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100 Years of Vitamins

In 1912, Casimir Funk introduced the term “vitamine” for at this time still mostly unknown micronutrients that are essential for life. The wealth of knowledge that has been gathered since then was a reason enough to celebrate the 100th anniversary in Basel, where vitamins played an important role in the scientific and industrial community.

During the first half of the 20th century 13 molecules have been described, isolated and chemically synthesized that fulfill various functions in the metabolism know today as vitamin A, D, E, K, B₁, B₂, B₆, B₁₂, folic acid, niacin, pantothenic acid, biotin, and C. Other compounds have been proposed to have similar or complementary functions, but none of them reached the criteria of essentiality. Thus, the question arises whether we know everything about vitamins or whether we still need to fill gaps in our understanding of their functionality.

The symposium covered, therefore, the three areas past, present and future of vitamin research. At the beginning of the 20th century, scientists had to fight against the dogma that diseases can only be caused by germs. Several decades later enthusiastic expectations of almost unlimited health benefits had to be dampened. Today, recommendations for the intake of micronutrients are revised with respect to the particular requirements of various age groups and of individual living conditions. The future will reveal whether we need to or whether we can go a step further and advice each individual according to their personal needs.

Although the lecturers of the meeting did not give an answer to all open questions, many suggestions or ideas for future research might be derived from this comprehensive compilation of the symposium.

U. Moser, SGE and I. Elmadfa, ÖGE

Original Communication

The Discovery of the Vitamins

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Abstract: The discovery of the vitamins was a major scientific achievement in our understanding of health and disease. In 1912, Casimir Funk originally coined the term “vitamine”. The major period of discovery began in the early nineteenth century and ended at the mid-twentieth century. The puzzle of each vitamin was solved through the work and contributions of epidemiologists, physicians, physiologists, and chemists. Rather than a mythical story of crowning scientific breakthroughs, the reality was a slow, stepwise progress that included setbacks, contradictions, refutations, and some chicanery. Research on the vitamins that are related to major deficiency syndromes began when the germ theory of disease was dominant and dogma held that only four nutritional factors were essential: proteins, carbohydrates, fats, and minerals. Clinicians soon recognized scurvy, beriberi, rickets, pellagra, and xerophthalmia as specific vitamin deficiencies, rather than diseases due to infections or toxins. Experimental physiology with animal models played a fundamental role in nutrition research and greatly shortened the period of human suffering from vitamin deficiencies. Ultimately it was the chemists who isolated the various vitamins, deduced their chemical structure, and developed methods for synthesis of vitamins. Our understanding of the vitamins continues to evolve from the initial period of discovery.

Key words: beriberi, discovery, pellagra, rickets, scurvy, vitamins, xerophthalmia

Nutrition at the beginning of experimental physiology

François Magendie (1783–1855), the pioneer of experimental physiology, laid the foundations for nutrition research in the early nineteenth century. Magendie conducted his research in Paris at a time when philanthropists were seeking ways to feed the poor. Chemists discovered that gelatin could be extracted from leftover bones. A possible solution to hunger was at hand: people of means could consume the meat, while the poor would receive a gelatin broth. However, the poor revolted against the unappetizing broth. The authorities appointed a committee, the Gelatin Commission, to evaluate gelatin.

Magendie advocated an experimentalist approach in which facts were established by direct observation. He raised two questions that paved the way for research on the vitamins and for nutrition in general: Are

foods that do not contain nitrogen (i. e., proteins) nutritious? Is gelatin a complete source of protein in the diet? In 1816, Magendie conducted studies in which he fed dogs nothing but sugar, gum arabic, or other foods that did not contain nitrogen [1]. The dogs lost weight, developed corneal ulcers, and subsequently died, a condition that resembles what is now known as human vitamin A deficiency. Charles-Michel Billard (1800–1832) reported similar corneal ulcers in abandoned infants under his care in Paris and remarked that the eye lesions resembled those in Magendie’s malnourished dogs [2]. After ten years of research, the Gelatin Commission concluded that gelatin was not a complete food. Some surprises came in regard to previous assumptions about food. “As so often in research, unexpected results had contradicted every reasonable expectation,” reported Magendie, “Have we not above all made it evident that science is still in its first steps in every aspect of the theory of nutrition?” [3].

By the late nineteenth century, the prevailing dogma held that there were four essential elements of nutrition: proteins, carbohydrates, fats, and minerals. Most work focused on proteins and calories. Justus von Liebig (1803–1873), and Carl von Voit (1831–1908), Max Rubner (1854–1932), and Russell Chittenden (1856–1943) were among the most influential proponents of these ideas. Different proteins were considered of equal value in nutrition. Likewise, fats – whether from lard, butter, or cod-liver oil – were considered interchangeable in nutrition.

From the germ theory of disease to nutritional deficiencies

The concept that diseases are caused by infectious organisms or toxins produced by these organisms – that is, germ theory – became the reigning principle in science. Louis Pasteur (1822–1895) and Robert Koch (1843–1910) were influential proponents of the germ theory of disease. Investigations identified the organisms responsible for anthrax, malaria, tuberculosis, cholera, leprosy, and diphtheria. Other diseases such as scurvy, beriberi, rickets, and pellagra – considered by some to be infections – continued to baffle scientists.

In the Dutch East Indies, Christiaan Eijkman (1858–1930) observed that a polyneuritis, the equivalent of human beriberi, developed in chickens fed rice that had been polished of its bran [4]. Eijkman concluded incorrectly that the starch in polished rice carries a toxin, while the bran neutralizes the toxin [5]. Gerrit Grijns (1865–1944) continued the investigations of Eijkman but concluded that beriberi was caused by the lack of a vital component in the diet: “There occur in various natural foods, substances, which cannot be absent without serious injury to the peripheral nervous system.” [6]. Grijns eventually convinced Eijkman that beriberi was caused by a nutritional deficiency. The contributions of Grijns were overlooked, most likely because he published his findings in Dutch.

The origins of the vitamin theory

In 1906, Frederick Gowland Hopkins (1861–1947) articulated what is now known as the “vitamin theory” during a speech given in London. Hopkins hinted at some dietary studies he had conducted that suggested: “...no animal can live upon a mixture of pure protein,

fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish.” He continued: “Scurvy and rickets are conditions so severe that they force themselves upon our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon *unsuspected dietetic factors* [emphasis added]...” [7].

There were previous expressions of the “vitamin theory” prior to Hopkins. Nicolai Lunin (1853–1937) conducted studies with mice and concluded: “Mice can live quite well under these conditions when receiving suitable foods (e. g., milk), however, as the above experiments demonstrate that they are unable to live on proteins, fats, carbohydrates, salts, and water, it follows that *other substances indispensable for nutrition* [emphasis added] must be present in milk...” [8]. His mentor, Gustav von Bunge (1844–1920) reiterated in 1887: “Does milk contain, in addition to [protein], fat, and carbohydrates, other organic substances, which are also *indispensable to the maintenance of life?* [emphasis added]” [9]. A study by another von Bunge student, Carl A. Socin, demonstrated that there was an unknown substance in egg yolk that was essential to life, and he raised the question of whether this substance was fat-like in nature [10].

Perhaps the earliest articulation of the “vitamin theory” came from the French chemist, Jean Baptiste Dumas (1800–1884). During the Siege of Paris (1870–1871), many infants and toddlers died when the city was cut off from the milk supply of the countryside. Some opportunists tried to manufacture an artificial substitute for cows’ milk, but this artificial milk failed to sustain the infants. Many children died. Dumas pointed out: [Since no] conscientious chemist can assert that the analysis of milk has made known all the products necessary for life... we must renounce, for the present, the pretension to make milk... it is therefore always prudent to abstain from pronouncing upon the identity of these *indefinite substances employed in the sustenance of life, in which the smallest and most insignificant traces of matter may prove to be not only efficacious, but even indispensable* [emphasis added]... The siege of Paris will have proved that we... must still leave to nurses the mission of producing milk” [11].

Further studies with milk

Wilhelm Stepp (1882–1964) conducted an important but overlooked study in which he mixed flour with milk

and formed it into dough, which he called milk-bread; the dough supported growth in young mice. After the milk-bread was extracted with alcohol and ether, mice could not survive beyond three weeks on the extracted milk-bread. When the substance extracted with alcohol and ether was added back to the extracted milk-bread and fed to mice, they survived normally. Stepp concluded that a fat-soluble substance was essential for life [12], thus: “certain lipid substances present in milk, soluble in alcohol-ether, are indispensable for the survival of mice” [13].

In 1912, Hopkins showed that young rats did not grow well when fed a basal ration of protein, starch, cane sugar, lard, and minerals. After a small amount of milk was added to the basal ration, they had normal growth. The unknown factors in milk that supported life were found in “astonishingly small amounts” and were termed “accessory factors” by Hopkins [14]. Casimir Funk (1884–1967) proposed the term “vitamine” instead of “accessory food factors” in 1912 for the deficient substances in the food as related to beriberi, scurvy, and pellagra [15]. Soon these unknown factors in foods became synonymous with both “vitamine” and “accessory food factors”.

New challenges to old dogmas

At Yale University, Thomas Osborne (1859–1929) refuted the half-century-old thesis of Liebig that four forms of plant protein – vegetable albumin, plant gelatin, legumin or casein, and plant fibrin – were identical to four animal proteins with similar names [16]. Osborne concluded that proteins of seeds are specific substances with distinctive amino acids, and even among closely allied species, seed proteins have pronounced differences. Another theory was soon overturned. German scientists believed that chemical analysis rather than actual feeding experiments could establish the nutritional value of foods. Edwin B. Hart (1874–1953) challenged this idea through what later became known as the “single grain ration experiment” conducted at the University of Wisconsin. Hart fed cows a diet of corn, wheat, oats, or a combination of the three, and according to the German theory, the cows should have fared the same. The results showed otherwise, as the corn-fed cows grew better and were much more healthy than the cows that were on wheat, oats, or a mixture [17].

Fats are not equivalent in supporting life

Another major dogma of the time was that all fats were equivalent in nutritional value.

This notion was challenged by Elmer McCollum (1879–1967) and his assistant Marguerite Davis (1887–1967) at the University of Wisconsin, who showed that young rats on a diet of casein, lard, lactose, starch, and salts grew normally if an ether extract from butter or egg yolk (ether mixed with either butter or egg yolk to chemically extract substances that were soluble in ether) was added to the diet. If a similar ether extract of lard or olive oil were added, the animals died. They concluded: “Our observation that ether extracts from certain sources improve the condition of animals on such rations, strongly supports the belief that there are certain accessory articles in certain food-stuffs which are essential for normal growth for extended periods” [18]. Osborne and his colleague Lafayette Mendel (1872–1935) reported that rats fed a basal diet of isolated proteins, starch, lard, and “protein-free” milk grew normally for about sixty days but then declined and died. The addition of butter or replacement of lard with butter in the diet allowed normal growth in young rats. They concluded: “In seeking for the ‘essential’ accessory factor we have, therefore, been led first to supply the cream component, in the form of butter...it would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter...” [19]. In contrast to the prevailing dogma, the fat in butter or egg yolk was not equivalent in nutritional value to the fat in lard or olive oil.

Scientific misconduct by Elmer McCollum

In his later writings, McCollum claimed that he discovered vitamin A with his study in 1913, and that “this observation was promptly verified by Osborne and Mendel” [20, 21]. This version of events is often accepted in the hagiography that surrounds McCollum. However, McCollum’s contention to have “discovered” vitamin A is based upon his observation that the unidentified factor was fat-soluble. Others had priority: Socin suggested that this unknown substance was fat-soluble in 1891 [10], and Stepp demonstrated that there was a factor that supported growth and concluded correctly that it was fat-soluble in 1911 [13]. In addition, the fat-soluble substance found in butter and

egg yolk actually contained three vitamins: vitamins A, D, and E, which were yet unidentified in 1913.

In 1917, McCollum left the University of Wisconsin for a new position at the Johns Hopkins University. His departure was clouded by accusations of academic misconduct that were aired in public in the journal *Science*. McCollum stole the research notebooks of his colleagues at Wisconsin, including the notebooks of his perceived rival, Harry Steenbock (1886–1953) and subsequently published Steenbock's work in two articles in the *Journal of Biological Chemistry*. McCollum violated university policy by publishing without approval of the station head and dean, and he sabotaged their animal experiments by releasing all the animals from their cages [22].

McCollum later reflected upon the controversy of the purloined notebooks and unauthorized publications in his autobiography with the prevarication: “unfortunately I failed to say that they were published with permission of the Director, as was customary in experiment station bulletins.” [21]. McCollum's account is directly contradicted by the “black book” diaries kept by Harry Russell, correspondence of Edwin Hart, Director of the Agricultural Experiment Station to Mendel, editor-in-chief of the *Journal of Biological Chemistry*, and a telegram from Russell to Steenbock in which he verified that he did not give McCollum permission to publish the papers [22].

Clinical observations of vitamin deficiencies

Masamichi Mori (1860–1932) made an early seminal description of vitamin A deficiency in fifteen hundred children in rural Japan. Cod liver oil proved to be an effective treatment for both the eye lesions and diarrhea. Contrary to the view of many physicians, Mori concluded that the disease was not infectious but rather was caused by the lack of fat in the diet. Joseph Goldberger (1874–1929) conducted rigorous epidemiological studies that showed pellagra was due to a defective diet rather than an unknown infection [26]. One of the most influential woman scientists in the early history of vitamins was Harriette Chick (1875–1977), a nutritionist at the Lister Institute. Chick served on the Accessory Food Factors Committee of Great Britain. She conducted important clinical investigations of rickets in Vienna following World War I at a time when many still believed that rickets was an infectious disease [27].

The importance of animal models

Fundamental insights into vitamin deficiencies came through studies with specific animal models: pigeons and chickens for beriberi, mice and rats for vitamin A deficiency, guinea pigs for scurvy, and dogs for rickets and pellagra. Most animals are able to synthesize vitamin C and cannot serve as models for vitamin C deficiency. In 1907, two Norwegian scientists at the University of Cristiania (Oslo), bacteriologist Axel Holst and pediatrician Theodor Frölich, developed an animal model that enabled a breakthrough in the study of scurvy [28, 29]. Edward Mellanby (1884–1955) made important steps towards the identification of vitamin D when he showed that a fat-soluble substance was responsible for rickets in studies conducted with dogs [30–32].

Isolation, description of structures, and synthesis of vitamins

Barend C. P. Jansen (1884–1962) and Willem F. Donath (1889–1957) crystallized thiamin in 1926 [33]. Robert Williams (1886–1965) described the chemical structure of thiamin and its synthesis in 1936 [34,35]. Albert Szent-Györgi (1893–1986) isolated a substance “hexuronic acid” [36], that was later confirmed as vitamin C by Charles Glen King (1896–1988) [37] and Szent-Györgi [38]. Norman Haworth (1883–1950) described the chemical structure of vitamin C and its synthesis in 1933 [39]. In 1936, Adolf Windaus (1876–1959) described the structure of both vitamin D₂ and, cholecalciferol, or vitamin D₃ [40, 41]. Harry Holmes and Ruth Corbet crystallized vitamin A in 1937 [42]. Conrad Elvehjem isolated nicotinamide from liver concentrates and used it to cure blacktongue in dogs, thus showing that niacin was the “anti-pellagra vitamin” [43]. Otto Isler synthesized vitamin A in 1947 [44].

The importance of basic research

The period of discovery of the vitamins paved the way for the development of dietary allowances, fortification of foods with vitamins, vitamin supplementation, and wider recognition of nutritional deficiency disorders. This succinct review has dealt with the five vitamins involved in scurvy, beriberi, rickets, pellagra, and xerophthalmia. Further details regarding the

discovery of each of the numerous vitamins and associated Nobel Prizes has recently been presented in a special issue [45].

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Original Communication

Micronutrients – a Global Perspective on Intake, Health Benefits and Economics

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Abstract: The link between a sufficient intake of vitamins and long term health, cognition, healthy development and aging is increasingly supported by experimental animal, human and epidemiology studies. In low income countries billions of people still suffer from the burden of malnutrition and micronutrient deficiencies. However, inadequate micronutrient status might also be an issue in industrialized countries. Recent results from nutritional surveys in countries like the United States, Germany, and Great Britain indicate that the recommended intake of micronutrients is not reached. This notably concerns certain vulnerable population groups, such as pregnant women, young children and the elderly, but also greatly influences the general healthcare costs. An overview is provided on the gap that exists between current vitamin intakes and requirements, even in countries where diverse foods are plentiful. Folic acid and vitamin D intake and status are evaluated in more detail, providing insight on health and potential impact on health care systems.

Key words: micronutrients, vitamin intakes, nutrition, economic burden, dietary survey, reference values, folic acid, vitamin D, deficiency

Before the discovery of vitamins, food was mainly viewed as a source of protein and energy. However, this has changed with the recognition that, given an adequate diet, it is possible to prevent diseases such as beriberi, scurvy and rickets [1]. For 100 years, after Casimir Funk introduced the term ‘vitamine’, researchers have discovered the structure, chemical and physical characteristics of the 13 vitamins and their importance for many fundamental metabolic processes [2, 3]. Consequently, dietary reference intakes (DRIs) have been established to define the intake at which health is optimal for the majority of individuals (generally 97.5 %) of a given population or group [4–9]. As the knowledge about the importance and functions of micronutrients is steadily increasing, inadequate vitamin intakes are still prevalent throughout

the globe, though by different degrees and due to different reasons.

Despite the global effort in the last decades to ameliorate micronutrient deficiencies and its consequences in the developing world, deficiencies for example from vitamin A are still affecting the lives and health of millions of people, especially the vulnerable population groups including children under five and pregnant women [10, 11].

Micronutrient deficiencies and inadequate intake compared to recommendations are also present in industrialized countries such as the United States and Europe [12]. Changed lifestyles and an increase towards the consumption of fast and convenience food with a low micronutrient density, have an impact on the quality of a persons’ daily diet and hence on their

nutritional status. Large-scale population based dietary intake surveys such as the German Nutritional Intake Study (Nationale Verzehrsstudie II) [13], the US National Health and Nutrition Examination Survey (NHANES) [14], the British National Diet and Nutrition Survey [15] and the Dutch National Food Consumption Survey [16] indicate that there is a gap between vitamin intakes and requirements for a significant proportion of the population even though diverse foods are available (Figure 1). For example more than 75 % of the population does not get enough vitamin E in the United States, Germany and Great Britain. Folic acid intake is especially low in Germany and in The Netherlands [12].

Recent reports state that non-communicable diseases (NCDs) accounted for two out of every three deaths worldwide by 2010 [17, 18]. Therefore, NCDs rank as the highest cause of human death and action is required to counteract this trend by improving lifestyles, with diets being an important component [19]. Even modest shortages of vitamins have been proposed to influence long-term survival and health. The Triage Theory provides a rationale on the suspected association of micronutrient shortages and the impact on long term health, a concept which is gaining scientific support [20–22]. The theory is well supported by a recent follow up of the Physician' Health Study (PHS). Within the PHS II, a large-scale, randomized, double-blind placebo-controlled trial in middle-aged and older men, a daily multivitamin supplement significantly reduced the risk of total cancer during a mean of 11 years of follow-up [23]. The vitamin/mineral supplement mix was given on top of a typical diet of American Health Professionals, which is assumed to be better than the diets consumed by many non-health professionals. Given that cancer is a leading cause of death worldwide, with more than 12.7 million new cancer cases diagnosed each year [24], this translates into a potential of about 1 million cancers prevented every year, and with it, all the burden of human suffering and health care costs.

Besides a combination of micronutrients, it has been suggested that even a single vitamin can have a tremendous impact on a number of body functions and health outcomes. For example, peri-conceptional folic acid supplementation has been shown to reduce the incidence of Neural Tube Defects (NTDs) by 20 % to 60 %. [25–28] However, although two thirds of pregnancies are reportedly planned, only about 4 % use an adequate prophylaxis [29]. Consequently more than 70 countries have implemented mandatory flour fortification with the result of a significant decrease in the prevalence of NTDs [30–34].

Vitamin D is another example, which has received increased attention over the past decade. The classical role of vitamin D is in bone health through its support of calcium absorption and deposition. Emerging health benefits of vitamin D include a reduced risk of falling by improving muscle strength, reducing the risk of multiple sclerosis, diabetes type I and II and infectious diseases such as tuberculosis, lowering of blood pressure and prevention of some cancers [35–37]. Although vitamin D₃, cholecalciferol, is synthesized in the skin by the action of ultraviolet light, reports from across the world indicate that hypovitaminosis D is widespread, even in sun-rich countries, and is re-emerging as a major health problem globally [38]. Older age, female sex, higher latitude, winter season, darker skin pigmentation, less sunlight exposure, dietary habits, and absence of vitamin D fortification are some factors that are significantly associated with lower levels of 25-hydroxyvitamin D (25OHD), which is an established marker of vitamin D status. Recently, the International Osteoporosis Foundation (IOF) developed a graphical illustration of global vitamin D status based on a systematic review (Figure 2). The findings show, that besides adults, widespread gaps exist especially in children and adolescents and rates of vitamin D deficiency are higher amongst women than men [39].

It has been estimated that an optimized vitamin D status in patients diagnosed with osteoporosis could reduce the number of fractures by 20 % [40]. For Germany that would be a total of 5478 hip fractures and 18420 vertebral fractures prevented every year. Taking direct and indirect costs into account this would lead to a net socio-economic benefit between 585 million – 778 million Euros. The magnitude of risk reduction for other associated diseases is assumed to be in the range of 50 % for multiple sclerosis, 25 % for diabetes, and 20 % for cardiovascular disease.

More studies are required to confirm these assumptions. However, policy makers and health insurance companies should engage to stimulate improvements in diets and adequate micronutrient intake.

Healthy nutrition is one of the global key challenges and opportunities

In 2012, an estimated 2 billion people worldwide suffered from hidden hunger, which means that people have enough calories however have inadequate vitamin intakes. In developing countries, intakes of nutritious foods are often not accessible to the poor,

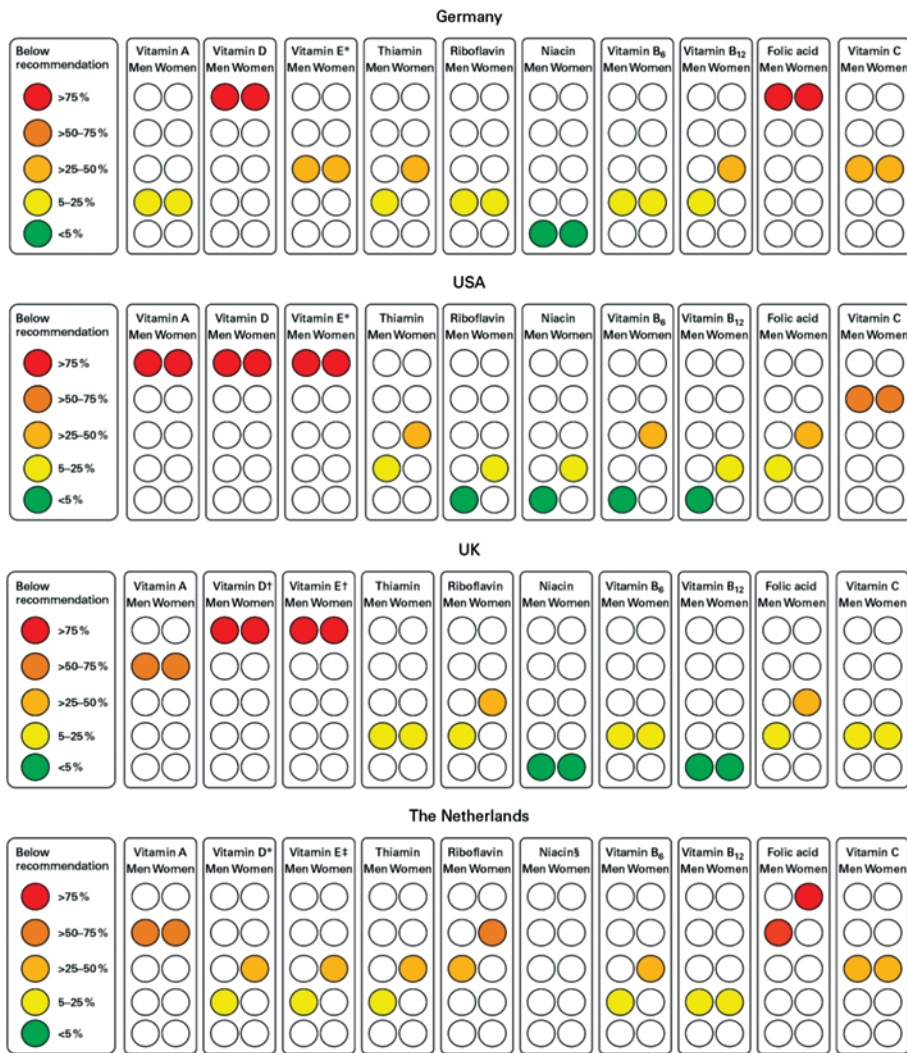


Figure 1: Adapted from Troesch et al. [12]. Population with intakes below the specific recommended reference value for the country. The level of recommendation covering the needs of 97.5% of the population was used where it existed. * Average nutrient requirement/ approximation. † No references exist, therefore, the Institute of medicine reference was used. ‡ 25–50% for men aged 19–30 years. § Data not available.

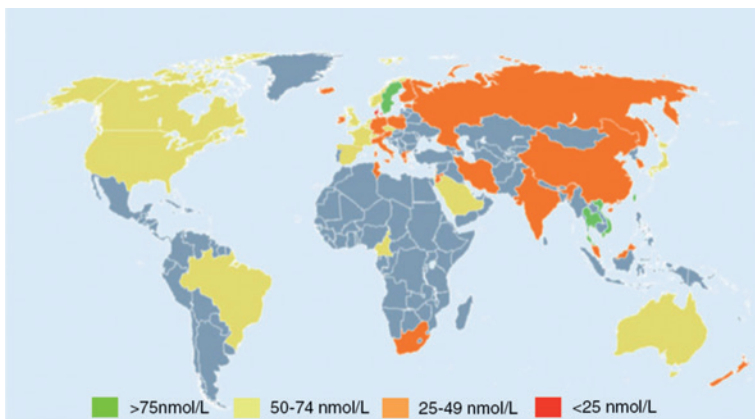


Figure 2: Vitamin D status in adults (>18 years) around the world when available; winter values were used to calculate the mean 25(OH) D levels [39].

whereas in industrialized countries poor diets, especially but not only, in lower socioeconomic groups affect micronutrient intake. Diet is one important factor

of non-communicable diseases worldwide, and even modest inadequate micronutrient intakes can impact long-term health. Ensuring adequate micronutrient

intake and status is not only a cost effective approach to reduce health care costs but also ensures a healthy and productive life of billions.

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Original Communication

Vitamin D – From Essentiality to Functionality

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Abstract: Vitamin D is essential in bone and muscle health. Severe deficiency (25-hydroxyvitamin D serum levels < 25 nmol/l) can result in rickets and osteomalacia, fractures, myopathy and falls. All recent recommendations on vitamin D agree that children and adults should reach a target 25-hydroxyvitamin D range of at least 50 nmol/l (threshold for normal vitamin D status) and 50 % of the population may be below that threshold. A vitamin D intake of 600 to 800 IU per day as recommended today will prevent about 97 % of children and adults from vitamin D deficiency. Notably, a higher 25-hydroxyvitamin D threshold of more than 60 nmol/l is needed for optimal functionality, fall and fracture in adults age 65 and older.

Key words: vitamin D, falls, muscle strength, bone density, fractures

Evidence linking vitamin D to bone health

Vitamin D is essential for bone growth [1, 2] and preservation [3], and higher 25(OH)D levels are associated with higher bone density in younger and older adults [4]. Severe vitamin D deficiency (serum concentrations below 25 nmol/l 25-hydroxyvitamin D) in children causes rickets, and secondary hyperparathyroidism, osteoporosis, and osteomalacia in the adult and senior population [5]. With respect to optimal functionality, vitamin D supplementation between 792 to 2000 IU per day (median: 800 IU per day), or a 25-hydroxyvitamin D status of above 61 nmol/l among adults age 65 and older, reduced hip fracture risk by 30 % [6].

Vitamin D and fracture reduction

In the largest individual subject level meta-analysis (31,022 individuals with mean age 76 years, 91 % women sustaining 1111 incident hip and 3770 non-

vertebral fractures) from double-blind RCTs of vitamin D supplementation available to date, the assessment of actual oral intake dose showed that fracture reduction is only present at the highest actual intake quartile of 792 to 2000 IU/day (median 800 IU/day) with a 30 % reduction at the hip and 14 % reduction at any non-vertebral site independent of vitamin D treatment, age group, gender, type of dwelling and study [6]. Actual doses up through 791 IU were ineffective and a dose of 800 IU or more per day (range 792 to 2000 IU/day) significantly reduced fracture risk in all subgroups of the senior population: men and women, community-dwelling and institutionalized, of any age 65+. This study offers as reliable an estimate of the dose requirements of vitamin D for fracture prevention as can be achieved with current data [6]. In the same analysis, 25(OH)D levels were available from 4383 participants at baseline who sustained 313 hip fractures during the follow-up. After adjustment for study, vitamin D treatment, age group, gender, and dwelling, hip fracture risk was 37 % lower in seniors with baseline serum 25(OH)D levels of at least 61 nmol/l compared to those with baseline levels of

less than 30 nmol/l (HR = 0.63; 0.46–0.87), and there was a significant trend for higher baseline quartile of 25(OH)D levels and lower hip fracture risk ($p = 0.02$) [6].

In contrast, three study-level meta-analyses [7–9] and one pooled analysis [10] suggested that vitamin D may have a neutral effect on total fractures [7], or may reduce hip fractures by 7 to 16 % independent of its dose if combined with calcium supplementation [7–9]. The discordant findings may in part be explained by different inclusion criteria of trials with respect to blinding and intake form (oral, injectable), or different accommodations for adherence and incorporation of actual dose (study medication plus supplements outside the study protocol). In fact, in all of these reports heterogeneity by dose may have been missed due to the inclusion of open design trials plus a dose evaluation that did not incorporate adherence. Biologically, the exclusion of heterogeneity by dose seems implausible even if a formal test of heterogeneity is not statistically significant. A dose-response relationship between vitamin D and fracture reduction was also documented in a 2009 trial level meta-analysis of double-blind RCTs [11] and is supported by epidemiologic data showing a significant positive trend between serum 25(OH)D concentrations and hip bone density [4] falls and lower extremity strength [12, 13].

The 25(OH)D threshold of at least 75 nmol/ml for optimal fracture prevention at older age is supported by the 2010 IOF position statement on vitamin D [14], the 2011 US Endocrine Society Task Force on Vitamin D [15], and the 2012 Swiss recommendations on vitamin D [16]. In contrast, the 2010 Institute of Medicine recommendations suggested that 50 nmol/l may be sufficient for bone health in the population [17].

Evidence linking vitamin D to muscle health

The beneficial effect of vitamin D on calcium absorption and bone mineral density may not be the only explanation for its protective effect against fractures [11]. In fact, vitamin D deficiency may cause muscular impairment even before adverse effects on bone occur [18], and vitamin D supplementation increased strength and reduced the risk of falling in double-blind RCTs of older adults [19]. Similar to bone health, also muscle health moved from essentiality to functional-

Four lines of evidence support a role of vitamin D in muscle health [19]

First, proximal muscle weakness is a prominent feature of the clinical syndrome of vitamin D deficiency [20]. Clinical findings in vitamin D deficiency myopathy include proximal muscle weakness, diffuse muscle pain, and gait impairments such as waddling way of walking [21]. *Second*, the vitamin D receptor (VDR) is expressed in human muscle tissue [22, 23], and VDR activation may promote de novo protein synthesis in muscle [24, 25]. Mice lacking the VDR show a skeletal muscle phenotype with smaller and variable muscle fibers and persistence of immature muscle gene expression during adult life [26, 27]. These abnormalities persist after correction of systemic calcium metabolism by a rescue diet [27]. *Third*, several observational studies suggest a positive association between 25(OH)D and muscle strength or lower extremity function in older persons [12, 13]. *Fourth*, vitamin D supplementation increases muscle strength and balance [28, 29], and reduces the risk of falling in community-dwelling individuals [29–31], as well as in institutionalized individuals [28, 32] in several double-blind randomized-controlled trials (RCTs) summarized in a 2009 meta-analysis discussed below [33].

Vitamin D deficiency and type II muscle atrophy in humans may be critical for falls at older age

Type II muscle fibers are fast-twitch fibers and therefore are the first to be recruited when fast reaction is needed, such as in the prevention of a fall. In fact, ageing itself has been associated with a decrease in type II fast-twitch relative to type I slow-twitch muscle fibers [34].

Muscle biopsy studies in humans suggest a potentially selective effect of vitamin D on type II muscle fibers. Patients with osteomalacic myopathy reveal type II muscle atrophy in muscle histology investigations [35] and in two smaller clinical trials treatment with 1-alpha-calcidiol [36] or vitamin D2 [37] increased type II muscle fibers in older adults.

Type II muscle atrophy in profound vitamin D deficiency fits well with the findings that high dose vitamin D supplementation (700 to 1000 IU per day) increased 25-hydroxyvitamin D levels to above 60 nmol/l and reduced the risk of falling by 34 % in a meta-analysis of 8 double-blind randomized controlled trials among adults age 65 and older [38].

Vitamin D and muscle functionality [19]

Most observational studies show a positive association between higher 25(OH)D status and better lower extremity function in older adults, a lower risk of functional decline [39, 40], a lower risk of future falls and a lower risk of nursing care admission [41], including two population-based studies from the US [42] and Europe [39].

Consistently, in several trials of older individuals at risk for vitamin D deficiency, vitamin D supplementation improved strength, function, and balance [28, 29, 31]. Most importantly, these benefits translated in a reduction in falls in some of the same trials [28, 29, 31]. In three recent double-blind RCTs supplementation with 800 IU vitamin D₃ resulted in a 4–11 % gain in lower extremity strength or function [28, 29], and an up to 28 % improvement in body sway [29, 31] in older adults age 65+ within 2 to 12 month of treatment. Extending to trials among individuals with a lower risk of vitamin D deficiency and including open design trials, a recent meta-analysis by Stockton identified 17 RCTs that tested any form of vitamin D treatment and documented a muscle strength related endpoint. The authors suggested that based on their pooled findings, vitamin D may not improve grip strength, but a benefit of vitamin D treatment on lower extremity strength could not be excluded ($p = 0.07$) among individuals with 25(OH)D starting levels of > 25 nmol/l and the authors report a significant benefit among two studies with participants that started with 25(OH)D levels < 25 nmol/l [43].

Guidelines on vitamin D and fall prevention

Several recent peer-reviewed meta-analyses of randomized, controlled trials have addressed the effect of vitamin D on fall risk reduction [33, 44–51], all of them suggesting a benefit. Thus, the Agency for Healthcare Research and Quality (AHRQ) for the U.S. Preventive Services Task Force [50], the 2010 American Geriatric Society/British Geriatric Society Clinical Practice Guideline [52], the 2010 assessment by the IOF [14], the 2011 recommendations on vitamin D by the Endocrine Society [15], and the 2012 Swiss recommendations on vitamin D [16] identified vitamin D as an effective intervention to prevent falling in older adults.

The Institute of Medicine did a thorough review on the effect of vitamin D on fall prevention. Their synopsis is that the evidence of vitamin D on fall prevention is inconsistent, which is in contrast to all pub-

lished and peer-reviewed meta-analyses [33, 44–51] and recent guidelines cited above. The IOM overall analysis of 12 RCTs ($n = 14,101$) showed a significant benefit of vitamin D on fall prevention (OR = 0.89; 95 % CI 0.80–0.99), as did the majority of their subset analyses, clearly supporting the use of vitamin D in the prevention of falling.

In their report, the Institute of Medicine (IOM) asked for a re-analysis of a 2009 peer-reviewed meta-analysis of 8 double-blind RCTs [33]. In the re-analysis [38], the authors confirmed their selection of trials and show a significant reduction in the odds of falling based on the primary analysis of the same 8 trials: OR = 0.73 [0.62, 0.87]; $p = .0004$. When the model was expanded to capture the impact of both high dose and low dose treatment, high dose vitamin D (700 to 1000 IU vitamin D per day) reduced the odds of falling by 34 % (OR = 0.66 [0.53, 0.82]; $p = .0002$), while low dose vitamin D did not (OR = 1.14 [0.69, 1.87]; $p = .61$) [38].

Desirable 25-hydroxyvitamin D status for muscle health

A threshold for serum 25(OH)D level needed for muscle function and fall prevention in older subjects has been evaluated in few studies. A dose-response relationship between lower extremity function and serum 25(OH)D levels has been found in two epidemiologic studies among older individuals [12, 13]. From these analyses a threshold of 50 nmol/l has been suggested for optimal function in one study [13], while in the larger study [12], a threshold beyond which function would not further improve was not identified, but most of the improvement was seen coming from very low 25(OH)D levels to a minimal threshold of 60 nmol/l [12]. Consistently, one meta-analysis of double-blind RCTs found a differential benefit of achieved 25(OH)D levels below 60 nmol/l versus levels 60 nmol/l or above, with a benefit demonstrated only in the higher group. Achieved serum 25(OH)D concentrations of 60 nmol/l or more resulted in 23 % fall reduction (pooled RR = 0.77; 95 % CI; 0.65–0.90), while less than 60 nmol/l resulted in no fall reduction (pooled RR = 1.35, 95 % CI, 0.98–1.84) [12].

Conclusion

Vitamin D is relevant to bone and muscle health with significant bone and muscle impairments resulting

from severe deficiency at levels below 25 nmol/l. Notably, beyond the correction of severe deficiency, a desirable 25-hydroxyvitamin D status above 60 or better 75 nmol/l may not only prevent disease but contribute to better functionality. The strong evidence available from clinical trials in the general population lends support to the correction of vitamin D deficiency for fracture and fall prevention as a public health goal.

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Original Communication

Vitamins – Wrong Approaches

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Abstract: Deficiencies of essential nutrients have been responsible for many epidemic outbreaks of deficiency diseases in the past. Large observational studies point at possible links between nutrition and chronic diseases. Low intake of antioxidant vitamins e. g. have been correlated to increased risk of cardiovascular diseases or cancer. The main results of these studies are indications that an intake below the recommendation could be one of the risk factors for chronic diseases. There was hardly any evidence that amounts above the RDA could be of additional benefit. Since observational studies cannot prove causality, the scientific community has been asking for placebo-controlled, randomized intervention trials (RCTs). Thus, the consequences of the epidemiological studies would have been to select volunteers whose baseline vitamin levels were below the recommended values. The hypothesis of the trial should be that correcting this risk factor up to RDA levels lowers the risk of a disease like CVD by 20–30%. However, none of the RCTs of western countries was designed to correct a chronic marginal deficiency, but they rather tested whether an additional supplement on top of the recommended values would be beneficial in reducing a disease risk or its prognosis. It was, therefore, not surprising that the results were disappointing. As a matter of fact, the results confirmed the findings of the observational studies: chronic diseases are the product of several risk factors, among them most probably a chronic vitamin deficiency. Vitamin supplements could only correct the part of the overall risk that is due to the insufficient vitamin intake.

Key words: vitamins, observational studies, randomized clinical trials, chronic diseases, Recommended Dietary Allowance, disease risk

During the late 19th century biochemical research was mainly focused at the unraveling of metabolic pathways. It was believed that carbohydrates, lipids, proteins and salts were the only required components of the diet. In 1881, N. Lunin at the university of Basel published the following feeding experiment with mice:

Two mice fed 2½ months with milk could be set in liberty in very good condition. 4 mice fed with distilled water only died after 3 or 4 days. 5 mice on an ash-free diet survived 11–21 days. 6 mice on the same diet receiving salts lived 20–31 days. He concluded that milk contains besides casein, fat, lactose and salts other substances that are essential for the nutrition. He suggested to trace these substances and to explore their importance for nutrition. However, his findings have been ignored since it was believed that toxins or germs only caused diseases. Even after the discovery

and description of vitamins by Casimir Funk in 1912 Emil Abderhalden wrote in 1913: There is no evidence for the hypothesis that there are unknown substances that are essential for survival. Thus, these authors obviously used the wrong approach for identifying essential nutrients.

During the first half of the 20th century vitamin deficiencies could then be linked to a specific disease (Table I).

Since vitamins are part of metabolic pathways, their requirement should be known in order to provide the best possible advice for a healthy diet. Therefore, the US Institute of Medicine started in 1941 to publish dietary reference intakes for vitamins and minerals (DRI) for advising the nation and improving health. These recommendations have been revised roughly every ten years, the last time in 2000. The aim of the

Table I: Vitamin deficiency diseases.

Vitamin	Disease
Vitamin A	Night blindness
Vitamin D	Rickets
Vitamin C	Scurvy
Vitamin B ₁	Beriberi
Vitamin B ₁₂	Pernicious anemia
Niacin	Pellagra
Folic acid	Megaloblastic anemia

The relationship between the nutrient and the disease is 1:1; e.g. the deficiency of one vitamin causes one disease.

Table II: US RDAs for adult males.

Year	Vitamin A µg	Vitamin E mg	Vitamin C mg	Folic acid µg
1941	1000		75	
1948	1000		75	
1957	1000		70	
1968	1000	30	60	400
1976	1000	15	45	400
1980	1000	10	60	400
1989	1000	10	60	200
2000	700	15	90	400

recommendation was until 1989 the elimination of vitamin deficiency diseases, but the scientific data did not allow to clearly define the requirements. This resulted sometimes in erroneous values as can be seen from Table II.

In the second half of the 20th century, large observational studies consistently pointed at possible links between nutrition and chronic diseases such as low intake of antioxidant vitamins and increased risk of cardiovascular diseases or cancer.

Table III: Lessons from epidemiological studies (* = ref 12).

	Favourable plasma level*	Intake (mg/d)*	US-RDA 2000 (mg/d)	DACH 2000 (mg/d)	France 2001 (mg/d)
Vitamin C	50 µmol/L	75–150	↓ : 90; ↓ : 75	100	110
Vitamin E	30 µmol/L	15–30	15 mg/d	↓ : 15–12; ↓ : 12–11	12
β-Carotene	0.4 µmol/L	2–4	↓ : 1.8 ↓ : 1.4 synt.; pro A	2–4	2.1

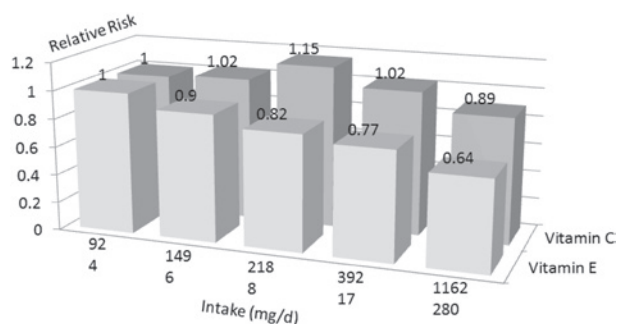


Figure 1: Health Professionals Study: relative risk of CHD in men.

Thus, the scientific rationale for conducting RCTs was excellent and the first outcomes very promising. In the Linxian trial, small but significant reductions of the total and cancer mortality have been observed in participants receiving daily 15 mg β-carotene, 50 µg selenium and 30 mg vitamin E for 5.25 years. The enthusiasm for using antioxidant vitamins as a cheap, nutritional concept for the prevention of chronic diseases weakened when the results of very large randomized intervention trials of western countries became available. The Heart Protection Study e.g. showed neither any benefit nor harm supplementing volunteers a high dose of a combination of vitamin E, vitamin C and β-carotene.

In order to understand the apparent discrepancy between the RCT and the observational studies the major outcomes of the latter will be shortly summarized.

In the WHO – MONICA study the difference of the CVD-mortality between 16 European countries could be explained by 90 % using plasma levels of vitamin E, vitamin C, carotene, cholesterol and blood pressure as predictors. The most important contributor was the plasma level of vitamin E with a range of 19–28 µmol/L between Northern- and Mediterranean countries. Several case control and prospective studies confirmed this relationship; however the risk predic-

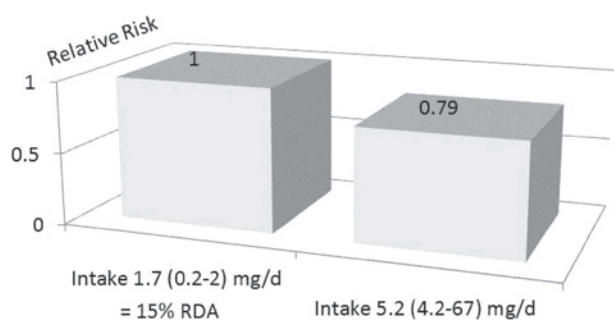


Figure 2: Nurses' Health Study: relative risk of CHD associated with intake of vitamin E.

tion was not as strong. In Scotland, e.g., the risk of angina pectoris was associated with low plasma levels of vitamin E (18.9 $\mu\text{mol/L}$ vs. 28.2 $\mu\text{mol/L}$), vitamin C (13.1 $\mu\text{mol/L}$ vs. 57.4 $\mu\text{mol/L}$) and β -carotene (0.26 $\mu\text{mol/L}$ vs. 0.68 $\mu\text{mol/L}$). Several very large observational studies reported intake data rather than plasma levels. In 39,910 U.S. male health professionals, the multivariate relative risk for coronary disease was 0.64 (95 percent confidence interval [95 % CI], 0.49 to 0.83) for men consuming more than 40 mg per day of vitamin E as compared with those consuming less than 5 mg per day. Vitamin C was not associated with CHD risk, however the consumption in the lowest quintile

was 92 mg/d what corresponds to the RDA (Fig 1).

From the figure it is evident that the risk is highest at an intake of 4 mg vitamin E per day that is roughly 25 % of the RDA. The 4th quintile corresponds to one RDA; unfortunately there are no indications whether there is a difference between the 4th and the 5th quintile. The multi-variance analysis reveals the following relative risks (95 % CI):

1st quintile: 1.0; 2nd quintile: 0.90 (0.71–1.14); 3rd quintile: 0.82 (0.64–1.07); 4th quintile: 0.77 (0.60–0.98); 5th quintile: 0.64 (0.49–0.83); p for trend: 0.003.

A similar study analyzing the dietary behavior of 87,245 female nurses found a relative risk of major coronary disease of 0.79 (95 % CI, 0.61 to 0.1.03) comparing the 5th to the 1st quintile. Although the trend is not significant (p=0.12) probably due to an intake of less than half of the RDA in the 5th quintile, there is still an increased risk at an intake significantly below the RDA (Fig. 2).

Several meta-analyses have combined results from case-control and cohort studies on vitamin E, C and beta.carotene. The outcome can be summarized as follows: studies with a vitamin supply significantly below the RDA are associated with an increased disease risk. If the lowest quintile is close to the RDA, there is no effect. A selection of studies is listed in Fig. 3.

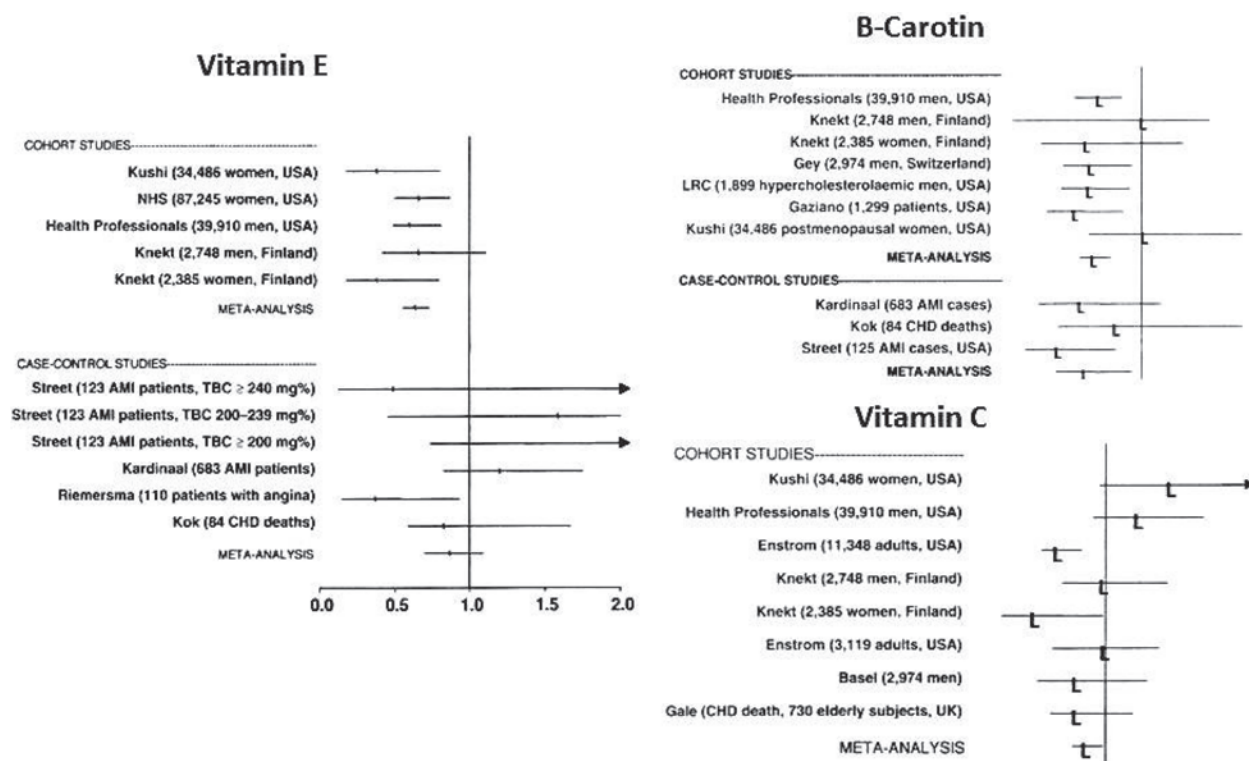


Figure 3: Epidemiological Studies, CHD.

Table IV: Plasma concentrations of antioxidants in placebo groups of several randomized trials.

	β -Carotene $\mu\text{mol/L}$	% of 0.4 $\mu\text{mol/L}$ ^{a)}	Vitamin E $\mu\text{mol/L}$	% of 30 $\mu\text{mol/L}$ ^{a)}	Vitamin C $\mu\text{mol/L}$	% of 50 $\mu\text{mol/L}$ ^{a)}
AREDS	0.48	120.0	39.0	130.0	58.1	116.2
ASAP male smoker	0.28	70.0	29.7	99.0	57.4	114.8
ASAP male non-smoker	0.39	97.5	33.5	111.7	68.1	136.2
ASAP female smoker	0.44	110	31.2	104.0	69.8	139.6
ASAP female non-smoker	0.59	147.5	35.4	118.0	82.5	165.0
ATBC	0.34	85.0	28.8	96.0		
CARET	0.32	80.0				
CHAOS			33.2	110.7		
HPS	0.32	80.0	27.0	90.0	43.2	86.4
Linxian: Dysplasia	0.21	52.5			22.5	45.0
Linxian: General	0.22	55.0			30.7	61.4
Skin cancer study	0.39	97.5				
SUVIMAX men	0.56	140.0	31.7	105.7	52.8	105.6
SUVIMAX women	0.76	190.0	31.7	105.7	61.3	122.6

^{a)} % of the recommended plasma levels according to ref. 12.

Table V: Major inclusion criteria of some RCTs.

HPS	Adults with coronary disease, other occlusive arterial disease, or diabetes were randomly allocated to receive antioxidant vitamin supplementation or placebo
ASAP	Smoking and nonsmoking men and postmenopausal women with serum cholesterol ≥ 5.0 mmol/L
HOPE	Women and men who were at high risk for cardiovascular events because they had cardiovascular disease or diabetes in addition to one other risk factor
SUVIMAX	Healthy French adults

In a consensus conference in Hohenheim, researchers came to the conclusion that an adequate plasma level should be achieved in order to benefit from the preventive potential of antioxidant vitamins. The following values have been proposed: 30 $\mu\text{mol/L}$ vitamin E, 50 $\mu\text{mol/L}$ vitamin C and 0.4 $\mu\text{mol/L}$ β -carotene. In order to reach these levels, they suggested an intake of 75–150 mg of vitamin C, 15–30 mg of vitamin E, and 2–4 mg β -carotene per day. These recommendations are in very good agreement with the RDAs in Europe and USA (Table III).

With this lesson in mind, it becomes evident that already the placebo groups of most randomized trials, where plasma levels have been reported, fulfilled this requirement with the exception of the Linxian trial (Table IV).

Thus, observational epidemiological studies always used a reference group that was close to deficiency. Randomized trials, however, answered only the question whether high dose supplements had an additional

effect on top of an adequate supply in particular risk groups (Fig 4).

The observational studies point at two major interrelations:

- A vitamin intake significantly below RDA might be a risk factor for chronic diseases
- Chronic diseases are the consequence of the occurrence of several risk factors, among them chronic vitamin deficiency.

To prove this interrelation a RCT should, therefore, test the hypothesis whether the elimination of a chronic vitamin deficiency reduces the risk of a chronic disease. However, from the inclusion criteria of some of the RCTs it is evident that such a hypothesis has, so far, not been tested (Table V). Hence, the wrong approach has been used for the design of RCTs that should confirm findings from observational studies.

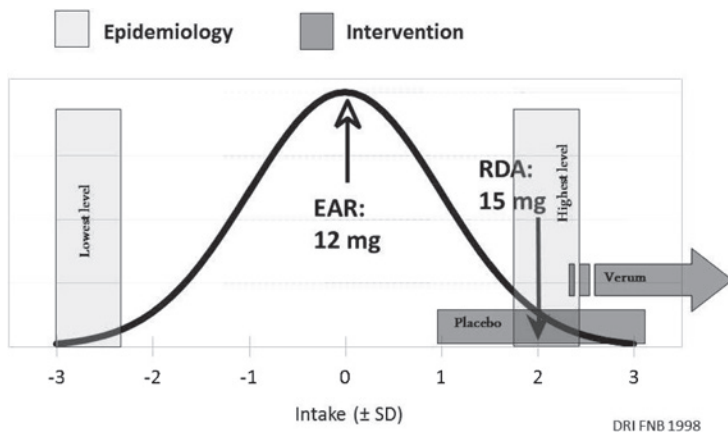


Figure 4: Discrepancy between observational studies and RCTs, e.g. vitamin E.

Conclusion

Wrong approaches have delayed the discovery of vitamins since a group of scientists argued that only germs and toxins can cause diseases, not deficiency of nutrients. Several diseases (scurvy, pellagra, etc.) are now known that are caused by a severe vitamin deficiency. Observational studies point at an association between low intakes of nutrients below RDA and part of the risk for a chronic disease. Although the aim of many RCT was to prove this association, it was only tested whether a high intake of a nutrient on top of an adequate supply (RDA) can reduce the disease risk. Obviously this is not the case, but the question is still open: are chronic vitamin deficiencies a risk factor for chronic diseases? The answer could only be given by correcting low vitamin intakes up to the RDA level and thus lowering the part of the disease risk that is due to the vitamin deficiency. However, this challenge might be too ambitious, more data could be gathered by observational- and mechanistic studies instead.

Abbreviations

AREDS	Age-Related Eye Disease Study
ASAP	Antioxidant Supplementation in Atherosclerosis Prevention study
ATBC	α -Tocopherol, β -Carotene Cancer Prevention Study
CARET	β -Carotene and Retinol Efficacy Trial
CHAOS	Cambridge Heart Antioxidant Study
HOPE	Heart Outcomes Prevention Evaluation Study
HPS	Heart Protection Study
MONICA	Monitoring of Cardiovascular Disease and Cancer

RDA Recommended Dietary Allowance
 SUVIMAX Supplémentation en Vitamines et Minéraux Antioxydants

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Original Communication

Discovery-Based Nutritional Systems Biology: Developing N-of-1 Nutrigenomic Research

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Abstract: The progress in and success of biomedical research over the past century was built on the foundation outlined in R.A. Fisher's *The Design of Experiments* (1935), which described the theory and methodological approach to designing research studies. A key tenet of Fisher's treatise, widely adopted by the research community, is randomization, the process of assigning individuals to random groups or treatments. Comparing outcomes or responses between these groups yields "risk factors" called population attributable risks (PAR), which are statistical estimates of the percentage reduction in disease if the risk were avoided or in the case of genetic associations, if the gene variant were not present *in the population*. High throughput metabolomics, proteomic and genomic technologies provide 21st century data that humans cannot be randomized into groups: individuals are genetically and biochemically distinct. Gene – environment interactions caused by unique dietary and lifestyle factors contribute to heterogeneity in physiologies observed in human studies. The risk factors determined for populations (i.e., PAR) cannot be applied to the individual. Developing individual risk or benefit factors in light of the genetic diversity of human populations, the complexity of foods, culture and lifestyle, and the variety of metabolic processes that lead to health or disease are significant challenges for personalizing dietary advice for healthy or medical treatments for individuals with chronic disease.

Key words: group level analysis, experimental design, systems nutrition research

Biological and biomedical research, like many established human activities, is based on conventions and practices that become standardized over time. Many of these conventions are decades old and do not account for the great advances in knowledge resulting from modern research. Some of the main practices of biomedical research are discussed herein, in an attempt to initiate a dialogue in the nutrition community about new concepts for personalized nutrition and health.

The Design of Experiments

The design of biomedical research experiments rests on standards developed almost 100 years ago. The methodology was codified in RA Fisher's *The Design of Experiments* [12] a 1935 treatise that described the key elements of good research protocols. These benchmarks are a valued requisite for determining the quality of experimental results and Fisher's experimental design is now considered the gold standard for human

Table I: Characteristics of Experimental Designs.

Randomization	Assign individuals at random to groups or to different groups in an experiment
Replication	Identify the sources of variation, better estimate the true effects of treatments, strengthen the experiment's reliability and validity
Blocking	Reduces known but irrelevant sources of variation between units for estimating the source of variation under study
Orthogonality	The forms of comparison (contrasts) that can be legitimately and efficiently carried out. Comparisons are uncorrelated and independently distributed if the data are normal.
Factorial Experiments	Evaluate the effects and possible interactions of several factors (independent variables)

biomedical research studies. Experiments designed according to Fisher's principles (see Table I), when applied to the appropriate scientific question, produce reliable evidence for improving public and personal health. More humans survive childbirth, infancy, childhood, and through advanced age than any time in our species history [44].

One of the key tenets of designing experiments is the requirement for randomization of subjects or other outbred animals to case and control groups. Randomization distributes unknown or unmeasurable characteristics between groups in an attempt to isolate and identify measurable variables that differ between or distinguish those groups. While this step was necessary in the 20th century, the underlying rationale for its use is no longer valid in the post-genomic era, especially for variables that produce small biological effects. For example, one of the key variables that could not be analyzed in sufficient detail was an individual's genetic make-up. However, genome technologies now allow for the characterization at the DNA sequence level (rev in [27, 34]) and soon at the epigenome (i. e., DNA methylation) level (e. g., [10, 11]). Genetic variation is no longer unmeasurable or unknowable. The necessity to analyze genetic makeup has been demonstrated from results of numerous genome sequencing projects: the latest data [8, 43] suggests that individuals differ from each other and reference genomes by about 3.5 million single nucleotide polymorphisms (SNPs), almost 1000 large copy number variants (CNVs), and large numbers of insertions and deletions (indels). An individual's genetic make-up differs from others and has to be measured and incorporated into biomedical research studies at the risk of producing unreliable and irreproducible results.

Randomization has serious consequences on the ability to interpret data from experiments where the response of a group exposed to an intervention is compared to a group not exposed (control). Genetic diversity will usually result in phenotypic diversity. Physiological variability has been recognized for mil-

lennia and summarized in the modern pre-genomic era by Williams in a 1956 book entitled *Biochemical Individuality* [46]. The ability to account for and measure individual phenotypic variation, however, has only become possible over the past two decades: high throughput tools and methods such as mass spectroscopy are now available to quantitatively measure human physiology, including in response to food (e. g., [5, 47]). A major challenge remains assessing environmental, cultural, and psycho-social variation but modern technology in the form of smart phones and tablets is increasingly used to measure an individual's activities, although linking these data to a common research database remains a challenge [42].

The edifice of statistical methods for group level research must also be re-evaluated:

- Comparing the average measure or response of one group (cases) against those measures in a second group (controls) yields the population attributable risk (PAR) [20, 26, 40]. Hence, PARs are population based – the average of a group – and these values do not apply to individuals because every individual is genetically distinct. PARs are applicable for large effect sizes (e. g., malaria, cholera) but have decreasing reliability as the difference in measures between groups decreases. No statistic is available to assess when PARs may provide knowledge that can be applied to all individuals in the population. The era of personalization requires the development of more personalized risk and benefit factor analysis.
- Power calculations (e. g., [13, 23–25]) do not consider genetic, phenotypic, and environmental diversity. Power analysis is used to calculate the minimum sample size required for detecting an effect of a given size. Alternatively, power calculations also allow one to determine the effect size that may be observed given the number of samples. Regardless of the approach, power analysis for studies of outbred species do not account for genetic, phenotypic, and environmental (such as diet) variation

between individuals. An unintended consequence of increasing the sample size to adequately power an experiment is that increasing the sample size of human studies results in increasing the “noise” while decreasing the signal within each group since each individual added to the study introduces differences in genetic, environmental, lifestyle, and cultural factors. When these factors are not measured in the study, this additional variability can only be considered as “noise” in the analysis.

Analyzing Variables One-By-One

Beadle and Tatum [1] published a seminal research concept and method in 1941 that is summarized as the one gene – one enzyme hypothesis. Although their experimental paradigm revolutionized biomedical research by demonstrating that a mutation in a single gene could alter enzyme activity, their complete hypothesis better reflects biological processes. Their treatise stated that although there would be simple one-to-one relationships, they also predicted that “...relations of great complexity” would also exist. However, their experimental results focused almost exclusively on auxotrophic mutations in *Neurospora*, which could be rescued by supplementing the media with the appropriate compound. The general applicability of their method contributed to the analysis of single compounds or genes. Even many genome wide association studies (GWAS) rely upon mathematical tools and criteria that analyze the association of multiple but independently-tested SNPs with some complex phenotype [33]. That is, each SNP is considered independently of interactions with other SNPs or genetic variants.

Most biological processes however, are relationships of great complexity and cannot often be characterized or understood by analyzing the effect or activity of a single gene, RNA, protein, enzyme, or metabolite. Organisms consist of systems consisting of interactions with the environment, interactions between organs, interactions between cells, and within cells of interconnected pathways, networks, and relationships. The complex organism is often studied by removing a component (e.g., metabolite or gene) from the cell system. Depending upon the importance of component, removing it from the system may force the system to channel flux through alternate pathways and networks. These concepts were also extended by Hartwell and coworkers [16–18] to the “normal” state in that flux through pathways is constrained by the

requirements of life. If one reaction of the system is fast, another reaction may be slow, so that the overall flux is held within a tolerable range.

With this inherent interactivity of biochemical systems, it is difficult to obtain a clear understanding of the system by analyzing each constituent in isolation. Network-based approaches are well adapted for this purpose, as they consider these interactions as the basic framework for the analysis. The variety of approaches for network analysis considers the available data and the goal of the analysis. If high throughput measurements of system elements are available (such as transcriptomics or proteomics), network analysis can be used to identify the most active regions of the system with respect to a given intervention or phenotype. Alternatively, if kinetic information is available for the interactions in the system, dynamic simulation can provide predictions for system behavior following a given perturbation of interest.

System biology

Aristotle wrote “...the whole is greater than the sum of its parts” (rev. in [47]) thousands of years ago. The general concepts from such thinking were further developed in von Bertalanffy in *General Systems Theory* [2], the foundation of modern systems biology concepts [3]. Three key concepts of systems theory are emergence, openness, and concurrency. Emergence is an ability of the system to have capabilities greater than the sum of the parts – an automobile moves only because of the combination and integration of the parts, much like Aristotle’s dictum. Machines have utility as metaphors but also do not capture other aspects of system thinking. For example, general systems theory also holds that biological systems are not “closed” like a machine but open [2]. That is, a biological system interacts with and adapts to its environment. A third related concept is concurrency, which describes interacting systems within machines or systems (e.g., a subsystem) that have internal distributed systems/embedded systems/sensor networks. The behavior of the subsystems is determined by the interaction of their internal state with the environment. Similarly, organisms interact with and respond to their environments.

Nutrigenomics describes this type of open, concurrent system by the term gene – environment interactions, classically defined as the statistical main effect of the interaction term [37]. Dominant mutations that cause catastrophic breaks in the system (e.g., inborn errors of metabolism [7]) and environments that over-

ride biological processes (such as cholera) are the exceptions to this rule. Biological systems respond to the signals from the environment such as nutrients, toxins, light exposure, but the system also adapts metabolism to respond to the environmental conditions by changes in transcriptional regulation or intracellular signaling that ultimately affect phenotype [21, 32, 36]. Embedded in this concept is that open systems are dynamic, changing due to changing interactions between environment and host.

The challenge of system thinking is defining the system. An analogy is Google Map: the ability to zoom from an earth view to a street view. Metabolites and proteins, that is reactions, may be considered the street view. The whole system or phenotype is the planet view. In between are layers of organization from the neighborhood of pathways to communities, counties,

states, and nations in modules, networks, organ systems. Each layer provides information that can be used to produce some understanding of the biological processes. The current state of system research and analysis focuses on components and interactions such as between pathways or network-network interactions. The results are (usually) maps that attempt to distill knowledge of emergent properties of the biological systems that cannot be directly inferred from the properties of the constituent parts. High-throughput data generation provides the input for systems analysis, permitting a more complete assessment of functional intermediates (i.e., transcripts/proteins/metabolites) between genotype/environment and phenotype. Environmental factors such as diet and lifestyle and cultural and psychosocial dynamics have been notably absent from most biomedical research programs.

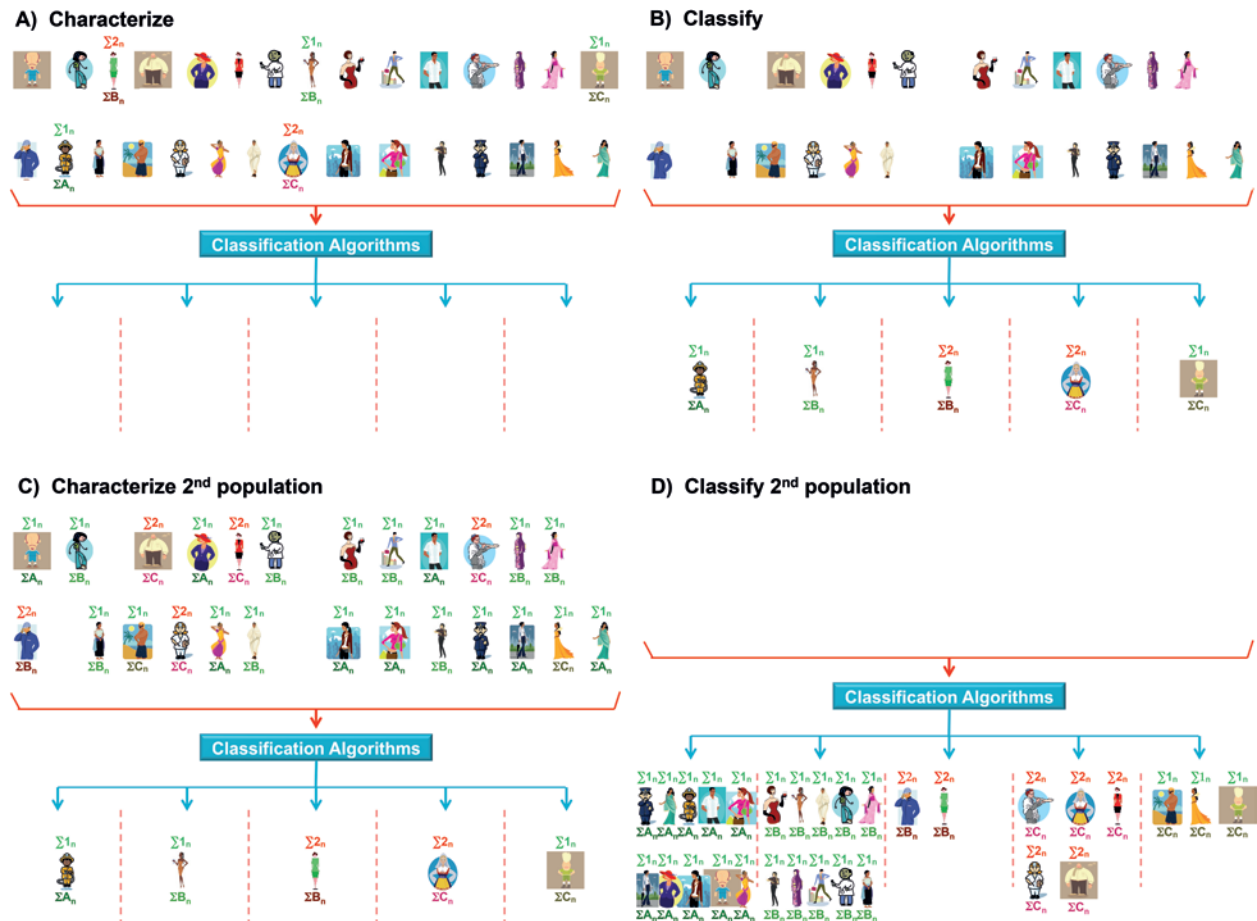


Figure 1: Conceptual strategy for n-of-1 studies. (A) Individuals in a population are “completely” characterized genetically and physiologically and environmentally. The genetic characterization is the sum $[\Sigma]$ of all genetic variations A, B, C and the physiological and environmental characterization is the sum $[\Sigma]$ of metabolomics, proteomic, lifestyle and other designated by numbers. After characterization, (B) classification algorithms are used to sort individuals into response groups that are empirically defined. To test these assignments, (C) individuals in a second population are analyzed as in (A) and then (D) classified as in (B). Each round of analysis and classification improves the probability that an individual is placed within the appropriate response or metabolic group.

While system thinking is essential for understanding biological processes, in most experimental cases, the data for the analyses are limited to metabolite, RNA, and protein in the blood, mRNA or microRNA from selected tissues, and genomic data. Only recently have attempts been made to integrate high dimensional data with measured environmental factors such as nutrient intake and physical activity, as well as multivariate (i. e., well-defined) phenotypes. That is, all of the components of the system are typically not analyzed, and perhaps, may never be analyzed. The final “systems” networks from such analyses can only be representations of the data acquired in the experiment, and as such, cannot be used to fully describe the final phenotype. Nevertheless, such representations better represent the data than two-dimensional, one-to-one relationships depicted in linear maps.

N-of-1 System Biology

These system biology concepts and technologies are beginning to alter experimental design and analyses (e. g., [15, 29–31]). However, most research conducted in the 21st century continues to be reductionistic, focusing on the effects of an individual nutrient or predicted consequences of an individual polymorphism in a gene to explain complex processes.

Strategies for analyses of individual data (often called n-of-1 data [35]) for identifying homeostatic groups and metabolic response groups (e. g., [19, 38]) may circumvent the limitations of case-control experimental designs. In n-of-1 designs, individuals are analyzed completely, or as completely as possible, and then analytical methods are used to find those of like response or characteristics (Figure 1). Such study designs are well suited to the use of unsupervised multivariate statistical approaches for class discovery from high throughput data, paired with network analysis for assessment of the functional context of observed patterns of variation. An interesting possibility from such analyses is that the grouping patterns of individuals at baseline may be different from the grouping patterns following a given intervention. From a translational perspective, these response groups will most likely help to inform optimal dietary interventions on an individual-specific basis. However there is more work to be done in terms of applying appropriate study designs and analytical strategies in order to understand nutritional systems.

Our approaches uses a cyclic method of analyzing a subset of individuals (Figure 1A) and classifying

them (Figure 1B). This approach allows the same data sets to be aggregated and used for analysis of population level results [35]. A distinct challenge for this experimental design is the level of data necessary to characterize the system. For example, while it has been long known that humans (and other animals) are hosts to a large variety of microorganisms, the ability to analyze microbial niches in and on the human body became possible only with the advent of high throughput analytical technologies. Mammals are “holobiont” – defined as a multicellular eukaryote plus its colonies of persistent symbionts [14, 41]. Organisms present in gastrointestinal, oral mucosa, urogenital, skin, and airways are being counted, but their functions are challenging to study [22]. The view of n-of-1 has to be expanded to include the microorganisms who live with us.

Systems Nutrition Research

Nutrition research, defined here as the entire environment that a holobiont is exposed to, is more challenging to study. Clinical research methods can be used – that is, experiments conducted in highly controlled settings. However, translation of results from such studies is challenging because real life has a wider range of variables than laboratory or clinical settings. One approach to conduct translational n-of-1 research is through community-based participatory research study [28]. CBPR’s central focus is developing a partnership among researchers and individuals in a community that allows for more in depth lifestyle analyses but also translational research that simultaneously helps improve the health of individuals and communities. As importantly, the unmeasurable factors or influences are incorporated into the overall physiological measures. Describing the setting of the research is metadata, which puts the research results into context even if not a part of the formal analytical process. While CBPR has been gaining much interest in the social and nutritional sciences fields [6, 39], relatively few studies have used this method for biomedical research [4, 9, 45]. CBPR is a process whereby the participants provide information and biological samples on an ongoing basis, and the biomedical researcher provides existing knowledge as well as results from the ongoing study. Research is “personalized” since one individual is assessed and informed even in the community setting. The applications are more immediate than population based methods and are targeted to the community and individual. Since genetic

SCOPE OF GENOTYPE ANALYSIS

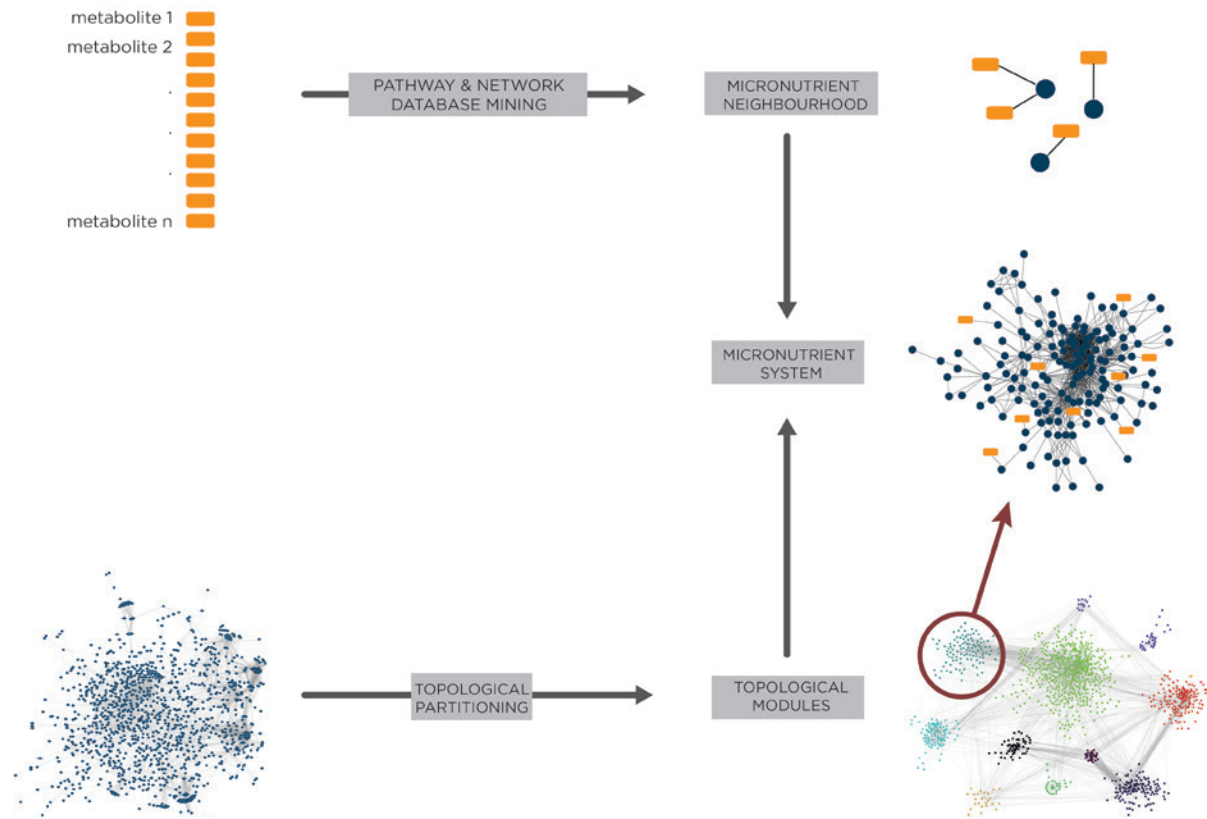


Figure 2: Middle Out Genotype Analyses. Two approaches were developed for the analyses of genotypes associated with metabolite levels or patterns. The top left shows metabolites that may be analyzed from subjects. An example may be vitamins in the blood samples. The pathways and networks that interact with, metabolize, or regulated by these metabolites are identified from pathway databases. This set of pathways and genes is called a neighborhood, and in this case, a micronutrient neighborhood. However, the pathways and networks may be unconnected due to the nature of pathway databases. A second approach uses a global interaction network derived from protein-protein interaction data. After topological partitioning, the function of the modules (subnetworks) are determined by pathways and genes within them (lower right). Modules which are enriched for micronutrient genes identify those systems most affected by changes in vitamin (in this example) status. In some cases, the neighborhood genes and one or more of the global network modules overlap as is shown in the micronutrient system in the middle right of this figure. Modules and subnetworks differ from pathways in that related functions are clustered. These representations provide a different approach to representing biological knowledge.

and omic data developed from population studies can not yet be reliably associated with health outcomes in individuals, the initial information flows between researcher and community collaborator focused on nutritional assessments and dietary advice. As more gene – nutrient or omic – nutrient associations are proven, the information flow will include biomedical data and results.

We applied this strategy to children and teens (ages 6 to 14), offering improved nutritious breakfast, lunch, and two healthy snacks during a 5-week summer day camp. Data aggregation (i. e., population level) results for nutrient intakes, healthy eating index scores, metabolite levels in plasma, and population genetic results

were analyzed (Monteiro et al, in preparation). The n-of-1 analyses identified metabolic groups consisting of patterns of plasma vitamin levels and red blood cell metabolites. We also developed a middle-out (as opposed to top-down or bottom-up) procedure (Figure 2) to analyze genotype data by defining and testing associations of single nucleotide polymorphisms of genes involved in micronutrient metabolism, related gene networks, and their protein-protein interactions (Morine, Monteiro, et al, in preparation). Topological partitioning and enrichment analysis identified modules (subnetworks) enriched in genes encoding proteins that participate or regulate pathways associated with micronutrients. One of these micronutrient

modules was significantly enriched with SNPs that differed statistically between groups defined by patterns of metabolite levels.

Our n-of-1 experimental design accounts for individual genetic and lifestyle (including dietary) differences and produces data that can be analyzed at the population level, metabolic group level, and individual level. It has not escaped our attention that this experimental design can be applied to a variety of biomedical research questions.

Summary

The advent of technology permitting the more complete characterization of biological systems has provided data demonstrating the genetic, physiological, and biochemical diversity in the human (and other animal) specie. Analyzing complex processes cannot reasonably be done using approaches of measuring the effects of a single gene, protein, RNA, or enzyme or how a single nutrient or chemical alters the system. New experimental designs that account for the diversity of genotype X environment interactions need to be developed in order to improve personal and public health.

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Original Communication

Vitamins for the First 1000 Days: Preparing for Life

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Abstract: Vitamins are essential nutrients for many body functions and particularly important during growth. Adequate supply in pregnancy and in early infancy is therefore crucial, but there is still a lack of knowledge about the needed amounts of vitamins of children older than six months and also during pregnancy. Recommendations for intake levels are generally derived by extrapolation from data for infants based in turn on the contents in breast milk and those for adults. A vitamin of particular importance in pregnancy is folic acid due to its role in the development of the brain and nerve system and the prevention of fetal neural tube defects (NTD). Mandatory fortification of flour and certain other grain products in many countries has been associated with a reduction in NTD incidence. However, other deficiencies or suboptimal status of B vitamins, especially B₆ and B₁₂ have been repeatedly reported in pregnant women also in high-income countries. Vitamin A is one of the three most critical micronutrients globally and pregnant women and young children are especially vulnerable to deficiencies. Night blindness, anemia, and immunodeficiency are major consequences of inadequate supply in these populations. Much attention has recently been accorded vitamin D that is also critical in pregnant women and young children for instance because of its involvement in bone mineralization but also its more recently discovered immune-modulating function that is thought to prevent development of autoimmune diseases like diabetes mellitus type I. A healthy balanced diet provides the best basis for optimal pregnancy outcome, lactation performance, and complementary feeding. However, supplements or fortified foods may be needed to cover the high requirements especially of critical vitamins such as vitamin D and folic acid and to correct unfavorable dietary patterns in women or to adapt foods to the needs of young children.

Key words: vitamins, pregnancy, lactation, development, infants

Introduction

As the foundations for a healthy life are already laid in the womb and early infancy, adequate nutrition in this period is of great importance. With their numerous functions in the body, vitamins are essential for growth and development, and special requirements arise during pregnancy, lactation, and infancy. However, there is still a lack of concrete recommendations for these life phases due to a scarcity of direct

studies in the respective population groups that is among other reasons ethically founded. Furthermore, assessment of vitamin status can be impeded by alterations in biomarkers. This is especially the case during pregnancy, when the physiological increase in blood volume causes a hemodilution leading to decreased plasma levels of certain vitamins while others are unaffected due to increases in carrier proteins [1]. A recent Danish study found that the reduction in serum cobalamin associated with pregnancy was

accounted for by lower levels of haptocorrin-bound cobalamin while the biologically available form bound to transcobalamin was unchanged compared to non-pregnant women. Vitamin B₁₂ absorption showed no alterations either [2].

Requirements and recommendations for intake

In the case of most vitamins, current recommended daily intake levels for pregnant and lactating women as well as older infants (6 months to two years of age) are extrapolated from those for non-pregnant women or older children, respectively, or derived from the composition of breast milk [3]. However, especially in the latter case, the wide individual variation of nutrient contents in breast milk has to be considered [4].

Accordingly, the recommended intake levels vary between different national nutrition entities. However, all recommend higher intake levels for vitamins

in pregnancy and lactation apart from vitamin K and a few other exceptions in some countries (see Table I for some examples).

Vitamin A

Vitamin A is best known for its role in color and night vision, its aldehyde 11-cis retinal being an integral component of the photoreceptor pigment rhodopsin. According to WHO worldwide estimates in 2009, vitamin A deficiency affects about 190 and 19 million young children and pregnant women, respectively, mainly in the African and South-Asian regions. It is a major cause of xerophthalmia, night blindness, and anemia [5]. Moreover, it is essential for immune functions and mucosal integrity, and inadequate supply is associated with a higher susceptibility for intestinal and respiratory infections [6, 7]. Through its role in gene regulation, vitamin A is involved in fetal development, organogenesis, limb formation, and body symmetry. Upon binding the active form retinoic acid,

Table I: Recommended daily intake levels of some vitamins from selected nutrition and health organisations (references [16–19]). Changes compared to levels recommended for non-pregnant women are given in parentheses.

	Vita- min A µg/d	Vita- min D µg/d	Vita- min E mg/d	Vita- min K µg/d	Vita- min B1 mg/d	Vita- min B2 mg/d	Vita- min B6 mg/d	Vita- min B12 µg/d	Folate µg/d	Vita- min C mg/d
FAO/ WHO 2001										
Pregnancy	800 (60)	5	*	55	1.4 (27)	1.4 (27)	1.9 (46)	2.6 (8)	600 (50)	55 (22)
Lactation	850 (70)	5	*	75–90	1.5 (36)	1.6 (45)	2.0 (54)	2.8 (17)	500 (25)	70 (56)
IOM, 1998/2000/ 2001/2010										
Pregnancy	750–770 (7–10)	15	15	75–90	1.4 (27–40)	1.4 (27–40)	1.9 (46–58)	2.6 (8)	600 (50)	80–85 (13–23)
Lactation	1200–1300 (71–86)	15	19 (27)	75–90	1.4 (27–40)	1.6 (45–60)	2.0 (54–67)	2.8 (17)	500 (25)	115–120 (60–77)
D–A–CH, 2000 (2010)										
Pregnancy	1100 (37.5)	5/20 [#]	13 (8)	60	1.2 (20)	1.5 (25)	1.9 (58)	3.5 (17)	600 (50)	110 (10)
Lactation	1500 (53)	5/20 [#]	17 (42)	60	1.4 (40)	1.6 (33)	1.9 (58)	4.0 (33)	600 (50)	150 (50)
NNR, 2004										
Pregnancy	800 (14)	10 (33)	10 (25)	n.d.	1.5 (36)	1.6 (23)	1.5 (15–25)	2.0	500 (25)	85 (13)
Lactation	1100 (57)	10 (33)	11 (37.5)	n.d.	1.6 (45)	1.7 (31)	1.6 (23–33)	2.6 (30)	500 (25)	100 (33)

Bold: differing from levels for non-pregnant/non-lactating women

* no evidence for altered requirements

revision of 2010 gives 20 µg/d for all population groups under the assumption of no sunlight exposure

the nuclear retinoic acid receptors (RAR) and the retinoid X receptors (RXR) build heterodimers with each other and, in the case of RXR, also with other nuclear receptors like PPARs or vitamin D receptor, and act as ligand-activated transcription factors via retinoid acid response elements (RARE). During fetal development, they regulate cell differentiation through morphogenetic processes based on local concentration gradients [7]. Deficiency during pregnancy can thus result in teratogenic effects on various organs and tissues such as the heart, eyes, the circulatory, pulmonary, urogenitary and central nervous systems, especially the hindbrain. However, excessive exposure to vitamin A shows teratogenic effects as well, and both, deficiency as well as overload, lead to malformations like spina bifida, hydrocephalus, palate cleft, anophthalmia and limb deformities [8, 9].

Vitamin D

Vitamin D has received much attention over the last years, as new functions beyond its role in bone metabolism have emerged. Indeed, vitamin D receptor is expressed in many tissues of the body and inadequate status of 1,25-(OH)₂-cholecalciferol has been associated with higher risk for cardiovascular disease and certain cancers, insulin resistance and disturbed glucose metabolism. Vitamin D has immune-modulating effects and is a regulator of gene expression [10]. In early life, the role of vitamin in bone formation is obviously of great importance. As status in pregnant women is often suboptimal and not sufficient to assure adequate concentrations in breast milk, supplementation of infants with 10 µg/d is advised [11]. Another crucial role of vitamin D in early life is related to its role in directing the immune system towards a more tolerogenic behavior, by stimulating the differentiation of naive CD4⁺ T cells to regulatory T and T helper 2 cells, thus preventing the development of autoimmune diseases [12]. Indeed, a relationship between low 25-OH-D₃ status and the incidence of diabetes mellitus type 1, multiple sclerosis, and rheumatoid arthritis has been observed in epidemiologic studies [13]. In a Norwegian study, the odds of developing diabetes mellitus type 1 were more than twofold higher in children of mothers with the lowest 25-OH-D₃ levels (≤ 54 nmol/L) compared to children of mothers with the highest levels (> 89 nmol/L) [14].

So far, intake recommendations for infants are mainly based on the needs for bone development and the avoidance of rickets while recently discovered functions of vitamin D have so far not been taken

into account in light of the scarcity of scientific data [15–19].

Folate

Folate is among the first vitamins that come to mind in the context of pregnancy and fetal development due to its role in the prevention of neural tube defects (NTDs). Indeed, a reduction of the incidence of NTDs was the motivation to implement mandatory fortification of cereal products like flour or bread with folic acid in 72 countries worldwide, high- and low-income alike, especially on the American continent and in the Eastern Mediterranean region. This measure was accompanied by a notable decrease in the incidence of NTDs [20–22].

The main function of the various folate metabolites is the provision of single carbon (C1) units for the synthesis of purine and pyrimidine and amino acid metabolism. In this way, it is essential for DNA replication and the reconversion of homocysteine to methionine. However, as a major source of methyl groups, folate is also involved in epigenetic methylation reactions. Effects of maternal folate status on epigenetic patterns and the phenotype of the offspring have been reported [23].

Besides folate, vitamin B₂, B₆ and B₁₂, being cofactors of enzymes catalyzing C1 transfer reactions, are essential for growth and development [24].

Critical vitamins in pregnancy and infancy

The particular role of and higher requirement for certain vitamins in pregnancy and infancy make pregnant and lactating women and young children prone for deficiency states. Inadequate supply with vitamins in pregnancy and during lactation exhausts the mother's body stores. Indeed, consecutive pregnancies further deplete low status due to malnutrition with detrimental consequences for mother and fetus [25]. Maternal micronutrient status determines that of the infant allowing the build-up of stores during the first four to six months of life [26].

As mentioned above, at the global level, vitamin A is among the most critical nutrients, but less so in high-income countries [5]. In these latter, focus is rather on folate and vitamin D supply. A low intake of folate has repeatedly been reported as indeed consumption of its main sources, leafy green vegetables, pulses, wholegrain cereal, some fruits like citrus fruits, and liver, is insufficient.

The Austrian Nutrition Report 2008 showed inadequate intake of vitamin D, folate and vitamin B₆ from food in the participating pregnant women across all age groups. A marginal intake was seen for vitamin B₂ in women aged less than 25 years. However, in a Viennese subsample, biochemical folate status was satisfactory in more than 70 % of the women and markedly low in less than 5 %. In turn, vitamin B₆ status assessed through the activation of erythrocyte glutamate oxaloacetate transaminase (EGOT) was insufficient in about 85 % with markedly low levels in 25 %. Vitamin D status was marginally low in about 30 % [27].

In the German National Health Interview and Examination Survey for Children and Adolescents (KiGGS), 69 and about 64 % of non-immigrant boys and girls aged 1–2 years, respectively, had sufficient vitamin D status (> 50 nmol/L), moderate and severe deficiency were found in about 7 and 0.5 % of boys, respectively, and in 6 and 1.3 % of girls. Deficiency was more common in children from immigrant families, especially in girls [28].

Improving vitamin supply in early life

As outlined before, optimal vitamin supply is particularly important during early life as it lays the foundations of a future health and wellbeing. Measures to improve vitamin intake in pregnancy and infancy therefore deserve high priority in public health.

Generally, pregnancy has been shown as a period of greater health awareness in women making them more amenable to lifestyle and diet changes [29]. However, considering the increased requirements for vitamins during pregnancy and the difficulties often seen with the implementation of dietary changes, supplementation of critical nutrients presents a convenient approach to ensure adequate supply. In the Austrian Nutrition Report 2003, use of nutritional supplements was reported by 89 % of pregnant women between the 21st and 30th gestational week and 94 % between the 31st and 40th. In both groups, multivitamin products were most used [30]. Intake of folic acid supplements, either alone or in combination with other nutrients, was associated with a better status of B vitamins (B₂, B₆, B₁₂, folate) in Norwegian pregnant women [31]. Supplements contribute notably to vitamin D intake in pregnancy although the proportion of women not meeting the recommended intake level remains high even among supplement users. In turn, other vitamins

might be supplied in excess through supplements so that careful administration is warranted [32].

Fortified foods are another source of vitamins during pregnancy and lactation especially if women are not willing or able to take supplements as evidenced in the case of folate [20]. They are of particular relevance in low-income countries and women with low socio-economic status [33].

Fortified foods are also a major source of vitamins in infants and toddlers. In the former, infant formula supplies the increasing nutrient requirements no longer met by exclusive breastfeeding. In turn, as toddlers are gradually fed regular foods, a careful selection has to assure adequate vitamin intake. A US survey revealed a high contribution of fortified foods to vitamin supply in toddlers suggesting unfavorable dietary patterns such as a low vegetable intake often reported in toddlers [34–35]. In accordance with these findings, in German children aged 1 year, mean folate intake was below the age-specific recommended intake level. Consumption of fortified foods, particularly infant formula and weaning foods as well as breakfast cereals, was associated with higher folate intake [36].

Conclusion

Adequate vitamin status is a prerequisite for healthy development in early life that also influences adult health and wellbeing. A healthy balanced diet during pregnancy, lactation, and infancy is the best source of these essential nutrients making nutritional counseling for pregnant women and mothers a public health priority. However, supplementation and/or fortification can make a contribution when the high demands for growth and development are difficult to meet through food alone.

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Original Communication

Nutrition Throughout Life: Folate

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Abstract: Scientific evidence supports a number of roles for folate in maintaining health from early life to old age. Folate is required for one-carbon metabolism, including the remethylation of homocysteine to methionine; thus elevated plasma homocysteine reflects functional folate deficiency. Optimal folate status has an established role in preventing NTD and there is strong evidence indicating that it also has a role in the primary prevention of stroke. The most important genetic determinant of homocysteine in the general population is the common 677C→T variant in the gene encoding the folate-metabolising enzyme, MTHFR; homozygous individuals (TT genotype) have reduced enzyme activity and elevated plasma homocysteine concentrations. Meta-analyses indicate that the TT genotype carries a 14 to 21 % increased risk of CVD, but there is considerable geographic variation in the extent of excess CVD risk. A novel interaction between this folate polymorphism and riboflavin (a co-factor for MTHFR) has recently been identified. Intervention with supplemental riboflavin targeted specifically at individuals with the *MTHFR* 677TT genotype was shown to result in significant lowering of blood pressure in hypertensive people and in patients with CVD. This review considers the established and emerging roles for folate throughout the lifecycle, and some public health issues related to optimising folate status.

Key words: folate; folic acid; neural tube defects (NTD); cardiovascular disease (CVD), personalised medicine

Introduction

Natural folates are a mixture of reduced forms of the vitamin, typically involving one-carbon substitution of the pteridine ring (most predominantly 5-methyltetrahydrofolate) and usually in the polyglutamylated form containing a variable number of glutamate residues (Figure 1). Folic acid refers to the synthetic form, a monoglutamate, found in the human diet only in fortified foods and supplements but readily converted to the natural co-factor forms after ingestion. Folic acid is fully oxidised; natural food folates in comparison are inherently less stable and show incomplete bioavailability.

Folate is required for one-carbon metabolism. This involves the transfer and utilisation of one-carbon units in pathways related to amino acid metabolism,

methylation processes and in DNA and RNA biosynthesis [1]. Folate status is routinely assessed by measurement of folate concentrations in serum/plasma or in red blood cells. Red cell folate is considered to represent folate stores and is the best index of longer term status (i. e. over the previous 3–4 months), whereas serum folate reflects recent dietary intake [2]. Folate, along with related B vitamins (namely vitamin B12 and vitamin B6), is required for the metabolism of homocysteine. When folate status is low or deficient, plasma homocysteine concentration is invariably elevated. Apart from its utility as a sensitive (albeit non-specific) functional biomarker of folate status, there has been considerable interest in plasma homocysteine as a potential risk factor for cardiovascular and other diseases of ageing.

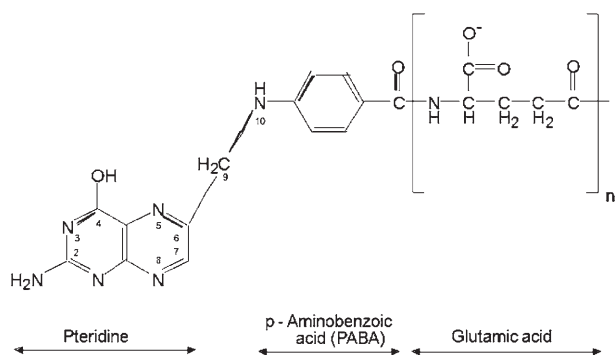


Figure 1: Structure of pteroylglutamic acid (i.e. folic acid), the folate form found in supplements and fortified foods.

Folate deficiency

Megaloblastic anaemia is the clinical sign of folate deficiency. This clinical manifestation is the result of an underlying biological perturbation of impaired synthesis of DNA. Megaloblastic anaemia is characterised by larger than normal red cell precursors (megaloblasts) in the bone marrow, macrocytes in the peripheral blood and giantism in the morphology of proliferating cells [3]. Deficient or low folate status is not uncommon even in otherwise well-nourished populations. Folate deficiency can arise as a result of increased requirements or decreased availability, with clinical folate deficiency more likely to be present when both occur simultaneously.

Pregnancy is well recognised as a time when folate requirement is increased to sustain the increased demand for folate related to rapid cell replication and growth of foetal, placental and maternal tissue. The discovery of folate is in fact attributed to reports that cases of macrocytic anaemia in pregnant Indian women were responsive to treatment with crude liver or yeast extract [4]; the active component in rich supply in both food sources was later recognised as folate and successfully synthesised. Folate deficiency in pregnancy is an important consideration because it is associated with a number of adverse outcomes such as early pregnancy loss, foetal growth retardation, low birth weight, pre-term delivery and neonatal folate deficiency [5]. Thus although public health efforts globally are focused on folate needs specifically to prevent neural tube defects (NTD) in early pregnancy, folate plays an important role throughout pregnancy in maintaining maternal and neonatal health and in preventing maternal folate deficiency and adverse outcomes of pregnancy. Apart from pregnancy, folate requirements are increased in some pathological conditions including certain anaemias, malignancy

and in patients on renal dialysis [6]. Several commonly used drugs (e.g. some anticonvulsant drugs and sulphasalazine used in inflammatory bowel disease) can also increase folate requirements because they interfere with folate metabolism in some way although the precise mechanisms are not well understood.

Some malabsorptive conditions can decrease folate availability even in the face of adequate dietary intake [6]. For example, coeliac disease (also known as gluten enteropathy), a genetically-determined chronic inflammatory intestinal condition induced by the ingestion of gluten, commonly leads to folate depletion in undiagnosed patients and in those found to have persistent mucosal damage despite apparently following a gluten free diet [7]. Chronic alcoholism is associated with severe folate deficiency, the causes for which are numerous, including poor dietary intake, intestinal malabsorption, impaired hepatic uptake with reduced storage of endogenous folates and increased renal excretion [8].

Low folate status can however arise even in the absence of disease or other factors that increase requirements or cause intestinal malabsorption of folate. Dietary folate intakes can be considered suboptimal in the diets of many people in both developed and underdeveloped countries in that, although they may be adequate in preventing megaloblastic anemia, they are often insufficient in achieving a biomarker status of folate associated with the lowest risk of NTD and potentially other folate-related disease. This under-provision of folate is generally attributed to the poor stability and incomplete bioavailability of natural food folates when compared with the synthetic vitamin folic acid [9].

Role of optimal folate in maintaining health throughout the lifecycle

Emerging evidence supports a number of roles for folate in maintaining health, from maternal and foetal health in pregnancy [10] through childhood to preventing chronic disease in middle and old age [11–13]. Figure 2 shows potential roles for folate in various stages of the lifecycle, along with an indication of the extent of the current supporting evidence. The protective role of folate in each of two lifecycle stages is considered below.

Neural tube defects (NTD)

Because of conclusive evidence of the protective effect of folic acid against NTD, women of reproductive age

Role	Evidence
Maternal health* in pregnancy	conclusive
Foetal development	conclusive
Cognitive health in childhood	early evidence
Prevention of heart disease/stroke	convincing
Cancer prevention	promising
Bone health	possible role
Cognitive function in ageing	possible role

*Prevention of maternal folate deficiency (anaemia)

Figure 2: Role of optimal folate status in maintaining health throughout the lifecycle.

worldwide are recommended to take 400µg/day folic acid from preconception until the end of the first trimester of pregnancy. Despite such universal agreement on the appropriate recommendations, the proportion of women who take folic acid supplements from before conception as recommended is estimated to be only 20–30 % [14, 15]. One large multicentre study examining 13 million birth records from 9 European countries without population-based folic acid fortifica-

tion, showed that there was no detectable impact on the incidence of NTD in any country over the 10-year period from 1988 to 1998, covering the time before and after current folic acid recommendations were introduced and actively promoted for women of reproductive age (Figure 3) [16]. In contrast, NTD rates declined markedly by between 27 % and 50 % in the USA and Canada coinciding with the introduction of mandatory folic acid fortification of food which led to population-wide improvements in folate status [17, 18]. Thus folate status in many otherwise well-nourished populations is generally high enough to prevent overt deficiency (i. e. megaloblastic anaemia), but in the absence of mandatory folic acid fortification is insufficient in protecting against the occurrence of NTD.

Cardiovascular disease (CVD)

There has been much interest in the potential protective effect of folate and metabolically related B-

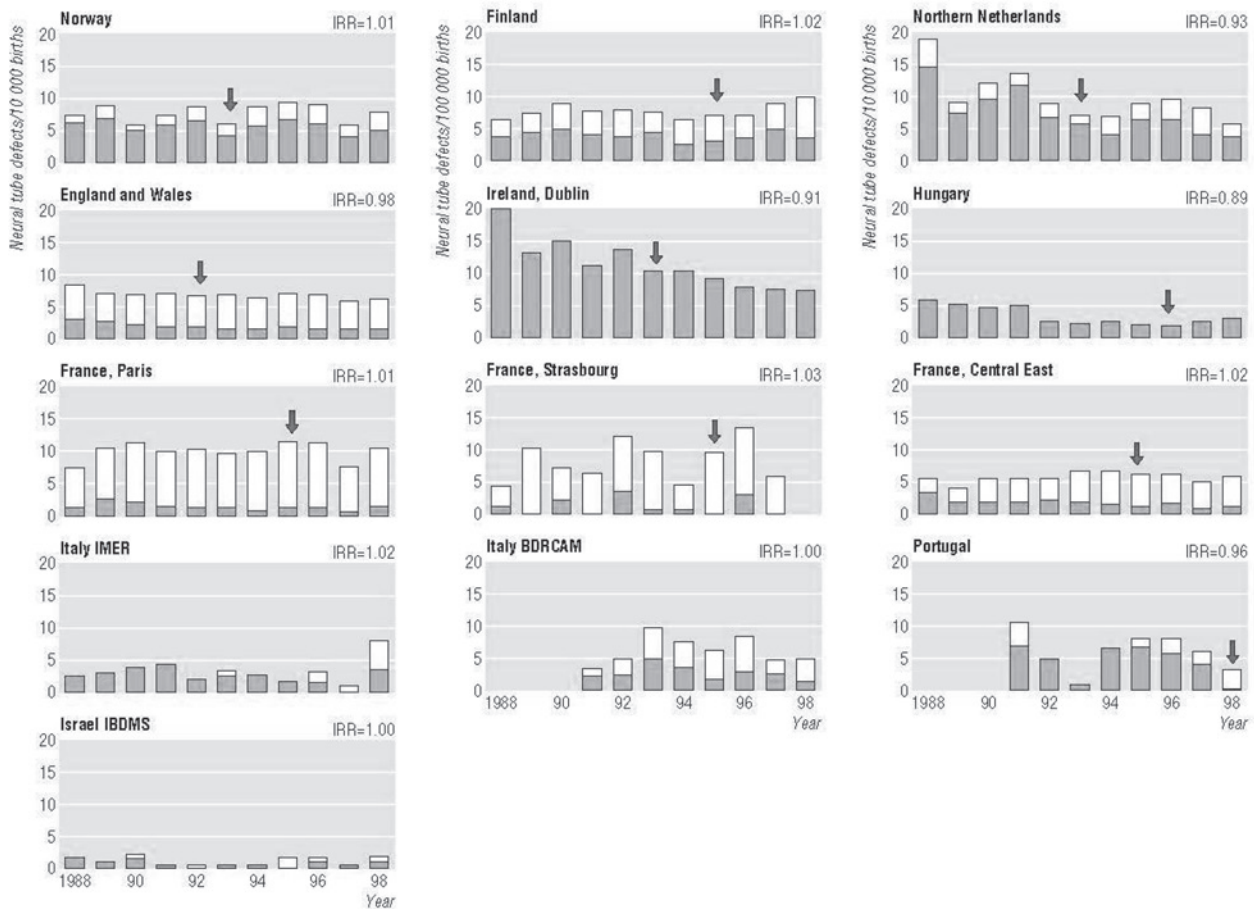


Figure 3: Rates of NTD per 10,000 births (1988 to 1998) [16]; Open top portion indicates terminated pregnancies. Arrow indicates time of recommendations in each country. IRR=average rate of change from one year to the next.

vitamins in CVD, an effect which may or may not be mediated via the role of these nutrients in maintaining plasma homocysteine concentrations within a desirable range. Predictions from epidemiological studies estimated that lowering plasma homocysteine by 3 $\mu\text{mol/l}$ would reduce the risk of coronary heart disease by 11–16 % and stroke by 19–24 % [19, 20]. The secondary prevention trials in at-risk patients published 2004–2012, however, failed to demonstrate a benefit of homocysteine-lowering therapy with B-vitamins on CVD events generally [21]. All of these trials were performed in CVD patients with advanced disease. Thus, what the current evidence suggests is that intervention with high dose folic acid is of no benefit in preventing another event or the progression of existing pathology. It remains possible however that folic acid plays a protective role against the initial development of CVD in healthy people. In addition, the evidence for a protective effect is generally stronger for stroke than for heart disease, with meta-analyses of randomised trials showing that folic acid reduces the risk of stroke, particularly in people with no history of stroke [22, 23]. Thus future trials to address the effect on CVD risk of intervention with folic acid, should investigate those without pre-existing disease and in addition focus on populations most likely to benefit, i. e. those with evidence of sub-optimal folate status. Exposing people with optimal biomarker status of folate to further increases is likely to be ineffective [22, 23] or even undesirable [24].

Genetic studies provide convincing evidence to support a causal relationship between sub-optimal folate status and the development of CVD. Meta-analyses report a 14 to 21 % higher risk of CVD in people homozygous (TT genotype) for the 677C→T variant in the gene encoding the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR), but there is considerable geographic variation in the extent of excess CVD risk linked with this common polymorphism [25–26]. A novel interaction between this folate polymorphism and riboflavin (a co-factor for MTHFR) has recently been identified, with recent evidence showing that supplemental riboflavin targeted specifically at individuals with the *MTHFR* 677TT genotype results in marked lowering of blood pressure [27]. Of note this effect, shown both in high risk CVD patients and in people with hypertension, appears to be independent of the homocysteine-lowering effect of riboflavin also seen only in the TT genotype group [28]. The role of this novel gene-nutrient interaction in blood pressure may thus provide insights as to the mechanism linking this

folate polymorphism with CVD generally. In particular the new evidence suggests that the reported excess risk of CVD is explained by higher blood pressure in individuals with the TT genotype rather than their elevated plasma homocysteine (i. e. the typically described phenotype).

Achieving optimal folate status

Red cell folate, widely considered the most robust biomarker of long term status, is found to be strongly correlated with habitual folate intake when the latter is expressed as Dietary Folate Equivalents (DFEs) [29]. The DFE, as used in the United States to express folate recommendations, was introduced to allow the much greater bioavailability of folic acid compared with naturally occurring food folates to be taken into account and is calculated as: μg natural food folate + 1.7 times μg added folic acid. In most European countries however, this conversion factor is not applied and dietary folate intake is expressed simply as total folate in $\mu\text{g/d}$, an approach that makes any comparison of folate intakes and recommendations across different countries inherently complicated. Also by disregarding the known differences in bioavailability between the natural food forms and folic acid, the relationship with biomarker status of folate is found to be far weaker than that observed where folate intake is expressed as DFEs [29].

There are potentially three options to achieve optimal folate status for nutrition and health benefits: increased intake of natural food folates, folic acid supplementation, folic acid fortification. The potential to optimise folate status by means of natural food sources is very limited as food folates may be unstable during cooking and show incomplete bioavailability once ingested [9]. Folic acid supplementation is a highly effective means to optimise folate status in individual women who take supplements, but the timeframe for preventing NTD presents a practical challenge to achieving this benefit on a population-wide basis. The neural tube closes 21–28 days after conception, a time when a woman may not necessarily be aware that she is pregnant and particularly so if the pregnancy is an unplanned one. Recent evidence from almost 300 pregnant women sampled at 14 weeks showed that 84 % were taking folic acid in the first trimester of pregnancy, but only 1 in 5 had commenced folic acid before conception as recommended [15]. Folic acid fortification, like folic acid supplementation, is highly effective as a means of

optimising folate status in individuals [30], and has the advantage over folic acid supplementation that it is also highly effective for populations. In populations with voluntary fortification in place, folic acid-fortified foods can have a very significant impact on folate intakes and biomarker status of consumers of these foods [29]. Mandatory folic acid fortification is now in place in over 60 countries worldwide; a measure that has reduced the incidence of NTD in the United States [17] and Canada [18] by between 27 % and 50 %. There is also some evidence that there has been an improvement in stroke mortality in North America as a result of this measure [31].

The folic acid dose required to achieve optimal folate status in relation to any health benefits may be lower than is generally perceived. Recent evidence showed that a dose of folic acid as low as 200 µg/d (over and above current dietary intakes) can, if administered for a prolonged period of 6 months, maximally lower homocysteine concentrations regardless of initial plasma homocysteine, suggesting that higher doses are unnecessary [32]. Several previous trials concluded that doses of >800 µg/d were required, but had probably underestimated the effect of folic acid at lower doses because of treatment durations that were too short to allow the maximal plasma homocysteine response to be observed.

Conclusion

Optimal folate has a proven effect in preventing NTD and a probable effect in preventing stroke, while the evidence for other protective roles through the life-cycle is still emerging. Outside of the United States and other countries worldwide with mandatory folic-acid fortification, achieving optimal folate status can be problematic because of the instability and incomplete bioavailability of folate from natural food sources. Thus folate intakes in many European countries are typically insufficient for the achievement of optimal folate status especially where access to fortified foods, even on a voluntary basis in some cases, is limited. Compared with natural food folates, folic acid offers a very stable and highly bioavailable vitamin form and has proven to be an effective means of increasing folate status in populations when it is delivered via fortification. Future randomised trials to demonstrate health benefits should target those at risk of sub-optimal folate status or with impaired folate metabolism as a result of genetic variants.

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Original Communication

The Role of Vitamins in Aging Societies

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Abstract: Raising numbers of elderly lead to a dramatic shift in demographics, accompanied by an increase in non-communicable diseases such as cancer, cardiovascular disease and dementia. All these conditions are thought to be modifiable by diet to some degree and mounting evidence indicates that improved intakes of certain vitamins can slow their progress. Strong evidence exists for the beneficial effect of vitamin D on the risk of bone fractures. Moreover, as chromosomal damage is a risk factor for dementia, supplementation with nutrients preventing these impairments are thought to have a beneficial effect on cognitive decline. However, the aging progress strongly affects nutrient intakes and utilisation due to social, physical and psychological changes. Data from dietary surveys suggest that many of the elderly in Europe have intakes for various vitamins that are well below the recommendations. The situation appears to be even more critical for elderly in institutions such as care homes. Given the increasing number of elderly and the importance of an adequate supply with vitamins, more research is warranted to find nutritional solutions to improve their wellbeing and health – which in the long run can be expected to contribute to reduce the ever increasing health care costs.

Key words: vitamin, aging, elderly, non-communicable diseases, inadequate intake

Century of aging and its consequences

Based on current estimates, in 2040, more than one in four European will be ≥ 65 and one in seven ≥ 75 years [1]. The 21st century was coined the *century of aging* as life expectancy increased during the past 100 years by >30 years due to declining mortality rates, which lead, combined with reduced fertility rates, to a dramatic shift in demographics [2]. As a result of this global phenomenon, non-communicable diseases (NCD) such as cardiovascular diseases, dementia and cancer are becoming more prevalent [1]. This is reflected in the 8 to 10 years difference between health and life expectancy, indicating that the last decade of life is marked by an increasing rate of disability [2]. Due to their chronic nature, NCD place a heavy burden on the economy, affecting increasingly also low and

middle income countries [3]. This means demand for health and long-term care as well as expenses for pensions and social security will increase [4]. Moreover, the prolonged illness also leads to a reduction in the quality of life [5].

Nutrition plays an important role in maintaining health throughout the lifecycle: According to the Institute of Medicine, “*nutrition is a key component to promoting healthy and functional living among older adults [6]*”. Bruce Ames formulated the so-called triage theory, which postulates that during times of nutritional scarcity, evolutionary priority was given to short-term survival at the expense of damage evident in older age [7]. Mounting evidence indicates that some of these adverse effects can be countered by adequate nutrition.

In elderly women in nursing homes, for example, supplementation with vitamin D and calcium de-

creased the risk of falling by 49 % compared to calcium supplementation alone [8]. It also led to a reduction in body sway, a risk factor for hip fractures, by 9 % [9]. A recent meta-analysis including >31.000 persons >65 years reported a 30 % reduction of hip fractures and a 14 % decrease of any non-vertebral fracture in the group with a median daily intake of 800 International Units (IU) vitamin D [10]. Consequently, it is now widely recommended that all postmenopausal women and men over the age of 60 should receive at least 800 IU of vitamin D supplements per day to minimise the risk of falls and fractures [11]. This was recently acknowledged in an 14.1 EFSA claim [12]. However, vitamin D deficiency is thought to be widespread among the elderly due to reduced production in the skin and renal insufficiency [13] as well as decreased absorption due to a reduced number of vitamin D receptor in the intestine [14].

Impact of aging on micronutrient intake and utilisation

Age and the often closely linked pathologies result in a multitude of physiological and social changes that affect food intake and utilisation. Decreased income after retirement, lack of mobility and social contacts lead to a decrease in food intake [13]. The decline in the effectiveness of detecting and reacting to hunger makes the elderly particularly vulnerable to malnutrition [15]. Moreover, appetite decreases due to declining taste and smell sensitivities, various pathological conditions or medications and impaired chewing due to ill-fitting dentures [16].

The efficiency of the stomach is often impaired due to decreased secretion of gastric acid, pepsin and mucus as well as a reduction in gastric emptying and blood supply [17]. Malabsorption is often a result of surgery or pathologic conditions such as Crohn's, Whipple's or celiac disease, infections with *Helicobacter pylori*, alcoholism or certain infections [17–19]. With age, the gut microbiota shifts towards an increase in enterobacteria at the cost of anaerobes and bifidobacteria, which increases the vulnerability to diarrhoeal diseases [20]. At the same time, constipation is widespread due to lack of exercise, dehydration, a diet low in dietary fibre and drug treatment [21].

While body mass increases from early adulthood to about 70 years mainly due to increase in visceral and subcutaneous fat, it decreases afterwards as lean mass and some subcutaneous fat is lost [22]. A decrease in circulating hormones involved in muscle metabolism

and damage due to oxidative stress and inflammatory processes affecting fat and protein metabolism further contribute to the loss of muscle tissue [23]. Whether caused by voluntary or involuntary factors, these changes were found to be associated with increased risk for malnutrition [22]. It is therefore thought that optimising food quality and increasing level of physical activity can improve nutrient status of elderly nursing home residents [24].

Micronutrients and non-communicable disease

Of the 10 leading causes of death, the six most important ones are NCD accounting for 70 % of deaths in the ≥65 years old in the US [25]. According to WHO, 36 out of 57 million global deaths in 2008 were due to NCD and 80 % of premature heart disease, stroke and diabetes can be prevented by modifying factors such as nutrition [26]. Due to the multifactorial nature of factors affecting the development of NCD and their long latency period, it is difficult to establish a clear cause-effect relationship between nutrition and chronic diseases [27]. Much of the evidence is derived from epidemiological studies as randomised control trials (RCT) are often not practical due to the long latency period of the effect and the ethical problems using no or insufficient intakes of nutrients as a 'true' placebo controls [28]. Due to these and a host of other reasons, the evidence from RCT is considered inconsistent. However, a recently published RCT with a follow-up of more than a decade showed that long-term supplementation with multi-micronutrients led to a significant reduction of overall cancer risk by 8 % [29]. According to the authors, effects in the same subjects on the risk for cardiovascular events, eye diseases and cognitive decline will be published separately. The mounting evidence of the beneficial effect of various dietary interventions with micronutrients or associations thereof with health outcomes makes a strong case for the importance of micronutrients at old age.

Micronutrients and cognitive functions

Even though a strong correlation exists between aging and dementia, it is clearly a pathological condition caused by various genetic and environmental factors, including nutritional components [30]. It is thought

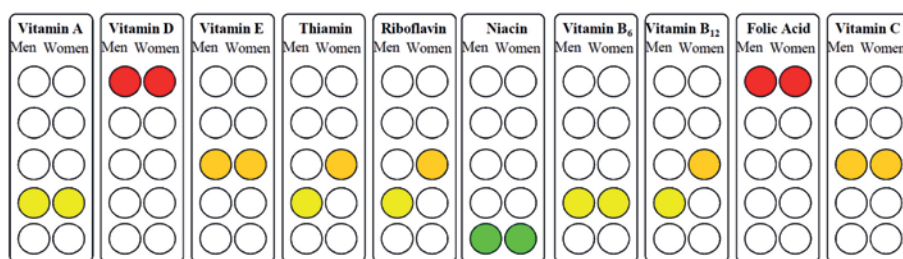


Figure 1: Elderly (65–80 years) in Germany with intakes below the specific recommended reference value for men (n= 1469) and women (n= 1562) [42]. • <5 %, • 5 % to 25 %, • >25 % to 50 %, • >50 % to 75 % and • >75 % of DACH recommendations [43, 44].

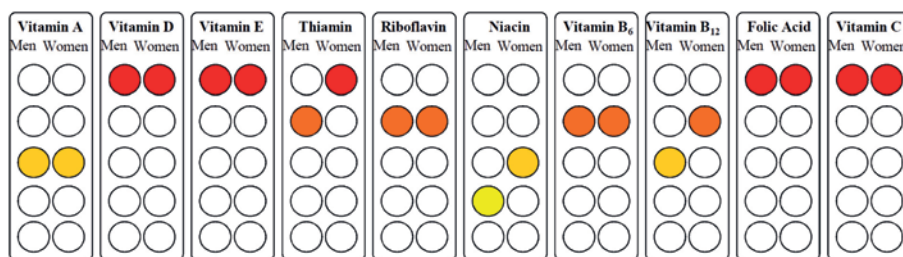


Figure 2: Institutionalised elderly (≥65 years) in Germany with intakes below the specific recommended reference value for men (n= 148) and women (n= 606) [40]. • <5 %, • 5 % to 25 %, • >25 % to 50 %, • >50 % to 75 % and • >75 % of DACH recommendations [43, 44].

that there is an association of increased homocysteine alone or combined with decreased levels of folate, vitamin B6 and/ or B12 and the development of dementia [30]. Low vitamin B12 status was connected with cognitive dysfunction [31], which improved with supplementation [32]. Chromosomal damage is associated with accelerated ageing and neurodegenerative diseases [33, 34]. Consequently, the impact various nutrient deficiencies are thought to have on the chromosomes, is likely affecting the development of dementia [35]. A recent meta-analysis found evidence that supplementation with multivitamins reduces the odds of cognitive decline, potentially also linked to the development of Alzheimer's disease [36]. Multivitamins were reported to slow down the progression of cognitive decline in elderly women [37]. Moreover, increased intakes of vitamins were also linked to alertness, general well-being and a reduction in negative mood-swings in elderly men [38]. Finally, in an RCT, high doses of folic acid, vitamin B6 and B12 were shown to significantly reduce the rate of age-related loss of brain tissue in elderly (>70 years) with cognitive impairments [39].

What do we know about micronutrient intakes in the elderly?

Data collected in the frame of a recent German nutrition survey shows that intakes for several vitamins are inadequate in the elderly (Figure 1). While vitamin D and folic acid appear to be the most critical, vitamin

E and C are low in up to half the elderly. Moreover, these data do not take into account reduced absorption of vitamins due to aging, but also concurrent diseases. The situation becomes even more critical when assessing the vitamin intakes of institutionalised elderly [40] (Figure 2). Similar results were reported in a recent report on the benefit of oral nutritional supplements for the elderly [41]. The authors concluded that the issue of malnutrition in the elderly needed to be addressed urgently and that nutritional interventions were shown to improve health outcomes and to be cost effective.

Conclusions

The rapid demographic shift we are witnessing is a global phenomenon and the resulting consequences, in particular for the growing segment of the elderly, are a challenge. As physiology changes with aging, the nutritional needs do too! A number of studies report benefits on various health outcomes of micronutrients including on cognition, cardiovascular disease and cancer. On the other hand, it is alarming that the micronutrient intake in significant parts of the elderly is well below current intake recommendations. We think this field of research should be given much more attention as there is encouraging evidence that an appropriate micronutrient status will contribute to wellbeing and health outcomes in the elderly – which can also be expected to help to reduce the ever increasing health care costs.

Abbreviations

IU International Units

NCD non-communicable disease

RCT randomized controlled trial

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Original Communication

Vitamins: Preparing for the Next 100 Years

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Abstract: The insights gained from the last 100 years of vitamin research and applications have contributed substantially to our fundamental understanding of biology and importantly to the promotion of human health. There is no reason to think that the next 100 years will be any less fruitful if we are committed to preparing for them, particularly by changing four critical nutrition paradigms. First, we must move beyond the concept of preventing vitamin deficiencies and inadequacies to establishing health and, further, to creating optimal physiological functions. Each essential vitamin possesses different concentration thresholds for its variety of effects and the required balance necessary to achieving each has yet to be fully defined. Second, we must apply the research approaches and methods of “-omics,” systems biology, and imaging technologies to define the dynamic role of vitamins and their broad array of genomic, molecular, biochemical, and functional interactions. Such work is necessary to understand the multiplicity of vitamin actions and ultimately apply them directly at the level of the individual. Third, we must revise the concept of evidence-based nutrition away from its current hierarchical system to recognize in a comprehensive and integrated way the attributes of each type of approach to research. To adhere to a single gold standard of the randomized clinical trial ignores both how we have moved forward so productively during the last 100 years and the vital information to be gained from basic research and other human studies; further, it acts to stifle innovation in both scientific and regulatory affairs. Fourth, we must understand that changes in the supply and distribution of food during the next century are likely to be at least as dramatic as those which have occurred over this last one. For example, inevitable environmental constraints will require more food protein be derived from plant than animal sources, a shift that will directly impact the dietary sources for vitamins. To meet the challenge of achieving global health in 2113 among a population of 9 billion people, effectively managing these four changes demands new and creative ways in which those in academia, government and non-government organizations, and industry must work together.

Key words: evidence-based nutrition, deficiency, optimal health, requirements, vitamins

Vitamins: From Preventing Deficiencies to Promoting Optimal Health

Important benefits to public health have been achieved from what we have first learned about vitamins and then applied that knowledge to individuals and their communities since Casimir Funk first described “vi-

tal amines” and “vitamines” in 1912 [1]. The insight gained from vitamin research has also contributed substantially to our fundamental understanding of biology and now suggests innovative new ways to continue the quest to promote human health and reduce our risk for common diseases. There is no reason to think that the next 100 years will be any less fruitful if we

are committed to preparing for them. However, this is no small challenge as it would be hubris to presume that our foods – and way we grow, process, transport, prepare, and cook them – will be the same as today in the next 50 or 100 years. Moving forward, we need to consider the ways in which we should define the role of vitamins beyond their original concept as dietary substances essential to preventing nutrient deficiencies.

Classical definitions viewed vitamins as substances essential in minute amounts for normal growth and activity of the body and obtained from plant and animal foods. Most vitamins were discovered because they were so closely associated with a corresponding deficiency disease, as was noted for conditions like beriberi, scurvy, rickets, and pellagra [2]. Thus, vitamins were first identified as useful to treat an established deficiency syndrome. Later, applications of vitamins for preventing an expected deficiency were found, including the fortification of milk with vitamin D and of refined flour with folic acid to reduce the incidence of rickets and neural tube birth defects, respectively. Similarly, administration of vitamin K to babies is now a standard approach to preventing hemorrhagic disease of the newborn. Today, we are beginning to appreciate the role of vitamins in the continuum of health from treating the very sick with total parenteral nutrition, to maintaining vitamin status sufficient to reduce the risk of deficiency in a population, to enhancing innate defenses against environmental and physiological stressors, and, ultimately, to promoting personalized optimal health and performance.

The dietary requirement for a vitamin is dependent on which criterion is selected to establish adequacy [3]. However, definitions of nutrient adequacy are neither fully clear nor consistent between countries or over time. For example, if the criterion selected for vitamin C is prevention of scurvy, then an adequate intake would be defined as 10 mg or less per day. However, if the criterion were the amount of vitamin C at which ascorbate is first excreted in the urine (as an approximation of saturation of body stores), then an adequate intake would be defined as a much higher value. Requirements for vitamin C today vary from 40 to 100 mg. Indeed, it is possible to have multiple recommended intakes, each corresponding to a different indicator or criterion of biochemical, cellular or physiological function. Considered in this manner, it can be appreciated that vitamins have multiple sites of action and, thus multiple thresholds for different functions. So, it is possible to have abnormal function in some parameters while other parameters for the same vitamin appear within normal range at lower doses. Indeed, the recognition of this dose-dependent

relationship between a vitamin and its many functions is one reason that recommended dietary allowances for most vitamins have changed over the last 60 years. Theoretically, a dose-response relationship might be developed between a vitamin and its associated capacity for reducing the risk of chronic diseases. For example, the dose of folic acid necessary to prevent megaloblastic anemia could represent one measure of adequacy, while a higher intake would be necessary to prevent neural tube birth defects, and higher intakes yet to reduce the risk of colorectal cancer, stroke or Alzheimer's disease. A similar dose hierarchy might be created for functional outcomes of folic acid like pyrimidine nucleotide biosynthesis, cervical dysplasia, homocysteine status, immune responsiveness, and cognitive performance. Importantly, simple relationships between a vitamin and its biochemical and cellular functions are rare.

Future research on vitamins needs to better appreciate the interrelationship between vitamins such that they are needed in the "right balance" for both efficacy and safety. Ready examples of this balance include the dependent interactions between folic acid, vitamins B2, B6, and B12, and choline in supporting one carbon metabolism [4] and between vitamins C and E as well as minerals like manganese, selenium and zinc and non-vitamins such as carotenoids and polyphenols in maintaining the antioxidant defense network [5]. Beyond the achieving the right balance between vitamins in determining their requirements for optimal health, is their further complex interplay with personal attributes like age, sex, and body composition as well as external factors like lifestyle (e. g., alcohol, physical activity, smoking) and exposure to environmental toxicants.

Emerging Approaches and Methods for Vitamin Research

Life expectancy has substantially increased over the last century due to reduced childhood mortality and improvements in sanitation, the advent of vaccinations, and improved medicine and nutrition. However, substantial benefits may still be achieved from optimizing nutrition to reduce the incidence of acute infectious diseases and chronic conditions like obesity, type 2 diabetes mellitus, cardiovascular disease, and cancer. This potential cannot be achieved without the application of recent advances in "-omics," systems biology, and bioinformatics. These and other technologies can expand our understanding of the actions of vitamins

and allow us to apply them to patients in hospital and apparently healthy individuals at home as well as more broadly to population groups, particularly vulnerable communities. We should be able to elucidate the direct and indirect roles by which vitamins interact with genes to alter functional outcomes (genomics), induce changes in gene expression through methylation and other reactions (epigenomics), influence gene expression as ligands for nuclear receptors (transcriptomics), participate in the post-translational modification of proteins (proteomics), and change the profile of the vast array of circulating metabolites (metabolomics) to affect phenotype [6]. As these data are generated, they allow for the application of interactomics where the interactions between all these processes and the consequences of these interactions can be examined as a broad network (interactome). This presents a top-down approach to systems biology allowing an overall view of an organism, leading to hypotheses that can be tested by new experiments.

Imaging technologies – including computed tomography, functional magnetic resonance imaging, position emission tomography, and single photon emission computed tomography – now allow for whole body dynamic, non-invasive detection of body composition, gene regulation, and molecular tracers and probes. The application of these technologies, particularly in research on cognitive function, should provide a greater depth of information by following vitamin actions in location and time. As a part of this approach as well as in research using -omics techniques, there is a need to better incorporate observations that examine how vitamins can prevent or correct not just single points in biochemical pathways but the imbalances of overarching processes such as inflammatory stress, metabolic stress, oxidative stress, and psychological stress [6]. When exploring these clusters of biomarkers, it is also useful to do so in the context of homeostatic adaptability, where physiological systems are perturbed by some of these stresses and their attenuation by vitamin intervention followed [7].

We are still in the early stages of exploring the interactions between nutrition and the gut microbiome. However, it is clear that the nutritional value of food is partly influenced by the complex operation of the gut microbial community and that food in turn shapes the microbiota and, thus, the profile and function of its genes. This relationship is significant because intestinal microbiota have the capacity to synthesize vitamin K and several B vitamins, including B3 (niacin), B5 (pantothenic acid), B6 (pyridoxal phosphate), B12 (cobalamin), biotin, and tetrahydrofolate (Leblanc et al. 2012). Over 80 % of non-absorbed dietary vi-

tamin B12 is converted to bioactive corrinoids like *p*-cresol and methyladenine. The ability of the gut microbiota to produce folate and cobalamin could affect host DNA methylation patterns, but little is yet known about how these relationships between the host, host diet, and microbial community evolve and shape physiological phenotypes. However, it is interesting to note that vitamin A has the potential to modulate immune responses through direct interactions with immune cells as well as indirectly by modulating the composition of the microbiota (Cha et al. 2010). Research is needed to identify new host and microbial biomarkers and mediators of vitamin status to better establish the nutritional and health value of various foods. This approach would allow for a more rigorous definition of nutritional health and serve as a basis for preventive and therapeutic recommendations for vitamin consumption. Detailed investigation of the microbiome component of nutrition will help characterize an axis of our microbiome evolution that reflects our changing dietary habits and perhaps even uncover new aspects of the pathophysiology of ‘Western diseases’ to yield new microbiome-based strategies for disease prevention and treatment (Kau et al. 2013).

Evolving Paradigms for Evidence-Based Nutrition

We face an enormous challenge in trying to devise new ways to provide healthful food to a human population predicted to grow to 9 billion by 2050. Our success in developing new translational pipelines for defining the nutritional value of foods we consume now and those that we envision creating in the future depends on an evolution of the paradigm of evidence-based nutrition. This paradigm must now extend beyond the standard medical model using criteria more pertinent to treatment of the signs and symptoms of disease by drugs than to promoting health and wellness through our diet. Over the last decade, randomized clinical trials (RCTs) of vitamins and other nutrients for major disease entities have largely resulted in null or negative outcomes despite positive results from *in vitro*, animal model, and observational studies [12]. Because RCTs have traditionally been accepted as the “gold standard” for establishing cause-and-effect relationships, these studies have led to skepticism about the importance of supplemental vitamins alone or in combination with other nutrients in health and disease by clinicians, researchers, funding agencies, and the public. Nonetheless, the foundation of RCTs in evidence-

based medicine has now been wholly adopted in the creation of nutrition and science policy despite distinct differences between the evidence needed for testing of drugs versus that needed for the development of nutrient requirements and dietary guidance [13]. So, there is a need to better define the types of evidence necessary for developing dietary guidelines and recommending nutrient interventions, including those of vitamins, than that used for drug efficacy and safety. For example, unlike drugs, vitamins and other nutrients work in complex networks, are homeostatically controlled, and cannot be contrasted to a true placebo group (i. e., people not exposed to the intervention) because virtually everyone has been and continues to be exposed through their diet.

Although RCTs present one approach toward understanding the efficacy of vitamin interventions, the innate complexities of nutrient actions and interactions can only rarely be adequately addressed through a single research design. Further, action to define nutrient requirements or dietary recommendations to promote health should be taken at a level of confidence that is different from that needed in the evaluation of drug efficacy and safety in the treatment of disease [14, 15]. Moreover, in assessing the balance between the potential harm of making or not making a nutrient or dietary recommendation, appropriate educational strategies will be necessary to convey the varying levels of the strength of the evidence [16].

Requiring RCT-level evidence when this design is ill-suited or not available impedes the application of vitamin research to public health issues. Moreover, failing to act due to the absence of conclusive RCTs jeopardizes the potential for achieving substantial benefits with little risk and low cost. Interestingly, the L.E.A.D. Framework (for Locate Evidence, Evaluate Evidence, Assemble Evidence, Inform Decisions) to inform decision-making uses a broader base than an exclusive reliance on the requirement for RCTs to address the urgent need to bridge the evidence gap regarding obesity prevention [17]. It is noteworthy that the L.E.A.D. Framework was designed as an innovative way to consider and research complex questions that need to be answered soon. This approach to research specifically addresses deviations from the status quo with respect to methods considered to be the gold standard but which are responsibly designed to inform policy. Advancing evidence-based nutrition from its current version to one based upon more relevant and realistic criteria will depend upon research approaches that include RCTs but go beyond them. Achieving this goal will require new models of coop-

erative rather than adversarial interactions between industry, government, and academia.

The Future of Food Production and Vitamin Needs

Changes in the supply and distribution of food during the next century will be driven by several major factors, including the adverse impact of climate change, the declining availability of clean water, and the growth of the human population. Feeding a world populated by over 9 billion people will require us to double our food production on a planet where virgin land available for new farming is limited and concerns exist about overfishing of the oceans. More than another “green revolution” with new hybrid seeds and chemical fertilizers will be required to meet the demand for more food. Food, farm, and water technologists are exploring not only novel ways to produce more food but to examine less common and new kinds of food to eat. Part of this exploration must include considerations for the content and/or changed requirements of micronutrients like vitamins associated with these new foods.

Entomophagy, the consumption of insects and arachnids as food, is neither new nor novel as it is common to several cultures in developing regions of Africa, Asia, and elsewhere. It is estimated that 1400 species are edible to man. Entomophagy as a source of protein is cited as an ecologically sound concept providing greater efficiency, lower resource use, and environmental sustainability compared to traditional livestock. However, little attention has been directed to insect consumption as a source of micronutrients and how these foods might need to be fortified with vitamins to ensure adequate intakes.

Algae are also consumed in several cultures, particularly in Asia where it has long been a staple. There are about 145 species of brown, green and red seaweed currently used as food and, as they are grown in the ocean, generally obviate the need for land and fresh water. Like insects, algae can also be formulated into a variety of types of food products and fortified to ensure healthful intakes of vitamins.

A potentially novel source of food is that of artificial or *in vitro* meat, sometimes called shmeat, cultured from stem cells biopsied from livestock. While still in its early development, this approach to protein food could also be ecologically sound with less energy and water consumed and less greenhouse gases produced than associated with raising cattle, hogs, and sheep.

Based on techniques being tested to build tissue and organ structures for the purpose of regenerative medicine, experiments are currently underway to employ three-dimensional bioprinting to create meat products. This process builds the meat with minute droplets of cultured cells “printed” layer by layer via a controlled jet nozzle. Again, the micronutrient content of meat products that might be produced in this manner has not been considered.

Genetically engineered foods are not uncommon today and this method may find even more application in the future. To date, this approach is more frequently employed to increase agricultural productivity than to address the need for nutrients like vitamins. Importantly, achieving the benefits of both traditional and novel foods in the future will not be possible without employing and integrating the full capabilities of modern agriculture and aquaculture, food and pharmaceutical companies, and retail industries that distribute and market foods and food ingredients to consumers.

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