3 Are dietary nitrates a threat to human health?

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Summary

Two major charges were levelled at nitrates about thirty years ago: infant methaemoglobinaemia ('blue-baby syndrome'), and a greater risk of cancer in adults. These were either presumptions or hypotheses.

The many scientific studies carried out over the last few decades allow us to conclude that neither of those grievances were founded. Dietary nitrates pose no threat to human health.

Nitrates present in blood plasma have two sources. One is exogenous, from food: 80 per cent of these alimentary nitrates come from vegetables, and 10 to 15 per cent from drinking water. The other source is endogenous, provided in similar quantities, of cell origin, and involves the amino acid L-arginine and nitrogen monoxide (NO). In addition to passive urinary excretion, there are two active secretions of nitrate (NO_3) from plasma: colonic secretion and salivary secretion. Salivary secretion reintroduces NO_3 ions for a second time in the mouth.

Directives issued in 1962 by the UN's World Health Organisation (WHO), and the Food and Agricultural Organisation (FAO), and in 1980 by the European Economic Community are now redundant. Moreover, the directive on drinking water is very costly. These directives will inevitably need to be repealed.

Introduction

From the 12th to the 19th century, nitrates (NO_3) were used as medicines, sometimes in very large doses, for a wide and sometimes surprising range of symptoms.

At the beginning of the 20th century, the development of aspirin followed by the introduction of corticoids meant the end of therapeutic use of nitrates. By the 1950s, the growing incidence of infant methaemoglobinaemia in some rural areas of the United States, together with the discovery of the carcinogenic properties of many nitrosamines in animals, contributed to suspicions about the effect of dietary nitrates on humans.

In 1962, these doubts led the Committee of Experts on Food Additives of the WHO and the FAO to set an acceptable daily intake (ADI) level for man at 3.65 mg/kg of NO_3, and in 1980, the European Community issued a directive which established a limit for NO_3 in drinking water of 50 mg/litre, above which the water was deemed unfit for human consumption.

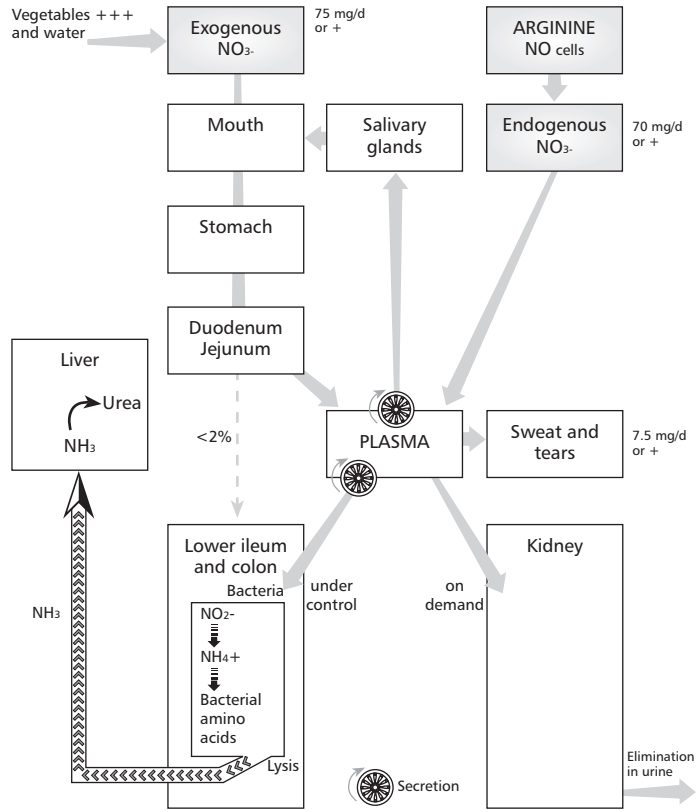
Some 37 years after the WHO and the FAO's decision and 18 years after the European Community's directive, the scientific perspective has changed completely. Many studies and experiments have been conducted; any suspicions which were legitimate a few decades ago can no longer be justified.

The metabolism of nitrates

Figure 1 gives an overview of the metabolism of nitrates. Nitrates are always present in our bloodstream, at levels normally ranging between 1 and 3 mg/litre before meals. In normal conditions, two sources of nitrates coexist – the exogenous source from food and water, and the endogenous source from cell activity; they each provide 70 to 75 mg per day each.

When humans ingest nitrates from food sources, 80 per cent ingested normally come from vegetables, and 10 to 15 per cent from drinking water. When swallowed, these nitrates pass into the stomach as NO_3, before being quickly and almost completely

Figure 1 **The metabolism of nitrates in man**



absorbed in the upper section of the small intestine. Therefore less than 2 per cent of all nitrates ingested reach the large intestine.[1]

The endogenous source of nitrates has been known since 1985, when research was carried out by Stuehr and Marletta.[2] The metabolic process of the amino acid L-arginine releases a nitrogen atom

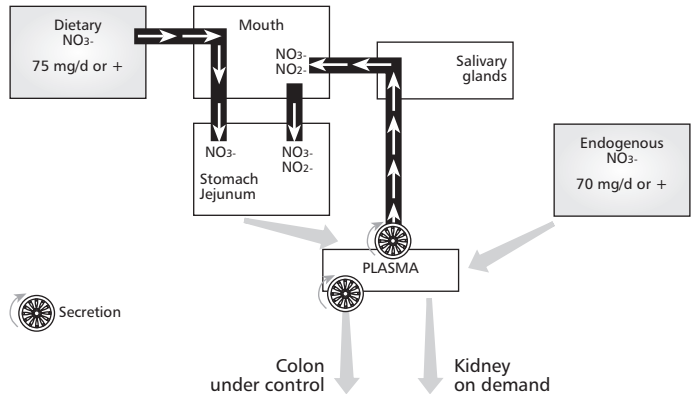
at cell level, which forms a molecule of nitrogen monoxide, NO. Outside the cell, the NO molecules combine with oxygen to form various molecules including nitrates, nitrites and nitrosamines. Many physiological activities, such as running and cycling, and pathological conditions, such as infections, lead to cell stimulation and thereby contribute to increasing this endogenous synthesis of nitrates.

Thereafter, the fate of plasmatic nitrates from exogenous and endogenous sources is rather unusual:

- A small proportion of plasmatic nitrates – approximately 10 per cent of the quantity of nitrates ingested – is eliminated through sweat and tears.
- A majority of plasmatic nitrates are passively excreted in urine, this excretion being based only on NO_3 plasmatic concentrations.
- Two active phenomena also have a significant impact from a physiological perspective: the colonic and salivary secretions of nitrates.
- One of the functions of the columnar cells of the colonic epithelium is to draw NO_3 ions from the plasmatic sector towards the colon's light through an active capture phenomenon. The purpose of this colonic secretion of NO_3 ions is most probably to ensure the nutrition of the colonic bacterial flora.
- The cells of the salivary acinus also actively draw NO_3 ions from the plasmatic sector and release them into the saliva, its secretion product. At times, levels of salivary nitrates are between 6 and 30 times higher than for plasmatic nitrates.

Thereafter, these salivary nitrates remain for some period in the mouth, and come under the influence of bacterial enzymes produced from a relatively abundant physiological bacterial flora. Some of these salivary nitrates (NO_3^-) thereby turn into salivary nitrites (NO_2^-).

Figure 2 **The duality of nitrates in the mouth**



Dietary nitrates and nitrates secreted by the salivary glands are two distinct entities. Only NO_3- ions secreted by the salivary glands, as NO_3- precursors, can induce nitrosamines. By contrast, NO_2- ions from food reach the stomach intact, i.e. without turning into NO_3^2- and therefore without any risk of turning into nitrosamines.

As figure 2 shows, NO_3^- ions pass through the oral cavity twice, the first time as dietary nitrates, the second time as salivary nitrates. Only the latter process induces the formation of a certain amount of salivary nitrites, which reach the stomach when the saliva is swallowed.

The role of this salivary secretion of nitrates merits clarification. It is quite possibly a preliminary stage in the digestion of proteins, as salivary nitrates have the ability to make food proteins more sensitive to the subsequent action of proteolytic enzymes (pepsine and trypsine). Another study in 1994 showed that in an acid medium, ingested salivary nitrates which have reached the stomach release NO and thereby destroy organisms such as *Candida albicans* and *Escherichia coli*, thereby promoting host defence against ingested pathogens.[3]

Grievances against nitrates – their refutation

In the 1950 and 1960s, alimentary nitrates aroused disquiet on two counts. They were thought to be responsible for methaemoglobinaemia in infants, and people wondered whether they might not also induce the onset of some cancers, in particular stomach cancer. However, numerous studies conducted in the last thirty years now allow us to conclude that the role of nitrates in causing these health impacts lacks scientific basis.

Dietary methaemoglobinaemia in infants – blue baby syndrome

Methaemoglobin is an oxidised derivative of haemoglobin, which loses its ability to carry oxygen molecules. In the physiological state, 1 to 2 per cent of haemoglobin in the red cells is in the form of methaemoglobin. Clinical disorders, in this case cyanosis, appear if methaemoglobinaemia levels exceed the 10 to 20 per cent threshold. Any increase reaching 70 to 80 percent can be fatal.

The transformation of haemoglobin into methaemoglobin in red cells is due to the action of nitrites rather than nitrates, which are very powerful oxidants. Babies under six months of age are most at risk, because they have not yet fully developed a protective enzyme system (reductase methaemoglobin or NADH-cytochrome b5 reductase). Beyond the age of six months, the risk of pathological methaemoglobinaemia no longer exists.

Prior to 1984, it was generally thought that nitrates in feeding bottles turned into nitrites in the baby's colon, after contact between nitrates and the colon's large bacterial flora, as only bacterial enzymes are capable of reducing NO_3 nitrates into NO_2 nitrites. However, since Bartholomew's studies in 1984 showed that 98 per cent of alimentary nitrates are absorbed in the upper section of the small intestine[4], this explanation is no longer valid.

Some scientists then wondered whether the nitrates-nitrites transformation might take place in the baby's stomach, as a result of a colonisation of the stomach by micro-organisms of enteric origin, under a hypochlorhydric effect of the gastric juices. However, studies on this subject are not conclusive, as Walker showed in 1990[5]; the secretion of gastric acid in infants is actually sufficient to prevent any significant bacterial colonisation.

In reality, as some authors had indicated 25 to 30 years ago[6], the nitrates-nitrites transformation, responsible for infant methaemoglobinaemia, occurs in the feeding bottles if basic hygiene rules are not followed when the bottles are being prepared, thereby causing microbe pullulation.

A number of clinical observations have confirmed this; these include the sudden and unexpected nature of cyanosis, when large amounts of nitrated foods (carrot soup, spinach) have been ingested during the days or weeks preceding the incident without giving rise to the slightest clinical anomaly, the rapid onset of cyanosis 15 to 20 minutes after feeding, and the lack of correlation between the level of methaemoglobinaemia and the amount of nitrates intake through food.

Simply to limit nitrate levels at 50 mg per litre of NO_3 in drinking water does not constitute an appropriate preventive response with regard to infant methaemoglobinaemia.

Nitrates will continue to be a potential element of infant food via the intake of water used to prepare formula milk, and through consumption of vegetables. The only effective preventive solution consists in acting on the bacterial element; all risks of bacteria pullulation must be eliminated in feeding bottles, whether or not they contain nitrates, by following a few basic hygiene rules when the bottles are being prepared. In the case of carrot soup, it should be boiled for a few minutes and fed to the baby shortly thereafter, and in no circumstances should the soup be left to stand at ambient temperature for more than six to eight hours.

What causes methaemoglobinaemia in infants therefore is not alimentary nitrates, but rather the nitrites formed in the feeding bottles, after the reduction of nitrates into nitrites as a result of an unfortunate microbe pullulation in the bottles. It is the latter phenomenon which should be prevented at all cost. Infant methaemo-

globinaemia caused by food has been virtually eradicated in developed countries, where people are familiar with basic hygiene rules for preparing bottles of formula milk.

Cancer

As shown above, the salivary glands draw nitrate ions from the blood plasma; salivary nitrates partly turn into nitrites in the mouth, and when swallowed these salivary nitrites reach the stomach.

Salivary nitrites then react with various amines in the stomach to form nitrosamines. Ninety per cent of nitrosamines tested in experiments are known to be carcinogens in animals. It was deduced from this that nitrates have a potential carcinogenic power, and this presumption has now been hanging over them for almost forty years.

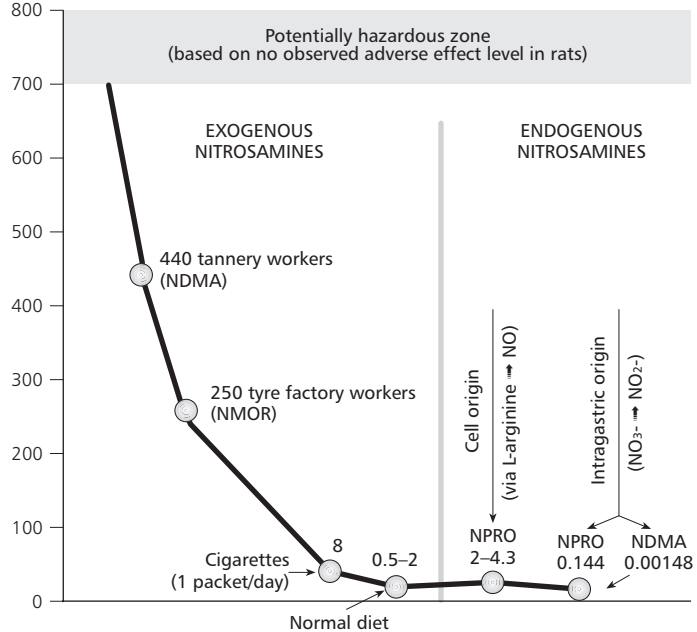
On analysis, however, this suspicion proves unfounded:

1 As figures 1 and 2 show, nitrites in the stomach do not come directly from alimentary nitrates; they come from plasmatic nitrates on which the salivary glands have had a very specific action.
2 The amount of nitrosamines thereby formed in the stomach through the metabolism of nitrates is very tiny.

Figure 3 compares levels between nitrosamines produced from endogenous synthesis, i.e. via the body's cells through the metabolic process involving L-arginine; those brought into the human body by direct exogenous intake via food; and those introduced exogenously by extra-dietary means (tobacco, tyre factories, tanneries), compared to levels of exposure in animals where no effects are observed.

Many foods, in particular beer and seasoned cooked meat, contain nitrosamines. Levels of intake by direct dietary means are several tens or hundreds of times higher than for nitrosamines

Figure 3 **Comparison of endogenous syntheses and exogenous intakes of nitrosamines**



NDMA: nitrosodimethylamine, NMOR: nitrosomorpholine, NPRO: nitrosoproline.

formed in the stomach through the metabolism of nitrates. If the precautionary principle were applied, it would involve the introduction of restrictive measures to prevent ingestion of nitrosamines of direct dietary origin. This would involve restrictive measures against a number of foods.

In fact, such restrictive measures are not necessary as these endogenous and exogenous nitrosamines remain confined to very tiny amounts compared to the theoretical toxicity threshold. The

level of direct dietary nitrosamines intake is several hundreds of times lower than the potential toxic level, and the amount of nitrosamines formed in the stomach during the metabolism of nitrates is several tens of thousands of times smaller than the potential toxic level.[7]

3 All experimental studies of a link between nitrates and cancer have proved negative in animals. No study conducted on rats or mice has succeeded in showing that even a considerable and prolonged intake of nitrates results in an increase in the incidence of cancers.
4 Since 1945, some twenty epidemiological studies have attempted to clarify the possible correlations in humans between nitrate intake and the incidence of stomach cancers. Only two out of twenty show a positive correlation. Seven out of twenty even point to a statistically significant negative correlation. Such a negative correlation should not surprise us at all; the favourable impact of vegetables on the incidence of cancer in general, is universally acknowledged[8]; and as we know, 80 per cent of ingested nitrates come from vegetables.

Therefore, as stated by the European Commission's Scientific Committee for Food in its 'Opinion on Nitrate and Nitrite' in 1995: "Epidemiological studies thus far have failed to provide evidence of a causal association between nitrate exposure and human cancer risk".[9]

In conclusion, the amounts of nitrosamines formed in the stomach during the metabolism of nitrates are actually very tiny; in no way are they capable of increasing the incidence of cancer pathology in humans.

Other grievances

Other, less serious charges have been levelled at dietary nitrates: an increase in the risk of foetal death, an increase in the risk of congenital malformation, a tendency towards enlargement of the thyroid gland, and an early onset of arterial hypertension.

There have been few studies on these issues, and some of them contain a number of methodological flaws. As a result, these ancillary grievances cannot be sustained legitimately as they lack a sound, documented scientific basis.

Conclusions

Whether they are considered major or secondary, no grievances against dietary nitrates in food can stand up to analysis. Scientific knowledge leads to the following conclusion: in the short, medium and long term, nitrates from food and from drinking water have no negative impact on human health.

Consequently, the directive drawn up in 1962 by the Committee of Experts on Food Additives of the WHO and the FAO on an acceptable daily intake (ADI) level for humans is now redundant; so is the 1980 directive from the European Community on the quality of water intended for human consumption (80/778/EEC), which set a limit of 50 mg/litre for NO_3 for water, above which it is no longer deemed fit for human consumption.[10]

The implementation of this latter directive on drinking water is particularly costly for the citizens of the European Community. Its repeal is necessary and inevitable.

Notes

1 Bartholomew (1984).
2 Steuhr and Marletta (1985).
3 Benjamin, et al. (1994).
4 Bartholomew (1984)
5 Walker (1990)
6 Knotek (1964), Simon (1966), Dupeyron (1970), L'Hirondel, J. (1971)
7 L'Hirondel and L'Hirondel (1996).
8 WHO (1990).
9 European Commission (1995)
10 European Commission (1980)

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