Proc. Estonian Acad. Sci. Ecol., 1993, 3, 4, 161—165
https://doi.org/10.3176/ecol.1993.4.01

INVESTIGATION OF NITRATE EXCRETION IN HUMAN ORGANISM

Maarja ROOMA, Ingeborg VELDRE, and Svetlana KARLOVA

Estonian Institute of Experimental and Clinical Medicine, Hiiu St. 42, EE-0107 Tallinn, Estonia

Presented by J. Martin

Received February 8, 1993; revised version received April 15, 1993; accepted May 11, 1993

Abstract. In Estonia the daily load of nitrates on the human organism depends on the nitrate content in drinking water rather than in vegetables. A strong positive correlation between the nitrate concentration in the drinking water and amounts of nitrates in urine and saliva was found. The rural inhabitants get more nitrates from the drinking water than the city dwellers. As the amount of nitrates in the drinking water is big the nitrate excretion in urine does not reflect how much nitrate is ingested with food. Thus a high nitrate concentration in drinking water does not permit to use the measurement of nitrate excreted in urine for the estimation of the role of nitrate ingested with food.

Key words: nitrates, drinking water, urine, saliva.

Nitrate in vegetables and in drinking water constitutes the main nitrate load of the human organism. The maximum permissible intake of nitrates established by WHO/FAO (1979) is 5 mg NO_3^- per 1 kg body mass. Analyses of nitrate contents in food give some idea about the nitrate load in the human organism. Products containing nitrate above the permissible level are not allowed for sale. Drinking water is often used in spite of its high nitrate concentration. It is not possible to analyse the whole agricultural produce and drinking water; moreover, analytical methods do not always give objective results.

Is it possible to evaluate the population's nitrate load on the ground of vegetable analyses? The average data of the nitrate content in different kinds of vegetables enable only to evaluate the trends of nitrate levels over years and can serve as guidelines for the farmer. Such analyses offer no data for estimating the nitrate dose which actually affects the human organism.

The toxic effect of nitrates on human organism has been investigated in Estonia for about twenty years, but there have been no epidemiological studies. Apparently, epidemiological studies of drinking water nitrate content could be conducted only when there is a large number of persons who drink water with various nitrate contents.

Nitrates are usually not regarded as xenobiotics. They are easily available for plants from soil. Part of their total amount is immediately used in the life cycle, and the remainder is stored in plant cells. The human organism is in contact with nitrates during its whole existence. Nitrates are also formed by endogenous synthesis in the human organism (Green et al., 1981; Mirvish, 1983). Endogenously synthesized nitrates have not been included in the investigations of the toxic and carcinogenic effects of nitrates. Up to now there is no explanation why nitrates are synthesized in the organism. Endogenously synthesized nitrate concentration can even exceed the level of the exogenous one. It has been suggested that the synthesis of nitrates is activated by bacterial infections, lambliosis, opistorchiasis (Siranujata et al., 1987; Wettig, Uhlig et al., 1989; Веттиг et al., 1990). The physiological role of enhanced nitrate in biosynthesis remains unclear. In case of acute diarrhoea in children Hegesh & Shiloah (1982) found that the content of methaemoglobin could increase even to 8% of haemoglobin. It has been suggested that acute diarrhoea rather than nitrate contained in water is the determinative factor in most cases of infantile methaemoglobinaemia (Hegesh & Shiloah, 1982).

Wettig, Schulz et al. (1989) estimated that 75% of the ingested nitrate was recovered as urinary nitrate and only 25% of the total dose stayed in the organism having a potential harmful effect and being a risk factor on normal human health. Nitrate excretion in urine is the result of intake with vegetables and drinking water and endogenous synthesis, minus metabolic losses (Green et al., 1981).

The excretion of nitrate in urine is also influenced by various exogenous and endogenous factors. In severe cases of inflammatory processes the endogenous nitrate may be an essential additional load on the human organism (Wettig, Schulz et al., 1989; Веттиг et al., 1990; Mirvish, 1983; Rooma & Kann, 1990; Роома & Канн, 1990).

Methods of indirect analyses of average nitrate doses in vegetables cannot be used to get results of satisfactory exactness. Therefore, it is necessary to work out for epidemiological studies a model for investigating the nitrate excretion in urine, in which the daily endogenous nitrate synthesis and retention in organism are taken into consideration.

The effect of different exogenous and endogenous factors on the amount of nitrate in urine and saliva has been studied in Estonia for about four years. In the present investigation we tried to assess the role of the consumption of nitrate with drinking water and vegetables in the whole daily nitrate load. In our previous investigations we observed the effect of diet, sex, age, health, the use of medicines and vitamins, smoking and other habits, contact with toxicants, and other factors in the formation of the nitrate level in urine and saliva (Rooma & Kann, 1990; Роома & Канн, 1990).

METHODS

Estonian rural and urban inhabitants, altogether 662 persons, were investigated. The aim of the study was to clarify the role of nitrates in drinking water and vegetables as environmental factors on the excretion of nitrate in urine and to determine the content of nitrates in saliva. Nitrate intake was estimated by the measurement of the morning urinary nitrate, as these concentrations are in positive correlation with nitrate in the daily urine and with the total amount of ingested nitrate (Wettig, Schulz et al., 1989; Wettig, Uhlig et al., 1989). Nitrate content in water was determined using the salicylate method; in urine and saliva with the reduction of nitrate in cadmium column with the Griess reagent (Rooma & Kann, 1990; Wettig, Schulz et al., 1989). Samples of urine and saliva vere taken in sterilized glasses and were analysed, as a rule, in two hours. When necessary, the samples were preserved and stored in a deep freezer. Data on the investigated persons were obtained from a questionnaire (Роома & Канн, 1990).

The samples of drinking water, urine, and saliva were collected in 11 regions — 4 rural and 7 urban ones. To evaluate the influence of nitrate from vegetables on the nitrate excretion in urine and saliva, 330 persons were chosen from Tallinn, where the nitrate concentration in drinking water was low (below 1 $mg \cdot l^{-1}$) and thus its effect was negligible. The effect of the drinking water nitrate on the organism was studied in 332 persons. Statistical analysis and estimation of significance were performed using a computer.

RESULTS AND DISCUSSION

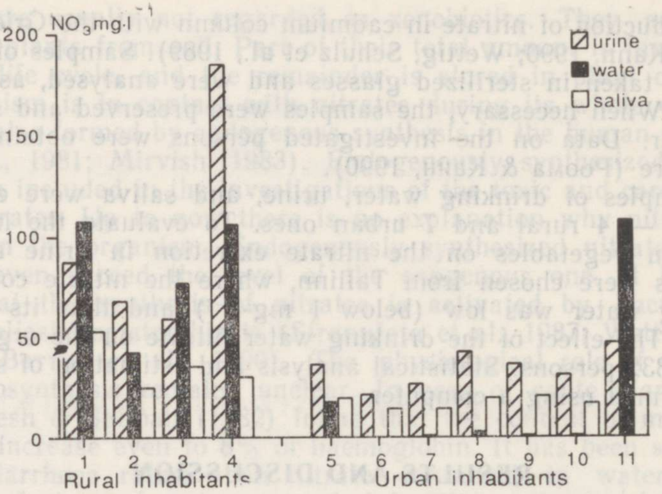
The results of the correlation analyses are given in Table 1. Positive correlation between urinary and salivary nitrate content was observed in case of all the 662 persons studied. A strong positive correlation ($P<0.01$) was found between the drinking water nitrate concentration and levels of nitrate in urine and saliva ($n=332$). Between nitrate in vegetables and nitrate in urine there is only a tendency ($P<0.1$) toward correlation ($n=330$). A positive correlation ($P<0.05$) was observed in our previous studies (Роома & Канн, 1990) when the subjects had consumed vegetables with high nitrate contents (beet, garden radish, greenhouse cucumber, etc.) the previous day.

Table 1

Correlation coefficients between nitrates in drinking water, urine, saliva, and vegetables

	Nitrates in water	Nitrates in urine	Nitrates in saliva	Nitrates in vegetables
Nitrates in water		0.4014 $P<0.01$ $n=332$	0.4211 $P<0.01$ $n=332$	
Nitrates in urine	0.4014 $P<0.01$ $n=332$		0.5584 $P<0.001$ $n=662$	0.0989 $P<0.1$ $n=330$
Nitrates in saliva	0.4211 $P<0.01$ $n=332$	0.5584 $P<0.001$ $n=662$		0.0123 $P>0.05$ $n=330$
Nitrates in vegetables		0.0989 $P<0.1$ $n=330$	0.0123 $P>0.05$ $n=330$	

A comparison of nitrate levels in drinking water, saliva, and urine in different regions is given in the Figure. In Table 2 the nitrate content in the drinking water of rural and urban inhabitants as well as the content of nitrates in their urine and saliva are compared. The nitrate content in water was about 2.7 times higher in rural regions than in urban places.



Content of nitrates in drinking water, urine, and saliva of subjects from different regions. 1 — Vandjala, 2 — Viiratsi, 3 — Rannu, 4 — Pajusi, 5 — Saue, 6 — Pärnu, 7 — Nõmme, Tallinn, 8 — Lasnamäe, 9 — Mustamäe, 10 — Tartu-Ropka, 11 — Tartu-Ülejõe.

Table 2

Content of nitrates in drinking water, urine, and saliva of rural and urban persons, mg·l^{-1}NO$_3^-$

Parameter	Rural persons $n=156$ mean ±SD	Urban persons $n=176$ mean ±SD
Nitrates in drinking water	88.7±13.6	33.1±4.6
Nitrates in urine	96.2±6.5	34.9±2.2
Nitrates in saliva	35.0±2.3	17.4±1.3

The influence of nitrate intake with vegetables and drinking water on the urinary excretion of nitrates was observed separately for urban and rural inhabitants. Both groups were divided into two subgroups. The first subgroup comprised persons who had eaten vegetables with high nitrate content (beets, radish, greenhouse cucumber, lettuce, spinach, etc.) the previous day; in the second subgroup were persons who had consumed only vegetables with low nitrate levels (potatoes, carrots, etc.), fruit, and berries, or not eaten vegetarian food at all. The results show that the nitrate content in the drinking water of persons who had consumed food with high and low nitrate content was quite similar in both rural and urban groups. It was proved that in urban people the food nitrates increased the nitrate excretion in urine to a great extent (1.6 times). However, in some rural areas of Estonia the effect of drinking water nitrates on the nitrate excretion was so significant that the food nitrates were of relatively small importance (Fig.) though rural people eat more vegetables than urban inhabitants.

We can conclude that in Estonia the level of urine nitrates depends more significantly on the nitrate content in drinking water than on that in vegetables as the correlation between the urine nitrate excretion and drinking water nitrates is stronger than the correlation between the urinary nitrate excretion and food nitrates. So the importance of drinking water nitrates in the daily nitrate load is higher than that of vegetables.

The results of our previous investigations (Роома & Канн, 1990) as well as the data of other authors have proved that when the amount of nitrate ingested is less than 50 mg per day it does not affect the nitrate content in urine and saliva (Mirvish, 1983; Wettig, Schulz et al., 1989). Our study showed that extremely high exogenous nitrate levels do not enable to estimate the amount of daily nitrate dose by means of measuring the nitrate excretion in urine. In our previous investigations we have found a number of factors which influence the nitrate excretion in urine such as age, health conditions, use of medicines and vitamins, smoking, contact with toxicants, etc. However, in case the nitrate level in drinking water is high, the effect of these factors is not evident. High nitrate content in drinking water increases the exogenous nitrate content to such a level that the endogenously synthesized nitrates are overshadowed (Rooma & Kann, 1990).

CONCLUSIONS

The results of our research allow of the conclusion that urine and saliva nitrate contents reflect the condition of the organism only when the nitrate intake is moderate. Urinary nitrate content cannot be used as a criterion for evaluating extremely high or low levels of exogenous nitrates. Still, the measurement of nitrate excretion in human organism can be used as a simple direct method to evaluate the load of various exogenous sources of nitrate instead of analysing vegetables and drinking water for nitrates.

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