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Selected B vitamins and their possible link to the aetiology of age-related sarcopenia: relevance of UK dietary recommendations

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Abstract

The possible roles of selected B vitamins in the development and progression of sarcopenia are reviewed. Age-related declines in muscle mass and function are associated with huge and increasing costs to healthcare providers. Falls and loss of mobility and independence due to declining muscle mass/function are associated with poor clinical outcomes and their prevention and management are attractive research targets. Nutritional status appears a key modifiable and affordable intervention. There is emerging evidence of sarcopenia being the result not only of diminished anabolic activity but also of declining neurological integrity in older age, which is emerging as an important aspect of the development of age-related decline in muscle mass/function. In this connection, several B vitamins can be viewed as not only cofactors in muscle synthetic processes, but also as neurotrophic agents with involvements in both bioenergetic and trophic pathways. The B vitamins thus selected are examined with respect to their relevance to multiple aspects of neuromuscular function and evidence is considered that requirements, intakes or absorption may be altered in the elderly. In addition, the evidence base for recommended intakes (UK recommended daily allowance) is examined with particular reference to original datasets and their relevance to older individuals. It is possible that inconsistencies in the literature with respect to the nutritional management of sarcopenia may, in part at least, be the result of compromised micronutrient status in some study participants. It is suggested that in order, for example, for intervention with amino acids to be successful, underlying micronutrient deficiencies must first be addressed/eliminated.

Key words: Micronutrient status: Sarcopenia: Neurological integrity: Neuromuscular function: Recommended daily intake: Ageing: B vitamin intake

Context

The term 'sarcopenia' was coined in 1989 to describe an age-associated process characterised by a decline in human skeletal muscle mass[1]. This definition has been extended to include the associated loss of muscle function (strength and power), which represents the primary qualitative concern due to its link with frailty[2–4]. Sarcopenia is also described by a measure of total lean mass ≥2 SD below that of a comparable young adult, distinguished by a reduction in muscle fibre number and cross-sectional area[2]. This loss is often described as irreversible and associated with a significant functional deficit, leading to a loss of physical independence[4,5]. Sarcopenia is associated with an increased risk of falls and related fractures[6,7].

The population in the UK is ageing; the elderly sector of the population is increasing independent of the overall increase in population[8]. Similar shifts are being observed in other European Union (EU) countries. Taken together, 18–21 % of the European population were found to be aged 65 years or over and this range is expected to rise to 19–31 % by 2035, which translates to >25 % of all Europeans being aged 65 years or over by that date[8]. There are at present few initiatives or therapeutic protocols in place to manage sarcopenia at the level of the general population. Research is emerging that indicates that the lean tissue decrements and increased frailty associated with ageing may be relatively manipulable phenomena, with nutrition as a key modifiable variable.

Nutritional interventions, for example with essential amino acids, have produced rather variable results, with some interventions appearing effective and others of limited value[9–11]. In such research, elderly sarcopenic subjects frequently present with multiple, potentially confounding factors. In this connection, the present discussion will examine the potential role of selected B vitamins in normal and pathophysiological modes of neuromuscular function. Several key themes will be explored,

Abbreviations: 5,10-MTHF, 5,10-methylenetetrahydrofolate; CD, cobalamin deficiency; CNS, central nervous system; COMA, Committee on Medical Aspects of Food and Nutrition Policy; EAAA, erythrocyte aspartate aminotransferase activity; EFSA, European Food Safety Authority; EU, European Union; FD, folate deficiency; hcy, homocysteine; holoTC, holo transcobalamin; HR, hazard ratio; MMA, methylmalonic acid; ND, niacin deficiency; NDNS, National Diet and Nutrition Survey; NHANES, National Health and Nutrition Examination Survey; PARP, poly (ADP-ribose) polymerase; PD, pyridoxine deficiency; PLP, pyridoxal 5′-phosphate; PNS, peripheral nervous system; PteGlu, pteroylmonoglutamic acid; RDA, recommended daily allowance; RNI, reference nutrient intake; ROS, reactive oxygen species; SIRT, sirtuin; TC, transcobalamin; TD, thiamin deficiency; THF, tetrahydrofolate; TKA, transketolase activity; TPP, thiamin pyrophosphate.

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notably issues associated with the adequacy of the knowledge base regarding older adults' requirements and intakes for B vitamins, the similarities between certain deficiency and frailty states associated with older age and finally the potential for suboptimal micronutrient status to mimic/mask features of sarcopenia.

Nutritional challenges in later life

Age-related features underlying compromise of intake or processing of nutrients are manifold and range from the psychosocial through to the physiological. In later life, issues of reduced income, mobility and socialisation may conflate to produce a reduction in energy and nutrient intake *per se*[12,13]. Problems with dental health may impair intake regardless of food availability[14,15], while changes in taste perception can also occur with ageing[16]. In addition, the elderly may experience difficulty interpreting labelling information[17], which, combined with economic limitations, may present the older adult with fewer appetising meal options. Many elderly regularly take medications and these can alter the uptake, metabolism and/or excretion of key nutrients[18,19]. Disease states such as cancer, diabetes and gastrointestinal inflammatory disorders also make a direct impact on nutritional status. There is considerable evidence that the elderly are at significant risk of two or more micronutrient deficiencies[20–23] and it has been established that there is a clear association between multiple micronutrient deficiencies and frailty[24,25]. The elderly therefore represent an 'at-risk' group for malnutrition and deficiency states that may have causal or aggravating connections to sarcopenia. With respect to the very elderly (aged 85+ years), a study in the UK found lower than estimated average requirements for energy and highlighted the lack of dietary intake data and the uncertainties about dietary reference values for very old adults[26].

B vitamins: rationale for review

The rationale for selecting B vitamins is as follows. First, B vitamins are involved in multiple aspects of energy and protein metabolism, and also in multiple aspects of neural integrity and function. Second, deficiencies of B vitamins may manifest in obvious neuromuscular problems (for example, beri beri) and/or neurological symptoms (for example, pellagra, peripheral neuropathies). Some of these signs and symptoms could mask or exaggerate key features of a number of age-related syndromes such as sarcopenia. Finally, in older adults, possible suboptimal dietary intakes of B vitamins, issues of impaired absorption and age-related anorexia potentially combine to produce a sector of the population for which B vitamin deficency is a reality. The long-term effects of subclinical deficiencies remain poorly understood.

The aim of the present text therefore is to review known functions of selected B vitamins in connection with sarcopenia and to assess the likelihood of an interactive or additive effect of suboptimal B vitamin status in the initiation, progression or extent of age-related decrements in muscle mass and function. The B vitamins that will be examined were selected on the basis of their known roles in relevant physiological and metabolic processes and their potential for suboptimal intake or status in older adults.

With respect to determination of sufficiency or otherwise, issues of intake are considered alongside biochemical indices where available, and contextualised through the lens of UK recommended daily allowances (RDA), which are the values that form the basis of advice and evaluation by most of the UK-based science, health and public health organisations. Differences exist between the UK RDA and the European Food Safety Authority (EFSA) recommended daily intakes of B vitamins for the elderly, with EFSA values being higher in almost all cases. Differences also exist between the values cited by EFSA and several European national guidelines, including those set by the partner institutions of the German Nutrition Society (Die Deutsche Gesellschaft für Ernährung e. V. (DGE)), the Austrian Nutrition Society (AGE) and the Swiss Nutrition Society (SGE). The present review therefore explores the rationale for the current UK RDA in particular.

The UK National Diet and Nutrition Survey (NDNS)[27], with respect to the elderly, presents varying degrees of detail in terms of B vitamin status, which is in some cases described by intake, in other cases by plasma levels and in others by functional assays. While the latest data appear to suggest a generally positive picture in terms of B vitamin sufficiency for those living in the UK, there are some important caveats. First, while the NDNS provides important, up-to-date insight into the nutritional status of the UK population, its findings are given meaning by national dietary guidelines, which are often at considerable variance with estimated requirements for nearby, comparable, modern industrial societies. Second, whilst the NDNS groups 'the elderly' within the single age bracket of ≥65+ years, in many instances the authors acknowledge age-related declines in the nutritional status of older adults, i.e. from 65 years onwards. As such, the stated guidelines for older adults would appear to lose applicability with advancing age. In this connection, the latest survey (2014/2015) does not include samples from non-community dwelling (i.e. institutionalised) elderly populations; the data presented for this survey are only for free-living individuals. This may in part explain the discrepancies that exist between the latest results and the results of the previous study (which indicated a greater prevalence of deficiency), since the latter included individuals living in institutions as well as the free-living. As there is an increasing number of elderly individuals in care, and since the non-community-dwelling elderly represent a group at high risk of malnutrition, this may well constitute an important oversight; one that may distort the perceived landscape of nutritional health in the UK elderly population.

More generally, assessment of nutritional status in the elderly is for the most part based on nutritional intake data, for example, from FFQ, food diaries and food purchasing data. Whilst such methods have utility in assessing patterns of consumption and estimating energy intake, their application in determining micronutrient status in the elderly is limited as follows. In general terms, these methods are not reflective of intra-population requirements or rates of utilisation, do not account for the significant changes in micronutrient availability

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due to variation in storage, processing or cooking methods, nor for antagonisms between different nutrients and/or non-nutritive substances in food. These methods may also be limited in terms of accuracy, since they may rely either on recall (FFQ), present a high respondent burden (food diaries) or represent an estimation (purchase data). In addition, dietary records and questionnaires do not accurately capture supplement use in the elderly[28], which, research suggests, may be substantial[29–31]. Thus it is possible that reported average B vitamin intake, at least in some studies, may be misleading.

More important perhaps is the conceptual framework by which the reference guidelines seek to specify deficiency prophylaxis. There may well be important differentials between levels of intake associated with deficit symptom prevention and optimal metabolic status and function. Analyses using additional biochemical measures have shown nutrient intake data to lack validity in determining micronutrient status in elderly populations[32].

Sarcopenia appears to be a multi-factorial pathological process, which, although associated with age, is not a *de facto* outcome of ageing. Since B vitamins are directly and indirectly involved in the operation of an array of biological systems, and since these systems appear to be sensitive to even relatively short-term deficiencies, vitamin contribution to the complex aetiology of sarcopenia is likely to be significant and may be modifiable in relation to its progression.

Thiamin (vitamin B_1)

Functions and the effects of deficiency

Important food sources of vitamin B_1 include yeasts, whole-grain cereals (B vitamin content being concentrated in the outer germ layer of grains), nuts and legumes, pork meat and organ meat (particularly the liver, kidneys and heart)[33,34]. Vitamin B_1 is present in the human body in different forms. The best characterisation of vitamin B_1 is as thiamin pyrophosphate (TPP) or thiamin diphosphate (ThDP) in its twice-phosphorylated, activated, cofactor form. Poorly characterised forms include adenosine thiamin triphosphate (AThTP), thiamin monophosphate (ThMP) and thiamin triphosphate (ThTP). As TPP, vitamin B_1 is principally regarded as a key enzymic cofactor in oxidative metabolism[35]. More specifically, vitamin B_1 is known to function in twenty-four enzymic reactions, most importantly pyruvate dehydrogenase (for energy production via the Krebs cycle), transketolase (for lipid and glucose metabolism, production of branched-chain amino acids, and production and maintenance of the myelin sheath) and 2-oxoglutarate dehydrogenase (for synthesis of acetylcholine, γ-aminobutyric acid (GABA) and glutamate)[36].

Vitamin B_1 is known to be directly associated with nervous system function, primarily as a result of observation of rapid structural and functional declines in deficiency states and in alcoholism[37,38]. Whilst the sensitivity of the nervous system to vitamin B_1 is largely attributed to the heavy reliance of this system on oxidative metabolism[39], region-specific sensitivity to vitamin B_1 deficiency has been observed in neuronal tissues that have otherwise comparable metabolic profiles[40]. This sensitivity may in part be explained by the additional, non-coenzyme functions of vitamin B_1, particularly as an antioxidant, i.e. in relation to varying regional susceptibility/exposure to oxidative stress[41,42].

Chronic vitamin B_1 deficiency is associated with several potentially life-threatening neurological disorders. Whilst vitamin B_1 deficiency is frequently associated with the central nervous system (CNS), the most advanced neurological changes have been shown to occur in the periphery – particularly in the lower limbs[43]. This finding mirrors an aetiological feature of sarcopenia, for which muscle mass and function in the lower limbs are relatively more compromised[44,45]. Since muscle fibres function within the context of motor units, such an overlap is unsurprising.

Early signs of vitamin B_1 deficiency are cognitive decline, loss of appetite, weight loss/loss of lean mass, reduced walking speed, abnormal gait and muscle weakness/tremors[46]. Though poorly delineated, these symptoms are relevant and represent the least easily detected (by clinical appraisal) and most likely manifestations of vitamin B_1 deficiency in otherwise healthy elderly. Vitamin B_1 deficiency is being increasingly associated with loss of vibratory sensation in the lower extremities[47], a higher incidence of falls over time[48] and depression[49]. A low vitamin B_1 status is also increasingly implicated in other age-related neurodegenerative disorders such as Alzheimer's disease[50,51].

A number of different mechanisms have been put forward to explain neuronal loss/damage during thiamin deficiency (TD). The mechanisms proposed relate chiefly to the reduced activity of vitamin B_1-dependent enzymes in mitochondria and include impairments in oxidative metabolism[52], and mitochondrial function[53], resulting in inflammatory responses associated with microglial activation[54,55], increased production of reactive oxygen species (ROS)[56], and later, glutamate receptor-mediated excitotoxicity[57,58]. These mechanisms have been elucidated through investigations using cell lines, following/leading to observations of CNS damage during experimental vitamin B_1 depletion in animals.

In addition to providing mechanistic insight, these studies have elucidated important temporal dimensions in TD, and suggest a need for further research into the effects of episodic and cumulative suboptimal vitamin B_1 status in human subjects. For example, Ke *et al.*[54] found that a moderate-low vitamin B_1 intake in mice triggered an immune response, activating microglia and resulting in widespread loss of neural tissue (29 %) in vitamin B_1-sensitive regions within 9 d of TD. Whilst intervention with vitamin B_1 on day 8 of TD was shown to prevent further damage, the commitment of neuronal cells to apoptotic pathways appeared to be irreversible by day 10–11, when a 90 % loss of neural tissue was observed[54].

Prevalence of deficiency

The UK NDNS reports that the average intake of vitamin B_1 exceeds the reference nutrient intake (RNI) across all age groups[27]. Audits in other European countries have largely produced similar findings. However, recent large-scale, cross-European investigations have indicated important regional

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variations[59,60]. Other large-scale and smaller-scale studies of free-living elderly populations demonstrate significant numbers who have inadequate intake (up to 60 %)[61–64], possibly due to more rigorous methodological approaches or biases resulting from the age ranges selected, socio-economic or geographical factors[65]. Inadequate intake in non-free-living elderly populations is reported to be widespread in industrialised nations: between 33 and 94 %[66–70]. Where subject age is reported as a continuum, vitamin B_1 intake appears to be negatively correlated with this variable[47,71]. Interestingly, supplemental intake, which has been reported to make a major (>50 %) contribution to overall intake[72] is often not included in dietary intake studies.

As with other B vitamins, variation in the reported prevalence of deficiency is partly due to the use of different referencing guidelines, for example, minimum requirements *v.* two-thirds of the RDA[21]. Furthermore, assessments of intake frequently do not take into account a potential relative deficit of vitamin B_1 induced by consumption of refined carbohydrates, which require vitamin B_1 for their metabolism[39,73]. Storage, cooking methods and co-ingestion of foodstuffs such as alcohol, sulfites, tannins and *o*-diphenols (from coffee), and widely used prescription drugs such as diuretics[74], that reduce vitamin B_1 content and bioavailability[75–77] are seldom considered. It is unsurprising therefore that studies comparing assessments of intake with biochemical markers find little correlation, with one study demonstrating biochemical deficiency (TPP effect >14 %) in more than 50 % of a sample who had an intake exceeding guideline requirements (RNI)[78].

Biochemical data from elderly populations are scant and mainly related to specific pathological states. Results from the UK NDNS indicate that less than 1·2 % of the elderly are deficient in vitamin B_1, based on a measure of transketolase activity (TKA); erythrocyte transketolase activation coefficient of >1·25[27]. This cut-off point is less conservative than that suggested by other authorities, including EFSA (>1·15)[79]. Studies on free-living elderly populations, which employ a cut-off point of >1·15, have indicated a greater prevalence of vitamin B_1 deficiency: between 10 and 47 %[29,32,48,80,81]. The reported range does not appear to be greater in non-free-living elderly populations. In view of these findings, it is interesting to note that whilst most reports, including that of the UK NDNS, consider 'the elderly' as a single age bracket having reduced requirements, biochemical data indicate a significant age-related decline in the vitamin B_1 status of even apparently 'healthy elderly'[47].

UK dietary recommendations

Guidelines for vitamin B_1 intake in the UK currently indicate a requirement of 0·9 and 0·8 mg/d for men and women aged ≥50 years, respectively[82]. These represent the RNI, and have largely been determined according to measures of minimum vitamin B_1 requirements/4184 kJ (1000 kcal) energy intake[82]. Whilst these recommendations are safeguarded by established minimum vitamin B_1 requirements, associated urinary output measures for the prevention of beri beri and/or signs of clinical TD, not enough consideration appears to have been given as yet to the elderly in this respect, and only one of the studies referred to appears to have included elderly participants. More importantly perhaps was the nature of this study: experimental depletion/repletion of a small sample (*n* 21) of institutionalised male psychiatric patients[83]. Given the known association of neuropsychiatric illness with vitamin B_1 status, a history of mental illness at study baseline may limit the broader contemporary relevance of this work. On the other hand, this research does present some interesting suggestions in that while small age-related differences in glucose absorption rates were found, functional differences in vitamin B_1 status (via lactate and pyruvate accumulation) were in evidence according to younger–older subject status. The study does not, however, go on to discuss any precise mechanistic role for vitamin B_1 status to explain these observations. In the absence of such, and given the limited scope of the measurements, derivation of age-specific dietary requirements may be in need of review. Finally, it is interesting to note that the study found the association between vitamin status and the urinary output of vitamin B_1 to be aberrant[83].

Recommendations made by other major industrialised countries, including Germany, Switzerland, Australia, New Zealand and the USA, are between 10 and 25 % higher than the UK guidelines[46,84,85]. Part of this difference may be accounted for by use of different paradigms, whereby guidelines are developed in relation to points of saturation rather than minimum requirements for disease prevention. There is some epidemiological evidence to indicate that an intake of vitamin B_1 at a level of about 0·9 mg/d appears to be sufficient to sustain a good level of health in elderly Italian women[86], an observation-based indication of vitamin B_1 sufficiency at a level 12·5 % higher than the UK RNI.

With respect to the use of the TKA assay as a reflection of vitamin B_1 sufficiency it should be noted that in the elderly: (a) age-related reductions in TKA activity are known to occur (probably due to depressions in apoenzyme levels and potentially confounding the interpretation[87]; (b) prolonged vitamin B_1 deficiency also induces a lowering of basal and stimulated transketolase activities, possibly through the same mechanism[88]. Therefore the absence of baseline TKA data may be misleading with regard to sufficiency. It is of note that syndromes of polyneuritis, as commonly observed in the sarcopenic elderly[89], are also associated with reduced TKA, potentially masking deficiency states.

Niacin (vitamin B_3)

Function and effects of deficiency

Important food sources of vitamin B_3 include: yeasts, teas and coffees, whole-grain cereals, dark-green leafy vegetables, poultry and meats, fish (especially varieties which have 'red' meat, for example, tuna), nuts and legumes and organ meat (in particular, liver)[34,90].

Niacin (nicotinic acid), and its derivative nicotinamide, are principally understood as components of the coenzymes NAD^+ and $NADP^+$, which have related functions. NAD^+ and $NADP^+$, which may be reversibly reduced to NADH and NADPH, are

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known for their role in energy metabolism: the transfer of hydride ions (H^-) within dehydrogenase–reductase systems. These two co-enzymes mediate, and have impact on, a wide range of processes within the body. These include, but are not limited to, Ca homeostasis[91], gene expression[92], mitochondrial function[93,94], anti-oxidation[95] and immune function[96].

Potential relationship with sarcopenia

Chronic vitamin B_3 deficiency (niacin deficiency; ND) is known to result in pellagra[97]. Although the neurodegenerative symptomology in pellagra is well established, the neuropathological component of pellagra (*per se*) has had relatively little attention. Neuropathological observations from anatomical studies in human subjects in the 1930s include the chromatolysis of motor neurons, beginning at the level of the CNS (for example, Betz cells in motor cortex)[98,99], with clear implications for neuromuscular function. With regard to disease progression, human depletion studies have indicated that the clinical symptoms of pellagra can manifest in subjects on a diet low in vitamin B_3 (about 4·3–5·7 mg/d) and tryptophan (178–230 mg/d) in 4–6 weeks, and that it is possible to reach a critical state within a further 4–8 weeks[100,101].

Symptoms associated with vitamin B_3 deficiency include neuromuscular deficits such as muscle weakness and wasting, gait and truncal ataxia, peripheral neuritis, limb areflexia and myoclonus[102–104]. Although non-specific, our understanding of the early/less acute clinical manifestations of ND largely derives from behavioural changes associated with a diminished metabolism and nervous system dysfunction, for example, anorexia, weakness, inactivity, a decline in nerve transmission velocities, fatigue, anxiety, irritability and depression[105,106]. It is interesting to note a degree of similarity between the neurological and neuromuscular deficits observed in ND states and the frailty of sarcopenia. In this connection, it is important to be aware that sub-acute ND is poorly characterised in the literature, seemingly due to the variable and non-specific nature of associated symptoms.

Potential mechanisms for neuromuscular damage can be discerned from experimental depletion of cellular NAD^+ that induces oxidative damage and mitochondrial instability. Such models may be of particular relevance since they mirror key processes underlying cellular senescence and which are associated with the pathogenesis of sarcopenia[107].

NAD^+ is a cofactor for poly (ADP-ribose) polymerases (PARP), which carry out DNA base excision repair processes and/or mediate cell death in response to oxidative damage, ischaemia and excitotoxicity[108,109]. When DNA damage is increased following ROS insult, cytosolic NAD^+ is rapidly depleted. Such events have been demonstrated to initiate PARP-mediated apoptosis in myocytes *in vitro*, as well as in neurons, if NAD^+ status is not restored within hours[110,111]. In terms of the precise concentration–effect relationship, it is noteworthy that PARP complex formation appears to cease with only a 50 % reduction in cellular NAD^+[112]. Importantly, the administration of 'supraphysiological' doses of vitamin B_3 (≥500 mg/kg), to allow for a surplus of $NAD^+/NADP^+$, has been demonstrated to prevent these specific events and the subsequent loss of neuronal tissue during experimentally induced ROS insult *in vivo*[113]. Following on from this line of enquiry, and since ROS production is known to increase with age along with mitochondrial dysfunction, investigations aimed at re-examining requirements according to such parameters as the maintenance of cellular NAD^+ seem warranted.

A number of additional mechanisms by which low levels of NAD^+ may contribute to cell death have been identified and it appears that the apoptotis-inducing events following PARP-induced NAD^+ depletion can manifest independent of PARP activation, when NAD^+ levels are depleted[111]. With regard to the latter point, mitochondria are known to require relatively high concentrations of NAD^+ to drive ATP production, and to re-coup NAD^+ from reduced NADH formed during glycolysis (in cytosol). Subsequently, low levels of NAD^+ have been shown to result in a 'cellular energy crisis' caused by mitochondrial dysfunction. This dysfunction has been further shown to result in mitochondrial depolarisation and the release of mitochondrial apoptosis-inducing factor, as in PARP-1-mediated cell death following NAD^+ depletion[111]. Though these effects have been observed in neurons, which are highly glycolytic, they may also apply to muscle tissue, which periodically enter phases of high glycolytic production of ATP.

In addition, Gomes *et al.*[114] demonstrated in animal models that the age-related decline in nuclear NAD^+ induced pseudo-hypoxia by disrupting mitochondrial homeostasis through a shift in PGC-1α/β-independent pathways associated with nuclear–mitochondrial communication (and secondary to hypoxia-inducible factor 1-α (HIF-1α) accumulation). This communication change alters mitochondrial function through loss of mitochondrial DNA coding for specific subunits required for oxidative phosphorylation. Since mitochondrial dysfunction is now well established as one of the hallmarks of sarcopenia and ageing in general, the maintenance of mitochondrial function should represent a fundamental consideration in the establishment of nutritional requirements.

Gomes *et al.*[114] demonstrated that normal mitochondrial function can be restored in ageing mice, relative to young mice, simply by increasing cellular concentrations of NAD^+ via normal dietary means. This effect appears to be sirtuin (SIRT) 1 mediated. Since the SIRT family of enzymes are also NAD^+ dependent, low levels of NAD^+ have been shown to have a negative influence on their activities[115,116]. The better-characterised of these, SIRT1 and SIRT2, have been implicated in cell survival (and human longevity) due to their role in the regulation of programmed cell death[117]. Importantly, recent experiments in mice have indicated that when NAD^+ levels within the cell are limited, the stimulation of SIRT1 in neuronal tissue can hasten action in apoptotic pathways. However, when NAD^+ levels are high, or with increasing concentrations of nicotinamide (a SIRT1 inhibitor), neuronal tissue has been observed to be protected from apoptotis-inducing events[115]. Similarly, there is now good evidence to support the importance of maintaining cellular nicotinamide and NAD^+ concentrations in order to prevent SIRT2-mediated apoptosis[118].

There is evidence that age is an important determninant of cellular NAD^+ levels. A recent study on Wistar rats demonstrated that, starting in adulthood, ageing is associated with a

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significant decrease in intracellular NAD^+ levels ($P \leq 0{\cdot}01$), and a decrease in the NAD^+:NADH ratio ($P \leq 0{\cdot}01$)[119]. These changes have been shown to be consistent with increasing ROS production and DNA damage, resulting in decreased SIRT1 ($P \leq 0{\cdot}01$) and increased PARP ($P \leq 0{\cdot}01$) activity, as well as a decrease in ATP production ($P \leq 0{\cdot}01$), indicating impaired mitochondrial function. This finding appears to translate to humans. Massudi *et al.*[120] have shown a significant age-related decline in cellular NAD^+ levels in both men ($P = 0{\cdot}001$; r –0·706) and women ($P = 0{\cdot}01$; r –0·537). Interestingly, Massudi *et al.*[120] found that the change in cellular NAD^+ concentration over time was consistent with the increased activation of PARP in men ($P \leq 0{\cdot}0003$; r –0·639) and a decline in SIRT1 activity ($P \leq 0{\cdot}007$), but not in women[120]. These results demonstrate an increasing requirement for NAD^+ with age, and whilst the mechanisms for NAD^+ depletion may appear to differ between the sexes, this only strengthens the need to review vitamin B_3 requirements in the elderly in particular.

Prevalence of deficiency

There is a paucity of recent biochemical data on vitamin B_3 status in the elderly (UK) population, and from elsewhere in Europe and North America. This may be attributable to the relative difficulty in assessment, with the urinary output of associated metabolites representing the only universally accepted method. Subsequently, assessments of vitamin B_3 status are widely based on intake.

The 2014 update to the UK NDNS suggests a mean intake of vitamin B_3 above the RNI for all age groups[27]. Recent large-scale European and North American studies have recently suggested that ND is not widespread in free-living elderly populations[121,122]. Interpretation of such is difficult as larger-scale intake studies suggesting adequacy are often presented in terms of mean intake for an entire cohort. However, intake studies specifically focused on the elderly have indicated that dietary intake of vitamin B_3 in free-living elderly populations decreases significantly, typically between the ages of 50 and 90+ years[71,123,124]. Intake data from institutionalised elderly populations in the Western world indicate a broad range of inadequate intakes, between 0 and 26·7 %[125–127]. It is important to note, however, that intake data frequently do not correlate with biochemical determinants of status[128]. Numerous factors, including genetic variability[129], have been indicated in this regard. In addition, it should be noted that the primary source of vitamin B_3 for most populations is mature staple cereal grains, in particular wheat and corn, which contain high concentrations of glycosides. These bond with vitamin B_3 in a manner which significantly reduces its bioavailability[130].

UK dietary recommendations

The UK RDA (RNI) for vitamin B_3 is 16 and 12 mg/d, for men and women aged 50+ years, respectively[82]. This determination is based on data from an early human depletion study, indicating mean vitamin B_3 intake requirements (a) to alleviate the clinical signs of pellagra, and (b) to 'normalise' urinary output of *N*-methyl-nicotinamide and its downstream metabolite methyl-pyridone carboxamide, whilst in energy balance[82]. This recommendation, apparently derived from the one study referenced[131], is 5·5 mg/4184 kJ (1000 kcal). The UK RNI is inclusive of a 10 % CV and yields an RNI of 16 and 12 mg/d for men and women, respectively (assuming set levels of energy expenditure in individuals in energy balance). Also included in this RNI is the contribution of niacin derived from the endogenous conversion of tryptophan to niacin in the ratio 60:1, giving a stated requirement for niacin or nicotinic acid equivalents. Whilst it is suggested that tryptophan to niacin conversion alone may satisfy vitamin B_3 requirements, based on assessments of dietary protein intakes[82], the conversion ratio is dependent on the intake, and relative availability of, multiple nutrient factors, including the availability of all other relevant cofactors (for example, pyridoxine)[132]. The study of Hokwitt *et al.*[131] included only three elderly subjects who were reported as sedentary. Taken together, this gives limited scope to interpret requirements in the elderly and further research appears warranted.

Pyridoxine (vitamin B_6)

Function and effects of deficiency

Vitamin B_6 is naturally abundant in meat and poultry, including lean muscle meat, and can also be found in nuts and legumes and in fortified foods[133]. Vitamin B_6 refers to a class of N-containing compounds, which comprise pyridoxine, pyridoxamine, pyridoxal and their phosphate derivatives. In physiological terms, vitamin B_6 is principally understood to function in amino acid metabolism[134] as the cofactor pyridoxal 5′-phosphate (PLP) and is associated with a wide range of physiological processes, most of which are biosynthetic, being (for example) involved in the synthesis of haeme[135], several neurotransmitters, including serotonin[136], purines[137], several hormones, including steroid hormones[138] and fatty acids[139]. Clinical vitamin B_6 deficiency (pyridoxine deficiency; PD) may therefore manifest as a diverse symptom-set, affecting multiple systems.

Potential relationship with sarcopenia

Clinical indicators of marked PD in adults are largely neurological and epithelial/mucosal aberrations, which mimic deficiency of other vitamins within the B complex. The majority of neurological symptoms (particularly in cases of mild PD, which is more commonplace) appear to affect the peripheral nervous system (PNS) and are associated with loss of motor function[140]. These include numbness/paresthesia in the extremities which may progress to a loss of distal sensation, motor ataxia, weakness and loss of deep tendon reflexes[141–143]. Non-specific symptoms such as nausea, gastrointestinal disturbances, vomiting and mood/behavioural changes (for example, anorexia, apathy, depression and fatigue) have also been described.

Work in this area has been carried out with rats, with one seminal investigation demonstrating axonal degeneration akin to Wallerian degeneration, as primary in the development of

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peripheral neuropathy characterised by ultrastructural changes, including swelling of local (axonal) mitochondria, the disruption of axoplasmic ground substance, and the accumulation of dense bodies within neurons[144]. Pathological disturbances have also been noted in and around Schwann cells and within the nucleus[145]. Motor neurons, and in particular those which feed more distal muscles, appear most affected by this process, which leads to an increasing denervation of muscle fibres[146]. These findings are consistent with case studies of isoniazid (isonicotinic acid hydrazide, a vitamin B_6 antagonist) toxicity in humans, in which motor symptoms are reportedly predominant[140]. Interestingly, however, the morphological changes discussed earlier have been shown to be present in cases without any clinical signs of neuromuscular dysfunction. Therefore, studies in which isoniazid has been administered to animals with the aim of developing an understanding of early changes in neuropathies may provide valuable insight. Noting from previous studies that functional disturbances take 7–8 months to manifest[147], Hildebrand *et al.*[148] administered isoniazid to rats for 3–13 d and found a significant loss of neural tissue, a reduction in conduction velocity and wide-spread muscle atrophy (by day 3), followed by extensive myelin disruption, fragmentation of intramuscular nerve fibres and a reduction in muscle action potential (by day 10). Whilst reactive collateral branching of motor fibres appeared to be sufficient to normalise most of the assessment parameters 37 d after ceasing the intervention, a significant proportion of the damage to peripheral nerve fibres was still apparent and the pattern of atrophy in large-group muscle fibres showed no improvement[148]. At present a mechanistic model for these observations has not been established, and whilst it is difficult to exclude the potential contribution of direct toxicity from isoniazid on nerve axons, it seems probable that vitamin B_6 deficiency is indeed implicated. With regard to muscle atrophy, studies elsewhere have observed reduction in skeletal muscle protein synthesis in rats with a marginal vitamin B_6 intake[149].

The effects of a chronic, suboptimal vitamin B_6 status in the elderly are poorly understood. Epidemiological associations between low vitamin B_6 intake and frailty/disability are, however, widely reported – particularly in women. Amongst these, a prospective (3 years) study of 643 free-living elderly (≥65 years) women, into the possible relationship between specific micronutrient status (biochemical) and risk of developing disability, found that those with a plasma PLP level of >4·4 ng/ml (17·8 nmol/l) had a significantly greater risk of developing disability (hazard ratio (HR) 1·31; 95 % CI 1·03, 1·67; $P=0·02$) than those with PLP levels exceeding this threshold[150]. In direct relation to sarcopenia, the Maastricht Sarcopenia Study, which comprised 227 free-living elderly (>60 years) individuals indicated that intakes of vitamin B_6 are reduced by 10–18 % in individuals with sarcopenia, relative to non-sarcopenic controls[151].

In this regard, it is interesting to note that inadequate vitamin B_6 intake has been linked with an increased risk of osteoporotic fractures and related falls. Since osteoporotic fractures are associated with concurrent sarcopenia and since the pathogenic mechanisms underlying both sarcopenia and osteoporosis may be inter-related[152], this association should be considered in view of a more holistic approach. With this in mind, it is important to note the likelihood of sex-based differences here, since the risk/progression of osteoporosis is more marked in older women. This is attributed to the relative deficit in bone mineral homeostasis incurred as a result of the decline in the oestrogen axis post-menopause[153]. To illustrate, a large-scale (*n* 63 257) prospective investigation (follow-up time about 13·4 years) into the association between dietary B vitamin intake and the risk of hip fracture in men and women aged 45–74 years found a statistically significant negative correlation between the incidence of hip fractures and energy-adjusted vitamin B_6 intake in women ($P=0·002$)[154]. Women whose vitamin B_6 intake represented the lowest quartile (mean pyridoxine ≤1·30 mg/d) had a 22 % increased risk of hip fracture, relative to those in the highest quartile (mean pyridoxine ≥2·03 mg/d) (HR = 0·78; 95 % CI 0·66, 0·93). This is consistent with other similar investigations, including the Rotterdam Study, which found women in the highest quartile to have a significantly reduced risk of fragility fractures (HR = 0·55; 95 % CI 0·40, 0·77; $P=0·004$) and non-vertebral fractures (HR = 0·77; 95 % CI 0·65, 0·92; $P=0·005$)[155]. Importantly, the same study identified a significant risk reduction following supplementation with vitamin B_6, which took place independent to any change in BMD. Whilst the specific mechanism(s) underlying this effect are at unclear at present, it is known that vitamin B_6 is required for the maintenance of the collagen matrix supporting bones[156]. Subsequently, an increased rate of collagen synthesis has been suggested as one potential mechanism for this effect[155].

Elsewhere, studies which have observed a correlation between hyperhomocysteinaemia and a decline in BMD, as well as deleterious architectural changes, have put forward the B vitamin deficiency (vitamins B_6, B_9 and B_{12})-induced build up of this metabolite as another possible mechanism, in addition to the direct role these B vitamins play in bone metabolism[157,158]. A recent review of this area suggests that while these and other B vitamins (for example, vitamin B_1) are clearly involved in bone homeostasis, clinical trials are needed to determine the efficacy of supplementation protocols[159].

Whilst such data help inform the relationship between poor vitamin B_6 nutrient status and frailty, evidence for optimal status/intake remains incomplete.

Prevalence of deficiency

The UK NDNS suggests a mean intake of vitamin B_6 above the RNI for all age groups[27]. However, the UK RNI is lower than those established by other authorities such as EFSA[160]. Data from other industrialised nations suggest considerable variation in intakes. Whilst some large-scale investigations report adequate intake of vitamin B_6 amongst free-living elderly populations, these often contradict smaller-scale studies, which typically employ more time-intensive methods and suggest a high prevalence of inadequate intake[21,161,162]. Outside the UK, PD was suggested in 1990 to have reached epidemic proportions in the total US population (affecting 71 and 90 % of men and women, respectively) and this was significantly associated with ageing ($P<0·001$)[163]. In fact, a significant age-related decline in intake is almost always observed where an extended

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age range is considered[31,72,164–166]. Inadequate intake is more consistently reported in non-free-living elderly populations[66,167,168]. This includes an extensive, cross-sectional assessment of vitamin B_6 intake across eleven long-term residential facilities in Canada, which reported inadequate intake in more than 50 % of the total participant group[125].

As with other B vitamins, data on intake are typically revealed to provide a more optimistic picture than biochemical data[169]. Amongst these, Kjeldby *et al.*[170] were able to identify a biochemical deficiency (using PLP values) in up to 49 % of an elderly population with an 'adequate' (mean) intake. Past NDNS (1994–1995) had indicated PD (plasma PLP < 30 nmol/l) to be endemic in the UK elderly population, being 49 % in community-dwelling and 75 % in the institutionalised elderly (>65 years)[171]. The 2014 NDNS report – the first since 1999 to include biochemical data on the elderly – suggests no indication of deficiency despite using the same measures[27]. Only the mean value for the entire cohort is provided, however. Elsewhere, the range of reported prevalence of PD according to biochemical indices is typically between 26–59 % in free-living[29,32,81,172] and 5·3–49 %[126,170] in non-free-living elderly populations in industrialised nations.

Assessment according to functional biomarkers, such as erythrocyte aspartate aminotransferase activity (EAAA), and levels of circulating metabolites, such as cystathionine and taurine, which are known to build up in the deficient state, appear to indicate an even greater prevalence than PLP measures[137,173]. Review data suggest that a combination of functional biomarkers are required for assessments in the elderly, due to confounders such as changes in renal function, increased inflammation, serum albumin and inorganic phosphate[174].

UK dietary recommendations

The RDA (RNI) for vitamin B_6 intake in the UK is set at 1·4 and 1·2 mg/d for men and women aged ≥50 years, respectively[82]. This determination is based on a small number of studies focused on functional parameters related to protein metabolism: namely, changes in tryptophan and methionine metabolism in response to variation in protein intake, and during depletion[82]. The RDA (RNI) is based on an assessment of the quantity of vitamin B_6 required to metabolise recommended (or slightly greater) levels of protein intake (about 50–150 g protein/d) whilst maintaining protein homeostasis, according to select outcome measures (for example, urinary output of xanthurenic acid). The RDA is therefore not in fact absolute, and is delineated as 15 μg/g protein[82]. Even if these parameters are sufficient in specifying intake requirements (according to protein intake), there may be issues with respect to their wider application, since determinations are based on small sample groups (*n* 6–13) comprised of young men (≤31 years)[175–178].

Whilst it is true that elderly populations are widely reported to have a reduced intake of protein, an increased requirement for protein in elderly individuals (1–1·2 g/kg per d) is now being recognised[179,180]. Subsequently, there is a growing impetus to increase protein intake in this population. On this basis alone, the existing guidelines for vitamin B_6 (although not absolute) may be outdated. The extent of this is perhaps best elucidated by the fact that the 25–50 % increase in protein requirements not reflected in the RNI for vitamin B_6 represents more than the difference between the current estimated average requirement and RNI, implying that >50 % of elderly individuals would not be able to match vitamin B_6 requirements with protein intake. It should also be noted that recent investigations do not in fact support any association between protein intake and vitamin B_6 status in both elderly men and women[172].

Data from more recent depletion/repletion studies in the healthy elderly indicate an extended physiological range of function for vitamin B_6 that is observed in response to increased levels of intake (15 μg/kg body weight per d – 50 mg/d). Evidence for this includes a significant decrease in urinary output of xanthurenic acid post-tryptophan loading (5 g) in elderly women and other direct outcomes in immune system function, including lymphocyte proliferation in response to peripheral mitogens[181]. Another study examined requirements in the elderly in relation to normalising multiple associated physiological outcomes, including EAAA and output of xanthurenic acid[182]. These authors took into account different levels of protein intake and suggested that the minimum requirement for vitamin B_6 in the elderly is 1·96 and 1·90 mg/d, for men and women, respectively[182]. Several noteworthy studies indicate still greater requirements. Amongst these, Morris *et al.*[169] differentially examined (for the first time) the correlation between vitamin B_6 intake and plasma PLP levels, using data from a US population-representative sample of over 6000 participants in the National Health and Nutrition Examination Survey (NHANES; 2003–2004). Their study also examined the possible relationship between plasma PLP and homocysteine (hcy). The findings indicated that the elderly have a markedly increased requirement for vitamin B_6, an intake of 3–4·9 mg of vitamin B_6/d was needed simply to avoid a low plasma PLP (<20 nmol/l) and hyperhomocysteinaemia[169]. More importantly perhaps, vitamin B_6 requirements were not found to be reduced in elderly individuals with a low protein intake (54 g/d)[182], which may suggest that the parallel with protein intake may have limited utility in the assessment of vitamin B_6 requirements in the elderly.

Folate (vitamin B_9)

Function and effects of deficiency

Important sources of vitamin B_9 include green leafy vegetables, citrus fruits, nuts and legumes, and fortified foods[133]. Folate content in lean muscle meat is typically low, but high in organ meats, particularly the kidneys and in the liver[183]. Folate (vitamin B_9) describes collectively both natural folates and the synthetic folic acid/pteroylmonoglutamic acid (PteGlu), which are functional within the body as the cofactor tetrahydrofolate (THF). THF has important roles in one-carbon metabolism: as a methyl group donor. The first step in the methylation cycle, in which THF is transformed into 5,10-methylenetetrahydrofolate (5,10-MTHF), is catalysed by the PLP-dependent enzyme, serine hydroxymethyltransferase[184]. This highlights the functional interdependence of B vitamins. Viamin B_{12} is of particular interest in this regard, since there is considerable functional

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overlap with vitamin B_9 – such that one being in sufficient supply may mask a functional deficiency of the other, for example, prevent macrocytosis[185]. This can have implications for elderly populations which are not routinely screened, since the neurological effects of vitamin B_{12} deficiency are irreversible at an advanced stage when clinical symptoms are manifest. It is also well established that vitamin B_{12} deficiency leads to a functional vitamin B_9 deficiency (folate deficiency; FD)[186]. Whilst the precise mechanism is as yet unclear, the subsequent reduction in the activity of (the vitamin B_{12}-dependent enzyme) methionine synthase is known to result in a build up of 5-methyltetrahydrofolate (5-MTHF)[187], and therefore represents a popular hypothesis[188]. In all, the assessment of vitamin B_9 status should include where possible an estimation of vitamin B_{12} status also.

Potential relationship with sarcopenia

Whilst anaemia is the traditional association, FD may compromise the function of any system dependent on one-carbon metabolism, or affected by concentrations of associated metabolites. Subsequently, vitamin B_9 deficiency may contribute toward a range of pathologies, particularly if prolonged and in the presence of aggravating factors such as inadequate levels of vitamin B_{12}. Determination of the extended role of vitamin B_9 in human health may be difficult in clinical terms since the effects of marginal deficiency may be subtle or so indirect as not to be obviously tied to vitamin B_9 status.

One of the established pathophysiological outcomes of vitamin B_9 deficiency, with possible pertinence to sarcopenia, is the build up of hcy: hyperhomocysteinaemia. Hyperhomocysteinaemia, which is prevalent in the UK elderly population[189], has been shown both epidemiologically and experimentally to be tied more closely to FD than to other methyl group donors such as vitamin B_{12}[190,191].

With regard to possible long-term effects, large-scale longitudinal investigations (>15 years) have identified plasma hcy as an independent risk factor for future incidence of major, age-related, neurodegenerative disease[192,193]. Similarly, studies into the long-term relationship between hcy and physical function have identified significant associations between modest elevations in plasma hcy and a decline in important aspects of neuromuscular function in elderly individuals[194,195]. Whilst effects on skeletal muscle are relatively unexplored, using both cross-sectional and prospective data obtained from 1301 elderly (aged >65 years) participants in the Longitudinal Ageing Study in Amsterdam (LASA), Swart *et al.*[196] have demonstrated an association between elevated hcy and reduced grip strength in men, and increased functional limitations in both men and women. Elevated (total) hcy (≥15 μmol/l) and vitamin B_9 (but not vitamin B_{12}) deficiency (<3 ng/ml) have also been associated with markedly increased risk of brain atrophy[197].

Whilst the authors of these studies suggest hcy to be a causal factor and not a marker in neuromuscular decline, there is a dearth of mechanistic enquiry into this topic, particularly in relation to neuromuscular/musculoskeletal decline. Several plausible mechanistic links have, however, been demonstrated, for example, hcy induction of neurotoxicity in animals. It is notable that neural tissue appears to be more sensitive to elevations in hcy than endothelial smooth muscle cells[198], on which most research in this field has been focused. Relevant mechanisms in the CNS include: the inhibition of acetylcholinesterase activity (involved in neurotransmitter breakdown) in rats, *N*-methyl-D-aspartate (NMDA; glutamate) receptor interaction-induced excitotoxicity in neurons, and region-specific inhibition of Na^+/K^+-ATPase activity in the brain (resulting in inability to maintain cell polarity)[198]. In the PNS, hcy–NMDA receptor interaction has additionally been linked (in mice) to increased sensitivity to oxidative stress-induced inhibition of neurotransmitter release and associated loss of cell integrity at the level of the neuromuscular junction[199], as in motorneurone disease. Motorneurone disease is loss of motor neurons, so naturally there is degeneration of the neuromuscular junction.

Other mechanisms include the progressive depletion of NAD^+ in rats (secondary to DNA strand break-induced increases in PARP activity), subsequent mitochondrial dysfunction, and the activation of caspase-induced apoptosis[200]. This is in accord with findings elsewhere (in rats), which demonstrate that the selective inhibition of PARP not only prevents hcy-induced damage, but improves the condition of other tissue types[201]. Vitamin B_9 supplementation has proven efficacy in this regard: in addition to reducing hcy-associated PARP activity and inhibiting caspase-induced apoptotic pathways, treatment with vitamin B_9 but not vitamin B_{12} has been shown to increase expression of the anti-apoptotic B-cell lymphoma protein Bcl-2. In addition, enhanced vitamin B_9 supply inhibits expression of TNF-α, induces NO synthase and supresses the activation of glial cells in the CNS[202]. These effects have been demonstrated in knock-out models in mice that mimic action of a common genetic variant in humans that results in amyotrophic lateral sclerosis, a neurodegenerative condition in which elevated hcy is associated with the loss of motor neurons.

Stable isotope studies have provided valuable insights into the metabolism and relative bioavailability of synthetic PteGlu, compared with naturally occurring folates. In humans, as well as in other mammals, the metabolic path to which a high proportion of PteGlu is committed begins with conversion to the naturally occurring THF[203,204]. In this connection, it is important to note that the enzyme dihydrofolate reductase, which is expressed in the liver, and which is required for the conversion (reduction and methylation) of PteGlu into THF, is relatively limited in its activity[205]. This has led some to suggest that PteGlu is therefore less biologically active than naturally occurring folates. Also, due to the low rate of conversion, PteGlu supplementation can result in high concentrations of this circulating metabolite and potentially distort the accuracy of the assessment of vitamin B_9 status. Certainly the use of PteGlu as a 'reference folate' in studies of folate bioavailability has been questioned[204].

Vitamin B_9 has both a direct and an indirect role in DNA synthesis, methylation and repair processes, principally due to its function as a cofactor for specific enzymes known to be involved in the relevant pathways. Most notably, the (5, 10-MTHF) folate-dependent thymidylate synthases are known to catalyse the conversion of uracil into thymine[206].

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Correspondingly, low intracellular concentrations of 5,10-MTHF have been shown to result in a build up of uracil, an imbalance of the cellular deoxyuridine monophosphate:deoxythmidine monophosphate ratio, and the DNA polymerase-associated, mass misincorporation of uracil into cellular DNA[207–209]. Emerging evidence suggests that this frequently overlooked effect of vitamin B_9 deficiency is implicated in increased DNA deletions, chromosomal instability and damage to factors maintaining nuclear integrity[208,210–212]. Although currently this phenomenon has primarily been studied in relation to carcinogenesis, tissue-wide chromosomal instability has the potential to have a negative impact multiple systems, including neuromuscular systems.

The tissue-specific effects of this phenomenon have not yet been comprehensively delineated and the possible impact on the nervous system and, in particular, the musculoskeletal/neuromuscular system has not been explored in human subjects. This may represent an oversight, since animal studies suggest potentially severe consequences. With regard to the nervous system, where more research is in evidence, the dysregulation of the aforementioned pathway has been associated with neurodegenerative and neuropsychiatric dysfunction[213]. Knock-out models in mice have indicated impaired uracil repair and impaired DNA glycosylase activity, secondary to vitamin B_9 depletion that resulted in moderate deficiency in this vitamin. Overall in this situation, there is promotion of neurodegenerative processes resulting in neuronal death and an inhibition of neurogenesis, associated with a region-specific reduction in brain-derived neurotrophic factor and glutathione[213], and nerve growth factor[214]. Duthie[212] has further highlighted the relative contribution of vitamin B_9 deficiency, having observed that (moderate) FD, and not an inadequate status of other methyl-donors with overlapping functions (for example, choline and methionine), leads to progressive strand-breaking, DNA damage in lymphocytes (100 % after 8 weeks). Finally, vitamin B_9 deficiency has been shown to result in an increased frequency of large-scale deletions in mitochondrial DNA in older rats (1 year), compared with young weanling controls[215]. Interestingly, whilst this effect appears to be modulated by age, the association is lost following vitamin B_9 repletion; uracil levels in DNA are normalised upon restoring optimal vitamin B_9 status, and this is consistent with findings in other animal studies.

Animal studies into the musculoskeletal effects of the mass misincorporation of uracil into DNA are in shortfall. In addition to non-tissue-specific risks to myocytes, chromosomal instability would probably interfere with the formation of new myonuclei, which are required in the repair of skeletal muscle cells[216] and to facilitate muscle hypertrophy in animals[217] and humans[218,219]. The latter association may be interpreted within the context of the myonuclear domain theory, namely that additional mynucleii are required to facilitate hypertrophy[220]. In addition to compromising muscle repair processes, for example, following injury/extended bed rest, such a phenomenon may also compromise the prophylactic/therapeutic benefit of exercise/movement as a stimulus for increased muscle mass/strength. Damage to mitochondrial DNA within muscle fibres/cells is also likely to represent a limitation for maintaining muscle strength and function.

Prevalence of deficiency

The UK NDNS suggests a mean intake of vitamin B_9 close to or above the RNI for all age groups[27]. Data from other industrialised countries typically suggest a high prevalence of inadequate intake, with some reports ranging as high as 37–100 % in both free-living[23,221–224] and non-free-living elderly populations. Over time, there seems to be a reduction in reported prevalence of inadequate vitamin B_9 intake in community-dwelling elderly populations, probably due to the emergence of functional foods and food-fortification initiatives, but also in part due to the popularisation of vitamin supplementation in the general population[225], particularly in Europe. Data from the European Nutrition and Health Report 2004 indicate vitamin B_9 intake to be below acceptable levels across Europe[60].

The reported prevalence of biochemically determined FD typically ranges from 0 to 23·5 %[226–229] in free-living elderly populations and from 5 to 68 % in non-free-living/sick elderly populations[125,167,230,231] in industrialised nations. It is again difficult to extrapolate from these findings due to the differences (unknown or otherwise) in sample populations, reference parameters and methods of assessment. In particular, the percentage of participants with polymorphisms affecting the methylene THF reductase (*MTHFR*) gene is almost always unknown. The 2015 supplementary report on the UK NDNS presents an interpretation of serum and erythrocyte folate levels according to thresholds used by the WHO (<10 nmol/l and <340 nmol/l, respectively)[232]. This assessment finds the prevalence of FD in the UK elderly population (>65 years) to be 8·5 % in men and 12·4 % in women, according to serum total folate, and 7·3 % in men and 10·8 % in women, when determined by erythrocyte folate levels[232]. Whilst these data do present evidence for/against potential age-related differences in vitamin B_9 status for those aged over 65 years, similar investigations, which differentially assessed the over 65s, suggest that the prevalence of deficiency in this age group increases significantly with age[233]. It should be noted that supplement users were not excluded from the NDNS sample population, and as with other B vitamins, supplemental vitamin B_9 intake is often found in sample populations with a low prevalence of FD[126,234] and this probably distorts perception of dietary adequacy across a given population.

Metabolic markers of vitamin B_9 status (for example, hcy) are rarely employed in the assessment of prevalence, despite indications of greater sensitivity in terms of detecting deficiency and typically translating into marked increases in reported deficiency prevalence[235]. It should be noted that past national surveys, such as the 1994–1995 sample assessed by the UK Ministry of Agriculture, Fisheries and Food (MAFF), reported hyperhomocysteinaemia to be endemic in the British population[236]. The same report, which distinguished between free-living and institutional elderly populations, found serum vitamin B_9 to be relatively lower in those living in residential care, despite intakes exceeding requirements[236].

UK dietary recommendations

The RDA (RNI) for vitamin B_9 in the UK is 200 µg/d for both men and women aged 11+ years[82]. As such, the UK RNI for

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vitamin B_9 represents one of the lowest amongst EU member states[128]. The UK RDA is based on several considerations. First, the lower reference range is based on the quantities of PteGlu shown simply to reverse the signs of acute FD (megaloblastic anaemia), following experimental depletion in (a) an undefined sample[237] and (b) in a sample of seven moribund cancer patients[238]. Whilst it is not even possible to comment on the sample population for the first study, determining requirements through study of advanced-stage cancer patients is problematic as derangements in cell turnover are pathognomonic. Further work in understanding the lower threshold for vitamin B_9 requirements seems merited.

The determination of the upper reference ranges (RNI) for vitamin B_9 are based on findings of adequate liver vitamin B_9 stores (>3 μg) following post-mortem analysis of 560 Canadian samples, which represent a heterogeneous sample[239]. Since dietary intake is not included, the results are contextualised (by the Committee on Medical Aspects of Food and Nutrition Policy (COMA)) by allusion to an altogether separate investigation reporting a mean dietary vitamin B_9 intake of 150–200 μg/d, and adequate erythrocyte vitamin B_9 levels (>150 μg/ml), in about 90 % of a 1978 sample of the Canadian population[240]. COMA attempts to establish the relevance of these data to the UK population through reference to 'comparable' median intakes in 1990[241]. This is a rather indirect estimate of requirements. Additionally, it was noted in the said report, and in a subsequent analysis of the same dataset, that FD, as it was then defined (erythrocyte vitamin B_9 <170 μg/l (0·37 μmol/l)), was observed in 35 and 47 % of men and women, and so represented the most widespread deficiency of a nutrient known[242].

Elderly individuals have been shown to be more sensitive to marginal FD and relatively less responsive to repletion than younger adults[243], particularly when more rapidly responding biochemical parameters such as changes in DNA methylation are used in assessment[244]. Other such parameters indicate even higher requirements still. Amongst these, hcy has been observed not to reach its nadir until an intake of 400 μg/d has been achieved[245].

Finally, and as COMA later acknowledges, intake is typically observed to decline with age and vitamin B_9 status is further affected by age-associated medical conditions, and associated medications[82]. Common examples include: non-steroidal anti-inflammatory drugs (NSAIDs)[246], hydrogen receptor antagonists, antacids, proton pump inhibitors[247], insulin mimetics (biguanides, for example, metformin)[248], a wide-range of commonly prescribed antibiotics (for example, amoxicillin)[249], bile acid sequestrants (cholestyramine and colestipol)[250] and K-sparing diuretics (for example, triamterenes)[251]. Taken together, there appears to be a strong case for careful reappraisal of the area with the aim of developing population-specific guidelines.

Cyanocobalamin (vitamin B_{12})

Function and effects of deficiency

Since vitamin B_{12} is synthesised by bacteria living in the gastrointestinal tracts of animals[252], sources include all animal produce, to a lesser extent eggs and dairy produce, and fortified foods[253]. Unlike other B vitamins, vitamin B_{12} is stored in relatively large quantities in the liver[183].

Cyanocobalamin, or cobalamin (vitamin B_{12}), forms the central component of the two corrinoid coenzymes, methylcobalamin and *S*-adenosyl-cobalamin (cobamamide), which are unusual in that they are thought to have limited functions within the body. Methylcobalamin is known to be required for the methylation of hcy to produce methionine by way of methionine synthase[254]. *S*-adenosyl-cobalamin is required for the catabolic isomerisation of methylmalonyl-CoA into succinyl-coA by L-methylmalonyl-CoA mutase[255]. Inadequate vitamin B_{12} intake therefore results in a build up of hcy and methylmalyonyl-CoA. Finally, low levels of vitamin B_{12} are also known to result in a build up of glycine, since vitamin B_{12} is required for the synthesis of porphyrin rings from glycine and succinyl-CoA[256]. The build up of these metabolites results in a shortfall in methionine and related upstream products such as *S*-adenosyl methionine, which carry out a broader range of functions. Therefore, despite being involved in a limited number of reactions, the effects of vitamin B_{12} deficiency are extensive, affecting multiple systems. The manifestations of deficiency may be categorised as gastrointestinal disturbances, neuropathies and megaloblastic anaemia. Pernicious anaemia, which typically affects the elderly, is aetiologically distinct from megaloblastic anaemia, in that it refers to instances in which vitamin B_{12} absorption has been compromised[257].

Potential relationship with sarcopenia

In terms of neuromuscular dysfunction, vitamin B_{12} deficiency (cobalamin deficiency; CD) is associated with sub-acute combined degeneration of the spinal cord and polyneuropathies. Histopathological findings from post-mortem examinations indicate that CD results in extensive demyelination in the CNS – most prominently in the spinal cord – although focal demyelination is also notably apparent in the white matter of the brain[258–260]. Demyelination has also been observed in the PNS, along with signs of significant damage to nerve fibres, most notable of which is axonal degeneration in distal afferent fibres of dorsal root ganglion neurons[261,262]. The mechanism by which demyelination occurs is at present unclear.

Clinically, vitamin B_{12} deficiency is associated with an extensive range of neuromuscular symptoms, which become more severe as deficiency progresses, including paraesthesia, numbness in the trunk, muscle weakness, abnormal reflexes, tendon jerks, spasticity, gait ataxia, myelopathies and myeloneuropathies[263–266]. Although CD is primarily associated with macrocytic anaemia, it is noteworthy that neuromuscular symptoms, such as gait ataxia, represent common clinical associations and mark the earlier stages of deficiency. These symptoms are primarily connected with the loss of sensory rather than motor-unit function[264,267], specifically a decline in proprioceptive, vibratory, tactile and nociceptive sensation. CD has also been shown to have a significant negative impact on nerve conduction velocity[268], implying a direct detrimental effect on peripheral motor function.

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Given the extent of neuromuscular dysfunction observed in CD, it is unsurprising that recent studies have found significant associations between vitamin B_{12} status and frailty in old age. Amongst these, Matteini *et al.*[269] recently observed a 1·66–2·33 times greater risk of frailty ($P \leq 0{\cdot}02$) in elderly Caucasian women with elevated methylmalonic acid (MMA). Whilst not all studies report such an association, this again appears to be explained largely by use of different parameters and cut-off points, as indicated in a recent study by Oberlin *et al.*[270]. Oberlin *et al.*[270] carried out a retrospective examination of data on 3105 elderly individuals (≥60 years) from early NHANES (1999–2002), for which multiple measures of vitamin B_{12} status were employed. They found that CD was significantly associated with a much greater range of disabilities (for example, in social and leisure activity, in mobility, etc.) when based on functional biomarkers and/or higher cut-off points for serum cobalamin[270]. The inconsistency between cobalamin values and the functional outcomes of vitamin B_{12} status have been noted in seminal investigations into CD as discussed below[271,272].

Although investigations into the long-term effects of vitamin B_{12} supplementation in the elderly do not appear to have produced clinically significant outcomes that directly relate to sarcopenia (for example, time to/number of falls, muscle mass and strength), there has been indication of improvements in more general measures of physical function, such as walking speed (cumulative OR 1·3; 95 % CI 1·1, 1·5), which may indicate improved neuromuscular interactions[273]. A realistic approach to future research may utilise vitamin B_{12} data in conjunction with the many other known factors and cofactors involved in neuromuscular structure and function – particularly amino acids associated with muscle anabolism, such as leucine.

Indirect negative outcomes for neuromuscular function are also apparent. Amongst these, vitamin B_{12} status (as well as that of vitamin B_6) has been significantly associated with length of stay in rehabilitation following injury[230], which in turn was related to a decline in both muscle mass and function – muscle cachexia[9,274]. The relationship between mood disturbances (for example, depression)/neurocognitive decline related to CD[266,275,276] and the development and progression of sarcopenia requires further investigation since these are increasingly being established as significant contributors[277,278].

Finally, it is interesting to note that a recent study by Verlaan *et al.*[279] found intakes of vitamin B_{12} to be 22 % lower and serum cobalamin to be 15 % lower in a sample of individuals with sarcopenia relative to non-sarcopenic controls.

Prevalence of deficiency

The UK NDNS suggests a mean intake of vitamin B_{12} above the RNI for all age groups[27]. This is consistent with other large-scale pan-European studies on free-living populations which report mean intake[59]. However, as demonstrated in a recent systematic review and pooled analysis of observational cohort and prospective studies of intakes in the elderly (>65 years), even though mean reported intakes may be above the RNI, a large percentage of individuals (16–19 % in the case of vitamin B_{12}) had an intake below the UK RNI/EU estimated average requirement[64]. Inadequate intake is reportedly even greater in non-free-living elderly populations (>65 years) across Europe and other industrialised countries, particularly in the lower socio-economic strata and amongst migrants[29,167]. There is indication that a further 20–30 % would be deficient if not for supplementation[29,280]. In any case, intake in the elderly is unlikely to translate to metabolic sufficiency in many cases due to poor absorption associated with the combined issues of hypochlorhydria[281], gastritis and diminished intrinsic factor production[282–285], as well as the widespread use of antacids[18,286].

As for other B vitamins, vitamin B_{12} status has been shown to decline significantly with advancing age[81,233]. This finding is confirmed with respect to vitamin B_{12} in a study of an elderly population aged 85+ years in New Zealand[287].

By measure of serum cobalamin, the UK NDNS[27] has indicated that 5·9 % of elderly men and women are deficient (<150 pmol/l) in vitamin B_{12}. Although varied, estimates of the prevalence of CD from elsewhere in Europe tend to be much higher, ranging from 10 to 40 % for both community-dwelling and institutionalised elderly populations[266,284,288–292]. These typically use higher cut-off points and other/additional, functional measures that have been shown to demonstrate greater sensitivity[292]. To illustrate the extent of the potential discrepancy, 12 % of community-dwelling elderly in the Framingham Study were identified as vitamin B_{12} deficient according to serum cobalamin; however, follow-on investigations identified a further 50 % of the sample (having elevated serum MMA) to be deficient metabolically[235]. Similarly, Morris *et al.*[293] identified a further 15 % of the NHANES III sample population as being deficient when MMA was used as the criterion instead of serum cobalamin. Also holo-transcobalamin (holoTC) has been shown to demonstrate even greater sensitivity than serum cobalamin[294,295] and MMA[296], particularly in older populations[297]. Due to the lack of clear reference standards it is, however, unclear whether these differences are made up of false positives.

UK dietary recommendations

Vitamin B_{12} is distinct from other B vitamins in that, under normal conditions, there is a physiologically significant bodily store, with estimates ranging from 2 to 3·9 mg, most of which appears to be in the liver[298,299]. For this reason, and since the neurological effects of CD cannot be reversed, human depletion studies have never been carried out.

The RDA (RNI) for vitamin B_{12} in the UK is 1·5 μg/d for both men and women aged 15+ years[82]. Excluding Ireland, this value represents the lowest RNI in Europe[128], and stands in sharp contrast to the German Nutrition Society guideline, for which the RNI is set at 3 μg/d[300], and the EFSA guideline of 4 μg/d[301]. EFSA, which has based its recommendation on mean intakes associated with normal ranges of circulating functional markers (MMA, total hcy, serum cobalamin and holoTC), in fact reports that it is intakes between 4·3 and 8·6 μg/d that are associated with these values[301]. This is consistent with a recent, comprehensive analysis of vitamin B_{12} status alongside intake in Danish postmenopausal women, which considered

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serum cobalamin, MMA, holoTC, transcobalamin (TC) saturation (holoTC/total TC) and total hcy, as well as potential absorptive complications[280]. It was found that an intake of 6 μg/d was required to normalise all parameters in individuals with normal absorptive capacity[280]. Follow-up investigations into requirements in adulthood (18–50 years) have likewise suggested an increased requirement of between 4 and 7 μg/d, even in healthy individuals[302].

The UK recommendation is based on an assessment of the requirements for the prevention of megaloblastic anaemia as indicated by: (a) a small number of intake studies on narrowly defined groups of vegan/vegetarians in Sweden, Australia and South Asia; and (b) the haematological response to parenteral vitamin B_{12} administration in patients with pernicious anaemia[82].

There appear to be several limitations to the appropriateness of these data in determining the RDA for elderly populations. First, whilst it is widely held that haematological abnormalities represent the first symptom of CD, it has long been understood that neurological deficits often present first[291,303]. Moreover, experimental investigations from the 1980s and 1990s suggest that the absence of macrocytic anaemia is commonplace, relating to about one-third of cases, whilst the absence of neurological symptoms appears to be much less common[264,271,272]. Interestingly, severe incidence for one set of symptoms (for example, neurological symptoms) has been associated with absence of the other (for example, haematological symptoms)[264,267], and this inverse relationship is reflected in measures of vitamin B_{12} status[270,271,304] and vitamin B_{12} analogues[304]. This suggests that vitamin B_{12} utilisation in the deficient state is subject to antagonistic or compensatory mechanisms which are determined at the level of the individual. The relative proportion of individuals affected by each or both sets of symptoms is unclear from the literature, since there is a shortfall of relevant and epidemiologically representative data. With regard to the timeline of symptom development, it is again interesting to note that the great majority of those with only neurological symptoms (about 80 %) do not present until sometime between the fifth and the seventh decade[264,271,290].

Conclusions

Emerging perspectives on sarcopenia, in which declines in muscle mass and function in older age are seen as neuromuscular rather than simply muscular, have raised important questions in terms of research into potential therapies. Nutritional intervention strategies for sarcopenia have a strong theoretical basis and much work in terms of protein and individual amino acids is evident in the literature, with varying success reported. Elderly subjects frequently present with multiple, potentially confounding factors in terms of research, many of which may be nutritional in nature. In this connection the present discussion has examined the potential role of selected B vitamins in normal and pathophysiological modes of neuromuscular function. Several key themes emerge, notably those surrounding the adequacy of the knowledge base in terms of older adults' requirements for B vitamins and the clear similarities between certain deficiency and disease/frailty states associated with older age. It is possible therefore that compromised intake or function of the B vitamins discussed may induce, mimic or aggravate key aspects of neuromuscular compromise as observed in age-related sarcopenia.

The available evidence regarding the B vitamins considered and their potential role in the development of sarcopenia presents an interesting mix of clinical observations and biophysiological mechanisms. TD, for example, has long been characterised by reduced appetite, weight loss and neural and neuromuscular compromise mirroring aetiological features of age-related sarcopenia (including disproportionate effects in the lower limbs). Niacin deficiency has known associations with anorexia and with reductions in hydrochloric acid in gastric secretions, leading to specific and general nutrient deficiencies. Furthermore, low niacin intake can lead to motor neuron chromatolysis. With respect to vitamin B_6 deficiency, axonal degeneration and pathological disturbances in Schwann cells has been described as affecting distal motor neurons and is associated with loss of motor function. In FD, in addition to the correlation between hyperhomocysteinaemia and decline in neuromuscular function, it is possible that the induction of mass misincorporation of uracil into DNA as a result of low folate status and subsequent chromosomal instability could inhibit muscle synthetic and repair processes. Similarly, compromised vitamin B_{12} status is a known risk for muscle weakness, abnormal reflexes, spasticity, gait ataxia and myelopathies, probably due to demyelination in both the CNS and PNS.

It is concluded therefore that for any meaningful investigation of the development or treatment of sarcopenia, it is important to consider, and where possible eliminate, deficiencies of B vitamins, the lack of which may impinge on muscle, nerve or the neuromuscular nexus. While it is not possible to conclude that one or more B vitamin deficiencies are causative of sarcopenia, within the mélange of known and surmised risk factors, such deficiencies warrant serious consideration. This seems especially true given the ease and relatively low cost of addressing such nutritional inadequacy. The evidence base for recommended daily quantities of micronutrients could usefully be contemporised in terms of adequacy for an ageing demographic, especially where there is a paucity of (functional) metabolic data available. Finally, given the increasing number of individuals reaching their ninth decade or beyond, it is worth considering future recommendations for older adults nuanced beyond a 65-years-and-over category.

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