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Influence of Dietary Nitrate on Nitrite Level of Human Saliva

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Nitrates and nitrites are being a major problem in dietary toxicology because of a progressive increase in their general levels in the environment. High levels of nitrate are present in many vegetables such as radish, lettuce, red-beets, celery and carrot (Spiegelhalder 1976, Tannenbaum 1976, Tenovuo 1986). The general level of nitrate in vegetables has constantly increased, mainly due to the increased use of nitrate-based fertilizers but also due to its presence as a component of acid rain (Dickson 1980, Tenovuo 1986).

Nitrates and nitrites are also used to preserve ham and sausage. (Salam 1972, Sosis 1972).

The amount of nitrite in saliva depends directly on the amount of nitrate and nitrite ingested. Ingested nitrate and nitrite are absorbed by the upper gastrointestinal tract, concentrated from the plasma and excreted into the saliva by salivary glands. The presence of nitrate-reducing bacteria in the mouth caused nitrite to be formed, resulting in higher nitrite concentration.

In recent years it has been shown that the measurement of some drugs and agents in mixed saliva might be a reliable quide to blood or body levels of those agents.

In this present study the level of nitrite in mixed and parotid saliva in Eskisehir (Western part of middle Anatolia) and the correlation between sex, smoking and age was determined. The effects of drinking water and meat products on nitrite levels were determined.

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MATERIALS AND METHODS

One hundred healthy volunteers, composed of 50 males and 50 females, took part in this study. Their age and sex distribution are given in Table I. All volunteers were healthy as judged by normal routine clinical and biological examinations (blood counts, erythrocyte sedimentation rate, blood glucose and urea, serum transaminases and serum Na, K, Cl and Ca) given prior to entry into the study . No subjects were on medication including oral contraceptives. Care was taken to avoid testing on days of menstruation.

Mixed saliva samples were taken from 100 subjects 2 hrs after eating a standard breakfast. Parotid saliva samples were taken from 20 subjects selected from the main group.

After the first samples were taken 100 g of sausage and 100 g of ham was eaten by 15 volunteers. Second mixed saliva samples were taken 3 hrs post ingestion.

Subjects were asked to rinse their mouth with water before sampling. Mixed saliva samples were collected according to Kerr's method (Stephen 1976) with the head tipped forwards, and the mouth pointing downwards. Saliva accumulated behind the closed lips for 1 min. was spit into a test tube. Parotid saliva was collected by insertion of a polyethylene cannula into Stenson's duct. Lemon slices were used to stimulate saliva flow.

An additional experiment was carried out with 20 tap water samples that were taken from the region.

For nitrite analysis 0.2ml sample of saliva was taken, 1ml sulfanilic acid was added, the mixture was diluted with 20 ml distilled water and diazotized for 5 minutes. 1 ml naphthylamine hydrochloride and 1 ml sodium acetate (2N) were added, diluted to 50 ml with distilled water and measured with a spectrophotometry λ_{max}=520 nm within 10 to 30 minutes. Nitrite content of the samples were obtained from calibration graphs. A standard nitrite solution was measured before and after each experiment to confirm the reproducibility of the technique.

Statistical analysis was performed using students t test.

RESULTS AND DISCUSSION

The subjects were grouped acording to their sex, smoking and age (Table 1).

Table 1: Age and sex distribution of the subjects

Age groups	Sex		Total	
	Male	Female	No	%
10 - 20	18	12	30	30
21 - 30	16	17	33	33
31 - 40	8	10	18	18
41 - 50	4	6	10	10
51 - 60	4	5	9	9

The average nitrite levels in each group is given in (Table 2). While the differences were not significant between males and females, the levels were significantly higher in non smokers and the older group.

Table 2: Nitrite levels and statistical evaluation of the subjects grouped according to their sex, age and smoking.

	Nitrite levels mg/L	statistical values
Male	33 + 5	t= -0.83
Sex		
Female	28 + 4	p>0.05 ns
10-30	12 + 2	t= -5.73
Age		
31-60	41 + 5	p<0.001
+	21 + 4	t= -2.29
Smoking		
-	35 + 5	p<0.001

The nitrite levels were significantly increased by eating sausage and ham ($t=-8.78$, $p<0.001$).

Nitrite in parotid saliva varied between 0.17-2.2 mg./L.
Neither nitrite nor nitrate was detected in tap drinking water.

Our results showed that the nitrite levels of saliva are very high in our environment. The difference between the average values of male and female subjects was not significant as reported by others (Forman 1985, Tenovuo 1986, Broschinski 1987). It can be attributed to similar diets, drinking water and environment for both males and females.

The literature indicates that smoking has a consistent effect on salivary concentration of nitrite , with smokers always having lower levels than non-smokers. This obsevation has been explained by the presence of higher amounts of thiocyanate ions in the saliva of smokers where these ions are known to inhibit competetively the uptake of nitrites (Ruddell 1977). The salivary nitrite concentration of non-smokers was also found very high in our study.

The correlation of the age and salivary nitrite level is another subject investigated by many authors. We observed that increasing age is associated with an increase in the levels of nitrite as reported by Eisenbrand (1980), Forman (1985), Tenovuo (1986). Although Broshinski (1987) reported that there was not any relation between them, a statistically scientific difference was observed when the average results of 10-30 and 30-60 age groups were compared in our study. Forman explained these data as age-related changes in the active transport mechanism responsible for nitrate uptake into the salivary ducts and, for nitrite, in the density of nitrate-reducing bacteria in the oral cavity.

Several studies indicate that the level of salivary nitrite is strictly dependent on salivary nitrate with nitrate being partly converted into nitrite by oral microbial flora (Tesch 1976, Spiegelhalder 1976, Tannenbaum 1976). Salivary nitrite levels were higher in people with poor oral hygiene (Kühn 1974).

In agreement with the microbial requirement for salivary nitrite formation, Kühn(1974), and Tenovuo(1986) reported that pure ductal secretions contain nitrate, but not nitrite .

Fritsch et al.(1985) analysed the amount of nitrite and nitrate in the saliva, blood and bile of dogs. Thirty min. after iv. injection of sodium nitrate, nitrate levels increased in all samples. Nitrite levels in saliva increased with time, while it was relatively stable in blood and bile. The iv. injection of sodium nitrite increased the nitrite levels to a lesser degree.

In our study nitrite in pure ductal secretions of parotis gland was between 0.17 - 2.2 mg/L. This level suggests that nitrate derived nitrite was not the only source but some of the nitrite was dependent on the dietary intake of nitrite. It is reasonable to postulate that our subjects took a very large amount of nitrites in their diet continuously since it is known that nitrites are hardly secreted in the saliva after a large iv. injection of sodium nitrite (Fritsch 1985) .

It was reported that the main source of nitrates were vegetables and commercially available vegetable juices (Spiegelhalder 1976, Tannenbaum 1976, Ellen et al. 1982, Tenovuo 1982). Preusman (1988) noted that drinking water and meat products are also important sources besides vegetables. He analysed drinking water samples taken from different regions of different countries, and reported that some of them concentrated nitrites and nitrates in varying levels. Nitrates and nitrites were not detected in the drinking water samples of our region that were analysed. It was our pleasure to observe this result because detecting nitrates and nitrites in drinking water means direct contamination and polution.

We observed a statistically significant increase in the salivary nitrite levels in our ham and sausage administered group. It seems that some local producers use food additives higher than the amounts recommended in order to store them longer. Thus our data unequivocally established that the nitrite levels of saliva are very high in our region. Although the difference was not significant between males and females, the levels were significantly higher in non smokers and the older group. Whether people with high salivary levels of nitrites are prone to a higher risk of oral and gastric cancer and can be ascertained only by prospective analytical epidemiology.

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