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Vitamin requirements and vitamin enrichment of foods

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The question of whether or not vitamins should be added to food is reviewed. Special emphasis is given to the problem of unbalanced diets and to the possible preventive action of antioxidative vitamins on cardiovascular disease and cancer. Some general aspects of vitamin addition, of vitamin analysis, and of legislation for vitamin enrichment in Switzerland and in Europe are presented.

DOES OUR FOOD PROVIDE US WITH SUFFICIENT VITAMINS?

In our body, every vitamin is responsible for several functions rather than just for one only. This mainly applies to B group vitamins which, as co-enzymes, regulate important enzymes in all metabolic pathways. Thus, for example, biotin-dependent enzymes are essential for the synthesis for glucose and fat in the liver, for fatty acid synthesis in adipose tissue, or for glutamate to be synthesised in the central nervous system. Furthermore, the vitamins B_1, B_2, niacin and pantothenic acid are important for glucose, fatty acid and protein metabolism. Such examples could be multiplied at libitum. Even a slight 'marginal' deficiency of one of these vitamins can therefore lead to extensive metabolic damage which in turn is responsible for functional disorders in the body (Fig. 1). If, for example there is a marginal deficiency of even one of the B vitamins quoted, this can result in sleep interference, loss of appetite and body weight, increasing irritability, lack of concentration, greater tiredness and reduced immunocompetence (Heseker *et al.*, 1990). If the vitamin deficiency is not compensated for, these symptoms rapidly turn into the clinical deficiency symptoms connected with the individual vitamins proper. These extreme vitamin deficiencies such as scurvy, beriberi, rickets, etc. have ceased to play a major part in the industrialised countries, so that more attention needs to be given to the problem of marginal deficiencies (for a review see Brubacher (1989)). In this context, and with a view to keeping the population healthy even in the long term, the well-known question has therefore to be asked again whether our daily food provides enough of all the vitamins to secure our health or whether special measures have to be taken such as vitamin enrichment of various food products.

It is generally accepted that a so-called 'balanced food' is a mandatory condition for the supply of adequate levels of all vitamins. However, for example, people who omit meat, eggs, milk and milk products from their diet have an increased risk of vitamin B_{12} and folate deficiency. Lack of fruits and vegetables in the diet will lead to a deficit in vitamin C and β-carotene. One of the problems of our society today is that many people, for one reason or another, have a one-sided diet. Many studies have been carried out showing that special attention must be paid to the vitamin intake of expectant mothers; seniors; people on any kind of diet, e.g. slimmers, vegetarians; junk-food eaters, teenagers; sportsmen and other groups with a higher energy requirement; people with simple illnesses such as diarrhoea or other digestive problems. Such studies on risk groups enable the health authorities to draw the population's attention to an adequate vitamin intake and to issue nutritional guidelines. Apart from general menu changes (e.g. more vegetables, wholemeal bread, etc.), improvements can also be achieved by enriching those food products with vitamins which are important for the various risk groups.

It should be noted that the composition of our food is not the only criterion for an adequate vitamin supply. As will be shown during this symposium, vitamins may be lost during cooking due to water extrusion or may be destroyed due to extensive heat treatment. Furthermore, the bioavailability of certain vitamins can differ greatly according to the foodstuff. This is especially true for folate for which the bioavailability may be less than 50%. Also of special importance is the fact

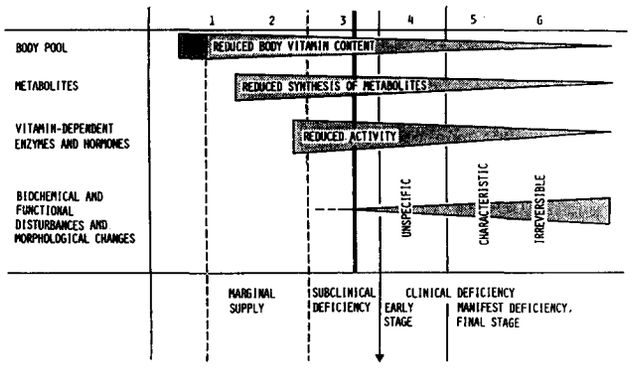

Fig. 1. Stages of vitamin deficiency. From Brubacher (1989), reproduced by permission of Hans Huber Publishers.

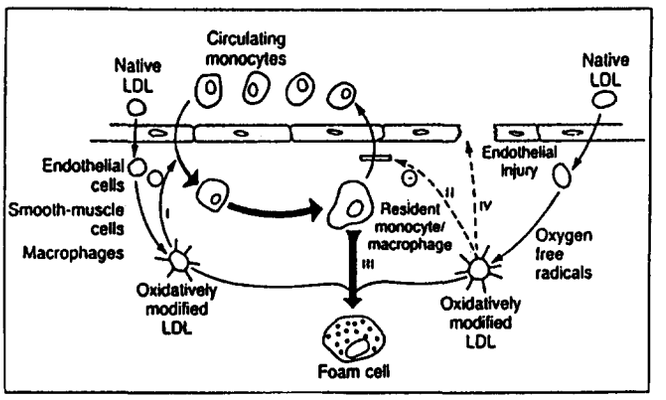

Fig. 2. Possible mechanisms for the contribution of oxidised LDL to atherogenesis. From Steinberg *et al.* (1989), reproduced by permission of *New England Journal of Medicine.*

that fat-soluble vitamins are only absorbed in significant amounts if the food taken up also contains lipids. A further aspect to be considered in connection with marginal deficiencies is the fact that our storage capacity varies for the different vitamins. In the case of vitamin B_1 our retention capacity lasts only for about 2 weeks whereas enough B_{12} can be stored to last for several years; for most of the other vitamins the storage capacity lasts several months. Since B_1 is easily extruded during cooking and heat treatment, marginal deficiencies of this vitamin are a special risk.

In order to assess vitamin supply and vitamin enrichment of food, a further aspect that has come up in recent years should be mentioned in detail. It concerns the possible preventive effect of antioxidative vitamins on cardiovascular disease and cancer. In the case of cardiovascular disease it is known that the pathogenesis is multi-factorial with a number of risk factors such as high blood cholesterol, high blood pressure, smoking, excess body weight, glucose intolerance and lack of exercise. Many of these risk factors overlap and there is a special high risk when several factors co-exist in a given individual. As for cholesterol, it has been shown that a high concentration of low-density lipoproteins (LDL), the main cholesterol carrier in the blood, can be correlated with maximum risk. High-density lipoprotein (HDL), on the other hand, which occurs in smaller quantities, acts as a protective factor against cardiovascular disease, because it is responsible for the removal of cholesterol from the tissues. As summarised by Steinberg *et al.* (1989) (see Fig. 2), LDL having undergone oxidative alterations can have various effects on atherogenesis. Circulating monocytes are chemotactically attracted by LDL which has suffered oxidative changes (I). The latter causes the monocytes or microphages to become immobile (II). The resident monocytes/macrophages which have become incapable of migration preferentially take up oxidised LDL and develop into foam cells which accumulate on the arterial walls forming a deposit (III). LDL which has undergone oxidative changes is cytotoxic and damages the endothelial cells (IV). It is thought today that native LDL only turns atherogenic if unsaturated fatty acids are oxidised by oxygen radicals. The intake of LDL by macrophages is controlled via a 'down regulation'; but this control mechanisms appears to fail if oxidative LDL are absorbed, so that these foam cells form.

What are oxygen radicals? They are reactive oxygen species which form as an intermediate product when molecular oxygen is reduced to water and which contain an unpaired electron in their outer orbital (Table 1). Thus, in the mitochondria, about 95–99% of oxygen is reduced in one step, i.e. with four electrons at the same time. However, about 1–5% of the oxygen we need is transformed in steps and forms radicals. Two of the oxygen species which occur in this step-by-step reduction, i.e. the superoxide radical and the hydroxyl radical, are especially reactive. The hydroxyl radical reacts with nearly all the molecules which are to be found in its surroundings, without being selective, and is therefore extremely toxic. In addition, there is an activated form of oxygen, the so-called singlet oxygen, which is also highly reactive. All these reactive oxygen species form in the normal metabolism, but they form in larger quantities during special stress, such as radiation treatment, ozone-rich air and maximum physical efforts (Seelert, 1991). The cumulative effect of such radical formations can lead to oxidations of cell membranes and organelles which can be destroyed during the process. Our body has special mechanisms designed to break down these radicals. Superoxide dismutase is one of these and is responsible for the destruction of the superoxide radical as well as a number of peroxidases which are responsible for the breakdown of the superoxide anion. Today, it is being assumed that also the antioxidant vitamins (β-carotene, vitamins C and E) play a decisive part in the process of detoxification of the radicals, especially of the hydroxyl radical and of

Table 1. Reactive oxygen compounds

Reduction of oxygen	
$O_2 + e^- + H^+ \longrightarrow O_2H\cdot$	Superoxyde radical
$O_2H\cdot + e^- + H^+ \longrightarrow H_2O_2$	Hydrogen peroxide
$H_2O_2 + e^- + H^+ \longrightarrow H_2O + OH\cdot$	Hydroxyl radical
$OH\cdot + e^- + H^+ \longrightarrow H_2O$	
Excited state of oxygen	
$O_2 \xrightarrow[\text{reactions}]{\text{Biological}} {}^1O_2$	Singlet oxygen

singlet oxygen (Di Mascio *et al.*, 1989). A recently published study by Riemersma *et al.* (1991) showed, on the basis of a sample of men aged 34–54 years suffering from angina pectoris who were compared with 394 controls, that there is a significant inverse correlation between the symptoms and the plasma vitamin C, E and β-carotene. More extensive epidemiological evidence stems from the Monica study (Gey *et al.* 1991) in which mortality rates as a result of coronary heart disease of 16 male European population groups were correlated with the standardised vitamin E serum concentrations, the cholesterol levels and the diastolic blood pressure. In 12 out of 16 populations with normal cholesterol μmol and blood pressure, there was no correlation between these two risk factors and the mortality rate due to coronary disease; both these cases showed a marked inverse correlation between the death rate and the plasma vitamin E content. When all 16 population groups were used, the mortality rate due to coronary heart disease was found to correlate best with the vitamin E status, once the statistical step-by-step regression was analysed.

A series of intervention studies is being carried out just now, but the results are not yet known. The only one which could be mentioned is the β-carotene study which was carried out in parallel to the USA doctors' aspirin study (Hennekens & Eberlein 1985). Three hundred and thirty-three doctors suffering from stenocardia symptoms or who had undergone a coronary bypass operation were given 50 mg β-carotene every other day. This double blind study which has been going on for 8 years showed that 44% of testees showed an improvement in their state of health. The protective effect of β-carotene was already significant after 2 years and further improved later on. Although this study is still insufficient to enable us to draw general conclusions, it nevertheless points to the potential of β-carotene in the treatment of coronary heart disease. It is being pointed out that an increase in the official recommended daily allowances (RDA) should be considered, whereby it is recommended to take 15 mg carotenoids, 36–60 mg E and 100–150 mg C (Esterbauer *et al.*, 1990).

In the case of cancer, a large number of prospective studies was published in connection with cancer mortality, all of which point to a potential protective effect of antioxidative vitamins for individual types of cancer (Gaby & Singh, 1991). Thus, in the Basel study (Stähelin, 1991) which has involved testing 2974 male volunteers since 1971, lung cancer, gastrointestinal cancer and stomach cancer showed a statistically significant inverse correlation with serum β-carotene; furthermore, serum levels of vitamin C and vitamin A were shown to be inversely correlated with stomach cancer. Since vitamin A is not an antioxidant vitamin, these results with cancer mortality indicate that other mechanisms exist as well. It is also quite likely that at least part of the β-carotene action may be mediated by its partial hydrolysis to vitamin A during digestion. Nevertheless, on the basis of the Basel study 'safe levels' of the antioxidant vitamins can be estimated which are similar to those postulated for the prevention of cardiovascular diseases (Stähelin *et al.*, 1989). More research is definitely necessary and we have to wait especially for the results of intervention studies being carried out at present.

The official RDA values are based on our knowledge of the minimal requirement for maximal protection for each vitamin dependent function (functional requirement). In order to compensate for individual deviations, a safety margin is included in each recommendation. Since our knowledge on the requirement of each vitamin to prevent marginal deficiencies is incomplete, these RDA values cannot be exactly determined. The RDA values therefore vary from country to country. In Table 2, the median and the maximum values recommended by 30 national and international bodies are given. Not all of these 30 countries or organizations have given recommendations for all vitamins. Therefore the number of countries that have contributed to these figures varies from six to 30. With the exception of vitamin K and folate, the ratios between the highest and lowest values lie between four and 10 showing certain conformity of the various recommendations. It is interesting to note that the 1980 US recommendations are almost identical to the median values.

If the results of the research on the preventive action of antioxidant vitamins on cardiovascular disease and cancer prove to be correct, then a new dimension has to be considered for an important revision of the RDA values. The question will then have to be answered as to whether it will still be possible to provide the higher amounts of vitamins C, E and β-carotene by our regular food. In my opinion, food fortified with vitamins or vitamin supplements will then be necessary to fulfil our needs.

Therefore, it can be concluded that, nowadays, food fortified with vitamins is important for the many risk groups we know today. Ongoing research on cardiovas-

Table 2. Median (minimum–maximum) values for recommended dietary allowances proposed by six to 30 national or international organizations (1 January 86)[a]

	Median[b]	Minimum–maximum[b]	
Vitamin A	800	360–1650	μg retinolequivalent
Vitamin D	5	2·5–20	μg cholecalciferol
Vitamin E	10	5–50	mg α-tocopherol equivalent
Vitamin K	140	30–3000	μg phyllochinon
Vitamin C	60	15–100	mg ascorbic acid
Vitamin B_1	1·2	0·5–2·2	mg thiamin chloride hydrochloride
Vitamin B_2	1·6	0·8–3·2	mg riboflavin
Vitamin PP	18	5·5–22·5	mg niacin
Vitamin B_6	2	1–4	mg pyridoxin
Folacin	210	100–2000	μg folic acid
Vitamin B_{12}	2	1–5	μg cyanocobalamin
Biotin	200	100–400	μg biotin
Pantothenic acid	7	3–14	mg panthotenic acid

[a] From Brubacher (1989), reproduced by permission of Hans Huber Publishers.
[b] The figures correspond to the recommendations or estimated requirements of moderately active male adults.

Table 3. Food enrichment: definitions

- *Restoration*:
  Addition of nutrients to a food to compensate for losses during processing and storage.
- *Fortification or enrichment*:
  (a) Addition of nutrients to a food above the level normally found in that food.
  (b) Addition to a food of nutrients which are not contained naturally in that food.

cular disease and cancer will show whether fortification of food with antioxidative vitamins will be an even more important issue in the future.

FOOD ENRICHMENT WITH VITAMINS

As shown in Table 3, we have to differentiate between restoration on the one hand and fortification or enrichment on the other. A typical example for restoration with vitamins is fine flour that has lost part of its vitamin B_1, B_2 and niacin content during production. These vitamins are added to the extent that the original content is restored.

More often food products are fortified or enriched by vitamins. In the case of milk, in many countries vitamins A and D are added above the level normally found. This addition also compensates for losses during the production, especially with respect to heating processes designed to give the milk a longer shelf-life. Vitamin C is quite often added to fruit juices above the natural level of this vitamin. Furthermore, food products can be fortified by additional vitamins originally not present in their components. Thus, in Switzerland, nearly all vitamins are added to food products such as health-food products, special nutrition for sporting activities, various slimming diets, and milk modifiers (Table 4).

A large number of technical problems is involved when vitamins are added to food. Losses of vitamins during processing as a result of heating, baking, stirring and the influence of light have to be accounted for.

Table 4. Food products fortified with vitamins in Switzerland

- Milk modifiers (all vitamins)
- Breakfast cereals (specially B-complex vitamins)
- Margarine (vitamins A, E and D)
- Plant oils (vitamin E, sometimes vitamin D)
- Flour for bread, biscuits and other products (vitamins B_1, B_2, and niacin)
- Mixed fruit juices (all vitamins, including β-carotene)
- Health food products, e.g. various processed seeds (vitamins E, B_1 and others).
- Special nutrition for sporting activities (very broad spectrum with all vitamins)
- Various kinds of slimming diets: 'easy-to-prepare', 'ready-to-serve', with all vitamins (in Switzerland by law)
- Confectionery (vitamin C)
- Yoghurts (various vitamins)
- Baby food, infant formula

Furthermore, losses during storage have also to be considered. An even distribution of the vitamins in the food and good bioavailability are also essential for each enrichment.

LEGISLATION OF VITAMIN ENRICHMENT

Switzerland has a longstanding tradition in the field of food enrichment with vitamins. The legal background dates back to 1957 when the regulations for the addition of vitamins were issued. According to these rules, vitamin amounts corresponding to either one RDA or to a third RDA can be declared for each portion of food, e.g. 500 g of milk, 50 g of margarine, 300 g of fruit juices. Up to three times the RDA can be added to compensate for losses during production and storage. In the case of vitamin D, excess addition is not allowed because of the toxicity of this vitamin. The manufacturers are responsible for the vitamin content and their products are taken from the market annually to be analysed by two specialised institutes, the author's laboratory in Basel and a second one in Lausanne. If the vitamin content is found not to be within certain defined limits, the official government agency in Berne will consider disallowing the sale of that specific product. These yearly controls, which are also being performed on products imported from other countries, provide a safe way to protect the consumer against false declarations and possible overdosages with toxic amounts.

At present, there are no regulations by the Common Market on food enrichment with vitamins and each country has its individual laws. In some countries, priority is given mainly to restoration, e.g. in flour, whereas in other countries vitamins can be added in a similar way as is being done in Switzerland. Furthermore, the addition of vitamins is compulsory practically all over the world for special dietary uses, for example, for baby food as well as for infants up to 6 months of age.

There are two council directives of the EEC dealing with vitamins. One is the directive 90-496-EEC on the labelling of foodstuffs. If vitamins are to be declared, at least 15% of the recommended daily allowance of the respective vitamin should be contained in a portion or in 100 g or 100 ml of the food. In the appendix of this directive, European RDA values are given. Only 2 years after the issue of this directive, these values are being rediscussed and it is likely that revised RDA values will become effective in 1994. Concerning the control of foodstuffs, the EEC council directive 89-397 says that each country is responsible for official inspection of the production procedure and of the products. Each manufacturer is therefore in fact responsible for its products and has to carry out proper quality control.

The analysis of vitamins in foodstuffs has made much progress in recent years. However, there still remain some problems especially with respect to the vitamins that occur in very small concentrations such as vitamins D_3 and B_{12}, folate and biotin. As

Table 5. Analysis of vitamins in food

Technique	Vitamin	
HPLC	A	
	B_1	
	B_2	
	C	
	D_2, D_3	
	E	
	β-carotene	
	K_1	
Chemical analysis	B_2	
	B_6	
	C	
	K_3	
	Ca-pantothenate	
Microbiological analysis	B_2	(*L. casei*)
	B_6	(*N. sitophila*)
	B_{12}	(*L. leichmanii*)
	Niacin	(*L. arabinosus*)
	Ca-pantothenate	(*L. arabinosus*)
	Biotin	(*L. arabinosus*)
	Folic acid	(*L. casei*)

shown in Table 5, various procedures are used for the determinations. For most of the fat-soluble vitamins, determination by high-pressure liquid chromatography (HPLC) is favoured. For the other vitamins, chemical methods and in some cases microbiological determinations are also required. The community bureau of reference of the Commission of European Communities (BCR) has set up a working group in order to compare the analytical methods used in different laboratories of the European countries. It is quite clear that considerable work lies ahead before all countries have the necessary competence to carry out high-quality analysis.

Since food patterns vary a great deal from country to country, the specific need for vitamin enrichment is also different. A strict legislation for the European Common Market countries may therefore not be desirable; however, some general regulations with respect to minimal and maximal values would be needed. A critical issue will also be whether the council directive on the official control of food stuffs (89-397) will be sufficient to provide an adequate food product quality throughout Europe. In our vitamin institute in Basel, we control not only the products of Switzerland but also those that are imported from other countries. The number of Swiss products for which the declared vitamin contents are not correct amount to less than 5% whereas for the products from other countries the percentage is considerably higher. It may be necessary, as in Switzerland, to introduce a mandatory annual control of the products on the market to protect the consumer effectively.

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