uptake of the thyroid gland was measured. On days 0, 14, 21, 28 and during the pre- and post-study screening blood samples were obtained for thyroid hormone analyses. On days 0, 7, 28 and during the pre- and post-study screening blood samples were taken for methaemoglobin analyses. On days 0, 7, 14, 21 and 28 body weight was registered. In addition the blood pressure and heart rate were registered on days 0, 1, 7, 14, 21 and 28.

Blood samples were taken to check nitrate and nitrite levels in plasma at the pre- and post-study screening and twice a week during the study. During the study period this occurred randomly and for every volunteer on different days. Only the investigators knew the exact days. 24 hr urine was collected on the day before day 0 (day -1) and two randomly picked days during the study. Before leaving the Clinical Research Unit the volunteers received their amount of bread for the day. Female volunteers were tested for pregnancy on days 0 and 28. In Appendix 7 the procedures are summarised in a table.

2.4.3 Diet

Dietary instructions (see Appendix 8) were given to avoid a substantial influence of dietary nitrate and iodide on the outcome of the study. The volunteers were asked to refrain from the consumption of nitrate-rich vegetables (beetroot, spinach, lettuce, endive, etc.) for three days prior to and during the study.

It is supposed that the effect of nitrate on the thyroid gland function is a competitive inhibition of the iodide transport into the thyroid gland. To rule out iodide intake as a strong confounding factor, the volunteers were placed on an iodide-restricted diet from two weeks prior to and during the study (see Appendix 8). In The Netherlands bread provides about half of the total iodine intake. To approach the average Dutch iodine intake, bread was distributed during this diet-period. Every day women received 3 or 4 slices of bread (containing circa 60 or circa 80 μg I) and men received 5 or 6 slices of bread (containing circa 100 or circa 120 μg I).

2.5 Thyroid ^{131}I uptake measurement

At the beginning of the study and after (nitrate) exposure the volunteers had a thyroid radioiodine (^{131}I) uptake measurement. For both measurements the volunteers received a capsule containing a tracer dose of 3.7 MBq ^{131}I-NaI (Mallinckrodt Medical b.v., Petten, The Netherlands). The ^{131}I uptake was then measured 5 hr and 24 hr after ingestion of the capsule. After exposure the volunteers had an extra measurement before the ingestion of the capsule to determine the retained activity (ra) of the first capsule. The ^{131}I uptake measurements were performed in a standardised manner (24). To measure the ^{131}I uptake a scintillation probe (Canberra 7350-PE collimator with a 2x2" NaI crystal) was positioned at the volunteer's thyroid region for 4 minutes, at a fixed distance of 25 cm. For background (BKG) correction the probe was positioned then for 4 minutes at the volunteer's thigh at the same distance. After correction for BKG, the activity (counts per minute, cpm) was measured from a standard solution containing 3.7 MBq ^{131}I ($cpm_{standard}$), placed in a perspex thyroid/neck phantom, and was corrected for room background (BKG) and decay. The percentage ^{131}I uptake (RAIU) by the thyroid gland, corrected by a decay correction factor (DCF) was for the measurement at the beginning of the study:

$$RAIU = [(cpm_{neck} - cpm_{thigh}) * DCF/(cpm_{standard} - cpm_{room\ BKG})] * 100\%.$$

For the measurement after exposure the retained activity (ra) had to be taken into account. Therefore cpm_{neck} was corrected for cpm_{ra}. The $cpm_{ra} = cpm_{neck} - cpm_{thigh}$ before the ingestion of the second capsule. The cpm_{ra} was corrected for the decay in time. The formula then used:

$$RAIU = [(\{cpm_{neck} - cpm_{ra}\} - cpm_{thigh}) * DCF/(cpm_{standard} - cpm_{room\ BKG})] * 100\%.$$

2.6 Laboratory analyses

2.6.1 Hormones

The following hormones were analysed: TSH, T_4, T_3, rT_3, and IGF I. T_4 and TSH were analysed on an AxSYM machine (Abbott Laboratories, Illinois, USA). The sensitivity of TSH was 0.06 mU/l and the sensitivity of T_4 was 13.5 nmol/l. T_3 was analysed with a competitive radioimmunoassay Coat-A-Count (Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity was 0.1 nmol/l and the interassay variation was 1.45 ± 0.12 (8.4 %, n = 23). Reverse-T_3 was analysed with a competitive radioimmunoassay of RADIM (Angleur, Belgium). The sensitivity was 0.05 nmol/l and the interassay variation was 0.42 ± 0.04 (10 %, n = 13). Part of the IGF I analyses was analysed with a two side immunometric assay with bioinylated capture antibody against C-term and a second antibody against N-term with chemoluminescence as marker and Streptavidin coated magnetic particle for separation (Nichols, Germany). The sensitivity of the assay was 6 ng/ml and the interassay variation was 227 ± 18 (7.8 %, n = 14). The other samples were analysed by an immunoradiometric assay (IRMA) done by extraction kit (code 40-2100, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The sensitivity was 20 ng/ml and the interassay variation was 8.8%, 8.5% and 6.1% at 70, 200 and 400 ng/ml respectively (n = 12). The correlation coefficient was 0.97 based on 57 samples between 40 and 1000 ng/ml.

2.6.2 Plasma nitrate and nitrite

Concentrations of nitrate and nitrite in plasma were determined by the Laboratory of Residue Analysis by means of a method based on High Performance Ion Chromatography (HPIC) with UV detection at 208 nm. Analyses of the plasma samples took place according to SOP ARO/414 (25). 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 (recoveries for nitrate and nitrite were within 100 ± 10%. The lower limit of quantification was 0.2 mg/kg for both plasma nitrate and plasma nitrite.

2.6.3 Urinary iodine

The method is based on the reduction of Ce^{4+} to Ce^{3+}, which is catalysed by I^-. The yellow colour of Ce^{4+} disappears the more cerium is reduced. The absorbance is measured at 405 nm. The rate of decrease of the colour is a measure for the amount of iodide. Analyses of the urine have been taken place according to ‘Determination of Iodine in urine with the

microtiterplate-reader' (26) with a microtiterplate-reader (Molecular Device, Napa Valley, USA).

2.6.4 Methaemoglobin

MetHb was analysed in an ABL520 Cooximeter (Radiometer BV, The Netherlands). After haemolysing the erythrocytes the spectrum is measured at specific wavelengths (535, 560, 577, 622, 636 and 670 nm). These wavelengths are chosen to enable differentiation among Hb-derivates. The fraction of the individual Hb-derivates (in this case metHb) was then calculated.

2.6.5 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 ESR, Hb, Ht, leukocytes (+differentiation), thrombocytes, sodium, potassium, Chloride, urea, calcium, creatinin, bilirubin, CPK, alkaline phosphatase, ASAT, ALAT, LDH, γGT and total proteins and albumin. 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.

2.7 Statistical analyses

All statistical analyses were performed with SPSS®. The uptake data (RAIU) and the hormone concentrations were log-transformed before analysis. Paired t-tests were performed to compare the thyroid radioiodine uptake after 4 weeks of exposure to nitrate or distilled water (day 28/29) with the thyroid radioiodine uptake on days 0/1. Differences between the nitrate and the control group were tested with a student's t-test.

Volunteers' hormone values on day 0 (and the pre-medical screening) were taken as their own reference values and were compared with the hormone values on days 14, 21 and 28. Therefore paired t-tests were performed. When the hormone concentrations were determined during the medical screenings (check-in and check-out), the volunteers did not follow the iodine-restricted diet, which they started two weeks before day 0 and continued till the last thyroid ^{131}I uptake measurement on day 29. Therefore the hormone concentrations at the medical screenings were not included in the hormone analyses.

Differences in plasma nitrate baseline levels between the nitrate and the control group were tested with an oneway ANOVA and a student's t-test. Differences were considered significant if $p < 0.05$.

2.8 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 preparation of the sodium nitrate solution (60 mg/ml) took place at the Central Pharmacy of the University Medical Center Utrecht (UMC Utrecht), the Netherlands.
- The thyroid gland iodine uptake measurements were performed by the Department of Nuclear Medicine of the University Medical Center Utrecht (UMC Utrecht), the Netherlands.
- The T_3, rT_3 and IGF-I analyses were performed by the Laboratory of Endocrinology of the University Medical Center Utrecht (UMC Utrecht), the Netherlands.
- The routine laboratory analyses of the blood samples obtained from the volunteers during pre- and post-study screening and the methaemoglobin, TSH and T_4 analyses were performed by the Department of Clinical Chemistry of the University Medical Center Utrecht (UMC Utrecht), the Netherlands.
- The analyses of the plasma samples for nitrate and nitrite were performed by the Laboratory for Residue Analyses (ARO) of the National Institute of Public Health and the Environment (RIVM), the Netherlands.
- The 24-hr urinary iodide excretion was analysed by the Department of Animal Sciences, Human and Animal Physiology Group, Wageningen University, The Netherlands.

3. Results

3.1 Medical screening

3.1.1 Check-in

Participating volunteers

In Appendix 9 the results of the medical screening of the participating volunteers are shown. Volunteers 1, 2, 5, 6, 7, 10, 11, 13, 16, 18, 20, 24, 25, 30, 32 could be directly included on basis of check-in. For some of the volunteers minor deviations from the normal range existed, but these were judged as of no clinical relevance by the physician, who performed the medical screening.

Volunteer 8 also had a high total proteins concentration of 86 g/l (normal range 65 - 81 g/l) plasma. A repeat test resulted in 84 g/l. This was still above normal, but judged as of no clinical relevance and volunteer 8 was included into the study.

Volunteer 14 had an elevated percentage of monocytes (14 %, normal range 3 - 10 %) in the leukocytes differentiation, so the leukocytes differentiation was repeated. The percentage of monocytes had been decreased to 12 % at a normal leukocytes count (5.3 * 10^9/l, normal range 4.0 - 10.0 * 10^9) and the volunteer was included into the study.

Volunteer 19's blood sample was haemolytic. Therefore all chemical and haematological parameters were repeated. In the first drawn blood sample the activity of ALAT was low (7 U/l, normal range 10 - 50 U/l). The concentrations of bilirubin (30 µmol/l, normal range < 17 µmol/l) and direct bilirubin (14 µmol/l, normal range 0-4 µmol/l) were high and also the activity of LDH was high (1007 U/l, normal range 300 - 620 U/l). Because of the elevated activity of LDH the LDH iso-enzymes were also analysed. In a second blood sample ALAT was normalised to 27 U/l and LDH was normalised to 503 U/l. No deviations were seen in the LDH iso-enzymes (isofr1 0.27 [normal range 0.16 - 0.36], isofr2 0.39 [0.33 - 0.41], isofr3 0.23 [0.17 - 0.29], isofr4 0.07 [0.05 - 0.13] and isofr5 0.04 [0.03 - 0.14]). Bilirubin was 18 µmol/l and direct bilirubin was 8 µmol/l. This was judged as of no clinical relevance by the physician who performed the medical screening. Something had gone wrong in the leukocytes analysis of the first blood sample (see Appendix 9). The second drawn sample gave the following results for leukocytes differentiation: eosinophiles 2 % (normal range 0 - 5 %), basophiles 1 % (0 - 2 %), neutrophiles 49 % (40 - 72 %), lymphocytes 40 % (20 - 45 %) and monocytes 8 (3 - 10 %). The urine test of this volunteer had to be repeated, because the first urine test showed some erythrocytes in the urine (about 50 erythrocytes/µl). The second test showed a minor amount of erythrocytes in the urine and a minor amount of proteins. No bilirubin was found in the urine and urobilinogen was normal. These parameters were looked into, because of the high bilirubin and direct bilirubin concentrations found in the plasma. A third test was done in the first week of the study. This time all urine parameters (glucose, proteins, blood and leukocytes) were negative or normal.

Volunteer 21 had elevated plasma activity for LDH (734 U/l, normal range 300 - 620 U/l), elevated bilirubin concentration (25 µmol/l, normal range < 17 µmol/l) and direct bilirubin

concentration (9 µmol/l, normal range 0 - 4 µmol/l). LDH was repeated (472 U/l) and LDH iso-enzymes were determined and they were within the normal range: isofr1 0.23 (normal range 0.16 - 0.36), isofr2 0.39 (0.33 - 0.41), isofr3 0.24 (0.17 - 0.29), isofr4 0.09 (0.05 - 0.13) and isofr5 0.05 (0.03 - 0.14). Also bilirubin (20 µmol/l) and direct bilirubin (8 µmol/l) were repeated. Because the bilirubin had been decreased, the direct bilirubin was not more than 40% of the bilirubin and LDH fell in the normal range, volunteer 21 could be included in the study.
Volunteer 23 had an ESR of 24 mm after 1 hour (normal range 2 - 12 mm after 1 hour) and the ESR was repeated. The ESR was decreased to 16 mm after 1 hour and the volunteer was eligible for study participation.

Excluded volunteers
Volunteer 9 had already been included in the study after the medical check-in, but her general practitioner objected for participation, because in 1996 she has had serious stomach complaints and bulbitis was diagnosed by endoscopy.
Volunteer 17 had a low haemoglobin concentration of 6.6 mmol/l (normal range 7.4 - 9.6), haematocrite fraction was 0.35 (normal range 0.36 - 0.46) and the ESR was 28 mm after 1 hour (normal range 2 - 12 mm after 1 hour). These parameters were repeated. The ESR had been decreased to 22 mm after 1 hour, but there had been no changes in Hb and Ht, so the volunteer was excluded from the study.
Volunteer 22 had a bilirubin concentration of 52 µmol/l (normal range < 17 µmol/l). A second blood sample was drawn, but the bilirubin concentration was still too high (49 µmol/l). The volunteer was therefore excluded from the study.
Volunteer 26 showed a strong vasovagal reaction during the blood sampling. Because in this study blood had to be taken frequently, she was excluded from the study. Further the EKG showed sinusbradycardia during the vasovagal reaction.
Volunteer 28 had erythrocytes (10-50 erythrocytes/µl) and leukocytes (about 75/µl) in the urine. Besides she had an elevated ESR (22 mm after 1 hour, normal range 2 - 12). She had had cystitis 5 days before the medical check-in. The leukocytes and leukocytes differentiation were within the normal range (leukocytes 8.3 * 10^9/l [normal range 4.0 - 10.0 * 10^9/l], eosinophiles 3 % [0 - 5 %], basophiles 1 [0 - 2 %], neutrophiles 66 [40 - 72 %], lymphocytes 22 [20 - 45 %] and monocytes 8 [3 - 10 %]. All these parameters were repeated. The leukocytes and differentiation were still within the normal range (leukocytes 8.7 * 10^9/l, eosinophiles 4 %, basophiles 1, neutrophiles 64, lymphocytes 23 and monocytes 8). The urine test showed hardly any erythrocytes and leukocytes, although ESR had raised to 25 mm after 1 hour. Therefore volunteer 28 was excluded from the study.

Other withdrawal from the study
Volunteer 3 had been included in the study after the medical check-in. On day 0 it turned out, she was not able to have the first I-uptake, because she had other obligations. It was decided to withdraw her from the study and continue with a volunteer from the standby-list.

Volunteer 4 withdrew himself from the study after signing the informed consent, but before he had had the medical check-in.
Volunteer 12 had been included in the study after the medical check-up, but she did not show up the bread collection day. Later it turned out she had been ill and had decided not to participate anymore.
Volunteer 27 had passed the medical check-in. The urine test had to be repeated, because there were too many erythrocytes in the urine (about 50 erythrocytes/µl), but the volunteer withdrew herself before the urine test was repeated.
Volunteers 29 and 31 were eligible for study participation, but withdrew themselves from the study.

3.1.2 Check-out

The following persons were discharged from the study without any peculiarities: 1, 6, 14, 16, 18, 20, 21, 24, and 32.
Volunteer 2 had a total proteins concentration of 89 g/l (normal range 65 - 81 g/l) and a slightly elevated ESR of 16 mm after 1 hour (normal range 2 - 12) during the medical check-out. These parameters were repeated and were then 80 g/l for total proteins and 10 mm after 1 hour.
Volunteer 5 had some erythrocytes (about 10/µl) in the urine. The urine test was repeated and no blood was found in the urine.
Volunteer 7 had a bilirubin concentration of 41 µmol/l (normal range < 17 µmol/l). This was not due to the study, because before the study she also had a bilirubin concentration of 41 µmol/l. She had also a positive urine test for proteins (< 30 mg/dl), blood (about 50 erythrocytes/µl) and leukocytes (about 10-25 leukocytes/µl). When this test was repeated the test was still positive for proteins (< 30 mg/dl), blood (about 50 erythrocytes/µl) and leukocytes (about 75 leukocytes/µl). The volunteer had no physical complaints. She was advised to see her general practitioner.
Volunteer 8 had some erythrocytes in the urine (about 10 erythrocytes/µl). The test was repeated and then all urine parameters (glucose, proteins, blood and leukocytes) were negative or normal.
Volunteer 11 had an ESR of 29 mm after 1 hour (normal range 2 - 12). The ESR was repeated after about 3 weeks and was then 11 mm after 1 hour.
Volunteer 13 had a TSH concentration of 0.26 mU/l (normal range 0.35 - 5.0). TSH and total T_4 were repeated and were respectively 0.83 mU/l and 130 nmol/l (normal range 50-150 nmol/l).
Volunteer 19 had a bilirubin concentration of 18 µmol/l and direct bilirubin of 8 µmol/l. This had not been changed since the medical check-in. Total proteins were 84 g/l (normal range 65 - 81 g/l). The volunteer had no physical complaints and since there were no clear changes compared to the medical check-in, no extra blood sample was taken. The general practitioner was informed about these findings.

Volunteer 23 had at the medical check-out 2.9 * 10^9/l leukocytes in the blood (normal range 4.0 - 10.0 * 10^9/l) with a normal leukocytes differentiation (see Appendix 9). LDH was 698 U/l (normal range 300 - 620 U/l) and the ESR 13 mm after 1 hour (normal range 2 - 12). Three weeks later leukocytes plus differentiation, LDH + LDH iso-enzymes, total proteins and ESR were repeated. The leukocytes had been normalised (5.2 * 10^9/l) (leukocytes differentiation eosinophiles 2 % [0 - 5 %], basophiles 1 [0 - 2 %], neutrophiles 56 [40 - 72 %], lymphocytes 33 [20 - 45 %] and monocytes 8 [3 - 10 %]) and LDH had been normalised (599 U/l) (LDH iso-enzymes: isofr1 0.26 [normal range 0.16 - 0.36], isofr2 0.36 [0.33 - 0.41], isofr3 0.23 [0.17 - 0.29], isofr4 0.10 [0.05 - 0.13] and isofr5 0.05 [0.03 - 0.14]). The ESR had been slightly increased to 16 mm after 1 hour, but was then the same as at the check-in and total proteins had slightly increased from 79 to 84 g/l. Volunteer 23 had had a throat inflammation. This was probably the cause of the slightly elevated ESR.

Volunteer 25 had a slightly elevated bilirubin concentration in the blood (27 µmol/l, normal range < 17 µmol/l) with normal direct bilirubin (4 µmol/l, normal range 0 - 4 µmol/l). Because it had not changed since the medical check-in (then the bilirubin concentration was 29 µmol/l and the direct bilirubin concentration was 5 µmol/l) and the volunteer had no physical complaints, no repeated analysis were performed. The general practitioner was informed about the elevated bilirubin concentration.

Volunteer 30 had blood (50 - 250 erythrocytes/µl) and leukocytes (75-500 leukocytes/µl) in the urine. The test was repeated and the erythrocytes were decreased to about 10 erythrocytes/µl, but the leukocytes had still been remained at 75 - 500 leukocytes/µl. The volunteer had also some complaints during miction and was therefore advised to consult her general practitioner.

3.2 Adverse effects

The volunteers themselves reported the tabulated adverse effects.

Before the study

Table 1 Self-reported adverse effects between 14 days before and at the start of the study

Volunteer number	Adverse experience	Time started (before start of study on day 0)	Duration	Intensity (mild/mode-rate/severe)
1	-	-	-	-
2	-	-	-	-
5	Headache	5 days (afternoon)	few hours	mild
6	Fever Fever Cold (nose and the first days also shortness of breath)	7 days 6 days 3 days	1 day 3 days 12 days	mild moderate mild
7	Coughing	1 day	1 day	mild
8	Headache	3 days (at 15.00 u)	5 hours	mild
10	-	-	-	-
11	-	-	-	-
13	-	-	-	-
14	-	-	-	-
16	Fever/cold	11 days	5 days	mild
18	-	-	-	-
19	Cold	8 days	8 days	mild
20	Fever	4 days (at 21.00 u)	± 1 day	mild
21	Fever Headache	9 days (evening) 1 day	1 evening ± 1 night	mild mild
23	Sore throat	10 days	6 days	mild
24	-	-	-	-
25	-	-	-	-
30	Tooth ache Tooth ache (2 tablets Ibuprofen taken)	13 days (afternoon) 6 days (morning)	1 afternoon 2 days	mild moderate
32	-	-	-	-

- = No adverse effects reported

During the study

Table 2 Self-reported adverse effects during the study

Volunteer number	Adverse experience	Time (expressed in days after the start of day 0)	Duration	Intensity (mild/mode-rate/severe)	Relation to test-product?
1	Sore throat	3 days (evening)	2 days	mild	no
	Sore throat	7 days	1 day	mild	no
	Feeling shiftless	6 days	3 ½ days	mild	no
	Aphthae	8 days	± 1 week	mild	no
	Headache	10 days (at 15.00 u)	17 ½ hours	moderate	possible
	Headache	11 days (at 8.30 u)	1 ½ days	mild	possible
	Headache	14 days (at 7.30 u)	1 day	mild	possible
	Tired	10 days (at 15.00 u)	5 ½ days	mild	no
2	Sore throat	4 days (early morning)	2 days	mild	no
	Sore throat	15 days (at 7.15 u)	± 1 day	mild	no
	From menstruation:				
	• Cramp in abdomen	22 days (at 17.00 u)	evening	mild	no
		22 days (in the night)	few hours	moderate	no
		23 days (morning)	± 2 days	mild	no
	• Back pain	22 days (at 17.00 u)	evening	mild	no
		23 days (morning)	± 2 days	mild	no
5	Painful hand (after stubbing)	3 days (at 17.00 u)	± 1 day	mild	no
	Back pain	8 days (afternoon)	1 ½ days	mild	no
	Not sleeping well	6 days	1 night	mild	no
	Not sleeping well	12 days	1 night	mild	no
	Not feeling well (stomach)	27 days (at 7.30 u)	½ hour	mild	no
	Stiff calf	27 days (at 8.30)	± 1 day	mild	no
6	Cold (nose, see also table 1)	3 days before	12 days	mild	no
	Coughing	3 days	6 days	mild	no
	Coughing	12 days (at 23.00 u)	2 hours	mild	no
	Rash on finger	3 days	1 day	mild	no

Table 2 Self-reported adverse effects during the study (continued)

Volunteer number	Adverse experience	Time (expressed in days after day 0)	Duration	Intensity (mild/mode-rate/severe)	Relation to test-product?
7	Pain in abdomen	1 day	1 ½ days	mild	no
	Cold (nose)	3 days	21 ½ days	mild	no
	Coughing	3 days	15 days	mild	no
	Coughing	16 days (at 16.00 u)	by periods	moderate	no
	Coughing	17 days (at 8.00 u)	period	moderate	no
	Coughing	17 days (afternoon)	4 ½ days	mild	no
	Coughing	22 days	3 days per-iods in the morning	mild	no
	Pain in abdomen	9 days (at noon)	2 ½ days	mild	no
	Sore throat	14 days (at 14.00 u)	2 ½ days	mild	no
	Diarrhoea	19 days (at 19.00 u)	1 time	mild	no
	Diarrhoea	21 days (at 8.00 u)	1 time	mild	no
8	Headache	28 days (15.00 u)	3 hours	mild	no
10	From menstruation: • Flushes • Headache • Cramp in abdomen	18 days (evening)	± 1 day	mild	no
	• Feeling weak	19 days (morning)	± 1 day	mild	no
	Cold (nose)	19 days (afternoon)	4 ½ days	mild	no
	Coughing	19 days (afternoon)	4 ½ days	mild	no
11	Burn on arm (wound was open in evening)	5 days (evening)	pain ± 1 day 1 morning	mild	no
	Headache	15 days (at 8.30 u)	3 hours	mild	no
	Cramp in abdomen (from menstruation)	15 days (morning)	± 3 days	mild	no
	aching muscles (especially in neck), swallowing hurts (throat/stiff jaw)	20 days (evening)		mild	no
13	From menstruation: • Headache • Nauseous • Cramp in abdomen	1 day (at 15.00 u)	afternoon + evening	mild	no
	Headache	3 days (at 17.00 u)	4 ½ hours	mild	no
14	Cold (nose)	3 days (afternoon)	1 ½ days	mild	no
16	-	-	-	-	-
18	Sore muscles	8 days (morning)	1 day	mild	no

- = No adverse effects reported

Table 2 Self-reported adverse effects during the study (continued)

Volunteer number	Adverse experience	Time (expressed in days after day 0)	Duration	Intensity (mild/mode-rate/severe)	Relation to test-product?
19	Cramp in calf, while walking	2 days (at 6.30 u)	5 minutes	moderate	no
		2 days (at 6.35 u)	2 hours	mild	no
	Cold (nose)	9 days (at 7.00 u)	1 day	mild	no
	Cold (probably allergy)	12 days (morning)	± 1 day	mild	no
	Sneezing	13 days (morning)	few times	mild	no
	Diarrhoea	14 days (at 5.30)	1 time	mild	no
	Sneezing (allergy)	24 days (at 21.30 u)	3 ½ hours	mild	no
	Running nose	25 days (at 21.00 u)	± 1 day	mild	no
20	Sore muscles	7 days (evening)	1 day	mild	no
	Vaginal fungal infection (used Gyno-daktarin-1)	16 days (night)	1 night noticeable	mild	no
21	Cold (nose)	7 days (afternoon)	1 ½ days	mild	no
	Headache	14 days (at 15.00 u)	1 hour	mild	no
	Headache	24 days	1 morning	mild	no
23	Sore throat	Evening of day 0	1 evening	mild	no
	Sore throat (inflammation)	25 days (at ± 13.00 u)	> 4 ½ days	mild	no
	Herpes (lip)	5 days (afternoon)	6 ½ days	mild	no
	Cold (nose)	25 days (at ± 13.00 u)	> 4 ½ days	mild	no
	Red eye (by H_2O_2)	28 days (morning)	Half the morning	mild	no
24	Nauseous (if not eaten a long time)	9 days	now and then	mild	no
	Nauseous	10 days (at 22.00 u)	± 1 day	mild	no
25	Cold (nose/sinuses)	6 days (at 24.00 u)	± 4 days	mild	no
	Sore throat	6 days (at 24.00 u)	1 day	mild	no
	Fever	8 days	1 night	mild	no
30	-	-	-	-	-
32	Feeling weak	5 days (evening)	3 hours	mild	no
	Black eye	8 days (evening)	1 - 2 weeks	mild	no
	Sore throat	27 days (at 22.00 u)	> 2 ½ days	mild	no
	Cold (nose)	27 days (at 22. 00 u)	> 2 ½ days	mild	no

- = No adverse effects reported

After the study

Table 3 Self-reported adverse effects between the end of the study and the medical check-out

Volunteer number	Adverse experience	Time started (after the study from day 29 on)	Duration	Intensity (mild/mode-rate/severe)	Related to test-product
1	-	-	-	-	-
2	-	-	-	-	-
5	Feeling ill (nauseous)	4 days	2 days	mild	no
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
10	Black spots haemorrhages	not known	continued till after check-out	mild	no
11	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
16	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-
30	-	-	-	-	-
32	-	-	-	-	-

- = No adverse effects reported

3.3 Thyroid ^{131}I Uptake

In Appendices 10a and 10b the individual 5-hrs and 24-hrs RAIUs are tabulated as well as the differences in RAIUs before and after nitrate intake. The average difference in 24-hrs RAIU in the nitrate group was 8 ± 11%. Table 4 shows the geometric (back-transformed from log-transformation) average RAIUs in the nitrate group and the control group. As can be seen in the 24-hrs RAIU on day 29 was about 1.5 times that of the 24-hrs RAIU on day 1. When this was tested with a paired t-test, the log-transformed difference between 24-hrs RAIU before and after nitrate exposure was not significant ($p = 0.056$). No significantly differences were found between the 5-hrs RAIUs in the nitrate group before and after nitrate exposure and between the 5-hrs RAIUs in the control group before and after nitrate exposure. Also the 24-hrs RAIUs before and after nitrate exposure in the control group were not found to be

significantly different. All differences between the nitrate group and the control group were not significant.

Table 4 Geometric (back-transformed from log-transformation) mean (95% confidence interval) thyroid ^{131}I uptake measured 5 hours and 24 hours after ^{131}I capsule intake before (day 0 and 1) and after (day 28 and 29) nitrate exposure.

		Day 0 or 1	Day 28 or 29	Day 28/29 : Day 0/1
5-hrs RAIU (%)	nitrate	13 (11 - 17)	14 (11 - 18)	1.1 (0.8 - 1.4)
	control	11 (7 - 16)	11 (8 - 15)	1.0 (0.8 - 1.3)
24-hrs RAIU (%)	nitrate	20 (14 - 29)	29 (24 - 36)	1.5 (1.0 - 2.2)
	control	22 (15 - 33)	25 (20 - 30)	1.1 (0.9 - 1.4)

3.4 Hormones

In table 5 the geometric (back-transformed from log-transformation) average hormone concentrations in the nitrate group and the control group are reported. In Appendix 11 the individual hormone concentrations are tabulated.

Table 5 Geometric mean (95% confidence interval) hormone concentrations in serum (1) / plasma (2) of the nitrate and control group measured before exposure (day 0) and during the study.

		Day 0	Day 14	Day 21	Day 28
TSH(mU/l) [1]	nitrate	1.46 (1.06 - 2.28)	1.03 (0.71 - 1.49)	1.14 (0.87 - 1.49)	1.27 (0.97 - 1.68)
	control	2.07 (1.49 - 2.89)	1.52 (0.95 - 2.43) [3]	1.38 (0.96 - 1.98) [3]	1.51 (1.13 - 2.02) [3]
T4 (nmol/l) [1]	nitrate	106 (89 - 126)	112 (94 - 133) [3]	110 (95 - 129)	110 (93 - 130)
	control	106 (92 - 121)	107 (93 - 122)	109 (95 - 124)	109 (96 - 123)
T3 (nmol/l) [1]	nitrate	1.9 (1.6 - 2.1)	1.9 (1.5 - 2.3)	1.8 (1.6 - 2.1)	1.9 (1.6 - 2.2)
	control	1.7 (1.4 - 1.9)	1.8 (1.5 - 2.0)	1.8 (1.5 - 2.1)	1.8 (1.6 - 2.0)
rT3 (nmol/l) [1]	nitrate	0.34 (0.30 - 0.38)	0.34 (0.30 - 0.38)	0.36 (0.31 - 0.41)	0.33 (0.31 - 0.36)
	control	0.36 (0.31 - 0.40)	0.35 (0.31 - 0.40)	0.35 (0.32 - 0.38)	0.35 (0.31 - 0.38)
IGF I (ng/ml) [2]	nitrate	234 (188 - 291)	274 (228 - 331)	257 (203 - 327)	257 (205 - 323)
	control	204 (162 - 258)	229 (181 - 291)	254 (213 - 302) [3]	234 (189 - 290) [3]

3: Concentrations are significantly different from the concentrations on day 0, when the log-transformed data are tested with a paired t-test ($p < 0.05$).

From the paired t-tests with the log-transformed data it appeared, that in the nitrate group the concentrations T_4 on day 14 were significantly different from the concentrations on day 0, $t = 2.68$, $p = 0.025$, though the concentrations T_4 on day 21 and day 28 were not significantly different from the concentrations on day 0. When the IGF I concentrations in the nitrate group on day 14 were compared with the concentrations on day 0, the p-value was 0.050 ($t = -2.27$). On day 21 and day 28 the IGF I concentrations did not significantly differ from the IGF I concentrations on day 0. In the control group concentrations TSH on day 14, day 21 and day 28 were significantly different from the TSH concentrations on day 0, respectively t

= - 2.68, p = 0.025, t = - 2.40, p = 0.040 and t = - 3.62, p = 0.006. Also the IGF I concentrations on day 21 and day 28 were significantly different from the concentrations on day 0, respectively t = 2.80, p = 0.021 and t = 2.28, p = 0.049, though not on day 14.

3.5 Iodide

The total amounts of iodide in the collected 24-hrs urine are tabulated in Appendix 12. Most volunteers had amounts < 2.0 μmol. Two volunteers had total iodide amounts > 2.0 μmol. UTN 8 had an amount of 24.98 μmol I^- in the first urine collection and an amount of 4.20 μmol I^- in the third urine collection. UTN 19 had an amount of 2.21 μmol I^- in the first urine collection.

3.6 Nitrate

The individual plasma nitrate concentrations are shown in Appendix 13. Plasma nitrate concentrations during the dietary period were measured on day 0 and further randomly twice a week (eight samples). On day 0 the nitrate concentration in the nitrate group did not significantly differ from the nitrate concentration in the control group. During the study the plasma nitrate concentrations in the nitrate group were significantly higher than those of the control group, when they were tested with an oneway ANOVA analysis ($p \leq 0.005$). After the nitrate exposure had been stopped the plasma nitrate concentrations of the nitrate group and the control group were not significantly different (tested with a student's t-test).

3.7 Nitrite

Though there was a slightly increased baseline nitrate concentration in the nitrate group, this did not lead to an increased baseline nitrite concentration (see Appendix 14). The lower quantification limit for nitrite in plasma was 0.2 mg/kg. Both in the nitrate group and in the control group the baseline plasma nitrite concentrations were around or lower than this quantification limit.

3.8 Methaemoglobin

As expected no elevation in the percentage of metHb was found after administration of three times the ADI of nitrate during a period of four weeks. The individual percentages are tabulated in Appendix 15.

4. Discussion

In this study the effect of nitrate on the thyroid gland was investigated by measuring thyroid radioiodine (^{131}I) uptake (RAIU) and by measuring the thyroid hormones T_4, T_3 and rT_3. Also the hormones TSH (influenced by T_4 and T_3) and IGF I (influenced by T_4) were measured. The theory is, that nitrate inhibits the thyroid iodine uptake. This may lead to a decrease in thyroid hormone (T_4, T_3) secretion, followed by an increase in thyroid stimulating hormone (TSH). In the end thyroid gland enlargement (goitre) may occur.

It was expected that if nitrate inhibited iodine entering the thyroid by competitive inhibition, the RAIU would be decreased after 4 weeks of nitrate exposure. Surprisingly the RAIU in the nitrate group had increased and was about 1.5 times the RAIU measured at the beginning of the study. So there were no signs of inhibition by three times the ADI of nitrate on iodine uptake in the way it is suggested by several investigators (12, 21, 27, 28, 29). The intra-individual variation was large in both the nitrate group and the control group. The overall precision of the uptake measurement is calculated on 2.06% (30), which cannot account for the large intra-individual variation. With an iodine-restricted diet it was tried to avoid excessive iodine intakes from for example fish, because excessive iodine intake can disturb the thyroid radioiodine uptake. The diet was checked by the urinary iodide excretion. From these data it appeared that no major dietary violations were made, except for one person, who had two of the three sampling times a large amount of iodide in the urine (24.98 µmol and 4.20 µmol). Wellner et al. (31) has developed an iodine metabolism model and studied the influence of physiological and pharmacological iodine amounts on the Iodine-131 uptake by the thyroid gland. From this model it appears that physiological iodine intakes can influence the RAIU. An intake of 20 µg iodine at the same time as the radioiodine intake can lower the RAIU with one tenth part compared to a 0 µg iodine intake. An intake of 100 µg iodine at the same time as the radioiodine intake can lower the RAIU with one third part compared to a 0 µg iodine intake (31). In our study it was tried to minimise iodine intake variations. From the urinary samples, however, average variation within persons were found from 18 to 111 µg iodide and an outlier of 2017 µg. So although the volunteers kept their diet well, it cannot be ruled out, that the iodine intake influenced the RAIU.

Other investigators who did find an effect of nitrate on the thyroid iodine uptake used large amounts of nitrate. Höring et al. (13) found a lower thyroid ^{131}I uptake (6 ± 2%) in rats that were exposed to drinking water with 6600 mg/l nitrate for six weeks compared with a control group of rats (25 ± 4%). While in experiments with lower nitrate concentrations (from 40 mg/l to 4000 mg/l) no effect of nitrate on the thyroid ^{131}I uptake was found (13). Bloomfield et al. (12) fed rats a corn-soybean oil meal with 0, 0.5, 1.0 and 2.5 percent KNO_3 (or 3069, 6139 and 15347 mg/kg nitrate) added. At all these high nitrate levels the iodine uptake of the thyroid gland was adversely affected (12). In a study with fish species first a decrease in thyroid radioiodine uptake was seen with water nitrate concentrations of 0.88 mg/l and 1.5 mg/l, but with a higher nitrate exposure (11 mg/l) an increase in thyroid radioiodine uptake was seen. They proposed a hypothesis that with higher amounts of nitrate the inhibition will

be stronger. When the fish body becomes depleted of iodine, the uptake mechanism (in the gills and digestive tract) is reactivated (14). However this hypothesis does not correspond with findings of Höring et al. (13) and Bloomfield et al. (12).

Also no effect of three times the ADI of nitrate was seen in hormone concentrations. From nitrate a decrease in T_4 was expected (17, 18). However, a significantly higher T_4 concentration on day 14 compared with the T_4 concentration on day 0 was found in the nitrate group. Also for T_3 a decrease in concentration was expected (17), though no differences were found in the measured T_3 concentrations in both the nitrate group and the control group. For rT_3 an increase was expected, which was not seen in both groups. Other expected hormone changes would be secondary effects from T_4 (TSH and IGF I) and T_3 (TSH). So it is not surprising that also for these hormone concentrations no effect from nitrate was seen. For IGF I a decrease in concentration would be expected from nitrate, while in the nitrate group the IGF I concentrations remained at the same level and in the control group an unexpected significant increase was observed. A rise in TSH concentration would be expected from nitrate, but both in the nitrate group and in the control group decreased TSH concentrations were seen during the study compared to the TSH concentration on day 0. In the control group these concentrations were significantly lower than the concentration on day 0, while in the nitrate group the decreases were not significant. For TSH intra-individual variations are not uncommon (32, 33) and also circannual rhythms are described for TSH (33). So although some differences in the measured hormones were found to be statistically significant, these differences could not be ascribed to nitrate exposure, but could be ascribed to normal intra-individual variation.

Remarkable is that when an effect of nitrate on thyroid hormones in animals was found, these animals had an extremely high nitrate intake. Jahreis et al. (15) fed piglets a diet containing 3% KNO_3 and compared these piglets with piglets eating the same diet without nitrate. After 5 weeks the serum T_4 concentrations of the nitrate-fed piglets was significantly lower than the T_4 concentration of the control piglets. Compared to a control group, that was fed ad libitum the nitrate-fed and the pair-fed piglets ate less and had a loss in weight gain. With feeding an excess of iodine (1 mg) for one week, the T_4 concentration in the nitrate-fed piglets was normalised. Compared to a control group of piglets, that was fed ad libitum the nitrate-fed and the pair-fed piglets ate less and had a loss in weight gain (15). In another study of Jahreis et al. (17) nitrate-fed (3% KNO_3) rats were compared with control rats. Both plasma T_4 and T_3 concentrations were lower in the nitrate-fed rats than in the control rats after 6 weeks. Weight gains of the nitrate-fed rats were decreased compared to the control rats, but the food intake of the nitrate-fed rats was also decreased (17). Also a decrease in T_4 concentration was found in bulls fed 100 to 250 g KNO_3 per day (18). Though in a research done with beagle dogs no significant effect was seen of nitrate on the thyroid gland hormones (T_4 and RT_3U). Different concentrations nitrate in drinking-water (0 to 1000 mg/l) were given to the dogs for one year (34).

Several investigators found an inhibiting effect of nitrate on the thyroid iodine uptake (27, 28). Eskandari et al. (1997) found with the rat thyroid Na^+/I^- symporter, that the nitrate-ion (NO_3^-) and (I^-) share the same transport mechanism into the thyroid gland. However the

affinity of the transporter is much larger for iodide and other anions than for nitrate (35). This larger affinity may be the cause that only an effect of nitrate on the thyroid gland is seen, when unrealistic high nitrate exposures are created. The larger affinity also corresponds with the finding that other anions than nitrate had larger goitrogenic potency (27, 28). Also Höring et al. (22) only found an effect of nitrate on the thyroid, when the iodine intake was marginal. The WHO has set a minimum limit of 0.78 µmol urinary I excretion per 24-hrs (21) and the WHO recommends an excretion of 1.0 µmol I per 24-hrs (23). In our study 50 % of the volunteers had an average total amount of iodide < 0.78 µmol and 70% of the volunteers had an average total amount of iodide < 1.0 µmol. That means, that most persons had a marginal iodine intake during the study period. Though unlike this marginal iodine intake no effect of nitrate on the thyroid gland was found.

In this study only young adults participated. However so far there are no indications, that the I-uptake mechanism (Na^+/I^- symporter) acts differently in older persons. Also in children the I-uptake will not be substantial different from that of young adults. From that point of view the competitive inhibition of nitrate will not substantially differ among age groups. So it is not expected that three times the ADI of nitrate will cause changes in thyroid function in children or elderly as long as they have a normal thyroid function.

Besides no effect on the thyroid gland, no other effects of nitrate were found. All the plasma nitrite concentrations were low during the study ($\leq$ 0.3 mg/kg) in both the nitrate group as the control group. In correspondence the percentage methaemoglobin remained normal in the nitrate group. So most likely the self-reported adverse effects were not to ascribe to the administered nitrate.

From this study it seems that a nitrate exposure of three times the ADI did not have any relevant effect on the thyroid gland function of young adults. If there would be any effect of nitrate on the thyroid gland function at least higher doses than three times the ADI of nitrate are needed to express this effect. So it seems that a dose of three times the ADI of nitrate is still safe for healthy people according to the thyroid gland function, since other factors (like iodine intake) have at least more influence on the thyroid iodine uptake than nitrate has.

5. Conclusions

The aim of this study was to investigate the effect of nitrate on the thyroid gland function. In order to investigate this ten healthy volunteers were administered a nitrate solution of three times the ADI of nitrate for four weeks and ten healthy volunteers were administered distilled water (control group). No effect of this nitrate exposure was found on the measured thyroid hormones T_4, T_3, rT_3, TSH and IGF I during the four weeks nitrate exposure. Also the thyroid iodine uptake was not affected by nitrate. So it is concluded that a nitrate exposure of three times the ADI will not cause changes in the thyroid gland function in a healthy population.

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Declaration of quality control

Undersigned states herewith that the research presented in this report has been carried out according to the European guideline 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:

QA Report publication date	QA Report inspection date
26 August 1998	24, 25 August 1998
26 August 1998	25, 26 August 1998
22 December 1998	15, 17 December 1998
24 March 1999	23 March 1999
17 May 1999	11, 12 May 1999

This report was inspected on 8, 10, 15, 22, 23, 29, 31 May and 4 July 2000.
QA report publication date: 24, 29, 31 May and 4 July 2000.

Quality control officer:

Name : A.W.M. Hofstee
Laboratory : National Poisons Control Centre, RIVM
Date : 5 July 2000
Signature :

Appendix 1 Mailing list

1-5 Inspectorate for Health Protection, Commodities and Veterinary Public Health, dr ir M.W.J. Wolfs
6 Inspectorate for Health Protection, Commodities and Veterinary Public Health, dr ir P.C. Bragt
7 Inspectorate for Health Protection, Commodities and Veterinary Public Health, drs H.J. Jeuring
8-12 Director of the Directorate of Health Policy, dr A.AW. Kalis
13 Directorate of Health Policy, drs J. de Stoppelaar
14 Directorate of Health Policy, ir R. Top
15 Acting Director-General of Public Health, drs N.C. Oudendijk
16 Inspectorate for Health Care, drs P.H. Vree
17 Inspectorate for Health Care, drs J.J.L. Pieters
18 Director of the Health Council of The Netherlands, prof dr J.J. Sixma
19 Directorate of Veterinary, Food Policy and General Environment of the Ministry of Agriculture, Nature Management and Fisheries, dr ir N.B. Lucas Luijckx
20 Medical Ethics Committee (METC), University Medical Center Utrecht
21 Depot of Dutch Publications and Dutch Bibliography
22 Directorate-General of the National Institute of Public Health and the Environment
23 Director Division Substances and Risks (Division 3/4), RIVM, dr ir G. de Mik
24 Laboratory for Health Effects Research, RIVM dr A. Opperhuizen
25 Laboratory for Health Effects Research, RIVM, dr A.B.T.J. Boink
26 Laboratory for Health Effects Research, RIVM, dr ir E.H.J.M. Jansen
27 Laboratory of Exposure Assessment and Environmental Epidemiology, RIVM, dr ir E. Lebret
28 Laboratory of Exposure Assessment and Environmental Epidemiology, RIVM, dr A.J.A.M. Sips
29 Laboratory of Exposure Assessment and Environmental Epidemiology, RIVM, dr ir M.J. Zeilmaker
30 Centre for Substances and Risk Assessment, RIVM, dr G.J.A. Speijers
31 Laboratory for Residue Analysis, RIVM, dr R.W. Stephany
32 National Poisons Control Centre, RIVM, drs I. de Vries
33 National Poisons Control Centre, RIVM, Ing A.W.M. Hofstee
34 National Poisons Control Centre, RIVM, drs G.A. van Zoelen
35 dr L.J. Schuddeboom
36-42 Authors
43 Public Relations Department, RIVM
44 Bureau Report Registration, RIVM
45 Library, RIVM
46-60 Sales Department
61-70 Reserve

Appendix 2 Deviations from study protocol

- Iodide analyses were performed by another laboratory, since it turned out that the laboratory described in the study protocol was not able to perform the analyses.

- UTN 20 started a 5-day trimethoprim (300 mg) treatment because of a bladder infection five days before the start of the study. It was judged that the influence during the study was minimal. On day 0 the plasma nitrate concentration was slightly raised (3.11 mg/kg), but decreased during the study.

- Before taking the second ^{131}I-capsule the volunteers had an extra measurement of the thyroid gland and thigh. This measurement was done to measure the retained activity of the first capsule.

- After the volunteers had taken radioiodine capsules before the nitrate exposure the thyroid radioiodine uptake measurements did not exactly take place after five and 24 hours for everyone. It was tried to have the same times in between the capsule intake and the uptake measurements after the nitrate exposure as the in-between times before the nitrate exposure. Therefore the volunteers got new scheme times, but also here were deviations again. The differences between the in-between times were for most volunteers less than 15 minutes. However, UTN 18 had the 5-hrs uptake measurement before nitrate exposure 5.01 hrs.min after the capsule intake and after nitrate exposure 4.38 hrs.min after capsule intake, so a time difference of 23 minutes. UTN 23 had the 5-hrs uptake measurement before nitrate exposure 5.00 hrs.min after the capsule intake and after nitrate exposure 3.40 hrs.min after capsule intake, so a time difference of 1 hour and 23 minutes. UTN 30 had the 5-hrs uptake measurement before nitrate exposure 4.43 hrs.min after the capsule intake and after nitrate exposure 4.59 hrs.min after capsule intake, so a time difference of 16 minutes. UTN 2 had the 24-hrs uptake measurement before nitrate exposure 23.18 hrs.min after the capsule intake and after nitrate exposure 23.40 hrs.min after capsule intake, so a time difference of 22 minutes. UTN 11 had the 24-hrs uptake measurement before nitrate exposure 24.05-hrs.min after the capsule intake and after nitrate exposure 24.50 hrs.min after capsule intake, so a time difference of 45 minutes. UTN 24 had the 24-hrs uptake measurement before nitrate exposure 24.40 hrs.min after the capsule intake and after nitrate exposure 24.01 hrs.min after capsule intake, so a time difference of 39 minutes. And UTN 30 had the 24-hrs uptake measurement before nitrate exposure 23.38 hrs.min after the capsule intake and after nitrate exposure 23.54 hrs.min after capsule intake, so a time difference of 16 minutes.

Appendix 2 (continued) Deviations from study protocol

- Two persons had a body mass index (BMI) > 24. Both persons were solidly built, so it was considered safe for these persons to get a somewhat higher amount of nitrate. One of these two ended up in the nitrate group. Two other persons had a body weight > 85 kg. These two persons were tall (193 and 199 cm) and had a BMI < 24, therefore it was considered safe to include these persons in the study.

- The lower limit for quantification of nitrite was not 0.1 mg/kg as described in the study protocol, but was 0.2 mg/kg.

- UTN 30 had no urine collection on day -1 and on day 0 blood sampling and measuring blood pressure and heart rate took place after the thyroid radioiodine uptake measurement, because this person had been on the reserve list and was called in on day 0. UTN 30 however had been following the diet as the other volunteers (i.e. two weeks prior to day 0 an iodine-restricted diet and three or more days prior to day 0 a low-nitrate diet).

- From the dietary records it appeared that in general only small dietary deviations had been made. More serious deviations the eating of seafood: UTN 13 ate some sea-grass on day -12, UTN 19 ate one gamba (shrimp) on day - 13 and UTN 24 ate two big spoons of crab on day -3. Further some volunteers had 2 or more milk-product consumptions too many: UTN 6 ate on day 24 two ice-cream scoops, UTN 14 had on day 1 about 500 ml milk(-products) too much and UTN 24 drank on day - 11 two glasses (about 400 ml) chocolate-milk too much. Only six volunteers (UTN 10, 11, 18, 20, 21 and 32) ate the amount of bread that was agreed on. All the others deviated from the bread-agreement one or more days by eating less slices, more slices or extra bread-products. The following volunteers did not follow the bread-instructions for more than two days. UTN 6 had on day - 10 one roll extra, had on days - 2, 6, 13, 18, 20, 24 and 27 three in stead of four slices and on day - 1 two in stead of four slices. UTN 8 had on days 2, 20 and 26 no bread in stead of five slices and on day 4 three in stead of 5 slices. UTN 14 had on day - 12 one croissant extra, on day - 10 two in stead of six slices, on days 2 and 15 four in stead of six slices and on day 7 five in stead of six slices plus one sausage roll. UTN 23 had on day 3 two in stead of three slices and had eaten one sausage roll, had on day 13 four in stead of 3 slices and had on day 21 two in stead of three slices. UTN 25 had on day - 10 nine in stead of six slices, on day - 9 four in stead of six slices plus two sausage rolls, on day - 8 two in stead of six slices, on day - 7 14 in stead of six slices, on day 1 five in stead of six slices, day 2 seven in stead of six slices and on day 7 four in stead of six slices.

Appendix 2 (continued) Deviations from study protocol

- A scheme had been made for blood sampling and urine collection as a diet-control. Most blood sampling and urine collections were according the scheme. Only minor deviations of 1 day had sometimes been made, because of difficult blood sampling or a forgotten blood sample, but since the volunteers did not know when the blood samples would be taken or the urine collection was planned, this will not have had any effect on the study.

- Three blood samples as a control on the low-nitrate diet were lost. One tube had been broken, one plasma sample had been thawed and one sample had been forgotten to draw. Because of the many other samples it was chosen not to replace these samples.

Appendix 3 Informed Consent

Afdeling: Nationaal Vergiftigingen Informatie Centrum (NVIC)
Hoofd: Dr. J. Meulenbelt

INFORMED CONSENT
Bewuste bereidverklaring

De vrijwilliger verklaart een exemplaar te hebben ontvangen van de "Informatie voor Deelnemers" betreffende het onderzoek getiteld:
“Het effect van nitraat op de schildklierfunctie in gezonde vrijwilligers: Een vier weken toxiciteitsstudie”
(projectnummer: 348801 007; project: 235802; datum 01-09-1998).
De vrijwilliger heeft een mondelinge toelichting op dit informatieformulier gekregen en is in de gelegenheid geweest iedere gewenste vraag te stellen.

De vrijwilliger verklaart dat hij/zij, na kennis te hebben genomen van bovengenoemde informatie wil deelnemen aan bovengenoemd onderzoek.
De vrijwilliger verklaart dat hij/zij naar eer en geweten alle vragen gesteld tijdens de medische keuring zal beantwoorden en geen informatie bewust heeft 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 vrijwilligers.

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 de vrijwilliger is medegedeeld dat bij vragen en problemen gedurende 24 uur per dag, 7 dagen per week direct contact 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 de vrijwilliger medegedeeld dat hij/zij zonder opgave van reden op elk moment deelname aan het onderzoek kan staken.

De vrijwilliger gaat akkoord met inzagerecht van persoonlijke (medische) gegevens door bij het onderzoek betrokken medewerkers van het Nationaal Vergiftigingen Informatie Centrum, de afdeling IC-1 en Klinische Toxicologie en medewerkers van inspecterende instanties en heeft begrepen dat de persoonlijke gegevens vertrouwelijk worden behandeld en 15 jaar worden bewaard.

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* (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
1	F	30	199	88	22.2
2	F	23	182	71	21.4
5	M	20	197	84	21.6
6	F	23	164	64	23.8
7	F	23	170	62	21.5
8	M	23	180.5	84	25.8
10	F	20	167.5	61	21.7
11	F	20	179	64	20.0
13	F	25	178.5	73	22.9
14	M	26	183.5	82	24.4
16	F	24	166	56	20.3
18	F	25	179.5	68	21.1
19	M	26	180	70	21.6
20	F	26	176.5	71	22.8
21	M	28	193	86	23.1
23	F	26	169	63	22.1
24	F	20	163	61	23.0
25	M	23	184.5	69	20.3
30	F	24	167	58	20.8
32	F	21	173.5	60	19.9
Total	20				
men	6				
women	14				
Mean		24	178	70	22
men		24	186	79	23
women		24	174	66	22
SD		3	10	10	2
men		3	7	8	2
women		3	10	8	1

*Age is the age at the start of the study

Appendix 5 Inclusion and exclusion criteria

Inclusion criteria

- female/male
- age 18-35 years
- body mass index (BMI) between 20-24 and weight not exceeding 85 kg
- 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
- normal functioning thyroid gland, based on the reference values of the following thyroid hormones:
  - T_4 (total) 50-150 nmol/l (serum)
  - T_3 1-3 nmol/l (serum)
  - TSH 0.35-5.00 mU/l (serum)

Exclusion criteria

- smoking
- chronic drug treatment (especially antacids)
- drug treatment within one week before the start of and during the study, except for oral contraceptives. This to the judgement of the physician and/or medical investigator.
- administration of radiographic contrast agents within the last three months or during the study
- use of narcotics
- use of vitamin and/or mineral supplements
- average of more than 3 units of alcohol per day
- blood donation within 30 days before the start of and during the study
- participation in another drug study within 30 days before the experiment or during the study
- pregnant or lactating females
- goitre
- mental illness
- any chronic illness
- family history positive for pernicious anaemia
- excessive pyrosis or history of gastritis or gastric or duodenal ulcer
- abnormal dietary habits as judged by a physician
- anaemia
- history of frequent cystitis

Appendix 6 Sodium nitrate dose of the nitrate group

Volunteer number	Weight (kg)	$NaNO_3$ (mg)
1	88	1320
5	84	1260
6	64	960
11	64	960
13	73	1095
14	82	1230
16	56	840
18	68	1020
24	61	915
25	69	1035

Appendix 7 Study procedures

Days	-1 and two randomly picked days	0	1	2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27	7, 14, 21	28	29
Activity							
Collection 24-hr urine	X						
Pregnancy test		X				X	
Adverse effects		X	X	X	X	X	X
Dietary record		X	X	X	X	X	X
Blood pressure/heart rate		X	X		X	X	
Body weight		X			X	X	
Blood samples		X	(X)	(X)	X	X	
Administration of treatment			X	X	X	X	
Iodine uptake		X	X			X	X

Appendix 8 Dietary instructions (in Dutch)

VOEDINGSVOORSCHRIFTEN JODIUMBEPERKT EN NITRAAT-ARM DIEET

De volgende voedingsvoorschriften moeten 2 weken voor de onderzoeksperiode en tijdens de onderzoeksperiode gevolgd worden.

Voedings-middel	Toegestaan	Niet toegestaan
Brood	Brood wat is verstrekt door de onderzoekers. Alle sneden moeten nog dezelfde dag gegeten worden.	Meer of minder sneden brood dan is verstrekt en bolletjes, broodjes, stokbrood, krentenbrood, croissants, roggebrood.
Beschuit/ Knäckebröd	Onbeperkt	-
Boter/Marga-rine/Olie	Onbeperkt	-
Kaas	1 snee brood met kaas (± 20 g kaas per dag) = 1 plak kaas	Meer dan 1 plak kaas per dag
Vleeswaren	Alle vleeswaren, behalve lever en producten, die lever bevatten	Lever and lever bevattende producten, zoals (lever)paté, Saksische leverworst, leverworst
Zoet beleg	Alle soorten, behalve zoetbeleg met de rode kleurstof erythrosine (E-127)	Zoet beleg met de kleurstof erythrosine (E-127) **Controleer de verpakking!**
Overig beleg	Onbeperkt	-
Eieren	Maximaal 3 eieren per week	Meer dan 3 eieren per week, eiersalade
Melk en melk-producten	Maximaal 2 glazen/bekers/schaaltjes (± 200 ml) per dag	Meer dan 2 glazen/bekers/schaaltjes (± 200 ml) per dag
Soep	Zelfgemaakte soep (bouillon) van toegestane ingrediënten	Kant-en-klare soep (uit blik/pakje)
Vlees	Alle soorten, behalve lever en lever bevattende producten	Lever en lever bevattende producten
Vis(produc-ten) en schaal-, en schelp-dieren	-	Alle Vis(producten) en schaal-, en schelpdieren zijn **niet** toegestaan, inclusief producten als vismeel, kroepoek, trassi, gomasio, tahin
Jus/saus	Jus en sauzen op basis van water	Jus en sauzen op basis van melk(producten)

Appendix 8 (continued) Dietary instructions (in Dutch)

Aardappelen/ pasta/rijst	Onbeperkt	-
Groente *	Alle soorten, behalve de "niet-toegestane"	Alle soorten groene sla, radijsjes (rood en zwart), spinazie, andijvie en rode bieten
Peulvruchten	Bruine bonen, doperwten	Alle andere peulvruchten, zoals witte bonen, kidney bonen, capucijners, linzen
Fruit	Alle soorten, maar bij voorkeur vers fruit	-
Zout	Nezozout (wordt door de onderzoekers verstrekt)	Jozozout, zeezout, dieetzout
Dranken (niet-alcoholisch)	Alle soorten, behalve met de kleurstof E-127	Dranken met de kleurstof E-127
Dranken (alcoholisch)	Maximaal 3 glazen per dag	Meer dan 3 glazen per dag
Koek(jes) en gebak	Onbeperkt	-
Snoep en chocola	Alle, behalve met de kleurstof E-127	(Rood) snoep met de kleurstof E-127
Tussendoor-tjes/snacks	(Aardappel)chips, rozijnen, krenten, waterijs	Softijs, roomijs, pinda's
Diversen	Suiker, kruiden, aromat, knoflook, mosterd, ketchup, ketjap, sambal, zoetstof, azijn	Levertraan

* Dit voedingsvoorschrift moet vanaf 3 dagen voor de onderzoeksperiode en tijdens de onderzoeksperiode gevolgd worden.

Als je twijfelt over (een) bepaald(e) voedingsmiddel(en), aarzel dan niet om het de onderzoekers te vragen!

Appendix 9 Medical screening

Volunteer	1		2		5		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	22-01-99	29-03-99	21-01-99	31-03-99	21-01-99	31-03-99	Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	112/73	120/65	118/67	128/64	124/62	129/69	(mmHg)
Heart rate	73	81	84	72	97	81	(b.p.m.)
Standing:							
Blood pressure	129/71	111/76	121/71	113/75	118/79	109/67	(mmHg)
Heart rate	92	129	94	90	97	92	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	8.3	9.1	9.3	8.8	9.5	9.9	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.41	0.42	0.46	0.41	0.46	0.46	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	5.8	5.7	5	7.8	4.2	4.4	(4.0-10.0*Exp9/l)
Eosinophiles	1	2	1	1	2	2	(< 5 %)
Basophiles	1	1	2	1	2	1	(< 2 %)
Neutrophiles	49	38*	58	68	55	47	(40-72 %)
Lymphocytes	38	48*	32	25	33	36	(20-45 %)
Monocytes	11*	11*	7	5	8	14*	(3-10 %)
Thrombocytes	220	204	261	323	222	210	(150-450 Exp/l)
ESR	5	6	9	16*	2	2	M: 1-5 mm/h F: 2-12 mm/h
Sodium	137	140	142	140	142	140	(136-146 mmol/l)
Potassium	4.0	3.8	3.4*	3.8	3.8	3.9	(3.8-5.0 mmol/l)
Chloride	102	105	104	102	102	102	(99-108 mmol/l)
Calcium	2.42	2.43	2.38	2.43	2.45	2.36	(2.20-2.60 mmol/l)
Urea	4.1	3.5	3.9	3.4	4.9	4.2	(3.0-7.5 mmol/l)
Creatinin	66	65	97	93	75	71	(50-120 µmol/l)
Bilirubin	12	10	14	14	21	16	(< 17 µmol/l)
CPK	49	64	86	112	106	88	(15-180 U/l)
LDH	457	419	340	387	528	508	(300-620 U/l)
Alkaline phosphate	46	44	53	57	74	81	(40-130 U/l)
γGT	22	21	10*	12*	24	32	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	22	23	25	26	29	31	(15-45 U/l)
ALAT	17	15	26	24	27	28	(10-50 U/l)
Total proteins	75	77	81	89*	76	72	(65-81 g/l)
Albumin	42.3	42.6	41.1	46	46.3	43.3	(35-50 g/l)
URINARY ANALYSES							
Proteins/albumin	w. pos*	neg	neg	neg	w. pos*	neg	Negative
Glucose	neg	neg	neg	neg	neg	neg	Negative
Leukoscreen	w. pos*	pos*	w. pos*	neg	neg	neg	Negative
Blood	neg	neg	neg	neg	neg	w. pos*	Negative

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

Appendix 9 (continued) Medical screening

Volunteer	6		7		8		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	27-01-99	01-04-99	25-01-99	29-03-99	21-01-99	31-03-99	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	126/61	121/68	123/70	117/67	129/75	134/74	(mmHg)
Heart rate	57	67	86	76	72	75	(b.p.m.)
Standing:							
Blood pressure	113/71	113/67	106/79	116/70	134/80	130/79	(mmHg)
Heart rate	84	97	96	96	78	78	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	8.8	8.3	8.5	9.0	8.4*	8.3*	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.42	0.38	0.41	0.40	0.44	0.41	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	6.7	10	4.7	5.9	4.3	3.6*	(4.0-10.0*Exp9/l)
Eosinophiles	1	1	3	3	1	2	(< 5 %)
Basophiles	1	1	2	1	1	1	(< 2 %)
Neutrophiles	53	61	49	57	61	49	(40-72 %)
Lymphocytes	40	33	40	31	30	35	(20-45 %)
Monocytes	5	4	6	8	7	13*	(3-10 %)
Thrombocytes	279	260	166	257	241	210	(150-450 Exp/l)
ESR	7	6	5	4	3	4	M: 1-5 mm/h F: 2-12 mm/h
Sodium	140	139	141	138	140	139	(136-146 mmol/l)
Potassium	3.6*	3.7*	3.6*	3.6*	3.8	4.1	(3.8-5.0 mmol/l)
Chloride	102	106	102	103	100	101	(99-108 mmol/l)
Calcium	2.45	2.36	2.52	2.40	2.42	2.38	(2.20-2.60 mmol/l)
Urea	2.9*	3.3	3.9	4	6.9	7.7*	(3.0-7.5 mmol/l)
Creatinin	74	82	78	73	91	87	(50-120 µmol/l)
Bilirubin	13	9	41*	41*	21*	14	(< 17 µmol/l)
CPK	102	85	109	98	113	99	(15-180 U/l)
LDH	438	407	468	408	401	379	(300-620 U/l)
Alkaline phosphate	51	49	61	59	67	52	(40-130 U/l)
γGT	13*	12*	17	15	18	16	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	24	26	35	30	22	22	(15-45 U/l)
ALAT	16	16	26	17	24	24	(10-50 U/l)
Total proteins	83*	79	80	78	86*	80	(65-81 g/l)
Albumin	41.9	39.6	45.9	45	48.8	45.6	(35-50 g/l)
URINARY ANALYSES							
Proteins/albumin	neg	neg	neg	w. pos*	neg	neg	Negative
Glucose	neg	neg	neg	neg	neg	neg	Negative
Leukoscreen	pos*	w. pos*	neg	w. pos*	neg	neg	Negative
Blood	st. pos*	w. pos*	w. pos*	pos*	neg	w. pos*	Negative

* = Out of normal range neg. = negative w. pos. = weak positive pos. = positive st. pos = strong positive

Appendix 9 (continued) Medical screening

Volunteer	10		11		13		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	27-01-99	01-04-99	22-01-99	31-03-99	25-01-99	26-03-99	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	112/61	125/65	109/64	112/60	129/071	125/70	(mmHg)
Heart rate	71	75	75	86	62	62	(b.p.m.)
Standing:							
Blood pressure	108/71	119/73	113/70	115/65	125/70	128/69	(mmHg)
Heart rate	82	79	84	102	73	72	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	7.7	8.1	7.8	8.3	7.8	8.2	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.37	0.37	0.38	0.39	0.39	0.39	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	6.3	6.5	4.6	7.4	5.7	8.6	(4.0-10.0*Exp9/l)
Eosinophiles	7*	4	1	2	3	2	(< 5 %)
Basophiles	2	2	2	1	1	0	(< 2 %)
Neutrophiles	32	44	44	47	52	63	(40-72 %)
Lymphocytes	47*	38	44	42	35	27	(20-45 %)
Monocytes	12*	12*	9	8	9	8	(3-10 %)
Thrombocytes	197	229	259	284	254	271	(150-450 Exp/l)
ESR	12	10	12	29*	8	7	M: 1-5 mm/h F: 2-12 mm/h
Sodium	138	137	139	139	141	138	(136-146 mmol/l)
Potassium	3.9	3.9	3.8	3.9	3.6*	3.9	(3.8-5.0 mmol/l)
Chloride	103	101	103	106	101	97*	(99-108 mmol/l)
Calcium	2.39	2.34	2.46	2.41	2.48	2.53	(2.20-2.60 mmol/l)
Urea	3.6	3.1	4.2	3.1	3.9	4.6	(3.0-7.5 mmol/l)
Creatinin	64	63	84	76	78	84	(50-120 µmol/l)
Bilirubin	15	14	12	9	11	12	(< 17 µmol/l)
CPK	318*	91	86	77	67	68	(15-180 U/l)
LDH	450	468	403	383	417	426	(300-620 U/l)
Alkaline phosphate	55	58	47	43	45	58	(40-130 U/l)
γGT	11*	11*	14*	12*	21	21	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	46*	25	21	20	26	30	(15-45 U/l)
ALAT	20	24	19	16	16	23	(10-50 U/l)
Total proteins	73	78	73	76	78	80	(65-81 g/l)
Albumin	38.3	40.6	39.1	39.6	43.8	42.1	(35-50 g/l)
URINARY ANALYSES							
Proteins/albumin	neg	neg	w. pos*	w. pos*	neg	neg	Negative
Glucose	neg	neg	neg	neg	neg	neg	Negative
Leukoscreen	neg	neg	neg	neg	neg	w. pos*	Negative
Blood	w. pos*	w. pos*	neg	neg	neg	st. pos*	Negative

* = Out of normal range neg. = negative w. pos. = weak positive pos. = positive st. pos = strong positive

Appendix 9 (continued) Medical screening

Volunteer	14		16		18		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	25-01-99	31-03-99	25-01-99	01-04-99	26-01-99	31-03-99	Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	118/69	129/68	119/67	107/65	121/64	121/63	(mmHg)
Heart rate	62	75	78	56	75	65	(b.p.m.)
Standing:							
Blood pressure	121/69	115/76	129/75	110/69	112/68	112/76	(mmHg)
Heart rate	79	85	99	80	92	82	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	9.8	9.4	9.3	8.3	8.8	9.0	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.47	0.44	0.44	0.39	0.43	0.43	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	4.3	4.5	5.9	10.1*	6.0	7.3	(4.0-10.0*Exp9/l)
Eosinophiles	5	4	1	2	2	2	(< 5 %)
Basophiles	2	2	1	1	1	1	(< 2 %)
Neutrophiles	50	54	53	76*	63	69	(40-72 %)
Lymphocytes	29	28	39	16*	26	22	(20-45 %)
Monocytes	14*	12*	6	5	8	6	(3-10 %)
Thrombocytes	217	182	280	260	281	296	(150-450 Exp/l)
ESR	2	2	5	4	6	7	M: 1-5 mm/h F: 2-12 mm/h
Sodium	140	141	140	139	139	141	(136-146 mmol/l)
Potassium	3.7*	3.9	4.0	3.9	3.8	3.9	(3.8-5.0 mmol/l)
Chloride	101	103	102	104	102	104	(99-108 mmol/l)
Calcium	2.45	2.38	2.46	2.24	2.39	2.33	(2.20-2.60 mmol/l)
Urea	4.9	5.4	3.6	3.3	4.3	4.5	(3.0-7.5 mmol/l)
Creatinin	92	78	61	64	80	80	(50-120 µmol/l)
Bilirubin	17	14	10	11	12	11	(< 17 µmol/l)
CPK	108	288*	68	90	106	93	(15-180 U/l)
LDH	392	500	451	408	419	461	(300-620 U/l)
Alkaline phosphate	47	47	79	66	77	76	(40-130 U/l)
γGT	14	12	36	24	20	25	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	20	31	33	26	24	25	(15-45 U/l)
ALAT	20	32	35	20	18	16	(10-50 U/l)
Total proteins	75	74	79	67	73	78	(65-81 g/l)
Albumin	42.3	43.1	43.0	34.1	38.8	40.1	(35-50 g/l)
URINARY ANALYSES							
Proteins/albumin	w. pos*	neg	neg	neg	neg	neg	Negative
Glucose	neg	neg	neg	neg	neg	neg	Negative
Leukoscreen	neg	neg	w. pos*	neg	neg	w. pos*	Negative
Blood	neg	neg	neg	neg	neg	neg	Negative

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

Appendix 9 (continued) Medical screening

Volunteer	19		20		21		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	26-01-99	31-03-99	26-01-99	26-03-99	26-01-99	01-04-99	
							Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	122/64	131/69	137/66	107/55	143/83	137/77	(mmHg)
Heart rate	62	65	74	54	57	60	(b.p.m.)
Standing:							
Blood pressure	140/70	131/76	127/84	111/70	130/80	130/82	(mmHg)
Heart rate	84	75	81	78	64	62	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	abnormal*	
BLOOD ANALYSES							
Haemoglobin	9.9	10.1	9.0	8.3	10.2	9.3	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.48	0.46	0.44	0.39	0.50	0.42	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	5.6	6.4	7.8	6.5	4.9	4.3	(4.0-10.0*Exp9/l)
Eosinophiles	0	3	4	6*	6*	11*	(< 5 %)
Basophiles	0	1	1	1	2	1	(< 2 %)
Neutrophiles	4*	62	60	55	43	43	(40-72 %)
Lymphocytes	1*	25	28	31	40	39	(20-45 %)
Monocytes	1*	9	7	7	9	6	(3-10 %)
Thrombocytes	198	220	312	267	190	171	(150-450 Exp/l)
ESR	3	2	12	12	2	4	M: 1-5 mm/h F: 2-12 mm/h
Sodium	136	140	138	139	139	141	(136-146 mmol/l)
Potassium	4.3	4.0	4.2	3.8	4.6	3.8	(3.8-5.0 mmol/l)
Chloride	99	100	97	100	99	103	(99-108 mmol/l)
Calcium	2.43	2.42	2.58	2.43	2.52	2.36	(2.20-2.60 mmol/l)
Urea	4.9	4.0	4.7	4.3	5.5	4.7	(3.0-7.5 mmol/l)
Creatinin	94	77	74	66	92	82	(50-120 µmol/l)
Bilirubin	30*	18*	13	10	25*	16	(< 17 µmol/l)
CPK	139	157	41	50	120	98	(15-180 U/l)
LDH	1007*	446	389	368	734*	428	(300-620 U/l)
Alkaline phosphate	71	67	65	58	54	66	(40-130 U/l)
γGT	21	14	17	14	22	20	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	43	25	24	21	45	28	(15-45 U/l)
ALAT	7*	22	17	19	50	35	(10-50 U/l)
Total proteins	86	84*	87*	82*	81	75	(65-81 g/l)
Albumin	48.0	48.1	47.3	42.5	46.1	42.0	(35-50 g/l)
URINARY ANALYSES							
Proteins/albumin	neg	w. pos*	neg	neg	neg	neg	Negative
Glucose	neg	neg	neg	neg	neg	neg	Negative
Leukoscreen	neg	neg	neg	w. pos*	neg	neg	Negative
Blood	st. pos*	w. pos*	neg	neg	neg	neg	Negative

* = Out of normal range neg. = negative w. pos. = weak positive pos. = positive st. pos = strong positive

Appendix 9 (continued) Medical screening

Volunteer	23		24		25		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	26-01-99	29-03-99	27-01-99	01-04-99	27-01-99	29-03-99	Normal range
PHYSICAL EXAMINATION							
Supine:							
Blood pressure	109/65	113/74	107/55	111/66	137/69	149/75	(mmHg)
Heart rate	60	68	68	74	78	67	(b.p.m.)
Standing:							
Blood pressure	101/67	103/66	110/60	105/69	133/70	134/81	(mmHg)
Heart rate	72	100	78	85	104	101	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	normal	normal	
BLOOD ANALYSES							
Haemoglobin	8.0	8.9	9.6	8.3	9.5	9.6	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.40	0.42	0.46	0.39	0.45	0.44	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	6.0	2.9	7.0	9.8	6.7	6.5	(4.0-10.0*Exp9/l)
Eosinophiles	2	2	2	1	2	2	(< 5 %)
Basophiles	1	2	1	1	2	1	(< 2 %)
Neutrophiles	64	48	64	66	50	48	(40-72 %)
Lymphocytes	25	38	25	25	38	38	(20-45 %)
Monocytes	8	10	8	7	8	11*	(3-10 %)
Thrombocytes	175	154	314	301	261	244	(150-450 Exp/l)
ESR	24*	13*	8	10	2	10*	M: 1-5 mm/h F: 2-12 mm/h
Sodium	138	140	142	138	143	142	(136-146 mmol/l)
Potassium	3.5*	4.0	3.7*	3.4*	4.0	4.0	(3.8-5.0 mmol/l)
Chloride	102	106	103	105	106	102	(99-108 mmol/l)
Calcium	2.39	2.38	2.42	2.29	2.43	2.47	(2.20-2.60 mmol/l)
Urea	4.1	4.3	4.2	3.9	7.5	6.9	(3.0-7.5 mmol/l)
Creatinin	59	65	91	77	82	84	(50-120 µmol/l)
Bilirubin	12	14	22*	21*	29*	27*	(< 17 µmol/l)
CPK	198*	170	73	127	356*	149	(15-180 U/l)
LDH	583	698*	436	452	498	442	(300-620 U/l)
Alkaline phosphate	60	50	71	62	61	65	(40-130 U/l)
γGT	13*	12*	18	16	13*	15	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	29	31	37	27	40	28	(15-45 U/l)
ALAT	26	17	26	28	29	20	(10-50 U/l)
Total proteins	82*	79	80	71	75	81	(65-81 g/l)
Albumin	41.1	40.3	44.8	39.6	43.1	43.1	(35-50 g/l)
URINARY ANALYSES							
Proteins/albumin	neg	neg	neg	neg	neg	neg	Negative
Glucose	neg	neg	neg	neg	neg	neg	Negative
Leukoscreen	neg	neg	w. pos*	neg	neg	neg	Negative
Blood	neg	pos*	pos*	neg	neg	neg	Negative

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

Appendix 9 (continued) Medical screening

Volunteer	30		32		
	Pre-study Screening	Post-study Screening	Pre-study Screening	Post-study Screening	
Date	04-02-99	01-04-99	04-02-99	29-03-99	
					Normal range
PHYSICAL EXAMINATION					
Supine:					
Blood pressure	118/56	102/52	113/67	114/56	(mmHg)
Heart rate	58	69	58	62	(b.p.m.)
Standing:					
Blood pressure	114/74	101/68	124/65	121/69	(mmHg)
Heart rate	66	155	70	74	(b.p.m.)
ECG-registration (normal/abnormal)	normal	normal	normal	normal	
BLOOD ANALYSES					
Haemoglobin	7.8	8.6	8.4	8.7	M: 8.6-10.7 mmol/l (F: 7.4-9.6 mmol/l)
Haematocrite	0.38	0.41	0.41	0.41	(M: 0.41-0.55 l/l) (F: 0.36-0.46 l/l)
Leukocytes	10.6*	6.1	6.8	7.8	(4.0-10.0*Exp9/l)
Eosinophiles	2	4	1	2	(< 5 %)
Basophiles	1	1	1	1	(< 2 %)
Neutrophiles	78*	62	70	73*	(40-72 %)
Lymphocytes	12*	26	19*	16*	(20-45 %)
Monocytes	7	7	9	8	(3-10 %)
Thrombocytes	251	272	341	331	(150-450 Exp/l)
ESR	16*	4	6	8	M: 1-5 mm/h F: 2-12 mm/h
Sodium	139	140	137	139	(136-146 mmol/l)
Potassium	3.5*	3.8	3.7*	3.8	(3.8-5.0 mmol/l)
Chloride	103	101	101	104	(99-108 mmol/l)
Calcium	2.25	2.36	2.34	2.37	(2.20-2.60 mmol/l)
Urea	3.5	3.8	4.9	3.7	(3.0-7.5 mmol/l)
Creatinin	62	74	81	77	(50-120 µmol/l)
Bilirubin	14	10	8	10	(< 17 µmol/l)
CPK	114	801*	91	113	(15-180 U/l)
LDH	419	450	431	508	(300-620 U/l)
Alkaline phosphate	64	58	48	67	(40-130 U/l)
γGT	13*	12*	20	25	(M: 15-70 U/l) (F: 15-45 U/l)
ASAT	23	44	23	40	(15-45 U/l)
ALAT	16	33	21	26	(10-50 U/l)
Total proteins	71	76	70	74	(65-81 g/l)
Albumin	41.3	44.2	37.5	39.4	(35-50 g/l)
URINARY ANALYSES					
Proteins/albumin	neg	neg	neg	neg	Negative
Glucose	neg	neg	neg	neg	Negative
Leukoscreen	neg	st. pos*	neg	neg	Negative
Blood	neg	pos*	neg	neg	Negative

* = Out of normal range neg. = negative w. pos. = weak positive pos. = positive st. pos = strong positive

Appendix 10a Thyroid ^{131}I uptake measured 5-hrs after capsule intake

	Before exposure			**After exposure**			
Volunteer number	Intake-time capsule (hrs.min)	Time 5-hrs measurement (hrs.min)	5-hrs RAIU (%)	Intake-time capsule (hrs.min)	Time 5-hrs measurement (hrs.min)	5-hrs RAIU (%)	Difference 5-hrs RAIU (%)
Nitrate group							
1	11.11	16.12	13	10.25	15.23	11	**-2**
5	11.25	16.19	11	12.15	17.00	18	**7**
6	11.26	16.21	13	11.04	16.01	20	**7**
11	11.30	16.39	21	10.55	16.05	14	**-7**
13	11.07	16.00	21	11.25	16.24	23	**2**
14	11.01	15.57	17	11.32	16.30	13	**-4**
16	11.15	16.10	13	11.54	16.54	18	**5**
18	11.33	16.34	8	12.07	16.45	15	**7**
24	11.15	16.19	13	10.54	15.53	11	**-2**
25	11.30	16.14	9	11.25	16.13	8	**-1**
Control group							
2	11.30	16.26	21	11.05	15.55	12	**-9**
7	11.30	16.04	14	11.15	15.40	13	**-1**
8	11.22	16.32	3	12.09	17.04	4	**1**
10	10.50	15.47	12	10.45	15.43	16	**4**
19	11.41	16.31	12	11.20	16.21	12	**0**
20	10.52	15.52	18	10.40	15.43	15	**-3**
21	10.52	16.01	11	11.30	16.30	17	**6**
23	11.08	16.08	16	12.30	16.10	9	**-7**
30	12.00	16.43	10	12.14	17.13	13	**3**
32	11.30	15.53	5	12.07	16.32	6	**1**

Appendix 10b Thyroid ^{131}I uptake measured 24-hrs after capsule intake

	Before exposure			**After exposure**			
Volunteer number	Intake-time capsule (hrs.min)	Time 24-hrs measurement (hrs.min)	24-hrs RAIU (%)	Intake-time capsule (hrs.min)	Time 24-hrs measurement (hrs.min)	24-hrs RAIU (%)	Difference 24-hrs RAIU (%)
Nitrate group							
1	11.11	11.13	8	10.25	10.27	26	**18**
5	11.25	11.26	9	12.15	12.15	37	**28**
6	11.26	11.23	26	11.04	11.00	41	**15**
11	11.30	11.35	30	10.55	11.45	23	**-7**
13	11.07	10.45	35	11.25	11.08	45	**10**
14	11.01	10.54	30	11.32	11.30	30	**0**
16	11.15	10.55	21	11.54	11.48	30	**9**
18	11.33	11.44	16	12.07	12.06	29	**13**
24	11.15	11.55	22	10.54	10.55	23	**1**
25	11.30	11.23	24	11.25	11.15	20	**-4**
Control group							
2	11.30	10.48	41	11.05	10.45	30	**-11**
7	11.30	11.13	28	11.15	10.56	27	**-1**
8	11.22	11.26	6	12.09	11.58	13	**7**
10	10.50	10.39	24	10.45	10.43	30	**6**
19	11.41	11.36	24	11.20	11.25	25	**1**
20	10.52	10.47	29	10.40	10.40	29	**0**
21	10.52	10.59	22	11.30	11.35	32	**10**
23	11.08	11.05	27	12.30	12.30	24	**-3**
30	12.00	11.38	32	12.14	12.08	27	**-5**
32	11.30	11.00	12	12.07	11.35	19	**7**

Appendix 11 Hormone concentrations

Serum TSH concentrations (mU/l), reference values: 0.35 - 5.00 mU/l						
Volunteer number	Pre-medical screening	Day 0	Day 14	Day 21	Day 28	Post-medical screening
Nitrate group						
1	1.6	3.1	2	1.9	1.8	4.4
5	0.5	1.1	0.64	0.88	1.1	1.5
6	0.65	0.74	0.45	0.71	1	0.68
11	1.8	5.2*	1.6	1.2	2.8	3.2
13	1.6	1.4	0.69	1.1	1.9	0.26*
14	0.79	0.9	0.99	1.1	0.85	0.52
16	1.2	1.2	1.1	0.96	1	1.2
18	1	0.94	2	2.5	0.99	1.2
24	0.88	1.1	0.7	0.94	1.3	0.99
25	1.4	2.2	1.4	0.95	0.97	0.71
Control group						
2	2.2	3.4	2.9	1.3	2.6	2.6
7	1.4	1.8	0.7	0.65	0.87	0.82
8	0.71	0.7	0.35	0.63	0.72	1.2
10	2.8	1.7	1.7	1.4	1.9	2.1
19	1.3	3	2.9	1.5	1.7	1.4
20	2.4	3.4	1.9	1.2	2.1	1.9
21	2	2.3	2.1	2.6	2.2	3
23	1.1	2.3	1.5	1.2	1.4	0.96
30	1.1	2.2	1.4	2	1.4	1.9
32	1.1	1.7	2.2	2.9	1.3	1.5

Serum T_4 concentrations (nmol/l), reference values: 50 - 150 nmol/l						
Volunteer number	Pre-medical screening	Day 0	Day 14	Day 21	Day 28	Post-medical screening
Nitrate group						
1	95	95	104	99	107	94
5	91	87	88	89	88	108
6	132	155*	162*	147	154*	137
11	125	112	117	110	128	125
13	111	124	133	124	126	120
14	82	84	81	95	81	92
16	144	150	145	135	146	149
18	99	112	130	151*	117	130
24	92	95	101	93	95	99
25	84	74	86	84	82	106
Control group						
2	110	116	111	120	127	136
7	97	79	87	86	117	98
8	109	108	97	101	96	107
10	142	140	142	156*	134	141
19	86	90	87	86	88	88
20	117	119	125	121	125	112
21	89	94	100	96	97	106
23	107	108	129	111	110	109
30	129	134	124	130	125	134
32	81	86	82	97	81	99

* = Out of normal range

Appendix 11 (continued) Hormone concentrations

Serum T_3 concentrations (nmol/l), reference values: 1 -3 nmol/l						
Volunteer number	Pre-medical screening	Day 0	Day 14	Day 21	Day 28	Post-medical screening
Nitrate group						
1	1.7	1.7	1.6	1.5	1.7	1.6
5	1.8	1.7	2.8	1.7	1.9	1.9
6	2.2	2.2	2.5	2.2	2.4	2.2
11	2.2	2.1	2.4	2	2.6	2.3
13	1.8	2.4	1.8	2.3	2.7	2.4
14	1.6	1.6	1.5	1.5	1.4	1.6
16	2.2	2.4	1.9	1.8	1.6	2.1
18	1.6	1.9	2.2	2.4	1.8	1.9
24	1.4	1.6	1.2	1.6	1.6	1.5
25	1.5	1.3	1.4	1.4	1.6	1.6
Control group						
2	1.7	2.1	2.2	2.2	2.2	2
7	1.6	1.3	1.8	1.4	1.7	1.3
8	1.6	1.6	1.4	1.8	1.6	1.6
10	2.6	2.1	2.4	2.4	2.2	2.7
19	1.6	1.3	1.4	1.2	1.7	1.4
20	2.2	1.9	1.9	1.9	2	1.5
21	1.8	1.4	1.4	1.8	1.5	1.2
23	1.6	1.9	1.8	1.4	1.7	1.4
30	1.9	2.1	1.9	2.1	1.9	1.6
32	1.3	1.2	1.6	1.8	1.6	1.8

Serum rT_3 concentrations (nmol/l), reference values: 0.15 - 0.54 nmol/l						
Volunteer number	Pre-medical screening	Day 0	Day 14	Day 21	Day 28	Post-medical screening
Nitrate group						
1	0.28	0.29	0.32	0.33	0.29	0.28
5	0.29	0.31	0.26	0.29	0.29	0.30
6	0.41	0.44	0.42	0.45	0.42	0.37
11	0.31	0.35	0.29	0.32	0.37	0.29
13	0.32	0.30	0.39	0.40	0.32	0.32
14	0.28	0.31	0.30	0.32	0.30	0.30
16	0.45	0.42	0.40	0.42	0.37	0.41
18	0.31	0.38	0.40	0.48	0.35	0.35
24	0.40	0.34	0.36	0.36	0.32	0.36
25	0.25	0.28	0.28	0.28	0.34	0.37
Control group						
2	0.32	0.36	0.34	0.36	0.39	0.40
7	0.35	0.36	0.31	0.31	0.45	0.34
8	0.36	0.45	0.42	0.40	0.34	0.33
10	0.37	0.39	0.40	0.42	0.37	0.37
19	0.28	0.32	0.31	0.32	0.28	0.30
20	0.33	0.34	0.35	0.36	0.34	0.32
21	0.32	0.29	0.35	0.32	0.31	0.31
23	0.32	0.38	0.44	0.37	0.37	0.32
30	0.34	0.46	0.37	0.39	0.35	0.35
32	0.22	0.26	0.24	0.28	0.30	0.25

Appendix 11 (continued) Hormone concentrations

Blood IGF I concentrations (ng/ml), reference values are age dependent[1]							
Volunteer number	Age (yrs)	Pre-medical screening	Day 0	Day 14	Day 21	Day 28	Post-medical screening
Nitrate group							
1	30	180	210	190	163	165	181
5	20	500	460	450	520	493	425
6	23	260	160*	300	181	247	181
11	20	310	300	300	280	273	322
13	25	260	260	310	257	345	377
14	26	170	170	180	240	223	176
16	24	190	200	310	192	215	168*
18	25	300	200	250	330	182	245
24	20	240	260	280	271	299	294
25	23	250	230	260	280	257	226
Control group							
2	23	270	210	150*	280	215	215
7	23	210	200	250	230	244	202
8	23	400	330	410	330	353	355
10	20	160*	160*	140*	220	142*	139*
19	26	170	120	230	210	193	204
20	26	190	130	180	204	162	168
21	28	260	250	260	249	221	200
23	26	190	230	200	180	297	216
30	24	280	290	300	374	308	349
32	21	230	220	290	330	302	220

* = Out of normal range

1. Reference values: 16 - 25 years, 180 - 780 ng/ml
25 - 39 years, 115 - 490 ng/ml

Appendix 12 Total amount of iodide in 24-hrs urine

First urine collection

Volunteer number	Collection day	Urine volume (l)	Concentration iodide (μmol/l)	Total amount (μmol)
Nitrate group				
1	-1	2.7117	0.31	0.84
5	-1	0.7436	1.14	0.85
6	-1	1.0843	0.59	0.64
11	-1	0.9225	0.89	0.82
13	-1	2.5121	0.15	0.38
14	-1	1.7481	0.67	1.17
16	-1	2.1472	0.12	0.26
18	-1	1.2357	0.08	0.10
24	-1	0.5087	1.34	0.68
25	-1	0.8885	0.71	0.63
Control group				
2	-1	0.8670	0.44	0.38
7	-1	1.6993	0.73	1.24
8	-1	1.2976	19.25	24.98
10	-1	0.8187	0.53	0.43
19	-1	1.3699	1.61	2.21
20	-1	1.8982	0.01	0.02
21	-1	0.9419	1.89	1.78
23	-1	3.0843	0.18	0.56
30	-	-	-	-
32	-1	2.8245	0.36	1.02

Second urine collection

Volunteer number	Collection day	Urine volume (l)	Concentration iodide (μmol/l)	Total amount (μmol)
Nitrate group				
1	11	3.8259	0.21	0.80
5	4	1.6501	1.08	1.78
6	3	1.6859	0.58	0.98
11	6	0.9824	0.60	0.59
13	5	1.8138 (1) / 1.3843 (2)	0.61 (1) / 0.37 (2)	1.11 (1) + 0.51 (2) = 1.62
14	11	2.0888	0.90	1.88
16	10	1.9396	0.40	0.78
18	5	1.2953	0.59	0.76
24	4	0.9420	0.54	0.51
25	14	1.4905	0.68	1.01
Control group				
2	6	1.2909	0.26	0.34
7	6	1.5359	0.67	1.03
8	3	1.6418	0.69	1.13
10	8	1.7275	0.99	1.71
19	6	1.8301	0.68	1.24
20	11	1.6718	0.63	1.05
21	9	1.5152	1.25	1.89
23	5	3.5364	0.18	0.64
30	11	1.8217	0.33	0.60
32	7	2.0385	0.49	1.00

Appendix 12 (continued) Total amount of iodide in 24-hrs urine

Third urine collection				
Volunteer number	Collection day	Urine volume (l)	Concentration iodide (µmol/l)	Total amount (µmol)
Nitrate group				
1	22	2.4295	0.11	0.27
5	21	1.0019	0.68	0.68
6	24	1.5003	0.29	0.44
11	20	1.1810	0.83	0.98
13	24	2.7799	0.11	0.31
14	27	0.9950	0.58	0.58
16	26	2.0127	0.60	1.21
18	15	2.5120	0.29	0.73
24	19	1.5036	0.69	1.04
25	23	1.3021	1.02	1.33
Control group				
2	19	1.5362	0.11	0.17
7	17	1.6844	0.37	0.62
8	23	1.4049	2.99	4.20
10	26	0.9308	0.83	0.77
19	19	0.9447	0.65	0.61
20	25	1.9539	0.21	0.41
21	20	2.2535	0.53	1.19
23	23	2.4868	0.32	0.80
30	20	2.6909	0.32	0.86
32	18	2.0750	0.68	1.41

Appendix 13 Plasma nitrate concentrations (mg/kg)

UTN	Pre-medical screening	Day 0	Control sample 1	Control sample 2	Control sample 3	Control sample 4	Control sample 5	Control sample 6	Control sample 7	Control sample 8	Post-medical screening
Nitrate group											
1	3.58	2.26	2.57	5.67	6.31	4.96	5.35	4.66	6.18	4.55	2.06
5	3.71	3.19	1.86	9.44	6.91	6.72	1.70	7.71	6.92	5.78	6.40
6	3.07	4.98	5.43	4.44	5.79	4.73	6.12	6.85	5.80	4.99	2.38
11	1.53	3.15	2.19	3.63	2.93	3.00	3.45	3.16	4.46	3.27	1.84
13	1.77	2.10	6.14	9.08	6.50	5.23	3.02	4.94	3.72	NA	7.22
14	2.23	2.19	5.18	5.89	4.53	7.02	5.07	4.63	5.39	6.36	1.66
16	4.62	1.95	4.45	2.37	2.18	2.00	3.44	2.20	4.03	2.18	0.86
18	1.02	2.23	6.10	5.27	4.20	3.35	3.34	NA	5.86	5.27	1.34
24	2.02	1.80	3.80	4.08	4.17	4.64	4.67	5.30	4.16	4.63	2.15
25	2.13	5.89	6.34	NA	6.66	4.99	7.29	7.97	7.04	6.82	3.37
Control group											
2	1.86	2.86	1.83	3.45	2.91	2.70	2.00	5.23	1.85	1.70	1.89
7	2.48	1.89	1.71	2.48	1.84	2.01	2.09	1.31	1.85	1.57	4.42
8	2.12	1.67	1.53	2.94	1.37	1.48	1.63	1.67	1.51	1.69	1.76
10	3.56	1.61	1.19	1.68	1.52	2.61	1.79	1.38	1.21	1.19	1.88
19	2.24	3.82	3.59	4.57	5.77	2.45	2.17	2.31	2.30	2.71	2.59
20	1.91	3.11	3.22	2.72	2.71	2.01	2.70	2.85	1.68	1.42	1.48
21	2.28	1.75	1.35	2.05	2.54	1.88	1.63	1.81	2.58	2.66	4.90
23	2.96	1.64	2.15	1.66	1.94	2.41	2.09	2.90	1.43	3.06	1.33
30	1.17	2.76	1.82	1.64	2.36	2.40	2.80	2.88	3.73	3.12	3.17
32	2.84	1.68	4.79	3.17	1.93	2.34	1.55	4.61	2.05	2.68	10.52

NA = Not analysed

Appendix 14 Plasma nitrite concentrations (mg/kg)

UTN	Pre-medical screening	Day 0	Control sample 1	Control sample 2	Control sample 3	Control sample 4	Control sample 5	Control sample 6	Control sample 7	Control sample 8	Post-medical screening
Nitrate group											
1	< 0.2	< 0.2	< 0.2	< 0.2	0.20	< 0.2	< 0.2	0.21	< 0.2	< 0.2	0.25
5	< 0.2	0.22	0.20	< 0.2	< 0.2	< 0.2	0.20	0.21	0.20	0.22	0.22
6	< 0.2	0.20	< 0.2	< 0.2	< 0.2	0.22	0.20	0.21	< 0.2	< 0.2	< 0.2
11	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.23	< 0.2	0.23	0.21	< 0.2
13	< 0.2	< 0.2	< 0.2	< 0.2	0.20	< 0.2	< 0.2	0.21	< 0.2	NA	0.21
14	< 0.2	< 0.2	< 0.2	0.21	< 0.2	< 0.2	0.29	< 0.2	< 0.2	< 0.2	< 0.2
16	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.23	0.23	0.22	0.24	< 0.2	< 0.2
18	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.22	< 0.2	NA	0.20	< 0.2	0.20
24	< 0.2	< 0.2	0.21	0.20	< 0.2	0.21	0.20	0.22	< 0.2	0.22	0.20
25	< 0.2	< 0.2	0.20	NA	< 0.2	0.20	0.21	< 0.2	0.32	0.20	< 0.2
Control group											
2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.22	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
7	< 0.2	< 0.2	0.21	< 0.2	0.21	0.22	< 0.2	0.20	0.21	< 0.2	0.20
8	< 0.2	< 0.2	< 0.2	0.24	< 0.2	0.22	0.22	< 0.2	< 0.2	< 0.2	< 0.2
10	< 0.2	< 0.2	0.24	< 0.2	0.21	< 0.2	< 0.2	0.21	< 0.2	< 0.2	< 0.2
19	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.21
20	< 0.2	< 0.2	< 0.2	0.20	< 0.2	0.36	0.26	< 0.2	< 0.2	0.21	< 0.2
21	< 0.2	0.20	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.20	0.24	< 0.2
23	< 0.2	0.26	< 0.2	0.25	0.20	0.20	< 0.2	0.20	< 0.2	< 0.2	< 0.2
30	< 0.2	0.21	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.21	< 0.2	< 0.2	< 0.2
32	< 0.2	< 0.2	< 0.2	< 0.2	0.24	< 0.2	0.25	0.25	< 0.2	< 0.2	< 0.2

NA = Not analysed

Appendix 15 Percentages methaemoglobin

Methaemoglobin percentages of total hemoglobin (%), reference value: 0 -1 %					
Volunteer number	Pre-medical screening	Day 0	Day 7	Day 28	Post-medical screening
Nitrate group					
1	0.2	0.2	0.6	0.2	0.3
5	0.4	0.3	0.4	0.4	0.3
6	0.3	0.4	0.4	0.1	0.4
11	0.5	0.3	0.4	0.2	0.2
13	0.3	0.3	0.2	0.3	0.3
14	0.3	0.4	0.3	0.4	0.6
16	0.2	0.4	0.4	0.1	0.3
18	0.4	0.3	0.5	0.3	0.4
24	0.3	0.4	0.3	0.2	0.1
25	0.1	0.1	0.1	0.3	0.2
Control group					
2	0.2	0.1	0.3	0.1	0.2
7	0.2	0.3	0.2	0.3	0
8	0.5	NA	0.4	0.3	0.4
10	0.1	0.4	0.2	0.3	0.3
19	0.5	0.3	0.4	0.3	0.5
20	0.3	0.3	0.3	0.2	0.3
21	0.4	0.3	0.3	0.1	0.3
23	0.3	0.3	0.4	0.5	0.2
30	0.2	0.1	0.2	0.1	0.3
32	0.3	0.4	0.3	0.2	0.1

NA = Not analysed