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DETERMINATION OF VITAMIN REQUIREMENTS IN HUMAN NUTRITION

DELIA M. FLINT,* MARK L. WAHLQVIST* and ELIZABETH A. McLENNAN*

Summary

Estimates of human vitamin requirements serve as a basis for dietary allowances. Where frank vitamin deficiency has been found, this has enabled some assessment of vitamin requirements, but ethical considerations limit controlled studies in man. Methods to assess particular vitamin functions should allow sub-clinical evaluation of vitamin requirements which will probably vary according to the function. Examination of the distribution curve of vitamin levels in tissues, blood or urine for a population together with information about nutrient intakes in that population should also assist the evaluation of vitamin requirements. Factors which may affect requirements include age, sex, physical activity, other nutrients, the form of the vitamin and its bioavailability, presence of antivitamins and drugs. Theoretically, balance studies could be helpful in the assessment of vitamin requirements, but adaptation to a lower or higher intake is possible.

I. INTRODUCTION

Vitamins are "chemically unrelated substances that are essential in small amounts for the maintenance of normal metabolic functions but are not synthesized within the body and, therefore, must be furnished from exogenous sources" (Goodhart, 1980). The International Union of Nutritional Sciences recognizes 14 vitamins (IUNS 1975). However, although exogenous sources are mainly necessary, vitamin D can be formed from 7-dehydrocholesterol, vitamin A from carotenoids and niacin from tryptophan; assessment of their requirements is complicated accordingly.

The exogenous sources of vitamin K and biotin include gut microflora as well as food and the former component is difficult to quantitate.

There is no single way to view optimal vitamin nutrition. In evolutionary terms, it is related to survival of the species and reflected man's dietary practice to eat a variety of foods with a range of nutrients. Now the quest is for maximal life span for the individual, as little morbidity as possible and for a sense of well being. It is no wonder, then, that difficulties have been met in efforts to establish requirements (Arroyave 1971; Marks 1975).

Vitamin requirements are used by national nutrition expert committees (Department of Health and Social Security (DHSS) 1979; The National Research Council (NRC) 1980; National Health and Medical Research Council (NH & MRC) 1979). Vitamin allowances include an amount above the estimated requirement to allow for variation between individuals and in the same individual from time to time as well as methodological inadequacies.

* Section of Human Nutrition, Deakin University, Victoria, 3217.

Because of limited information on requirements, especially insofar as bioavailability is concerned, allowances have not been developed for particular vitamins such as vitamin K and biotin.

With some vitamins, such as nicotinic acid (Wahlqvist 1980, McInnis, Wahlqvist and Balasz 1980), effects of therapeutic interest are seen with large intakes. These are considered pharmcological rather than physiological effects. There is increased interest in vitamin analogues, for example of vitamin A, which will allow larger doses to be given without deleterious side effects.

In the final analysis, the requirement for a vitamin may be a judgement about which of its functions should be fully expressed and which of its untoward effects should be avoided, given a reserve capacity to handle a range of intakes and an ability to adapt to the intakes.

II. ASSESSMENT OF REQUIREMENTS

(a) By Overt Clinical Deficiency

When vitamin deficiency is sufficiently severe, typical symptoms and signs are usually evident. The amount of vitamin to prevent or reverse these clinical features is an estimate, probably an underestimate of requirements. For many vitamins there is an heirarchy of emergence of symptoms and signs, so that the amount required to prevent one feature may not prevent another. In volunteers on a vitamin A deficient diet, follicular keratosis appeared ahead of impaired dark adaptation (Hodges and Kolder 1971). The sequence of events for folic acid deprivation is shown in Table 1.

TABLE 1. Experimental folate deprivation in a 35 year old, 77 Kg male.

Days of Deprivation	Signs detectable
22	Serum folate < 3ng/ml (normal > 7ng/ml)
49	Hypersegmentation of nuclei of neutrophils (to be average > 3.35)
95	High urine FIGLU (> 50 mg/ml 12 hr. after 20g histidine)
123	Low erythrocyte folate (< 20ng/ml)
127	Unequivocal macro-ovalocytosis
134	Unequivocal megaloblastic marrow
137	Frank anaemia (erythrocytes < 4.6 x 10^6, haemoglobin < 14g/100 ml).

(Data from Herbert 1967)

(b) By Functional Impairment

As the actions of vitamins are clarified so more possibilities for functional tests of requirements emerge. For example, Bartley, Krebs and O'Brien (1953) were able to promote wound healing with 20 mg ascorbic acid/day, whereas frank scurvy was prevented by 10 mg/day. Bleeding time or in vivo platelet adhesion could be helpful in the evaluation of the haemorrhagic diathesis of ascorbic acid deficiency (Strauss et al 1980). In the case of vitamin A, impaired dark adaptation can be assessed by electroretinography and is evident before xerophthalmia (Sauberlich, Skala and Dowdy 1974).

(c) Depletion of Body Pools

The assessment of a vitamin level in one body pool alone can, ideally, only be taken as an indication of whole body status if steady state conditions apply and if the factors regulating movement from one pool to another are defined. Vitamin depletion in a particular pool due to redistribution may, however, be meaningful if that is the pool relevant to the vitamin function in question.

One way in which tissue or blood levels are used to evaluate requirements is to take an apparently healthy population and determine the distribution of levels which may be unimodal (normal or log normal) or bimodal. With a unimodal distribution 2.5% of the population will be above 2 standard deviations (SD) from the mean and 2.5% below 2 SDs from the mean. It is highly likely that the vitamin intakes of the 95% individuals within the mean ± 2 SD are acceptable and this could be regarded as the requirement range for that vitamin.

Another approach is the balance study. If the intake is known and this is added to until the vitamin is excreted, then the intake above which excretion occurs could be regarded as the requirement. This presumes tissue saturation is optimal status. It also does not take into account adaptation to higher or lower intakes unless extended studies are undertaken.

Particular problems arise in regard to the different vitamins:-

(i) Ascorbic Acid

This can be measured in plasma, white cells, platelets and urine. In utilizing a particular blood ascorbic acid compartment, each with a different turnover rate, a change in dietary ascorbic acid will be evident at different times in each compartment (Strauss et al 1980). In some studies the platelet compartment may not have been separated from the leucocyte compartment.

(ii) Vitamin A

Plasma retinol concentrations will be influenced by retinol binding protein (RBP) and prealbumin concentrations

which may in turn depend on protein (Rodriguez, and Irwin 1976) and zinc (Wahlqvist, Flint, Prinsley and Dryden 1980) nutrition.

Analysis of biological fluids for vitamin levels require methods which are specific and sensitive. Methods may not differentiate between a vitamin and its precursors or metabolites and may require large volumes of plasma or serum. Vitamin A has caused many analytical difficulties. The traditional colorimetric analysis uses a toxic reagent (antimony trichloride), the colour is fleeting, it is non-specific since all carotenoids react, and it is insensitive requiring about 3 ml of plasma. Spectrofluorimetry is a sensitive assay for vitamin A which requires 100 µl of plasma; however at least one carotenoid, identified as phytofluene, a constituent of tomatoes, interferes (Thompson, Erdody, Brien and Murray 1971). Measurement of the fluorescence at two wavelengths and subsequent use of a correction factor may give satisfactory results, but since different correction factors are found phytofluene may not be the only interfering species (Bubb and Murphy 1973).

We have developed a specific and sensitive assay for plasma retinol by High Performance Liquid Chromatography (HPLC) (McLennan, Wahlqvist and Flint, to be published). It requires 200 µl of plasma. The retinol is extracted from plasma with hexane to which has been added an internal standard, n-octyl-α naphthyl urethane, synthesised for the assay. Carotenoids, retinyl ester and other related compounds do not interfere, but it appears that the method can be adapted for these compounds.

As the use of carotenoids as colourings in foodstuffs grows and as a possible protective role for vitamin A against certain neoplasias emerges (Chytil and Ong 1978), a specific and sensitive assay for plasma retinol becomes more important.

(iii) Thiamin

This is one of the most common and economically important nutrient deficiencies seen in Australia (Wood, Breen and Pennington 1977), yet how intakes related to deficiency syndromes has not been clear (Wood, Breen and Pennington 1975). A recent double blind study of partial thiamin restriction and repletion diets by Wood et al (1980) in Melbourne has provided guidelines under experimental conditions for assessment of thiamin status in population groups. For the blood, thiamin status was measured as erythorocyte transketolase activity; the method specified by the Interdepartmental Committee on Nutrition for National Defence (1963) was used to measure urinary excretion of thiamin.

(iv) Vitamin K

At the present time it is only possible to assess the status of this vitamin by its functions, in particular its role in the formation of coagulation factors (Olson 1980). However, NMR or radioimmunoassay may allow plasma vitamin K to be determined in the near future.

111. FACTORS AFFECTING REQUIREMENTS

For requirements to be meaningful, the circumstances of their assessment must be well-defined.

(a) Age

Infants and growing children have high vitamin requirements per unit body weight compared with adults. Blood ascorbic acid concentration tends to be lower in older than younger adults which may be physiological (Irwin and Hutchins 1976). Very little is known about vitamin requirements in old age and longitudinal studies are necessary to distinguish changes due to ageing from other age-related changes. Vitamin deficiency is however more common in old age (Flint et al 1979, Exton-Smith 1980).

(b) Sex

There appears to be a physiological difference in the metabolism of ascorbic acid in the two sexes (Burr et al 1974; Flint et al 1979) but little is known about actual requirements which may not be different.

(c) Other Food Constituents

(i) Vitamins

In vitamin B_6 deficiency, the conversion of tryptophan to niacin in humans is impaired, which therefore increases the need for dietary niacin (Miller and Linkswiler,1967).

(ii) Non-vitamins

The precursors of vitamin D, vitamin A and niacin could be regarded as non vitamins. The quantitative requirement for vitamin D is not well defined because it has not been possible to estimate the amount of vitamin D produced by the action of sunlight on the provitamin in the skin. The amount formed is dependant on a number of variables including length and intensity of exposure and colour of skin (National Research Council 1980).

In general, increased dietary intake of polyunsaturated fatty acids requires an increased vitamin E intake, met in part by the positive association between degree of unsaturation of vegetable oils and their tocopherol levels (Horwitt 1960).

Vitamins A and D require a moderate amount of fat for their absorption and therefore a very low fat diet could lead to a deficiency of these vitamins.

Thiamin requirement is closely related to energy intake because of its essential role in carbohydrate metabolism (Sauberlich, Herman and Stevens 1970).

Macrocytic anemia due to folate deficiency frequently develops in alcohol abusers. Decreased consumption, increased requirement and decreased absorption all appear to play a part in the development of thiamin deficiency in alcoholics. A study by Fazio, Flint and Wahlqvist (1980) indicated that ethanol may reduce the availability of ascorbic acid from food and predispose to ascorbic acid deficiency.

Vitamin A utilisation is closely related to the quantity and quality of dietary protein (Rodriguez and Irwin 1976).

The requirement for vitamin B_6 in man as in animals is increased when high protein diets are consumed and also when excessive amounts of leucine are consumed (Gopalin and Rao 1975).

(iii) <u>Anti-vitamins</u>

Thiaminases in food such as raw fish can increase thiamin requirements. Amprolium, a molecule structurally analogous to thiamin, has been used extensively in poultry industry as a feed additive to control coccidiosis, is a thiamin antagonist (Read 1978). Avidin in raw eggs is a biotin antagonist.

(d) <u>Intestinal Microflora</u>

There is some evidence that the requirements for some of the vitamins (Holtzel and Barnes 1966) are met mainly by intestinal bacterial synthesis e.g. biotin and vitamin K. Vitamin K deficiency can occur in the newborn before establishment of the intestinal flora.

(e) <u>Physiological State</u>

There is an increase in vitamin requirements during pregnancy and lactation because new tissue is laid down and the vitamin quality of the milk is dependant on the maternal vitamin intake.

During pregnancy plasma ascorbic acid levels fall, which may in part be due to plasma volume change and in part to physiological increased demands (Rivers and Devine 1975).

Folic acid turnover during pregnancy and lactation is increased. There is evidence that folic acid supplementation during pregnancy reduces perinatal complications (Rothman 1970).

During lactation the requirement for riboflavin is assumed to increase by at least the amount which is secreted daily in the milk (Brzezinski, Bromberg and Braun 1952).

Information on vitamin B_6 requirement during pregnancy is equivocal (Dempsey 1978).

Additional vitamin A is necessary for foetal development and liver storage, maternal formulation of colostrom and for lactation (Rodriguez and Irwin 1976).

Most studies suggest that there is a general relationship between the customary vitamin intake of lactating mothers and the vitamin content of the milk. However, little is known about the intake required to provide an "adequate" vitamin supply in the milk and to maintain optimal maternal nutrition.

(f) Bioavailability

Vitamins may not be available for absorption if linkages cannot be broken by mammalian digestive enzymes.

There are several forms of folic acid. Metabolic balance studies in man indicate intestinal absorption of 50% to 75% of hepatoglutamyl folacin (Butterworth, Baugh and Krumdieck 1969). Differences also exist in the relative availability of folacin measured in different foods because of the presence of conjugase inhibitors, binders and other unknown factors (Tamura and Stokstad 1973). The bulk of evidence suggests that 25% to 50% of dietary folacin is nutritionally available.

Most of the niacin in maize is unavailable as it is in the form of nicotinyl esters which are not hydrolyzed on digestion (Kodicek 1962). The traditional method of making tortillas from milled maize grain which has been steeped overnight in lime water in Mexico releases free nicotinic acid.

(g) Drugs

Many drugs can cause nutrient deficiency, and the drug interactions with vitamins has been extensively reviewed (Symposium, The Nutrition Society 1974). Table 2 summarises the general effects of some common medicinal agents on vitamin status.

TABLE 2 General effects of some common medicinal agents on vitamin status.

Medicinal agent	Effect on vitamin status	Possible mechanism
Hypocholesterolaemic agents	Decreases available fat soluble vitamins, vitamin B_{12} and folic acid.	increases excretion of bile acids and hinders fat absorption.
Anticonvulsant and oestrogens	Decreased absorption of dietary folic acid	Interferes with polyglutamate conjugase.
Amphetamines	General reduced availability of vitamins	Appetite suppression
Colchicine	General reduced availability of vitamins	Non-specific decreased absorption.
Antimicrobials	Decrease absorption of fat soluble vitamins, vitamin B_{12}, folic acid	Binds fatty acids and bile acids which may cause steatorrhoea.

(Data from Hartshorn 1977).

(h) Metabolic Diseases

There are some conditions which require a specific high requirement for a particular vitamin, for example vitamin B_6 dependancy syndroe-Hartnup Disease. Only one of the specific biochemical signs of vitamin B_6 deficiency is present. In this symdrome there is little tryptophan available for synthesis of nicotinamide because of the defect in intestinal absorption and renal resorption. When supplements of niacin are given, normal metabolism of tryptophan appears to occur (Barker and Bender 1980).

(i) Adaptation and Body Stores

The body is an adaptive system, with regulatory mechanisms which conserve nutrients when there is an inadequate intake. The body also stores nutrients when the amounts consumed exceed the immediate needs, but capacity varies for each nutrient. For example, fat soluble vitamin storage can be enough to meet the requirements for several months, but water soluble vitamins are not stored to the same extent.

(j) Lifestyle

Under acute emotional or environmental stress, increased intakes of ascorbic acid are required to maintain normal plasma levels of the vitamin. Cigarette smoking increases ascorbic acid requirement (Irwin and Hutchins 1976).

IV. REFERENCE VALUES

In assessment of vitamin requirements one of the problems is what reference standards should beused for interpretation of blood, tissue and urine concentrations (Nobile 1979, Sauberlich, Dowdy and Skala 1974). There is a need to establish interlaboratory quality control, as at present there appears to be no mechanism in Australia for laboratories to agree. Unless this occurs, the establishment of requirements will be difficult.

V. CONCLUSION

Vitamin requirements must be considered with respect to different individual needs, the function in question and according to the particular food system. No single approach to assessment of requirements is likely to be adequate, and wherever possible the functional role of the vitamin should be evaluated.

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