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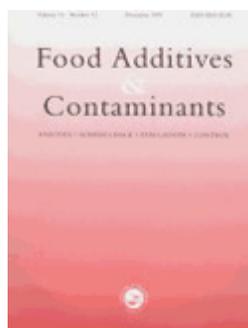
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Estimated dietary intake of nitrite and nitrate in Swedish children

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Abstract

This study examined the intake of nitrate and nitrite in Swedish children. Daily intake estimates were based on a nationwide food consumption survey (4-day food diary) and nitrite/nitrate content in various foodstuffs. The mean intake of nitrite from cured meat among 2259 children studied was 0.013, 0.010 and 0.007 mg kg⁻¹ body weight day⁻¹ in age groups 4, 8-9 and 11-12, respectively. Among these age groups, three individuals (0.1% of the studied children) exceeded the Acceptable Daily Intake (ADI) of 0.07 mg nitrite kg⁻¹ body weight day⁻¹. The mean intake of nitrate from vegetables, fruit, cured meat and water was 0.84, 0.68 and 0.45 mg kg⁻¹ body weight day⁻¹ for children aged 4, 8-9 and 11-12 years, respectively. No individual exceeded the ADI of 3.7 mg nitrate kg⁻¹ body weight day⁻¹. However, when the total nitrite intake was estimated, including an estimated 5% endogenous conversion of nitrate to nitrite, approximately 12% of the 4-year-old children exceeded the nitrite ADI. Thus, the intake of nitrite in Swedish children may be a concern for young age groups when endogenous nitrite conversion is included in the intake estimates.

Keywords: Nitrite, nitrate, dietary intake, Acceptable Daily Intake (ADI), cured meat, children

Introduction

Nitrite (NO_2^-) and nitrate (NO_3^-) are natural constituents of food but are also used as additives to improve food quality and protect against microbial contamination. However, there are health concerns due to their ability to form carcinogenic N-nitroso compounds (NNOC) and cause methaemoglobinaemia. Methaemoglobinaemia, manifested as cyanosis, has been associated with nitrate or nitrite intake since the 1940s (Comly 1945). Infants are known to be more susceptible to this adverse effect than older children and adults (Filer et al. 1970; WHO 2007). Nitrate can be partly converted to nitrite in the body and the toxicity of the two compounds is primarily ascribed to nitrite.

Nitrite in particular and sometimes nitrate are used as food additives in cured meat products due to their preservative, antibacterial, flavouring and colour fixative properties. The levels of residual nitrite and nitrate in these products are variable because they depend on the time and temperature used during processing and storing, the initial addition of nitrite and nitrate, the composition of the meat, pH, addition of antioxidant components such as ascorbate and the presence of micro-organisms (Gibson et al. 1984; Honikel 2008). Accordingly, there may be considerable depletion of nitrite in nitrite-cured meat products over time during storage (Merino, unpublished data; Pérez-Rodríguez et al. 1996). In addition to cured meat, nitrite can be detected in vegetables, especially after storage in conditions that favour bacterial growth. Some studies have also reported the presence of nitrite in other foodstuffs, e.g. cereals, cheese and bread, but the content of nitrite in these food groups is often lower than the limit of detection (LOD) (Knight et al. 1987; Dich et al. 1996; Petersen & Stoltze 1999; Jakszyn et al. 2006; Thomson et al. 2007; Menard et al. 2008; Griesenbeck et al. 2009).

Nitrate, which is ingested in much higher amounts than nitrite, is mainly found in vegetables, especially in green leafy vegetables such as spinach and lettuce (Petersen & Stoltze 1999; Tamme et al. 2006). Furthermore, nitrate is found in cured meat, either through

1
2 being used as a food additive or formed from conversion of initially added nitrite. Nitrate is
3
4 also present in limited amounts in other foodstuffs such as bread, cereals and dairy products
5
6 (Dich et al. 1996; Ysart et al. 1999; Menard et al. 2008; Griesenbeck et al. 2009). In addition,
7
8 nitrate is normally found in low concentrations in tap water, while private wells may contain
9
10 considerable amounts of nitrate. An acceptable daily intake (ADI) for nitrate of 3.7 mg kg^{-1}
11
12 body weight has been established by the World Health Organization (WHO) and the EU
13
14 Scientific Committee for Food (SCF). The ADI for nitrite is 0.07 mg kg^{-1} body weight
15
16 according to WHO (FAO/WHO 2003a) and 0.06 mg kg^{-1} body weight according to SCF (SCF
17
18 1995).
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22

23 The conversion of nitrate to nitrite in the body mainly takes place in the oral
24
25 cavity, and it has been estimated that 5-7% of nitrate ingested is converted to nitrite by
26
27 bacteria normally occurring in the mouth (Eisenbrand et al. 1980; FAO/WHO 2003b).
28
29 However, it has been reported that there are individuals with an even higher conversion rate,
30
31 up to 20% (EFSA 2008). In addition to the dietary intake, nitrate is formed endogenously,
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33 with nitric oxide as the precursor, at a daily rate of approximately 60 mg in adults, with
34
35 increasing amounts during inflammatory processes (WHO 2007).
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40 Most intake assessments made previously have considered adults. Therefore the
41
42 present study focused on the intake of nitrite and nitrate in Swedish children. The intake
43
44 estimates presented cover the consumption of cured meat, vegetables, fruit and drinking
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46 water.
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Materials and methods

Dietary survey

A nationwide dietary survey, including 56 Swedish counties, was performed in 2003 by the Swedish National Food Administration (NFA). The participants were 590 children aged 4 years, 713 school children aged 8-9 years and 956 school children aged 11-12 years, resulting in a total of 2259 children. The children aged 4 years were randomly selected from a register of families with children of this age, while the school children were randomly selected as whole school classes. To account for possible seasonal differences, the survey was conducted in two steps, one in spring and one in autumn. All food and drink ingested were recorded by the children or their parents in a food diary for four consecutive days. A picture book was used to help subjects estimate portion sizes. Prior to the study, all participants attended a meeting where they were informed about the procedures (Enghardt Barbieri et al. 2006).

Nitrite and nitrate levels in food and drinking water

Cured meat products

In 2008, the Swedish NFA analysed the contents of nitrite and nitrate in a total of 40 samples of cured meat products, which were randomly collected from different supermarkets in Uppsala, Sweden. Each analysed sample was pooled from two separate packages of the same product. The meat products were selected from a list of products preferentially consumed by children in Sweden.

All samples were analysed for nitrite and nitrate using a spectrophotometric method based on reduction of nitrate with zinc powder developed at the Swedish NFA (Merino 2009). The initial nitrite concentration and total nitrite after reduction are determined by the very sensitive and widely used diazotization-coupling Griess reaction. The results of a single-laboratory validation applied to five different matrices, including vegetables, meat

1
2 products and water, have shown that the method meets the international criteria for precision
3
4 and recovery (Merino 2009).
5

6
7 In addition, all samples were analysed in parallel by an HPLC ion
8
9 chromatography method (CEN 2005). For the analysis of meat products with low
10
11 concentrations of nitrite and nitrate, a comparison between the two methods showed that the
12
13 HPLC ion chromatography method had lower sensitivity and gave more biased results than
14
15 the spectrophotometric method. Hence, the nitrite and nitrate concentrations analysed by the
16
17 spectrophotometric method were used to estimate intake (Merino 2009).
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21 The content of cured meat in a variety of common dishes was calculated and
22
23 these data were included in the calculations (Table 1). When the meat product was
24
25 unspecified, e.g. 'sausage', the mean value of all sausages sampled was used. For products
26
27 that are normally boiled, fried or heated prior to consumption, possible losses of nitrite and
28
29 nitrate due to cooking were not included.
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32 33 *Fruit and vegetables*

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35 Data on the content of nitrate in common vegetables on the Swedish market were obtained
36
37 from HPLC analyses carried out at the Swedish NFA in 1995 (Merino et al. 1997; Merino et
38
39 al. 2000; CEN 1998). Since these analyses did not cover all vegetables, the database was
40
41 complemented with a list provided by the European Food Safety Authority (EFSA) containing
42
43 data on the nitrate content in various vegetables in 20 EU member states and Norway,
44
45 analysed between 2000 and 2007 (EFSA 2008).
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51 The definition of vegetables used for the intake calculations included raw and
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53 processed vegetables (e.g. mashed and cooked) but not vegetable dishes (e.g. gratins and
54
55 stews). The definition of potatoes included processed potatoes, as well as dishes with potatoes
56
57 as a main ingredient. The changes in levels of nitrate due to cooking were not included.
58
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60
There was no information on the content of nitrate in fruit in either the Swedish
NFA or EFSA compilation, so it was estimated at 10 mg kg⁻¹, based on analytical data

1
2 presented in an article by Sušin et al. (2006). Fruit was defined as a group that included fresh,
3
4 dried, frozen and preserved fruit. Juices and dishes that contain fruit were not included.
5

6 The contents of nitrite in fruit and vegetables reported in the literature show
7
8 great variability and are often reported to be lower than the LOD. Due to these inconclusive
9
10 data, the nitrite contribution from fruit and vegetables was not considered in the present intake
11
12 estimation.
13
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15 16 17 *Drinking water*

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19 Data on nitrate content in drinking water were obtained from the Swedish Water and
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21 Wastewater Association and contained analyses from 238 local water plants. No sample
22
23 exceeded the highest permitted value of 50 mg litre⁻¹ (Svensson et al. 2009). Among the
24
25 samples analysed, 46% had concentrations lower than the LOD (varying from 0.44 to 4.4 mg
26
27 nitrate ion/L) (VAV 1996). Samples with nitrate contents below the LOD were allocated a
28
29 value of half the LOD. In the calculations, a mean nitrate concentration of 3.2 mg litre⁻¹ was
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31 used and the daily intake of drinking water was estimated to be 1 litre in 4-year-old children
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33 and 1.5 litre in the older age groups.
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38 The concentration of nitrite in drinking water is concluded to be very low, and
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40 the nitrite exposure from water consumption was therefore excluded from the estimated
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42 dietary intake of nitrite.
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45 46 47 *Dietary intake assessment*

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49 The intake calculations were based on the consumption data from the food diaries and
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51 analytical data on nitrite and nitrate contents in different foodstuffs as presented above. The
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53 intake was calculated as the average daily intake for each respondent and expressed in mg
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55 day⁻¹ and mg kg⁻¹ body weight day⁻¹. The latter was based on individual body weight, which
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57 meant that children who had not reported their body weight were excluded from these
58
59 calculations, leaving 527, 644 and 912 children in age groups 4, 8-9 and 11-12 years,
60

1
2 respectively. Also, to investigate which category of food stuffs the children predominately
3
4 consumed, the consumption was calculated using the total weight of the ingested foods among
5
6 all children.
7

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9 To account for the total intake of nitrite from the diet, the estimated conversion
10
11 of dietary nitrate to nitrite was added to the direct intake of nitrite from cured meat products.
12
13 In the calculations, a conversion factor of nitrate to nitrite of 5% was used and adjustment was
14
15 made for the difference in molecular weight between nitrite and nitrate.
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19 There are two ADIs for nitrite, i.e. 0.06 and 0.07 mg kg⁻¹ body weight as
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21 established by SCF (1995) and WHO (2008), respectively. The toxicological data used to
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23 reach the early and lower ADI was later by WHO considered irrelevant (FAO/WHO 2003a).
24
25 In the present calculations, the newer ADI set by WHO was adopted. All values reported in
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27 the following sections are expressed as nitrate and nitrite ions.
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Results

Intake of nitrite from cured meat

A summary of the concentrations of nitrite and nitrate in the meat products most frequently consumed by Swedish children is presented in Table 1. Using these data, the mean intake of nitrite from cured meat products was estimated at 0.013, 0.010 and 0.007 mg kg⁻¹ body weight day⁻¹ in children aged 4, 8-9 and 11-12 years, respectively (Table 2). There was no considerable difference in nitrite intake between boys and girls. One child in each age group exceeded the ADI. In two of these three children, the major source of nitrite intake was chicken sausage, i.e. the product with the highest nitrite content, which was consumed at a rate of 35 and 90 g per day in the younger and older child, respectively. In the third child who exceeded the ADI, liver pâté was the main contributor to the high nitrite intake. All three children had a lower weight than average for their respective age. The mean body weights were 18.2 (range 11-29), 30.6 (range 18-59) and 42.4 (range 25-77) kg in age group 4, 8-9 and 11-12, respectively.

It has been suggested that nitrite intake estimations should include a calculation where children who did not consume cured meats are excluded (Pennington 1998).

Accordingly, separate calculations were made that included all participants, as well as only children who reported an intake of cured meat. However, the difference in nitrite intake between the calculations used was small. Therefore, only the calculations including all children are given in the present study.

The intake of cured meat products per kilogram body weight decreased with increasing age. The food group that included various sausages was the most consumed type of cured meat, and contributed approximately 77% of the total intake of nitrite from this food group. Liver pâté accounted for approximately 11% of the total nitrite intake, while ham and poultry products contributed approximately 5% each. Poultry products had the highest nitrite

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2 concentration, thus accounting for a relatively significant part of the total nitrite intake even
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4 though the consumption of such products was very small. In contrast, the consumption of ham
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6 was quite high, but the low concentration of nitrite in ham resulted in a low nitrite intake from
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8 this specific foodstuff.
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10 11 *Intake of nitrate from vegetables, fruit, cured meat and drinking water*

12
13 The contents of nitrate in Swedish vegetables and cured meat are presented in Table 1. Based
14
15 on these data, and previous presumptions on fruit and water levels, the total estimated intake
16
17 of nitrate from vegetables, fruit, cured meat and water is presented in Table 3. The daily
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19 intake per kilogram body weight decreased with increasing age. No individual exceeded the
20
21 ADI of 3.7 mg kg⁻¹ body weight day⁻¹. Drinking water contributed 21-26% of the total nitrate
22
23 intake. Of the nitrate intake from food excluding water, approximately 98% originated from
24
25 fruit and vegetables, while the remaining 2% came from cured meat products.
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31 Approximately 59% of the total nitrate intake from fruit and vegetables came
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33 from vegetables (excluding potatoes), 34% from potatoes and 7% from fruit. The total
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35 consumption of this food group was quite consistent among the three age groups, although the
36
37 intake of different items within the category of fruit and vegetables varied. Younger children
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39 consumed more fruit but less potatoes than older children, whereas the intake of vegetables
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41 was fairly constant throughout the ages.
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46 47 *Total intake of nitrite*

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49 Total nitrite intake, including 5% conversion of dietary nitrate from vegetables, fruit, water
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51 and cured meat and direct nitrite intake from cured meat, is presented in Table 4. In addition,
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53 a distribution diagram of the total nitrite intake among children aged 4 is presented in Figure
54
55 1. The results show that approximately 12 , 3 and 1% of the children in age groups 4, 8-9 and
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57 11-12, respectively, exceeded the ADI. The estimated contribution from the conversion of
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59 dietary nitrate was approximately 70% of the total nitrite intake.
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Discussion

A summary of previous intake estimations in different countries is presented in Table 5. However, the results of these studies are not easily compared due to differences in study design, food groups studied and selection of participants. For example, in the present study, analyses were performed on a small number of samples from each category of cured meat. Consequently, a single sample may have influenced the results. In any case, the estimated intake of nitrite from cured meat products was slightly higher in the present study than in a Danish study that included children of the same age groups (Leth et al. 2008). Conversely, the intake was three to five times lower than that reported in Estonian and Finnish children of comparable ages (Laitinen et al. 1993; Reinik et al. 2005).

The total daily intake of nitrate from food and water in the present study was half to one-third of the intake reported in Estonian and Finnish children (Laitinen et al. 1993; Tamme et al. 2006). The lower intake in the present study was probably due to the relatively low content of nitrate in Swedish vegetables, especially potatoes and tomatoes, in comparison with the levels reported in other countries. In addition, the reported intake of vegetables (excluding fruit and potato) was low in the present study, in comparison with the mean vegetable consumption in European 11-year-olds (Yngve et al. 2005).

When evaluating the risk of nitrite exposure, the issue is how to estimate intake correctly. Because all humans are concomitantly exposed to nitrate and nitrite, it seems logical that the ADI for nitrite should include both direct exposure to nitrite and endogenous conversion from dietary nitrate. The mean intake of nitrite from cured meat products alone ranged between 10 and 19% of the ADI for the age groups studied here, and only 3 of 2259 children exceeded the ADI for nitrite. The average intake of nitrate from food and water was 12-23% of the ADI and no individual exceeded the ADI for nitrate. However, when 5% endogenous conversion of dietary nitrate to nitrite was added to the nitrite intake from cured meat, it was found that 12% of the children in the youngest age group studied exceeded the

1
2 ADI for nitrite. It has been suggested that some individuals may convert up to 20% of dietary
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4 nitrate to nitrite (EFSA 2008), and if such a high conversion factor were used the majority of
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6 Swedish children would hypothetically exceed the ADI.
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10 In this study, the nitrite intake from cured meat alone was low and of no health
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12 concern in relation to the ADI. In fact, the nitrite intake from cured meat was overshadowed
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14 by the endogenous conversion of dietary nitrate to nitrite. However, when considering the
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16 nitrite intake from cured meat alone, it was shown that the ADI for nitrite could be exceeded
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18 if the average 4-year-old child, on a daily basis, consumed more than 35 g of chicken sausage,
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20 i.e. the meat product with the highest nitrite content. However, it is unlikely that the same
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22 individual would continually consume a high amount of the same meat product for a long
23
24 period of time.
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28 The observation in the present study of a high nitrite content in chicken sausage
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30 and smoked turkey prompted further analyses of cured poultry products. Analyses at the
31
32 Swedish NFA showed a higher average amount of nitrite in sausages containing poultry than
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34 in sausages made from red meat (unpublished observation). A higher level of residual nitrite
35
36 in products containing poultry has also been reported in a previous study (Cassens 1997). This
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38 may be an important finding to consider in future studies of nitrite intake because the use of
39
40 poultry meat may increase in various food categories.
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44
45 Another way to better evaluate the intake of nitrite and nitrate in future studies
46
47 would be to include the nitrite and nitrate from other food groups such as dairy products,
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49 cereals and bread, as well as nitrite in vegetables and water. Some studies have reported a
50
51 significant contribution from these food groups to the total nitrate and nitrite intake (Laitinen
52
53 et al. 1993; MAFF 1998; Jakszyn et al. 2006; Thomson et al. 2007).
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55

56
57 Even though nitrite derived from nitrate in various vegetables substantially
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59 increased the nitrite intake in the children studied here, it is known that vegetables are
60
beneficial to health for various reasons. In addition, vegetables contain compounds that inhibit

1
2 formation of NNOC (Dietrich et al. 2005). Therefore, it does not seem reasonable to limit the
3
4 intake of vegetables in general in order to lower the nitrate exposure. On the other hand, since
5
6 infants are more susceptible to methaemoglobinaemia, the Swedish NFA recommend
7
8 avoiding large quantities of spinach, beetroot, nettle, chard and celery in food given to
9
10 children during their first year. The ADI values on nitrite and nitrate are based on adverse
11
12 toxic effects in experimental animals and do not take into account possible beneficial health
13
14 effects of these compounds or transformation, e.g. blood pressure lowering, antibacterial and
15
16 anti platelet aggregation effects of nitric oxide (Lamas et al. 1998; Lundberg et al. 2008;
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18 Webb et al. 2008), However, there is no conclusive evidence to justify intakes exceeding the
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Conclusions

The intake of nitrite by Swedish children from cured meat products was generally low and the ADI was only exceeded in three of the 2259 children studied. This implies that the intake of nitrite from cured meat alone is tolerable. Furthermore, the intake of nitrate from food and drinking water combined was well below the safety margins covered by the ADI for nitrate. However, when an estimated 5% conversion of nitrate to nitrite was included, nitrite intake from cured meat contributed to only 30% of the total intake. It is noteworthy that 12% of children in the youngest age group (4 years) exceeded the ADI when the total exposure to nitrite was considered. Consequently, the intake of nitrite in Swedish children may be of concern for young age groups when endogenous nitrite conversion is included in the intake estimates.

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Table 1. Nitrite and nitrate concentrations (mg kg^{-1}) in a selection of Swedish vegetables and cured meat products.

Sample	Number of samples	NO_2^- Mean	NO_2^- Range	NO_3^- Mean	NO_3^- Range
Meat products					
Bacon	4	2.2	0.4-6.0	8.7	7.4-10.7
Sausage	10	7.7	0.5-16.5	15.3	6.1-44.9
Salami and sandwich sausage	6	0.7	0.3-1.2	6.2	0.0-17.7
Chicken sausage	1	34.1	34.1	12.6	12.6
Turkey (smoked)	1	23.5	23.5	6.9	6.9
Ham (cooked)	1	0.6	0.6	4.0	4.0
Ham (smoked)	2	1.1	0.8-1.3	3.5	3.2-3.8
Black pudding	1	0.6	0.6	6.9	6.9
Liver pâté	1	20.5	20.5	18.4	18.4
Vegetables					
Butterhead lettuce	9			1724	59-3332
Spinach (frozen, whole leaves, blanched)	3			1010	782-1161
Chinese cabbage	9			899	281-1456
Iceberg lettuce	10			864	98-2102
Leek	10			535	16-1000
Spinach (frozen, chopped, blanched)	6			486	268-812
Beetroot	9			486	102-1418
White cabbage	10			379	43-681
Broccoli	8			301	30-832
Cucumber	10			179	8-350
Carrot	10			165	0-280
Cauliflower	10			139	15-299
Potato	10			47	0-137
Tomato	10			4	0-16

Table 2. Nitrite intake from cured meat products by children in the three age groups studied

Age (years)	mg day ⁻¹		mg kg ⁻¹ body weight day ⁻¹	
	Mean	95 th percentile	Mean	95 th percentile
4	0.23	0.58	0.013	0.034
8-9	0.29	0.84	0.010	0.028
11-12	0.28	0.75	0.007	0.019

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Table 3. Total intake of nitrate from vegetables, fruit, cured meat and water by children in the three age groups studied

Age (years)	mg day ⁻¹				mg kg ⁻¹ body weight day ⁻¹			
	Mean		95 th percentile		Mean		95 th percentile	
	Incl. water	Excl. water	Incl. water	Excl. water	Incl. Water	Excl. water	Incl. water	Excl. water
4	14.9	11.7	28.4	25.2	0.84	0.66	1.62	1.42
8-9	20.3	15.5	38.1	33.3	0.68	0.51	1.24	1.06
11-12	18.4	13.6	36.9	32.1	0.45	0.33	0.92	0.78

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Table 4. Total exposure to nitrite of children in the three age groups studied, including the intake of nitrite from cured meat products and 5% conversion of nitrate to nitrite from other foodstuffs

Age (years)	mg day ⁻¹			mg kg ⁻¹ body weight day ⁻¹			Exceeding ADI
	Mean	95th percentile	Maximum	Mean	95th percentile	Maximum	
4	0.78	1.46	2.37	0.044	0.083	0.148	12%
8-9	1.05	1.88	3.78	0.035	0.061	0.145	3%
11-12	0.96	1.79	5.02	0.023	0.045	0.140	1%

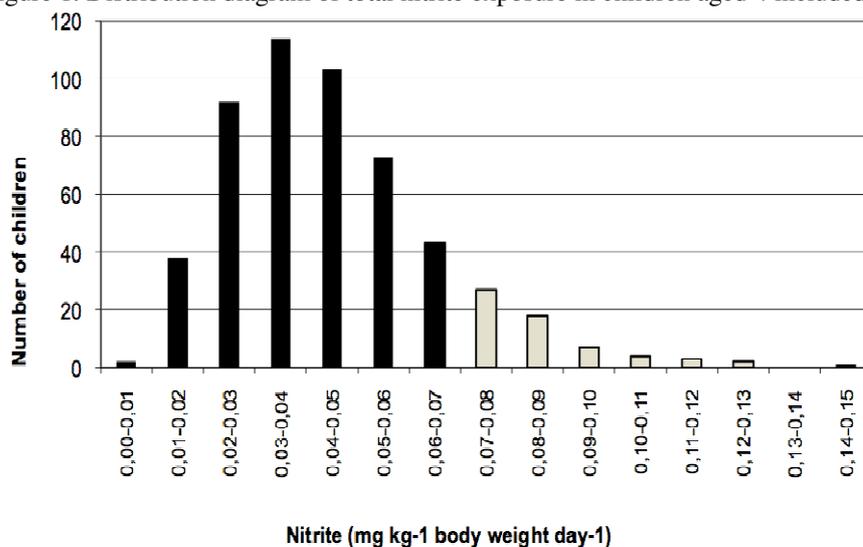
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Table 5. Estimated daily intake (mg kg^{-1} body weight day^{-1}) of nitrite and nitrate in children and adults in different countries

Study	Country	Sources of intake	Age (years)		Nitrite mg kg^{-1} bw day^{-1}	Nitrate mg kg^{-1} bw day^{-1}
Leth et al. 2008	Denmark	Meat products	4-5	Men	0.011	
				Women	0.009	
			6-14	Men	0.006	
				Women	0.005	
			15-75	Men	0.005	
				Women	0.002	
Reinik et al. 2005	Estonia	Meat products	1-16		0.028 ^{II}	
Jakszyn et al. 2006	Spain	Various foods	Adults		0.014 ^I	
Present study 2010	Sweden	Meat products (nitrite)	4		0.013	0.8
			8-9		0.010	0.7
		Various foods & water (nitrate)	11-12		0.007	0.4
Laitinen et al. 1993	Finland	Various foods & water	9-24		0.028 ^{III}	1.1 ^{III}
Dich et al. 1996	Finland	Various foods	Adults	Men	0.093 ^I	1.1 ^I
				Women	0.057 ^I	1.1 ^I
Thomson et al. 2007	New Zealand	Meat products & vegetables	Adults		0.009 ^{IV}	0.5 ^{IV}
MAFF 1998	UK	Various foods & water	Adults		0.020 ^I	1.3 ^I
Tamme et al. 2006	Estonia	Vegetables	Population			0.8 ^I
			1-3			1.7 ^V
			4-6			1.5 ^{VI}
Fernlöf & Darnerud 1996	Sweden	Vegetables	Adults			0.3 ^I
Petersen & Stoltze 1999	Denmark	Vegetables	Adults			0.6 ^I

^I Applying an average body weight of 70 kg. ^{II} Applying an average body weight of 30 kg. ^{III} Applying an average body weight of 51 kg. ^{IV} Applying an average body weight of 74.8 kg. ^V Applying an average body weight of 15 kg. ^{VI} Applying an average body weight of 20 kg.

Figure 1. Distribution diagram of total nitrite exposure in children aged 4 included in the present study (n=527)



Grey columns represent children with nitrite intake exceeding the ADI.

The upper limits of the units for each bar represent values “less (<) than”, e.g. 0.00-<0.01.