Nordic Biomarker Seminar

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Nordic co-operation

Nordic co-operation, one of the oldest and most wide-ranging regional partnerships in the world, involves Denmark, Finland, Iceland, Norway, Sweden, the Faroe Islands, Greenland and Åland. Co-operation reinforces the sense of Nordic community while respecting national differences and similarities, makes it possible to uphold Nordic interests in the world at large and promotes positive relations between neighbouring peoples.

Co-operation was formalised in 1952 when *the Nordic Council* was set up as a forum for parliamentarians and governments. The Helsinki Treaty of 1962 has formed the framework for Nordic partnership ever since. The *Nordic Council of Ministers* was set up in 1971 as the formal forum for co-operation between the governments of the Nordic countries and the political leadership of the autonomous areas. i.e. the Faroe Islands, Greenland and Åland.

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Preface

Biochemical indicators of dietary nutrient intake are called biomarkers. They may be used in clinical settings to assess deficiency or excess of nutrients like vitamin C and D. In nutritional epidemiology estimation of the dietary intake of foods and nutrients is done in order to classify subjects according to their nutrient intake and relate it to disease. Like for intake assessment methods there are pros and cons to be aware of when using biomarkers to estimate nutrient intake. These include the available tissue, nutrient metabolism, chemical method for analysis, specimen collection and storage and diurnal and biological variation.

There is current interest to use biomarkers not only for estimation of intake of single nutrients but also for assessing dietary patterns, like intake of fruits and vegetables. Suitable combinations of carotenoids and flavonoids may prove useful.

By the initiative of the Working Group on Diet and Nutrition (NKE) an expert seminar with the topic “Biomarkers of Nutritional Intake” was arranged on the 17-18th September 2004 in Helsinki. The aim of the seminar was to discuss and obtain information on research where biomarkers are used for assessing nutrient status. The focus was on nutrients relevant in the Nordic Countries. Although we are neighbours, our countries differ surprisingly much regarding life-style, dietary habits, geographical characteristics and chronic diseases. Research in nutritional epidemiology in the Nordic Countries should benefit from other’s experiences in order to maintain a high standard also in future research. The area of interest ranged from epidemiology to clinical interventions to validation of new biomarkers.

The Nordic Council of Ministers financed the meeting and made it possible to invite foremost experts within their field to give talks and participate in the discussions.

The organizing committee was composed of the following:

Georg Alftan	National Public Health Institute, Helsinki
Björn Åkesson	University of Lund, Lund
Rikke Andersen	National Food Administration, Copenhagen
Lene Frost Andersen	University of Oslo, Oslo
Antti Aro	National Public Health Institute, Helsinki
Iris Erlund	National Public Health Institute, Helsinki

I wish to thank the speakers, chairpersons and participants for their valuable contributions regarding the report, presentations and discussions and the organizing committee for their enthusiastic work in preparing the program of the seminar.

Georg Alfthan, chair of the committee

1. Biomarkers of Nutrient Intake

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My intension in this brief review is to consider why biomarkers of nutrient intake are useful and to explore some criteria for robust biomarkers; finally the potential for emerging biomarkers will be considered.

Biomarkers of nutrient intake provide information that is not collected by the individual under study and therefore not prone to under or overestimation associated with dietary records. They can inform about recent or long term intakes depending upon the choice of body compartment material for analysis. They are sometimes more reliable than dietary intake information (nitrogen excretion as a biomarker of protein intake for example) and they are often more specific and more sensitive than clinical information.

Let us consider what the role of the biomarker of nutrient intake is. Nutrient intake is an important determinant of the body pool of a nutrient and biochemical indices have been developed to reflect body pool status. These indices are in turn considered to reflect nutrient intake and represent the biomarkers of intake. The difficulty lies in the fact that various factors confound the relationship between nutrient intake and body pool such as bioavailability, disease and genotype. Similarly, numerous analytical issues confound the relationship between biochemical index and body pool. Thus, biomarkers are rarely wholly satisfactory.

Confounders of the Relationship Between Dietary Intake and Body Pool

1.1 Bioavailability

The magnitude of bioavailability effects on the relationship between nutrient intake and body pool largely depends on factors such as chemical form of the nutrient in the food matrix, interaction between nutrients in the lumen of the gastrointestinal tract, variations in gut flora and perhaps genotypic variation in transport proteins. For example, the availability of food folates is determined by the need for the removal of glutamates in

the gastrointestinal tract (1), whilst the absorption of iron is influenced by chelation by tannins, phytates, and ascorbic acid (2).

1.2 Disease

Disease per se can influence the relationship between dietary intake and body pool through interfering with absorption, transport, mobilisation from stores and excretion. Cystic fibrosis, for example, predictably leads to a malabsorption of fat and fat-soluble vitamins and biochemical indices underestimate intakes. Sub-clinical infection is perhaps the most important disease factor that can distort the relationship between nutrient intake and biochemical index of body pool, as in epidemiological studies, sub-clinical infection will go unrecognised. It has been shown very clearly that sub-clinical infection (raised CRP) is associated with an increase in plasma ferritin and plasma retinal thus leading to an overestimate of intakes (3).

1.3 Genotype

Genotype can influence the relationship between intake and body pool at several levels: absorption, transport, mobilisation from stores and metabolism. Common polymorphisms in genes expressing nutrient-relevant enzymes are of current interest. For example, homozygosity for a thermolabile variant of a gene expressing methylenetetrahydrofolate reductase (MTHFR C677T) is present in between 5-15% of the population. Subjects with this common TT polymorphism respond less well to the same folate intake, than other genotypes (4). This polymorphism therefore leads to an underestimation of folate intakes.

The second form of interference in the relationship between dietary intake and the biomarker relates to analytical issues. Biochemical indices are limited in their ability to reflect body pools for various reasons: the use of inappropriate body compartments for sampling, inappropriate sample handling, instability of the analyte, poor analytical precision and modulation by numerous non-nutrient factors (5). A biomarker of nutrient intake may therefore only be valid under specific circumstances.

For a biomarker of nutrient intake to be robust it should be sensitive (rapidly responsive to changing nutrient intake), valid (accurately reflecting intake), specific and precise. In this context biomarkers can be classified according to their ability to reflect nutrient intake. GOOD biomarkers would include nitrogen excretion as a marker of protein intake (6), sodium and potassium excretion as biomarkers of intake and membrane fatty acids as biomarkers of specific fatty acid intake. The majority of biomarkers however fall into the MODERATE category, which can pro-

vide useful information regarding whether consumers are low, intermediate or high consumers of individual nutrients. The validity of the biomarkers in this category can depend on the region of the intake range. For example, erythrocyte glutathione reductase activation coefficient (EGRAC) is responsive to changing riboflavin intake in the lower part of the normal range (7) whereas urinary riboflavin is only responsive in the upper part of intakes in the usual range.

The third category of biomarkers includes those that only poorly reflect intakes and this includes plasma calcium and plasma sodium and potassium, all of which are under strong homeostatic control and are therefore POOR biomarkers. Thus if plasma calcium concentration falls, PTH secretion increases with down stream effects leading to normalisation of plasma calcium at the expense of calcium in deeper sites.

1.4 Intermediate Biomarkers

There is increasing interest in the development of biochemical or physiological measures that reflect risk of chronic disease as well as informing about dietary exposure. These may be considered as intermediate biomarkers and their development is underpinned by emerging technologies. Uracil misincorporation into DNA is thought to reflect cancer risk and this measure has also been shown to be sensitive to folate intakes thus it could be classified as an intermediate biomarker (8).

1.5 Conclusions

Some broad conclusions can be drawn:

The habitual intake of a nutrient may be reflected in a biochemical indicator – a biomarker; numerous factors confound the relationship between intake and biomarkers; very few biomarkers are robust indicators of nutrient intake although many can usefully predict approximate intake and emerging technologies offer new opportunities for developing biomarkers.

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2. Biomarkers for Intake of Food

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2.1 Valid Biomarkers for Food Intake Are Important for Several Reasons

In nutritional epidemiology we are both interested in nutrient intake as well as the intake of whole foods because whole foods might provide bioactive factors in addition to the already known vitamins, minerals and phytonutrients.

We need valid biomarkers for validation of food intake from dietary assessment methods. Validation studies will give important insight regarding interpretation of dietary data.

Biomarkers for intake of fruit and vegetable, dairy products and fish have been proposed. A short review of the data published in this area will be presented. Moreover a discussion of the challenges and limitations with these biomarkers are included.

2.2 Biomarkers for Fruit and Vegetable

For fruit and vegetable several biomarkers have been proposed. We have included blood concentrations of carotenoids and folate in this review. Urinary flavonoids as a biomarker of fruit and vegetable are discussed elsewhere in this report.

The concentration of carotenoids in blood is a potential interesting biomarker since the most important source for carotenoid intake is fruit and vegetable. Several different types of studies have been conducted to validate blood carotenoids as a biomarker for intake of fruit and vegetable. A number of controlled feeding studies have shown increased plasma concentrations of carotenoids after high doses of single fruits and vegetables, indicating that carotenoid concentration in plasma could be biomarkers for the intake of fruit and vegetable (1-8). However, in a regular industrialized diet several different fruits and vegetables are usually eaten in varying amounts daily. Cross-sectional studies studying the correlations between intake of fruit and vegetable and plasma carotenoids have shown correlation coefficients in the low to moderate range (0.20-

0.50) (9-13). However, one limitation with this type of study is that the dietary assessment methods used may vary in validity and thereby attenuate the observed correlations. Three fully controlled intervention studies have focused on how plasma concentrations of carotenoids are affected by a mixed fruit and vegetable intake (2, 14, 15). In a Norwegian controlled intervention study, with the aim to test whether plasma concentration of carotenoids could be used to distinguish recommended consumption of mixed fruit and vegetable (five portions per day) from the common national intake of fruit and vegetable (two portions per day), we found that plasma alpha carotene, beta carotene and lutein may provide important information about self-reported intake of fruit and vegetable in national surveillance programs (14). These results were in accordance with the results observed by van Het Hof (15) and Broekmans (2).

There are several limitations with plasma carotenoids as a biomarker for intake of fruit and vegetable; 1). No general international carotenoid marker of fruit and vegetable exists. The relationship between different plasma carotenoids and the intake of different profiles of fruit and vegetable will depend on the amount and frequency of consumption of the different fruit and vegetable. Moreover, absorbed amount would be influenced by the cooking methods and season. 2). Plasma values are subject to day to day fluctuations and individual variation. 3). There are several other determinants of plasma carotenoids other than intake of fruit and vegetable e.g. gender, BMI, alcohol intake, supplements, serum triglycerol and serum cholesterol (16-20).

Fruits and vegetables are also rich sources of folate, and plasma concentration of folate may be another potential biomarker for the intake of these food items. In a Norwegian study, we have examined the association between plasma folate and intake of folate and fruits and vegetables in a large cohort of middle aged and old subjects (Brevik et al, unpublished). Folate from the combined intake of fruit, fruit juice and vegetable contributed with 32-38% of the total intake of folate. Among non-supplement users and supplement users the correlations between total intake of fruit, fruit juice and vegetable and plasma concentration were 0.22 and 0.18 ($p < .01$), respectively. These correlations are in the same range as reported for the association between dietary intake of fruits and vegetables and vitamin C and carotenoids (22-26). Brevik et al (unpublished) also observed a significant increase (38%) in plasma folate concentration between the lowest and the highest quartile of total intake of fruit, vegetable and juice. Several studies have investigated how dietary interventions with fruits and vegetables may increase folate levels in various fractions of the blood, and found similar results as in the Norwegian study (2, 15, 26, 27). There are several factors which may limit plasma folate function as a biomarker for intake of fruit and vegetable; 1). Plasma folate concentration probably reflects more recent folate uptake and not the usual intake, 2). Fortification with folic acid is common in several

countries, 3). Other determinants of plasma folate are e.g. other foods, supplements, alcohol etc.

2.3 Biomarkers for Dairy Products

Recent studies have suggested that the proportion of pentadecanoic (C15:0) and heptadecanoic (C17:0) acid in serum lipids and adipose tissue may reflect milk fat consumption (28, 29, Brevik et al unpublished). The fatty acids 15:0 and 17:0 are synthesized by the bacterial flora in the rumen of ruminants and can not be synthesized by humans. Thus, these odd numbered fatty acids may be good biomarkers for intake of dairy products. The correlations observed between the relative content in of fat in dairy products and C15:0 in serum cholesteroesters, serum phospholipids and total serum lipids vary from 0.34 to 0.50. Correlations observed with C15:0 in adipose tissue are even higher 0.52 and 0.75 (28, Brevik et al unpublished).

2.4 Biomarkers for Fish Intake

Several studies have found a positive association between fish intake, either as total fish or as fatty fish, and plasma phospholipids n-3 fatty acids, correlations ranging from 0.30-0.33 (30, 31). In a Danish study Marckmann and colleagues (32) found a highly significant correlation between total fish intake and DHA in adipose tissue ($r=0.55$), while an American study only found a correlation between total fish intake and DHA in adipose tissue of 0.15 (33)

2.5 General Limitations and Challenges

Several of the limitations and challenges are the same for biomarkers for food intake and biomarkers for nutrient intake e.g.; 1). There are other determinants of the biomarker than the food of interest, 2). Large intra- and inter-variation of the biomarker, 3) The biomarker for food can be used to categorise people according to intake but are not good enough at the individual level, 4). The food biomarkers are not quantitative markers of intake.

In summary, there are several biomarkers for intake of whole foods and these biomarkers may be valuable as a complement to traditional dietary assessment methods in epidemiological studies. Moreover, they may be useful as “objective reference methods” in validation of food intake from dietary assessment methods. However, we need further validation of the biomarkers for food intake.

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3. Biomarkers for Iodine

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Iodine deficiency is a large problem worldwide and iodine deficiency still exist in the Nordic countries especially in Denmark. The most obvious manifestation of iodine deficiency is goitre which is an enlarged thyroid gland. In addition to goitre iodine deficiency diseases include a spectrum of conditions that vary in severity. The most severe of these, cretinism with mental deficiency, retarded physical development e.g., does not exist in the Nordic countries. However, toxic and non-toxic goitre is seen especially in the elderly.

Iodine in significant amounts is only found in few foods. The main sources of iodine in the Nordic countries are milk and dairy products and fish. In the eastern part of Denmark tap water is also an important source. Furthermore, salt is iodized in the Nordic countries and contribute to the iodine intake at varying degree.

Some options for iodine biomarkers exist. These are:

Iodine excretion in 24-hour urine samples
Iodine excretion in a single urine sample

- expressed as a concentration
- expressed as the iodine-creatinine ratio
- expressed as estimated 24-hour iodine excretion

Serum thyroglobulin (TG) concentration
Serum thyroid-stimulating hormone (TSH) and serum thyroxine (T₄) concentration

3.1 Iodine Excretion in 24-hour Urine Samples

Urinary iodine reflects iodine intake since approximately 90 % of ingested iodine is excreted in the urine under stable iodine intake conditions (Nath et al 1992). Iodine content in 24-hour urine samples is a good measure for iodine intake and the level can be directly compared with iodine intake and with the recommended intake which is 150 µg per day. The 24-hour iodine excretion has been found to correlate with iodine intake ($\rho = 0.79$, $P < 0.001$) (Rasmussen et al 2002a) and there is a relation between iodine intake and iodine excretion within the same day. A disadvantage is the difficulty in collecting complete 24-hour urine samples. One 24 hour sample is insufficient to determine iodine status in an individual due to the large day-to-day variation (Rasmussen et al 1999).

3.2 Iodine Excretion in a Single Urine Sample Expressed as a Concentration

The concentration of iodine in casual urine samples is, by some researchers, the recommended measure when evaluating iodine deficiency in a population (Bourdoux 1993). The advantages are that only a single urine sample is needed from each person and only one analysis is needed. Iodine excretion given as a concentration correlates with 24-hour iodine excretion (Rasmussen et al 1999, Rasmussen et al 2002a). This indicates that it reflects iodine intake, although the correlation is not strong. Probably the biggest advantage by using the iodine concentration is that values for evaluating median iodine concentration are established. These values are based on a number of epidemiological studies and can be used to classify a population:

Population median value	Classification
< 20 µg/l	severe iodine deficiency
20-49 µg/l	moderate iodine deficiency
50-99 µg/l	mild iodine deficiency
100-199 µg/l	optimal iodine status
200-299 µg/l	more than adequate iodine status
> 300 µg/l	excessive iodine status

However, in a Danish population no relation between iodine excretion expressed as a concentration and thyroid volume or thyroid enlargement was found ($P = 0.4$ in multiple regression analyses) (Rasmussen et al 2002b). In addition, a disadvantage is, that the excretion given as a concentration cannot be related to the intake. Furthermore, the concentration is dependent on the dilution of the urine. The daily urinary volume vary appreciably from person to person. That is one reason why it is not a sensitive biomarker. Another point to pay attention to is that the iodine concentration seems to vary with time of the day, but results so far have not been consistent.

To conclude, the iodine concentration in one urine sample can be used to broadly classify a population as having a severe, moderate or mild iodine deficiency problem, but it cannot give the exact level of iodine intake in a population.

3.3 Iodine Excretion in a Single Urine Sample Expressed as the Iodine-Creatinine Ratio

Another way to express iodine excretion from a single urine sample is as the iodine-creatinine ratio. Creatinine is measured to take the dilution of the urine into account.

The main problem is, that creatinine excretion differs with gender and age, so the iodine-creatinine ratio in different groups cannot be compared.

For example women's iodine status will be overestimated compared with men's because the daily creatinine excretion is higher in men than in women.

Use of the iodine-creatinine ratio cannot be recommended.

3.4 Iodine Excretion in a Single Urine Sample Expressed as the Estimated 24-hour Iodine Excretion

Another way to use the iodine-creatinine ratio is to calculate the estimated 24-hour iodine excretion. This can be done by multiplying the iodine-creatinine ratio with the 24-hour creatinine excretion and thereby take the dilution of urine into account. Creatinine excretion is relatively constant from day-to-day. Values for 24-hour creatinine excretion in various age and gender groups have been published. The advantages are that the estimated 24 hour iodine excretion is comparable to the intake. Furthermore, it has been found to be highly significantly associated with thyroid volume and thyroid enlargement in a Danish population ($P < 0.001$ in multiple regression analyses) and to correlate with 24-hour iodine excretion (Rasmussen et al 2002a, Rasmussen et al 2002b).

In populations with protein malnutrition the creatinine excretion will be below normal values leading to an overestimation of the iodine intake. However, this is not a problem in a well-nourished population like the Nordic. The creatinine excretion has been found to vary throughout the day, but no clear diurnal pattern has been found. In population surveys these variations do not seriously influence the iodine excretion level. In conclusion the estimated 24-hour iodine excretion is useful in a healthy, well-nourished population.

3.5 Serum Thyroglobulin

Thyroglobulin is a glycoprotein that contains iodinated amino acids and is the storage form of the thyroid hormones in the thyroid gland. In iodine deficiency serum thyroglobulin increases. Serum thyroglobulin (Tg) may be a useful biomarker of iodine status. Serum Tg has been found to correlate strongly with thyroid enlargement in a population with mild to moderate iodine deficiency (Knudsen et al 2001). Likewise, serum Tg was found to be highly significantly associated with iodine excretion both expressed as a concentration ($P < 0.001$) and as estimated 24 hour iodine excretion ($P < 0.001$) in multiple regression models (Rasmussen et al 2002). Furthermore, serum Tg was highly significantly associated ($P < 0.001$) with iodine intake in the same population (Rasmussen et al 2002). Comparison of the results of different studies is hampered by the diffe-

rences in the performance of the assays, interference with endogenous Tg antibodies, and a lack of standardization (Spencer et al 1999).

In conclusion, serum Tg may be a sensitive biomarker of iodine intake. With newer assays with better standardization, higher sensitivity, less problems with interference from Tg Ab, and increasing automation, serum Tg may be a useful tool to determine iodine deficiency in a population although not in severe iodine deficiency. However, generally accepted normal values of serum Tg have not yet been published.

Serum thyroid-stimulating hormone (TSH) and serum thyroxine (T₄) concentration

The determination of serum TSH or T₄ provides an indirect measure of iodine nutritional status. The measure is particularly useful in neonates and pregnant women, and in areas with severe iodine deficiency, whereas in borderline and mild iodine deficiency the circulating levels of TSH or T₄ may still remain within the normal range and are insufficiently sensitive to be used as a biomarker of iodine status (Bourdoux 1993).

3.6 Conclusion

Estimated 24-hour iodine excretion based on iodine and creatinine in a single urine sample is probably the most useful biomarker for iodine status in countries like the Nordic countries. Iodine concentration in single urine samples can be used to broadly classify a population in regard to iodine deficiency. If the exact iodine intake has to be known, iodine excretion in 24-hour urine samples should be used. Serum thyroglobulin could be a good candidate for an iodine biomarker.

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4. How to Validate Vitamin D Status?

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4.1 Introduction

Vitamins are usually compounds that the body is not able to synthesize. However, vitamin D is unique since the body is able to synthesize an active vitamin D compound through sunshine. Due to this fact, dietary intake calculated by linking data from Food Composition Tables and Dietary Surveys do not reflect the amount of vitamin D the body has access to.

It is well known that vitamin D is essential for the development and maintenance of bones, and that low vitamin D status increases the risk of falls and osteoporotic fractures (Bischoff et al., 2003, Stein et al., 1999, Larsen, 2002, Trevedi et al., 2003). Besides, lack of vitamin D seems to be associated with a number of diseases including certain kinds of cancer (prostate, breast and colon), heart diseases, and infections and decreased immune defence (Zimmerman, 2003).

Recently studies have focused on the high degree of deficiency among population groups in the Nordic countries (Valimaki et al., 2004; Meyer et al., 2004; Brustad et al. 2004; Andersen et al., 2004), which enhance the need to solve the problems concerning common use of a biomarker for vitamin D status. The present short review focuses on these problems regarding such a biomarker.

4.2 Metabolism

The vitamin D biosynthesis and metabolism involve synthesis of vitamin D in the skin by exposure of the sun's ultraviolet B radiation with energies between 290-315 nm. Through this radiation 7-dehydrocholesterol in the skin is converted to pre-vitamin D₃, which further isomerises to vitamin D₃.

Vitamin D (vitamin D₂ and vitamin D₃) is a pro-hormone, and is not known to have any biological activity itself. It is hydrolysed in the liver

to 25-hydroxyvitamin D (25OHD) and afterwards in the kidney to the vitamin D hormone 1,25-dihydroxyvitamin D (1,25OHD), known as calcitriol. In the endocrine system vitamin D binding protein (VDBP) is carrier for vitamin D metabolites to the various target organs.

A strong regulation of the hydroxylation to 25OHD in the liver does not exist. From the liver 25OHD is rapidly released into the blood, where it circulates with a biological half-life of approximately 4 weeks. In contrast, the production of 1,25OHD is strictly regulated by parathyroid hormone (PTH), and 1,25OHD maintain calcium and phosphorous level in the blood, and as so the development and maintenance of bones.

4.3 Vitamin D Sources

For most people the main source of vitamin D is through sun exposure with vitamin D from diet as the secondary source. From October until March the production of vitamin D in the skin occurs little if at all in Nordic countries. Skin synthesis of vitamin D throughout the year is achieved as south as Africa at the latitude of 32°N.

The diet mainly contains two vitamin D compounds, vitamin D₃ and 25-hydroxyvitamin D₃. Fish, meat, eggs, milk, and dairy products are the main contributors to the intake. In nature vitamin D₂ is present in mushrooms but like 25-hydroxyvitamin D₂ the intake is negligibly unless dietary intake of mushrooms is relatively high.

Supplements were former mainly vitamin D₂, but nowadays vitamin D₃ is used as well, while vitamin D₃ normally is used for fortification.

Especially the content of 25-hydroxyvitamin D₃ in the diet is relative high compared to vitamin D₃, and as 25-hydroxyvitamin D₃ is absorbed better and faster from the diet than vitamin D₃ the bioavailability have to be taken into account for the calculation of dietary intake of vitamin D. However, bioavailability of the different vitamin D compounds differs, but studies in this area have so far not come to identical conclusions (Ovesen et al., 2003).

4.4 Vitamin D Intake in the Nordic Countries

In the new version of Nordic Nutrition Recommendations from summer 2004 (NCM, 2004), the recommended dietary intake of vitamin D has been increased from 5 µg/day to 7.5 µg/day for age groups between 2 and 60 years, while the other age groups, including pregnant and lactating women, are recommended 10 µg/day.

Dietary intake does not reach this level. Results for the calculation of dietary intake¹ in the Nordic countries are shown in table 1.

Table 1. The dietary intake in the Nordic countries

Country	Vitamin D, µg/10 MJ
Denmark	3.3
Norway	5.5
Finland	6.1
Sweden	6.3
Iceland	7.2

4.5 Biomarker for Vitamin D

The biological active vitamin D form, 1,25OHD in serum is usually normal or even slightly elevated in vitamin D deficiency, while concentration of vitamin D in serum reflects intake and skin-production of vitamin D, and therefore may vary greatly over a short time in an individual (Ovesen et al., 2002).

The fact that hydroxylation of vitamin D to 25OHD is not regulated, but act as the storage for 1,25OHD production, is utilised in vitamin D research. However, testing a biomarker for dietary intake demands limited sun exposure and the studies have to be performed during winter. Circulating 25OHD levels have been shown to reflect the amount of sunlight to which the skin is exposed, as well as the dietary intake of vitamin D (Brot et al. 2001, Heaney et al., 2003).

There is now a consensus that 25OHD concentration in serum is a good marker of internal vitamin D status (SCF, 2002), and do reflect an individuals dietary intake and cutaneous production. However, there is no agreement on cut-off levels for serum 25OHD for each step of vitamin D status: deficiency, in-sufficiency and sufficiency.

Little information is available for the optimal level of serum 25OHD to maintain normal calcium metabolism and to obtain optimal peak bone mass. However, vitamin D deficiency tends to decrease calcium level in blood, which results in secondary hyperparathyroidism. The measurement of intact PTH in serum has proven to be a valuable indicator of vitamin D status. The level of serum 25OHD above the level where further alteration in serum PTH occurs, could define optimal level of serum 25OHD.

This problem is emphasized in the published results of three research studies performed in the Nordic countries and published in 2004 dealing with, among other issues, deficiency. Independent of the analytical method used cut-off level between 20 and 37.5 nmol/l for S-25OHD is

¹ These calculations of dietary intake are based on food composition data derived from analysis for vitamin D using a biological assay which donot differentiate between the different vitamin D compounds, but utilise deficient rat to cure rickets.

used to define deficiency (Brustad et al., 2004; Valimaki et al., 2004; Andersen et al., 2004).

It is difficult to define the cut-off levels, which minimise risk of fractures or which could answer the question: “What is the optimal level to decrease development of the other diseases which vitamin D have shown to be involved in”?

4.6 Determination of Serum 25OHD

Collection of blood samples for measurement of 25OHD levels are usually taken from fasting volunteers. Most often serum is preferred but plasma may also be used.

Development of analytical methods used for determination of 25OHD has been a challenge since the discovery of 25OHD in 1969 (DeLuca, 1969). The first method was a competitive protein binding assay (CPBA) utilising vitamin D-binding protein as a primary binding agent and ^3H -25OHD₃ as a reporter (Haddad & Chyu, 1971). This assay included time-consuming chromatographic sample purification, which were overcome by the introduction of an antibody that was cospecific for 25OHD₂ and 25OHD₃ (Hollis & Napoli, 1985). Further development of this technique was the incorporation of ^{125}I as the reporter, which made the introduction of commercial radio-immunoassay (RIA) (Hollis et al., 1993). Since then manufacturers have introduced simple assays based on antibody (DiaSorin, Stillwater, MN, USA; IDS, Boldon, UK) and recently on CPBA (Nichols Institute Diagnostics, San Clemente, CA, USA) combining different detection system ^{125}I or chemiluminescence.

One of the essential parts of these assays is the quality of antibody/binding protein, which has to address equally to 25OHD₂ and 25OHD₃. On the other hand determination of 25OHD as the sum of 25OHD₃ and 25OHD also makes these assays non-specific.

In 1977 a specific HPLC-method was introduced, but the method was rather complicated and used 4 ml serum (Eisman et al. 1977). The analytical assay using HPLC and UV-detection have since the introduction been improved to use a more simple sample extraction as well as smaller sample size, 500 µl being the minimum at the moment (Gilbertson, 1977; Jones, 1978; Aksnes, 1994; Shimada et al., 1997; Alvarez & Mazancourt, 2001; Turpeinen et al., 2003). The benefit of these methods is the specificity i.e. the ability to quantify each metabolite.

Usually, HPLC-methods have been abandoned due to their requirement for expensive equipments, need for technical expertise and analytical run-time. However, a comparison study evaluating different assays for the determination of serum 25OHD indicates that performance of commercial assays is user-dependent and requires quality control to secure satisfactory results (Brinkley et al., 2004).

Furthermore, large variation between the assays has been demonstrated in the worldwide vitamin D assessment scheme – DEQAS (Charing Cross Hospital, London, UK) in which 90-100 laboratories do participate. Normally, the variation between methods is 20-30%, but analysis of samples containing 25OHD₂ and 25OHD₃ showed up to 43% of variation due to incomplete quantification of 25OHD₂ in some of the commercial assays.

4.7 Conclusion

There is consensus that 25OHD in serum is a good biomarker for exposure of vitamin D from sun and diet. Comparison of vitamin D status between studies is difficult due to large variation between analytical methods used. Therefore it is essential to agree on a reference method for fully utilisation of the biomarker for vitamin D status – serum 25OHD. We may choose between a commercial assay, which may change over time regarding antibodies or a specific HPLC-method, which enables a calibration on standards of 25OHD₂ as well as 25OHD₃.

Afterwards, cut-off levels for deficiency, insufficiency, and sufficiency have to be established to secure optimal vitamin D status throughout the year.

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5. Water Soluble Vitamins - Vitamin C

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5.1 Introduction

The necessity of vitamin C (ascorbic acid) for human health is firmly established. As humans are not able to synthesize ascorbic acid, they are dependent on their dietary intake. The dietary sources of vitamin C are fruits and vegetables, especially in uncooked forms. The historical discovery of the beneficial effects of fruits as food dates back to the Middle Ages. Scurvy was common among sailors during the long sea expeditions of the 15th and 16th centuries. The sailors suffered from symptoms of scurvy: capillary hemorrhages, bleeding gums and loosening of teeth, reduced rate of wound healing, depression and fatigue. Vasco da Gama, for example, lost about 100 of his 160 seamen in his India passage between the years 1497-1499. As late as 1740, the British admiral Anson lost five of his six ships and 1165 of 1500 seamen before reaching the coast of South America. Also during wars in the 19th century, when food shortage was acute, scurvy was a problem.

In 1753, James Lind published a book about scurvy. His classical study of prevention of scurvy is regarded as the first controlled clinical trial (1): He divided 12 sailors with scurvy into six groups to receive either wine, diluted sulfuric acid with ginger and cinnamon, vinegar, sea water, oranges and lemons or nutmeg and garlic daily. The result was that only the men receiving oranges and lemons recovered from the scurvy.

At the present time, scurvy is very rare. Fruits and vegetables are available throughout the year in every industrial country to prevent the clinical symptoms of scurvy. An adult requires 10 mg/day of dietary ascorbic acid to avoid scurvy (2). The U.S. Recommended Daily Allowance (RDA) is 75 mg/day for women and 90 mg/day for men, however tissue saturation appears to require an ascorbic acid intake of 100 mg/day (3,4). Nordic Recommendation is 60 mg/day for women and men, 70 mg/day during pregnancy and 90 mg/day during lactation. Recently published studies have been interpreted to provide evidence that vitamin C at inta-

kes higher than the current recommendations might improve a number of functions in the human body and might reduce the risk of some chronic degenerative diseases such as cataract, cancer and cardiovascular diseases (3,4,5). The mechanism of these beneficial functions of ascorbic acid has been proposed to be its ability to prevent or stop oxidative free radical attacks in the human body (6). Linus Pauling hypothesized that high doses of vitamin C might prevent colds and influenza (7), but this effect of ascorbic acid is still to be proved.

5.2 Chemical Structure of Ascorbic Acid

L-ascorbic acid is the naturally occurring form of ascorbic acid and has the most biological activity. The D-ascorbic acid and D-isoascorbic acid (erythorbic acid) have only marginal vitamin C activity. However, D-isoascorbic acid is used in the food industry as an antioxidant, even though it has only about 5% of the activity of L-ascorbic acid (1). The oxidized form of L-ascorbic acid is the dehydroascorbic acid (DHA). This is very unstable in aqueous solution and is degraded by hydrolysis to 2,3-diketo-L-gulonic acid. The fatty acid esters of ascorbic acid, particularly ascorbyl palmitate, are used as antioxidants in fatty foods because of their lipophilic character.

5.3 Biological Function of Ascorbic Acid

5.3.1 Reducing Properties of Ascorbic Acid

Ascorbic acid is a strong reducing agent. The predominant reaction is a radical chain-terminating one, for example with a hydroxyl radical ($\cdot\text{OH}$):

where AH^- is the ascorbate anion. $\text{A}\cdot^-$ formed in this reaction is the ascorbyl radical, which is reactive and can react with another radical to yield dehydroascorbic acid (A):

Thus two moles of hydroxyl radical are reduced for every mole of ascorbate consumed.

5.3.2 Ascorbic Acid as Prooxidant in Vitro

Ascorbic acid can in certain conditions act as a prooxidant and promote the generation of the same active oxygen species ($\cdot\text{OH}$, $\text{O}_2\cdot^-$ and H_2O_2) it is considered to destroy. It is presently generally agreed that this prooxi-

dant activity is derived from the ability of ascorbic acid to reduce transition metals Fe^{3+} or Cu^{2+} by a one-electron mechanism:

or by a two-electron mechanism:

The formation of Fe^{2+} (ferrous ion) and H_2O_2 gives rise to the Fenton reaction, where iron is oxidized and active hydroxyl radical is formed.

5.3.3 Ascorbic Acid as Prooxidant in Vivo

It has been suggested that the possible in vivo prooxidant effects of ascorbate are related to the availability of catalytic transition metal ions (8,9). The addition of vitamin C to meals increases non-heme iron absorption in patients with hemochromatosis or thalassemia, which might lead to increased iron-overload and deleterious clinical effects. Non-protein bound iron, if it exists in the human body, could induce lipid peroxidation especially if it is present together with the pro-oxidative ascorbic acid (reaction 3). Vitamin C ingestion enhances the iron absorption also in individuals with iron deficiency, but not in individuals with normal iron status (5).

5.3.4 Regeneration of Ascorbic Acid

When ascorbic acid becomes oxidized, the DHA that is formed can be reduced back to ascorbic acid in the presence of a suitable reductant (Figure 1). Two glutathione (GSH) molecules can reduce one DHA molecule to ascorbic acid since this reaction is energetically feasible.

Figure 1. Cyclic reactions between glutathione, ascorbic acid and tocopherol. NADPH=nicotinamide-adenine-dinucleotide phosphate, reduced form; ADP=nicotinamide-adenine-dinucleotide phosphate, oxidized form; GSH=glutathione; GSSG= glutathione disulfide; DHA=dehydroascorbic acid

GSH is an important antioxidant in cells. It maintains high concentrations of ascorbic acid in cells by reducing DHA to ascorbic acid. This leads to the accumulation of ascorbic acid in cells.

5.3.5 Ascorbic Acid as a Cofactor in Enzymatic Systems

Ascorbic acid is required for many hydroxylase enzymes in the human body. Ascorbic acid is needed for conversion of tyrosine to the neurotransmitter dopamine and further hydroxylation to adrenaline and noradrenaline, for synthesis of carnitine from lysine and probably for hydroxylation of steroid hormones. It is also known to participate in hydroxylation of aromatic drugs and carcinogens via microsomal monooxygenase systems of liver endoplasmic reticulum. Its role in the formation of collagen is thought to be to maintain iron in its ferrous state for an iron dependent proline hydroxylase, or to act as a direct source of electrons for reduction of O₂. Ascorbic acid has effects on endothelium. It scavenges the superoxide anion radical that destroys nitric oxide that is important in the relaxation of endothelium. It has also effects on lipid peroxidation and on atherosclerosis.

5.3.6 Ascorbic Acid as an Antioxidant for Lipid Peroxidation

In early atherosclerosis, changes take place in the endothelium, and monocyte/macrophages routinely penetrate the subendothelial space as part of their surveillance function. Macrophages can phagocytose low density lipoprotein (LDL) particles and form foam cells. However, it has been observed that macrophages in culture cannot be converted to foam cells by incubation with native, unoxidized LDL. Thus it has been suggested that LDL must first be modified before it can be recognized by macrophage scavenger receptors (10).

Ascorbic acid has been shown to inhibit lipid peroxidation in vitro in isolated plasma LDL (11,12). The effects of the supplementation of ascorbic acid on lipid peroxidation are rather conflicting. This is mainly due to the lipoprotein separation process which eliminates ascorbic acid from the sample. The measurement of the lipid peroxidation rate in whole serum is more reliable for assessing the effect of ascorbic acid (13).

5.3.7 Ascorbic Acid in Eastern Finnish Men

Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is a population study that was carried out between 1984-89. After that, the subjects were followed for their diseases and the follow-up is still going on. Plasma ascorbic acid value was available from 2580 men. Five percent of men had their plasma ascorbic acid concentration below 11.4 µmol/l,

20% had the value between 11.4 – 28 $\mu\text{mol/l}$ and 75% had the value over 28 $\mu\text{mol/l}$.

For this study, we followed the subjects for the average of 5.5 years. For statistical analysis we included only the subjects without previous coronary disease. We found that the relative risks for getting a myocardial infarction were about four times higher in the group of men with the lowest plasma ascorbic acid levels ($<11.4 \mu\text{mol/l}$) after adjustment for age, season, and year of examination (14). However, there were no differences in the risk in the quarters of ascorbic acid concentration above this limit.

We have followed 401 KIHDM men for several years and measured their plasma ascorbic acid concentration three times. At baseline the mean plasma ascorbic acid concentration was 46 $\mu\text{mol/l}$, 4.6 years later it was 53 $\mu\text{mol/l}$ and 6.8 years later it was 53 $\mu\text{mol/l}$. It seems that men had increased vitamin C in their diet after the baseline, in early 1990's.

The consumption of foods was assessed by four days food recordings. Of dietary factors, the intake of fruits and berries ($r=0.30$) and vegetables ($r=0.24$) had any notable correlations with plasma ascorbate concentration.

5.4 Smoking and Plasma Ascorbic Acid

Smoking is associated with reduced ascorbic acid plasma levels and that is thought to be due to either a decreased intake or higher consumption of ascorbic acid in smokers than in non-smokers (15,16). Ascorbic acid concentration is about 20% lower in smokers than in non-smokers. Dehydroascorbic acid proportion of the total ascorbic acid is higher in smokers than in non-smokers. We have found that during vitamin C supplementation, plasma levels of ascorbic acid increased to the levels of non-smokers, but there was no significant change in lipid oxidation resistance as measured in separated VLDL + LDL fraction (17). There is also a study concerning the effect of smoking cessation on plasma ascorbic acid values. The authors found that plasma ascorbic acid values were recovered to the levels of non-smokers after four weeks from stopping smoking (15).

5.5 Vitamin C Supplementation and Atherosclerosis

In the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study we assessed the effect of vitamin E and C supplementation on the progression of carotid atherosclerosis in 520 smoking and non-smoking men and women. The subjects were randomized in four strata by gender and smoking status. They were supplemented by 91 mg (136 IU)

α -tocopherol or 250 mg vitamin C or both or placebo, twice a day. The atherosclerosis was measured as the intima-media thickness of common carotid artery with an ultrasound technique. After 3 years supplementation, the atherosclerosis progression was significantly less in the C+E group than in other men. The covariate-adjusted intima-media thickness increase was reduced by sixty-four percent in smoking men and by 30 % in non-smoking men. However, Vitamin C alone did not have significant effect on atherosclerosis progression (18). The result was repeated after 6 years supplementation with vitamin C+E (19).

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6. Immunochemical Assay of Selenoprotein P and Glutathione Peroxidase-3 as Indicators of Selenium Status in Humans

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6.1 Introduction

More than 20 specific selenocysteine-containing selenoproteins have now been identified in animal tissue (Behne, Kyriakopoulos, 2001; Kryukov et al. 2003), but the concentrations and distribution of selenoproteins in different tissues are not well known. In human plasma, extracellular glutathione peroxidase (GSHPx) and selenoprotein P have been demonstrated (Takahashi, Cohen, 1986; Åkesson et al., 1994; Huang 1996; Persson-Moschos, 2000).

Extracellular GSHPx is produced in the kidney and placenta and also occurs in plasma, breast milk, aqueous humor, amniotic fluid, lung lavage, and the thyroid (Avissar et al., 1994; Howie et al., 1995; Avissar et al., 1996; Huang et al., 1997). Its postulated roles include control of peroxide transport at the membrane and of extracellular 'peroxide tone' (Brigelius-Flohé, 1999; Arthur, 2000). Selenoprotein P is the major form of selenium in the plasma and is involved in selenium transport (Åkesson et al., 1994; Burk et al., 2003). It is localised in the endothelium and may reduce peroxynitrite and phospholipid hydroperoxides (Arteel et al., 1998; Saito et al., 1999), and also form complexes with mercury and cadmium (Suzuki et al., 1998), binds to heparin and cell membranes (Burk et al., 2003), and may stimulate survival of nerve cells in culture (Yan, Barrett, 1998).

6.2 Survey of Results

Selenoprotein P and extracellular GSHPx were chromatographically purified from human plasma (Persson-Moschos et al. 1995A; Huang, 1996; Persson-Moschos, 1999). Radioimmunoassays were developed to assess the levels and activity of these selenoproteins.

In healthy Swedish subjects, selenoprotein P accounted for >40% of total plasma selenium, extracellular GSHPx for between 10-16% (concentration 3.3-4.1 mg/L), with the remainder probably being predominately protein-bound selenomethionine, mainly albumin (Huang, Åkesson, 1993).

Selenoprotein P levels have been measured in healthy adults from 17 European Regions, Fig. 1 (Marchaluk et al., 1996). Considerable variation between different regions was found. There was a good correlation between selenoprotein P and plasma selenium values with some indication of a plateau, although different studies have shown varying findings concerning the levels of the plateau, Fig 2. The correlation between GSHPx and serum selenium values in five different studies are shown in Fig. 3. It has been suggested that due to the different plateau attained by different selenoproteins they could represent useful functional markers of selenium status in different ranges of selenium status. (Marchaluk et al, 1996; Thorling et al, 1986; van Dokkum 1995).

Figure 1. Selenium status in different studies

In the Malmö Food Study (Åkesson et al., 1997) the relationship between the intake of different foods and selenium status was investigated. No significant correlations were observed in men but among women there were significant relationships between milk intake, and selenoprotein P and also urinary selenium, and between fish intake, and serum and urinary selenium.

In a study of Latvian fisherman (Hagmar et al., 1998) a highly significant relationship between fish intake and selenium status was demonstra-

ted and an inverse relationship between plasma selenium levels and thyroid stimulating hormone was found.

Figure 2. Overall correlation between selenium and selenoprotein P in plasma in different studies. $r=0.84$; $P<0.001$. Persson-Moschos, M. Ph.D. thesis, 1999.

Figure 3. Overall correlation between selenium and glutathione peroxidase in plasma in different studies. $r=0.58$; $P<0.001$. Huang W. Ph.D. thesis, 1996

Plasma selenium and cancer risk. There is some evidence showing an association between plasma selenium and future risk of cancer (Comstock et al., 1992). In the first study of this kind, the association between selenoprotein P in plasma and cancer risk was investigated, and in cancer of

the respiratory and gastro-intestinal tract this relationship reached statistical significance (Persson-Moschos et al., 2000).

Home parenteral nutrition patients with a variety of gastro-intestinal diseases have been found to have much lower levels of extracellular GSHPx, total selenium and selenoprotein P than control subjects (Ran-nem et al., 1996). During LDL apheresis using sulphated polysaccharide columns plasma SeP is depleted (Persson-Moschos et al., 1995B).

Selenium status and toxic metals. Studies of lead exposed children in Katowice, Poland, have shown inverse relationships between blood lead and levels of selenoprotein P and extracellular GSHPx (Osman et al., 1998). However, the direction of causality has not been established and it is not possible to conclude that lead exposure causes a decrease in selenoprotein concentrations or that poor selenium status increases susceptibility to high blood lead concentrations.

Selenium supplementation trials. Two trials conducted in Finland (Levander et al., 1983; Alfthan et al., 1991) comparing the responses to supplementation by 200µg selenium per day in healthy subjects have been performed. Selenoprotein P values before the introduction of selenium-enriched fertilisers plateaued within two weeks (Persson-Moschos et al., 1998). In a second trial after the introduction selenium-enriched fertilisers, no significant increase in selenoprotein P levels were observed nor were there any differences between groups given different forms of selenium (Persson-Moschos et al., 1998). Different modes of selenium supplementation have been reviewed (Johnsson et al., 1997).

6.3 Summary

Selenoprotein P has higher correlation to plasma selenium and extracellular GSHPx at low selenium status than at high selenium status;

At normal selenium status, selenoprotein P usually has a higher correlation to plasma selenium than extracellular GSHPx.

Selenoprotein P and extracellular GSHPx vary markedly in subjects from different regions and with different diseases.

There is limited data on the distribution of selenium among selenoproteins in human tissues other than plasma. Therefore, the immunochemical assay approach should be extended to determine the concentration of selenoproteins in other tissues.

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7. Fatty Acid Composition in Human Tissues as Markers for Dietary Fatty Acid Composition

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The fatty acid composition of our diet will directly affect the lipid composition of most tissues in the human body including all cell membranes, circulating lipoprotein lipids and storage fat. The fatty acid composition of the body tissues has profound effects on a number of vital functions such as gene expression, metabolic signalling, eicosanoid production, membrane properties and energy expenditure.

As dietary surveys are time consuming, expensive and notoriously flawed there is an ongoing search for some kind of objective markers to document dietary quality in a more objective way. The dietary fatty acid composition is to a large extent reflected in the body tissues. Analysis of the fatty acid composition of circulating plasma lipids, erythrocyte membrane lipids or the fatty acid composition of subcutaneous adipose tissue triglycerides offer possibilities to get objective information concerning some aspects of the dietary fat composition. The fatty acid composition can be very precisely measured by gas liquid chromatography using capillary columns. Detailed descriptions of the methodologies used are easily accessible in recent references.

As the turn over times of the lipids in plasma, red cell membranes and adipose tissue are very different the fatty acid composition of these tissues can give us some information on the average dietary fatty acid composition during weeks, months and half a year to a year before the sample was taken, respectively. Depending on the aim of the study a relevant tissue can be chosen. Thus, a measurement of the serum lipid fatty acid composition is usually chosen to monitor changes of the dietary fat composition during intervention studies while the fatty acid composition of adipose tissue triglycerides may be advantageous to study in a cross sectional, epidemiological study.

Although the fatty acid composition of different tissues can be precisely documented this does not mean that the fatty acid composition is directly mirroring the dietary fatty acid composition. While there are

rather strong relationships between the content of essential fatty acids in the diet and corresponding proportions in the tissues, these relationships are much weaker or absent for fatty acids which can be synthesised in the body. Thus, while strong relationships can be seen for e.g. linoleic acid (18:2 n-6) as well as long chained n-3 “fish” fatty acids in the diet, the relationships between saturated fatty acids in the diet and corresponding fatty acids in the tissues are weaker and the relationship between the major dietary fatty acid, oleic acid, and the corresponding proportion in the body tissues is usually very weak or absent. Other, usually minor, fatty acids in the diet which are not at all, or to a very limited extent, synthesised in the body are also well suited for studies by analyses of body tissue fat composition. This is true for different trans fatty acids, but also for other minor dietary fatty acids like some fatty acids with uneven chain length (e.g. pentadecaenoic acid 15:0 and heptadecaenoic acid 17:0). These latter fatty acids can not be made in the body and are nearly exclusively formed in the intestine of ruminants by specific bacteria. As they are found only in fat from ruminants they have proved to be useful markers for intake of dairy fat and can be used as tools in observational studies or to monitor dietary fat intake during interventions.

Although the fatty acid composition can be measured accurately the use of this technique and the interpretation of the data have to be done with care. The proportions of specific fatty acids differ in different tissues and do not directly reflect the proportions in the food. The proportions of the different fatty acids included in the chromatogram are highly correlated. A pronounced change of one major fatty acid may secondarily affect the proportions of the other fatty acids, as the total sum of all identified fatty acid peaks per definition is 100 %.

For the interpretation of the fatty acid spectra it is also important to remember that the fatty acid composition is not only influenced by dietary fat intake but also by endogenous synthesis and metabolism of fatty acids, genetic disposition and possibly early programming. Thus, while the proportion of linoleic acid (18:2 n-6) or docosahexaenoic acid (22:6 n-3) are rather closely related to the dietary intake the proportion of e.g. the more long chained and unsaturated fatty acids of the n-6 series is mainly reflecting endogenous metabolism of linoleic acid and not dietary intake.

In spite of several shortcomings, studies of the fatty acid composition of human tissues can give us much useful information. The biological reproducibility over time is surprisingly good, e.g. regarding the fatty acid composition of the serum cholesterol ester fatty acid composition in free living populations on their habitual diet. Using fatty acid composition in body tissues as dietary markers can be very useful in epidemiological cross sectional or prospective studies to investigate the relationships between dietary fat quality and the prevalence of disease or evaluate the importance of dietary fat quality in the development of different disor-

ders. By monitoring the plasma fatty acid composition it is possible to get information on patient adherence to the diet during intervention trials including changes of dietary fat quality. Important information can also be achieved about endogenous fatty acid metabolism or gene-nutrient interactions in the development of major diseases.

8. Iron Biomarkers in Children

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Worldwide, iron deficiency (ID) is the most common micronutrient deficiency. That is because certain population groups have high iron requirements, but insufficient iron intake or absorption to meet their needs. The relative iron requirement is greatest in infants and young children (aged 6-24 months) and adolescents (aged 12-16 years), which is explained by the food intake and rapid growth rate in these age groups.

8.1 Iron Absorption and Bioavailability

Iron homeostasis is maintained through absorption. Compared to many nutrients, iron is poorly absorbed, and another feature of human iron metabolism is the absence of an excretory pathway. The absorption in the intestine depends on the iron status of the body, the amount and type of iron in the diet and the composition of the meal (1).

In foods, there are two types of iron, haeme iron and nonhaeme iron. Haeme iron is mainly found in meat, where it accounts for about half the total iron. Iron in grains and other plant-derived foods is nonhaeme iron. Haeme iron is generally more efficiently absorbed than nonhaeme iron and is not subjected to the same regulation mechanisms. Absorption is increased in subjects with iron deficiency compared with normal subjects, i.e. it depends on body iron stores (2). Usually about 25% of the total amount of haeme iron is absorbed from food and in general not affected by food components, although reduced bioavailability of haeme iron due to interaction with calcium has been reported (3). The absorption of nonhaeme iron depends on the composition of meals. The absorption is enhanced by ascorbic acid, with the most pronounced effects at moderate intake, or up to 100 mg/day (4), and an unknown factor (MFP factor) found in meat, fish and poultry, which is thought to be cysteine-containing peptides, possibly with more factors involved (5). The absorption of nonhaeme iron is inhibited by phytates and its metabolites, iron-binding polyphenols like the tannins, and calcium (3,6,7,8). Manganese in larger amounts than can be obtained from food can compete with iron for absorption in the intestines (9). It appears that zinc does not compete with iron for absorption, at least not in the amounts usually obtained from

food (7), but interactions between iron and copper have been documented (10,11).

The influence of enhancing and inhibiting factors on iron absorption appears to be most marked in single meal studies, while studies of whole diets show varying results. Subjects with poor iron status seem to benefit from a diet rich in factors enhancing iron absorption.

8.2 Iron Biomarkers

Several indicators are used for detection of iron deficiency. S-ferritin is considered to be the best single indicator of iron status and is also the most widely used (12). It gives a good reflection of the size of the iron stores in the absence of infection and inflammation. WHO recommends 12 µg/L as the cut-off for children below the age of 5 years and 15 µg/L for males and females above 5 years (13). These cut-offs are built on global criteria that also include other races and countries, including developing countries. Lower s-ferritin values have been used for infants and young children, e.g. 10 µg/L in the Euro-Growth study (14). This reference value was also used for US children up to 5 years in a nation-wide study (15). Other indicators of iron status are s-transferrin (total iron binding capacity, TIBC), transferrin saturation (s-iron/TIBC) and s-TfR (serum transferrin receptor). While the transferrin saturation is less useful for detecting ID due to diurnal variations in s-iron, s-TfR is considered to be the single most sensitive indicator of functional iron depletion (16). The transferrin saturation is, however, very useful as a screening variable for hereditary haemochromatosis (13). In recent years s-TfR/s-ferritin has also been used as an indicator of iron status in scientific studies (17,18). Free erythrocyte protoporphyrin (or ZPP) and MCV (mean cell volume) become abnormal relatively late in the development of ID (16), so alone they would be relatively insensitive indicators of iron deficiency and are not often used in studies on iron status.

Anaemia is defined as a reduced concentration of Hb. According to WHO, Hb <110g/L should be used to diagnose anaemia in infants and children from 6 months up to 5 years of age, and 115 g/L for children up to 11 years (13). However, reference values for Hb (and also for other iron status variables) are poorly validated in infants and young children. In clinical practice as well as in research, the commonly used cut-off levels to identify iron deficiency and iron deficiency anaemia in infants (Hb <110 g/L and s-ferritin <10-12 µg/L) are in fact extrapolated from older age groups and there are indications that they may not be appropriate (19). Emond et al. suggested for 8-month-old infants a cut-off of 97 g/L for Hb (20). Others have used 105 g/L (21,22) and 100 g/L (19,23). Iron deficiency anaemia is defined as anaemia by Hb below given cut-off, along with abnormal iron status indicators. The number of iron status

indicators used for diagnosis varies, as well as their cut-offs used for iron deficiency. Sometimes only s-ferritin is used, but some studies have used the approach that when two out of three iron status indicators are below (or above) a given cut-off together with a Hb below cut-off, the individual is diagnosed with iron deficiency anaemia.

8.3 Studies on Iron Status in Icelandic Children

Iron deficiency anaemia in early childhood is known to have serious consequences for learning ability and mental and motor development (24,25). A full term infant of normal weight usually has enough iron stores for the first six months of life (26), but after that iron from dietary sources play increasing role in supplying iron for the body. Birth weight has been positively associated with iron status at 12 months (27) and even up to 18 months (28), and fast growth during the first year has been negatively associated with iron status (23,29).

The official recommendation on infants' diet in Iceland has been similar to that of most countries, however, Iceland earlier differed from most countries in its recommendation on weaning: cow's milk after the age of six months (30,31). Cow's milk is known to negatively influence the iron status in infants (32). It was therefore of great concern and theoretical interest to investigate how the frequent use of cow's milk for infants in the latter half of the first year affected the iron status at the age of 12 months (33).

Effects of growth and food intake on iron status at the age of 12 months, 2 years and 6 years has been investigated in Iceland, a population of high birth weight, high frequency of breastfeeding and at the time of the infant study with high intake of cow's milk during the weaning period. The findings of the studies have already altered the recommendations in Iceland and to the development of a special milkproduct for infants from 6 months of age and children up to 2 years.

The studies are based on two random cohorts, longitudinal study on infant nutrition (n=180) and a cross-sectional study of two year olds (n=130), >70% of eligible participants were involved in the iron status surveys at age 1, 2 and 6 years.

8.4 Infants

Every fifth one year old child was iron-deficient (SF<12µg/L and MCV<74fL) and 2.7% were also anaemic (Hb<105g/L). 41% of the one year old children were iron depleted (<12 µg/L). Higher weight gain from 0-12 months was seen in infants who were iron-deficient at 12 months (6.7±0.9kg) than in not iron-deficient infants (6.2±0.9kg)

($P=0.050$). Iron-deficient infants had shorter breast-feeding duration (5.5 ± 2.3 months) than not iron-deficient (7.9 ± 3.2 months) ($P=0.001$). Iron status indices were negatively associated with cow's milk consumption at 9-12 months, significant above 460g/day, but were positively associated with iron-fortified breakfast cereals, fish and meat consumption.

8.5 2-Year-Olds

Nine per cent of the two years old children were iron-deficient and 1.4% were also anaemic, while 27% of children were iron-depleted. Half of children consuming >500 g cow's milk/day ($n=10$) were iron-deficient, while one child in 58 consuming <500 g cow's milk/day had iron deficiency ($P<0.001$).

8.6 6-Year-Olds

No 6 year old children had iron deficiency or iron deficiency anaemia, but 16% were iron-depleted. Haemoglobin concentration at 6 years was negatively associated with length gain from birth to 12 months and proportional weight gain from birth to 12 months was higher among children with $SF<15$ $\mu\text{g/l}$ at 6 years than those with $SF\geq 15$ $\mu\text{g/l}$.

The studies showed that poor iron status among 12 months old was common in Iceland during 1996-1997, apparently related to high cow's milk consumption in the weaning period. The use of cow's milk at this time may also explain that longer duration of breastfeeding was found for those who had better iron status at 12 months. However, breastfeeding is also related to slower growth which can protect from worsening iron status (33,34). More than 500 ml/day of cow's milk was also found to have negative impact on the iron status among 2 years olds.

In conclusion, cow's milk above 500g/d should be avoided up to 2 years of age. Low iron status at 1 and 2 years might lead to slower growth up to six years of age. Faster growth in infancy and early childhood is related to lower iron status at 6 years.

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9. Bioavailability of Selected Flavonoids and the Usefulness of their Plasma Concentrations as Biomarkers of Intake

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9.1 Introduction

Flavonoids are polyphenolic compounds obtained from various fruits and vegetables. To date, over 6000 flavonoids have been identified, however, a much smaller number are important from a dietary point of view. One of the most studied flavonoids is quercetin, which is a compound that exhibits a wide range of different bioactivities *in vitro*. Quercetin is obtained from various fruits and vegetables, with onions, tea, and berries being particularly good sources. The flavanones hesperetin and naringenin are among the most important flavonoids from a quantitative intake point of view. Their intake is high because of the common use of citrus fruits and juices, which are their main dietary sources. For a recent review on the flavonoids see ref. 1. We developed analytical methods for the analysis of quercetin, hesperetin and naringenin in human serum (2) and studied their bioavailability. We also investigated their use as biomarkers of intake.

9.2 Quercetin

To evaluate the bioavailability of quercetin and its use as a biomarker of its intake, we analysed plasma quercetin from three human intervention studies. The first study was a two-period, single dose pharmacokinetics study performed in a cross-over design (3). Three different doses of quercetin and quercetin-3-rutinoside (8, 20 and 50 mg expressed as quercetin equivalents), similar to doses attained from a normal diet, were given in capsules to healthy volunteers. Quercetin was absorbed from both the aglycone and the rutinoside, and mean bioavailability was similar from

both sources. The elimination half-life of quercetin was fairly long. Bioavailability, evaluated by calculating area-under-curve (AUC) values, increased dose-dependently for both compounds. In the second study, 40 men consumed either 100 g/day of berries (lingonberries, bilberries and black currants) for two months, or their normal diets (4). Blood samples were collected at -2, 0, 2, 4 and 8 weeks. Plasma quercetin was 30-50 % higher in the subjects consuming berries compared to the control group, which indicates that quercetin is bioavailable from berries. In the third study, we investigated the effect of a diet rich in fruits and vegetables on plasma quercetin. In this study, 77 volunteers were allocated to different diets containing either 850 g of various fruits and vegetables daily or 170 g of them (5). After six weeks, plasma quercetin increased to 170 % compared to baseline in the high fruit and vegetable group. In the group consuming less fruits and vegetable, plasma quercetin decreased to 70 % compared to baseline (changes were statistically significant).

The results of the above-mentioned intervention studies show that quercetin is absorbed from capsules, berries and normal diets. Furthermore, plasma quercetin seems to reflect changes in intake and there seems to be a dose-response in these changes. Therefore we conclude that plasma quercetin can be used as a biomarker of its intake. We recently tested whether fasting serum quercetin can be applied as biomarker on a population level as well, with good results.

9.3 Hesperetin and Naringenin

Bioavailability of hesperetin and naringenin were studied in a pharmacokinetic study and a dietary intervention study. In the kinetic study, healthy volunteers ingested between 400 and 760 ml of orange juice or grapefruit juice once (6). Both compounds were well absorbed from the juices and the maximum plasma concentrations were relatively high (several $\mu\text{M/L}$). Mean urinary recovery for naringenin was 30 % from grapefruit juice and 1 % from orange juice. The corresponding value for hesperetin from orange juice was 5 %. The half-lives of the compounds were relatively short. In the dietary intervention study, healthy female volunteers consumed either a diet rich in fruits and vegetables, including citrus, or a diet low in such foods for 5 weeks (7). In the high vegetable and fruit group, the daily consumption of citrus was one glass of orange juice, one half of an orange and half a mandarin. After five weeks on this diet, hesperetin was detectable in fasting plasma of half of the subjects and naringenin in one fourth of the subjects. After the low fruit and vegetable diet, which did not include citrus, the compounds were not detectable in plasma. The results of the above-mentioned studies indicate that when attempting to evaluate the bioavailability of flavanones, multiple

blood-samplings, possibly combined with urinary data, are the best choice.

8.4 Conclusion

We conclude that the flavonoids quercetin, hesperetin and naringenin are bioavailable from the diet. Plasma quercetin appears to be a good biomarker of its intake, while for the flavanones, repeated blood and/or urine samples are preferred. Finally, it is emphasized that there is marked interindividual variation in bioavailability of flavonoids, as well as other polyphenols. Therefore, it is important to monitor bioavailability in human intervention studies investigating their effects. Poor bioavailability in some individuals may otherwise confound the results.

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10. Flavonoids –a Biomarker for Fruit and Vegetable Intake

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10.1 Introduction

Epidemiological studies have provided evidence that a high intake of fruits and vegetables is associated with a decreased risk of heart disease and some human cancers [1-3]. Current dietary assessment methods used in epidemiological studies are associated with random and systematic measurement errors, and therefore more objective ways are needed to more accurately assess the intake of foods important to human health. Reliable biological makers for the intake of vegetables or fruits are needed to get a better insight in the health effects of vegetables and fruits.

Flavonoids are a group of polyphenolic compounds ubiquitously found in commonly consumed fruits and vegetables. The flavonoids are therefore an obvious choice of compounds for the development of a new biomarker for intake of fruits and vegetables. The use of such a biomarker will provide a deeper insight in the nature of a group of dietary components that are may exert disease preventive effects. Furthermore, the individual flavonoids represent specific fruit and vegetable groups, e.g. phloretin occurs mainly in apple, the citrus flavonoids in citrus fruits etc., allowing the investigation of the protective potential of specific food items.

The daily intake of flavonoids is between 50 to 150 mg, which is comparable to the intake of other natural antioxidants, such as vitamin C (~ 80 mg/day), vitamin E (8.5 mg/day) and β -carotene (~2 mg/day) [4]. The widespread occurrence of flavonoids in the human diet and their ability to enter the systemic circulation, makes the determination of a representative group of flavonoids in biological samples interesting, not only for their potential health protective effects, but also as a very promising candidate to more accurately assess the intake of fruits and vegetables.

Plasma carotenoids have traditionally been used as markers for fruit and vegetable intake due to the ubiquitous distribution of these pigments in plants. However, plasma carotenoids have often shown only poor correlations with the intake of fruits and vegetables, probably due to indivi-

dual variations in response to intake and other dietary factors influencing digestion and absorption [5].

In order to analyse low levels of flavonoids in human urine and plasma samples, a very selective and sensitive LC-MS assay was recently developed in our laboratory [6]. By this methodology we are able to analyse for 7 different dietary flavonoids simultaneously in urine from human subjects. The flavonoids that are determined belong to the group of *citrus flavonoids*; (naringenin, and hesperetin), *flavonols*, widely distributed in e.g. onion, apples, tea, kale and wine (quercetin, kaempferol, isorhamnetin and tamarixetin), and a *dihydrochalcon*, primarily found in apples (phloretin).

Prior to the LC-MS analyses, the samples are enzymatically hydrolysed to liberate the flavonoids in urine from glucuronic acid and sulphate-ester derivatives [6].

The methodology was applied on urine samples from 94 subjects on their habitual diet and after eating a diet either high or low in fruits, berries and vegetables for 6 weeks [7]. In the intervention period with controlled dietary intakes, we found highly significant differences between the urinary excretion of all flavonoid aglycons included in the assay on the high fruit and vegetable diet compared to the low. The amount of flavonols (quercetin, kaempferol, isorhamnetin and tamarixetin) excreted in urine was much lower than the amounts of the citrus flavanones (naringenin and hesperetin) and of the chalcone phloretin, found in high amounts in apples. However, the diet did not contain higher amounts of citrus fruits or apples than of other fruits, berries and vegetables, so it seems as if these flavonoids, phloretin and the citrus flavanones are excreted and thus presumably also absorbed to a higher extent than the flavonols.

The correlation between the habitual intake of fruits and vegetable as determined by three days of food registration, with the total excretion of flavonoids was 0.35, $p < 0.001$. Again the excretion of the flavonols was much lower than of the other flavonoids included in the assay, and quercetin showed the poorest correlation with the habitual intake of fruits and vegetables. Thus, this study suggests, that other flavonoids from the diet may be more important to our health than the flavonols, and that it is quite unlikely that the beneficial effects of fruits and vegetables is due to quercetin, given this poor correlation. The correlation between intake of fruits and vegetable and total plasma carotenoids was only $r = 0.213$, and only $r = 0.157$ for β -carotene alone. Other studies are however, necessary to confirm these observations, before definitive conclusions can be drawn.

We have further validated the use of flavonoids as biomarker for intake of fruits and vegetables in two human intervention studies. These studies were designed to investigate the sensitivity of the flavonoids biomarker and its ability to distinguish between different intake levels of

fruits and vegetables in healthy human subjects. The first study was performed to evaluate whether urinary excretion of flavonoids could be used as biomarkers for a desired change from the Norwegian average consumption (2 portions pr day) to the recommended consumption of fruits and vegetables (5 portions pr day) [8]. In this study a significantly enhanced urinary excretion was observed of eriodictyol, naringenin, hesperetin, quercetin, kaempferol, isorhamnetin, and tamarixetin. The citrus flavonoids naringenin and hesperetin showed a steep dose-response relationship to dietary intake of fruits and vegetables, whereas the association to eriodictyol, quercetin, kaempferol, isorhamnetin, and tamarixetin was more moderate. The study demonstrated that urinary excretion of dietary flavonoids may be used to assess changes of mixed fruit and vegetable intake corresponding to an increase from the present national intake in Norway to the recommended amount of 5 servings of fruits and vegetables daily [8].

The second study investigated the ability of flavonoid excretion in both 24h and morning urine samples to reflect a low intake and moderate changes in fruit and vegetable consumption [9]. The subjects in this study received either a basic diet containing no fruits and vegetables, or the basic diet supplemented with 300g or 600g fruits and vegetables. The total excretion of flavonoids in 24-h urine samples increased linearly with increasing fruit and vegetable intakes ($r = 0.86$, $P < 1 \times 10^{-6}$). The total excretion of flavonoids in morning urine also increased, but the association was weaker ($r = 0.59$, $P < 0.0001$) [9].

10.2 Conclusion

Using new and sensitive methodologies it is now possible to determine low levels of flavonoids present in urine from normal subjects on their habitual diet [6]. This may be a very strong tool to provide more insight in the health protective effects of flavonoids, since e.g. flavonoid excretion levels could be correlated to disease incidence in cohort or case control studies. Furthermore, with the uncertainties related to food registration in epidemiological studies a valid biomarker for fruit and vegetable intake is greatly warranted. The flavonoids are present in almost all fruits and vegetables, and they may therefore be useful as a marker for fruit and vegetable consumption as indicated by recent findings.

There are however, several challenges associated with the use of flavonoids as nutritional biomarkers. Uptake of flavonoids is affected by the gut microflora, and a substantial inter-individual variation in uptake has been observed [10, 11]. Although the flavonoid uptake differs between subjects, the flavonoid biomarker reveals the true systemic exposure to flavonoids of individuals, which are the most central parameter in relation to the disease protective effect of dietary flavonoids. Despite these inter-

individual variations the flavonoid biomarker may still work in larger cohorts and thus be a useful alternative to current food registration methods or a supplement to these methods to validate and strengthen the information on fruit and vegetable intakes.

Future studies are needed to further investigate the usefulness of flavonoids as a biomarker for intake of fruits and vegetables, but present data demonstrate that the urinary excretion of flavonoids is a promising biomarker for the intake of fruits and vegetables.

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Concluding Remarks and Summary

Main goals in population health surveys are to monitor trends in disease risk factors in the general public and risk groups as well as to assess nutrient intake. Nutritional epidemiology deals with associations between nutrients and disease risk. Traditionally the 24-hour recall in combination with a food frequency questionnaire to collect food consumption data has proven to be a cost-benefit method in most instances. There are, however, nutrients for which dietary methods are less good. In these instances biomarkers may be a better or alternative approach. Biomarkers are not without certain limitations. One of the basic requirements of a biomarker is that it reflects the intake of a nutrient. Some do quite accurately, like beta-carotene, but in most instances the relationship is limited to some extent by homeostasis, metabolism, age, gender and nutrient interactions.

Presentations and discussion at the seminar focused on research dealing with nutrients important in the Nordic Countries. Estimation of the dietary intake is notoriously inaccurate for iodine, sodium, iron, folate and vitamin D. For monitoring trends, risk group behaviour and inter-country estimation of nutrient intake, the main goals are quantitative intake of vegetables and fruit, fat quality, sugar and vitamin D.

Summary of Presentations

There is current interest to use biomarkers to estimate intake of fruits, vegetables, dairy products and fish. Suitable combinations of carotenoids and flavonoids may prove useful to estimate the intake of fruits and vegetables. Odd numbered fatty acids may be good biomarkers for dairy products and adipose tissue docosahexaenoic acid for total fish intake.

Severe goitre does not exist any more in the Nordic countries, but toxic and non-toxic goitre can still be found in the elderly in Denmark. Urinary iodine excretion is the preferred biomarker either as a 24-hr sample or spot samples expressed in various ways. The biological function of iodine may be measured from serum as thyroglobulin, thyroid-stimulating hormone or thyroxine concentrations.

The intake of vitamin D is especially low in the Nordic countries since exposure to the sun is limited. In the new Nordic Nutrition Recommendations from 2004 the recommended dietary intake has been increased from 5 µg to 7.5 µg, which on average is not reached in any of the Nordic countries.

There is consensus that 25OHD in serum is a good biomarker for exposure of vitamin D from both sun and diet. There are, however, methodological issues involved- whether to measure specific vitamin D metabolites by HPLC or 25OHD with antibody based commercial kits.

Presently the vitamin C deficiency disease scurvy is very rare in the Nordic countries. Foods rich in vitamin C are available throughout the year and a low intake is due to bad food choices. The most common biomarker for vitamin C is plasma, but leucocyte vitamin C concentrations are also used because they reflect better body stores. Pretreatment of plasma and adequate storage is an absolute prerequisite for accurate data.

Usual biomarkers for selenium are plasma and whole blood selenium concentrations. More than 20 specific selenoproteins have been identified in animal tissue out of which extracellular glutathione peroxidase and selenoprotein P are found in human plasma. They are both considered markers of the biological function of selenium. A characteristic of both proteins is that the relation between selenoprotein and dietary intake of selenium grows weaker as the intake of selenium increases over the normal intake range. At a normal selenium status selenoprotein P is usually a better biomarker than glutathione peroxidase.

The fatty acid composition of the diet affects directly the lipid composition of most tissues in the human body. As dietary surveys are time consuming, expensive and dependent on data bases, objective biomarkers for fatty acids are needed to document dietary quality. The long chained n-3 fatty acids reflect well fatty acids of fatty fish. Recent research is focussed on fatty acids with uneven chain length, which are synthesized in the intestine of ruminants and have been proven to be useful biomarkers of dairy fat. Interpretation of fatty acid biomarkers is a challenge due to the influence of endogenous synthesis, metabolism and genetic disposition.

Worldwide iron deficiency is the most common micronutrient deficiency. The relative iron requirement is greatest during rapid growth up to adolescent age. Several types of biomarkers are available. Serum ferritin is considered the best single indicator of iron status reflecting body iron stores. Care must be exercised in interpretation due to its sensitivity to infection and inflammation.

Flavonoids are a large class of polyphenolic compounds found ubiquitously in plants. Their biological contribution to human health is still unclear, but their value as biomarkers for intake of vegetables and fruits is promising. Some flavonoids are specific for certain foods, eg. phloretin occurs mainly in apples and hesperetin and naringenin in citrus fruits. The uptake of flavonoids is affected not only by the type of flavonoid but also by the gut microflora. Recent developments in instrumentation and methods for measuring flavonoids and research of their metabolites promise a rapidly developing field. Under validation are many biomarkers of plant foods measured both from plasma or urine.

Sammandrag

Huvudmålen i populationshälsoundersökningar är att utreda trender i riskfaktorer för sjukdomar i allmänna befolkningen och i riskgrupper såväl som att uppskatta intaget av födoämnen. Nutritionell epidemiologi handlar om samband mellan födoämnen och risken för sjukdomar. Vanligtvis har 24-timmars recall metoden kombinerad med konsumtionsfrekvensen av livsmedel visats sig vara en bra metod ur cost-benefit synpunkt. Det finns dock födoämnen för vilka kostmetoder är mindre bra. I dessa fall kan användning av biomarkörer vara ett bättre alternativ. Biokemiska indikatorer av födoämnesintag kallas biomarkörer.

Användning av biomarkörer är inte problemfritt. En förutsättning för en biomarkör är att den bör återspegla intaget av ett födoämne. Somliga gör det noggrant som plasma betakaroten, men i de flesta fall är sambandet mindre bra på grund av homeostas, ämnesomsättning, ålder, kön och interaktioner mellan födoämnen mm.

Initiativet till ett seminarium om biomarkörer för intag av födoämnen fattades av Arbetsgruppen för Kost och Näring (NKE). Expertseminariet arrangerades i Helsingfors den 17-18 September 2004. Syftet var att diskutera och byta information om forskning kring biomarkörer. Föredragen och diskussionerna vid seminariet handlade om forskning särskilt viktig inom de Nordiska länderna. Uppskattning av intaget för jod, natrium, järn, folat och D vitamin är som känt speciellt svårt. För uppföljning av trender, risk grupper kostvanor och jämföring av födoämnesintag mellan länder är huvudintresset det kvantitativa intaget av vegetabilier och frukt, fettkvalitet, socker och D vitamin.

Denna rapport innehåller sammanfattningar av föredrag presenterade under seminariet. Rubrikerna varierar från nutritionell epidemiologi till kliniska interventioner till validering av biomarkörer.

Program of the Seminar

Nordic Biomarker

Biomedicum, Helsinki 17-18.9.2004

17.09 Friday 18.00 to 21.00

Get-together, refreshments

Speaker

20.00 Candle-light lecture

Biomarkers – a holistic view
M Mutanen, FI

18.09 Saturday

Chair G Alfthan FI

09.00 Biomarkers reflecting nutritional intake
HJ Powers, Sheffield
UK

9.30 Genetic biomarkers and predisposition tests
JT Salonen, FI

10.00 Biomarkers for intake of foods
LF Andersen N

10.30 Coffee

11.00 Critical nutrients in Nordic populations 15-20 min
Chair R Andersen DK

Iodine
LB Rasmussen DK

How to validate vitamin D
J Jakobsen DK

Water soluble vitamins-vitamin C
K Nyssönen FI

12.15-13.15 Lunch

Chair W Becker SE

Selenium status-selenium proteins

B Åkesson SE

Fatty acids

B Vessby, SE

Iron biomarkers in children

I Thorsdottir ICE

14.30 Future prospects:

Chair T Leth N

Plasma quercetin and hydroxycinnamates as biomarkers of intake I Erlund, FI

Flavonoids: a biomarker for fruit and vegetable intake
SE Rasmussen DK

15.15 Coffee

Chair A Aro FI

16-17 Workshop “Which are the good biomarkers –meaningful

for the Nordic countries?”

What demands on biomarkers are set by nutritionists
L. Johansson N

and epidemiologists?

The seminar is organized by KTL and sponsored by the Nordic Council of Ministers.