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ESPEN Guideline

ESPEN micronutrient guideline

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Pyridoxine
Biotin
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Vitamin C
Vitamin D
Vitamin E
Vitamin K
Carnitine
Choline
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SUMMARY

Background: Trace elements and vitamins, named together micronutrients (MNs), are essential for human metabolism. Recent research has shown the importance of MNs in common pathologies, with significant deficiencies impacting the outcome.
Objective: This guideline aims to provide information for daily clinical nutrition practice regarding assessment of MN status, monitoring, and prescription. It proposes a consensus terminology, since many words are used imprecisely, resulting in confusion. This is particularly true for the words “deficiency”, “repletion”, “complement”, and “supplement”.
Methods: The expert group attempted to apply the 2015 standard operating procedures (SOP) for ESPEN which focuses on disease. However, this approach could not be applied due to the multiple diseases requiring clinical nutrition resulting in one text for each MN, rather than for diseases. An extensive search of the literature was conducted in the databases Medline, PubMed, Cochrane, Google Scholar, and CINAHL. The search focused on physiological data, historical evidence (published before PubMed release in 1996), and observational and/or randomized trials. For each MN, the main functions, optimal analytical methods, impact of inflammation, potential toxicity, and provision during enteral or parenteral nutrition were addressed. The SOP wording was applied for strength of recommendations.

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Results: There was a limited number of interventional trials, preventing meta-analysis and leading to a low level of evidence. The recommendations underwent a consensus process, which resulted in a percentage of agreement (%): strong consensus required of >90% of votes. Altogether the guideline proposes sets of recommendations for 26 MNs, resulting in 170 single recommendations. Critical MNs were identified with deficiencies being present in numerous acute and chronic diseases. Monitoring and management strategies are proposed.
Conclusion: This guideline should enable addressing suboptimal and deficient status of a bundle of MNs in at-risk diseases. In particular, it offers practical advice on MN provision and monitoring during nutritional support.
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List of abbreviations

25-OHD	25-hydroxyvitamin-D
AA	L-ascorbic acid
AGP	Alpha(1)-acid glycoprotein
AI	adequate intake
ATP	adenosine triphosphate
CoA	coenzyme A
COPD	Chronic obstructive pulmonary disease
CoQ10	Coenzyme Q10
CRP	C-reactive protein
DFE	dietary folate equivalent
DHAA	dehydroascorbic acid
DRI	dietary reference intake
EAR	estimated average requirement
EN	enteral nutrition
FSMP	food for special medical purposes
HPLC	high-pressure liquid chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICU	intensive care unit
IM	intramuscular
IV	intravenous
LC-MS/MS	liquid chromatography tandem mass spectrometry
MADD	multiple acyl-CoA dehydrogenase deficiency
MN	micronutrient
NOAEL	no-observed-adverse-effect level
PN	parenteral nutrition
RBC	red blood cells
RBP	retinol binding protein
RCT	randomized controlled trial
RDA	recommended dietary allowances
TSH	thyroid stimulating hormone
UL	tolerable upper intake level

1. Introduction

Why are micronutrient guidelines needed? Most people would know that trace elements and vitamins, called globally “micronutrients”, are essential components of nutrition in health and disease. But specific knowledge remains limited among clinicians, the trace elements being even less known than vitamins. There are two levels of concern: 1) public health, and 2) individual health. For the general population, international recommendations are available under the form of RDA (recommended dietary allowances), or more recently, as DRI (Dietary Reference Intakes). These recommendations address the micronutrient (MN) deficiencies that constitute a worldwide health concern [1]: iodine, iron, vitamin A and zinc deficiencies are among the world's most serious health risk factors [2]. A large worldwide MN database has been constituted by the World Health Organization (WHO) and Food and Agricultural Organization (FAO) to address the public health issues and the frequent endemic deficiencies of some MNs that may require support by fortification [3].

While deficiencies in inpatients are more frequent than previously acknowledged [4], no procedures for determination of requirements or recommendations are available for patients with acute and chronic diseases. However, clinicians and their scientific societies have not remained inactive. To address the needs of specific pathologies, several societies and interest groups have attempted to generate guidance in different conditions. Recent international and ESPEN recommendations that include MN information are therefore available for parenteral nutrition (PN) [5–8], bariatric surgery [9], chronic intestinal failure [10], inflammatory bowel diseases [11], liver disease [12], surgery [13], major burns [14], cancer [15] and intensive care unit (ICU) populations [16]. Most of these texts, although signaling potential MN deficiencies, do not provide practical advice as to the diagnosis pathway, or about how to handle deficiency or toxicity.

The present document includes updated dose recommendations for both enteral nutrition (EN) and PN, the latter being called PN-daily recommended doses (PN-DR). Effective metabolism of the major nutrients for protein and energy provision requires an adequate supply of all essential trace elements and vitamins. Since most patients requiring nutritional support present with a variably depleted MN status, it is important to provide adequate amounts of all MN from the start of nutrition support [4,17].

The enteral feeding solutions generally comply with the above and deliver all MNs. The ESPEN recommendations are formulated for 1500 kcal/day because this is the most common target. But international surveys show that feed delivery is generally below the prescribed target, resulting in 1000 kcal/day being the most frequently delivered dose [18]. Since all the MN are incorporated in

the formula for EN products, the amount of each MN supplied depends on the volume of product provided. In patients receiving less than 1500 kcal, an additional enteral or intravenous provision of MNs at the start of feeding may be considered, especially if there is a recent history of poor intake [4,17].

In many clinical situations, for safety and practical reasons MNs can be provided orally or enterally to correct depletion or deficiency. Such provision of additional MNs may be in pill, tablet, or liquid form. The amount provided may need to take account of the possibility of impaired absorption. The parenteral route, intravenous (IV) or intramuscular (IM), may be indicated where absorption is poor, or for rapid correction of a deficiency.

The issue of diagnosis is rarely addressed and requires searching laboratory biochemistry sources. Therefore, when facing an abnormal laboratory result, such as a blood value below the reference range, clinicians may not know how to interpret it, as MN knowledge is even more limited than nutritional knowledge [19]. The rational interpretation requires an often forgotten important approach, i.e. the integration into the clinical assessment and laboratory investigation of the presence and magnitude of a concomitant inflammatory response [20].

The present document aims to provide practical guidelines to assist clinicians in the assessment of the MN status in adult patients, and about how to provide basic or increased amounts of each of the trace elements and vitamins, while covering the fields of EN and PN.

2. Methods

The ESPEN micronutrient-working group included representatives from different professions (physicians, dieticians, biologists, health scientists): patient representatives could not be found. The group attempted to apply the 2015 new standard operating procedures for ESPEN guidelines and consensus papers [21]. This rigorous methodology focuses on disease rather than the historical technique-based approach (enteral vs parenteral). The approach results in structured reports and depends on systematic review, relying on expert opinion only when the systematic approach is not possible or yields inconclusive results. This standard operating procedure is oriented on the methodology recommended by the Scottish Intercollegiate Guidelines Network (SIGN) [21].

The working group attempted to formulate PICO questions, i.e. questions related to the Patient/problem/population, to the Intervention, Comparison and Outcome. This was again difficult as rarely are the MN single problems but part of a complex clinical picture. This resulted in the formulation of 4 main topics that were addressed for each MN: 1) summary of main function with nutritional requirements in oral, enteral, and parenteral nutrition 2) Diagnostic tools, 3) when to treat deficiency or toxicity, and 4) how to treat, ending with 5) the recommendations. Emphasis was given to analytical aspects as the choice of the methods and matrix impacts on the validity of laboratory results.

The literature was searched for evidence regarding 1) different diseases (see § 3), 2) therapeutic interventions (EN, PN, renal replacement therapy), and 3) special periods of life (pregnancy, elderly). An exemplary search strategy is shown in appendix A. Each MN was under the initial responsibility of 2–4 members of the group, who generated the initial text, evaluated the relevant references, and recommendations. All texts were thereafter submitted to the entire working group for validation.

The group searched for physiological data (suggestion for harm caused by deficiency or overload), historical evidence (published before PubMed release in 1996), and observational studies, and incorporated the available randomised controlled trials (RCTs). The experts were confronted by a special difficulty: there is a very limited number of interventional trials for the individual MNs. For most of them only cohort observational studies and case reports were available. The SIGN evaluation system (Table 1) was applied to a limited number of references. The recommendations were created and graded into the four listed classes (A/B/0/GPP). When solid evidence coming from biochemistry and physiology has been extrapolated to clinical settings, it allows an upgrading of recommendation to an A or B level, enabling the use of “shall” or “should”. Dose recommendations based on existing Recommended Dietary allowances (RDA) are attributed a level A as they are based on internationally validated evidence, whereas those based on DRI are given a level B.

The present practical guideline consists of **29** recommendations. The recommendations 1–3 are single recommendations, whereas 4–29 are sets of recommendations for each of the 26 MN. These sets consist of up to 9 separate recommendations, resulting in 170 single recommendations in total. Many are supported by limited evidence, but they underwent a consensus process, which resulted in a percentage of agreement (%): the qualification as strong consensus required >90% of votes (Table 2). In case of agreement <90% during the Delphi vote, the recommendations were thoroughly reviewed and -if necessary-reformulated. All recommendations with substantial changes were voted on again.

Table 2
Classification of the strength of consensus.

Strong consensus	Agreement of >90% of the participants
Consensus	Agreement of >75–90% of the participants
Majority agreement	Agreement of >50–75% of the participants
No consensus	Agreement of <50% of the participants

Note: According to the AWMF methodology [22].

Table 1
The SIGN evaluation system and wording used for the recommendations [21].

SIGN scoring of evidence levels	SIGN Grade	Wording
At least one high quality meta-analysis, systematic review, or RCT rated as 1++ and directly applicable to the target population *or* A systematic review of well conducted RCTs or a body of evidence consisting principally of well conducted studies directly applicable to the target population and demonstrating overall consistency of results	A	shall
A body of evidence including well conducted cohort or case control studies directly applicable to the target population and demonstrating overall consistency of results *or* Extrapolated evidence from high quality or well conducted studies	B	should
Extrapolated evidence from studies rated as 2++ or 2+, or evidence level 3 or 4	0	can/may
The guideline group finds that there is an important practical point that they wish to emphasise but for which there is not, nor is there likely to be, any research evidence but only evidence from clinical experience	GPP	Shall, should, can/may

RCT, randomized controlled trial; SIGN, Scottish Intercollegiate Guidelines Network.

Table 3
Definition of the words related to assessment of status.

Status	Oxford definition	ESPEN Definition	Comments
Adequate	Enough in quantity, or good enough in quality, for a particular purpose or need	Blood/plasma concentrations are within local reference range (international range if no national reference is available), and Absence of any clinical signs or symptoms related to micronutrients. Status may be adequate despite a low plasma value in a patient with inflammation.	Normal values may be found when systematic monitoring is integrated in clinical practice Impact of inflammation needs careful assessment.
Depletion	The reduction of something by a large amount so that there is not enough left	Presence of an objective loss of a MN in body fluids, or intake below standard recommendation with blood/plasma concentrations below reference range (see below).	Clinical signs or symptoms are not present at this stage
Deficiency	The state of not having, or not having enough of, something that is essential	Evidence of objective loss of a MN in body fluids, or intake below standard recommendation **AND EITHER:** Presence of clinical signs or symptoms, compatible with a micronutrient deficiency **OR** Blood/plasma concentrations below reference range together with metabolic effects of inadequacy	Intake is not meeting losses Depending on the body stores, which vary for each MN, clinical signs of deficiency generally may require many weeks to become visible. Therefore, they are absent in acute conditions, such as intensive care. Example: B1 deficiency can occur in a very short period, whereas B12 deficiency can take months or years to appear
Overdose	To take too much of a drug at one time, so that it is dangerous	Detection (by monitoring of blood concentrations) of higher than upper reference values, associated with the administration or intake (accidental or intentional) of amounts greater than recommendations.	Generally asymptomatic May occur in context of repeated MN administration Particularly at risk in patients with diseases reducing elimination (e.g. liver and renal disease)
Toxicity	The fact of being poisonous	Presence of clinical signs or symptoms compatible with toxicity. The history can show the intake of amounts considered unsafe or toxic. Toxicity diagnosis is most frequently based on blood levels.	Clinical signs or symptoms are generally present. The risk is highest with intravenous administration as the intestinal mucosa, which acts as a filter or barrier, is bypassed
Reference ranges or values	None	Set of values that includes upper and lower limits of a laboratory test based on a group of otherwise healthy people	Ranges for normal values vary depending on patient populations and test assays used. Lab-to-lab variability can occur due to differences in testing equipment, chemical reagents used, and analysis techniques. Consequently, for most laboratory tests, there is no universally applicable reference value

Table 4
Established terms used to describe micronutrient requirements [23,39–41].

Terminology	Definition	Comment
EAR: Estimated Average Requirement	The average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group. It is equivalent to the term AR (Average Requirement) in the European Union (*)	The EAR is used to calculate the RDA
AI = Adequate Intake	The recommended average daily intake level is based on observed, or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate; used when an RDA cannot be determined.	The AI is expected to exceed the EAR and the RDA for a specified criterion of nutritional adequacy. This concept is like ARI (Adequate Reference Intake) in the EU.
RDA = Recommended dietary allowance	The average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97–98%) healthy individuals in a particular life stage and gender group. This concept is equivalent to PRI (Population Reference Intake) in the EU.	Applies to the general population
UL = Tolerable Upper intake levels	Daily MN doses that you can safely take without risk of an overdose or serious side effects The highest average daily nutrient intake level likely to pose no risk of adverse health effects to almost all individuals in the general population. EU includes another 3 terms (LOAEL, NOAEL and UF, see below).	Integrated in DRI. The potential risk of adverse effects increases with intakes > UL.The European values have been refreshed in 2018 [24].
DRI = Dietary reference intake	Set of reference values including: EAR, AI, RDA, UL, that, when adhered to, predict a low probability of nutrient inadequacy or excessive intake	DRIs are intended for the general population and will be used to indicate proportions of MNs used particularly in EN

Table 4 (*continued*)

Terminology	Definition	Comment
ESADDI = estimated safe and adequate daily dietary intake	Reference value of daily dietary intakes of nutrients for which there is insufficient evidence to determine average requirements and reference intakes.	
NOAEL = No Observed Adverse Effect Level	NOAEL is the highest intake of a nutrient at which no adverse effects have been observed	EFSA terminology [40]
LOAEL = lowest of Adverse Effect Level	The lowest intake at which an adverse effect has been demonstrated	EFSA terminology [40]
UF = uncertainty factor	The individual uncertainty factors may be combined into a single composite uncertainty factor for each nutrient and applying this (composite) uncertainty factor to a NOAEL (or LOAEL) will result in a value for the derived UL that is less than experimentally derived NOAEL, unless the uncertainty factor is 1.0	EFSA terminology [40]
PN-DR = PN Daily recommended doses	The doses used in PN are extrapolated from RDAs, bioavailability studies and long-term follow up of patients on home PN. They aim at covering basal needs in most patients. Individual patients may have increased or decreased needs.	The commercially available preparations contain variable amounts of MNs. Growing data indicates that many are outdated providing either too much or too little of particular MNs.

Table 5
Wording used to describe the type of prescription. Based on [40–43].

	Oxford definition	Definition	Comment
Complement	The act of adding to something in a way that improves it or makes it more attractive, complete	Complementation will be used to indicate the delivery of micronutrients *to cover basal needs* (e.g. to complete enteral feeds [4], or PN).	This action is likely to be needed to cover basal needs in case of progressive or insufficient EN
Repletion	The act to make something full again by replacing what has been used	Doses aiming to *restore a normal status*, and where the deficit is known. Sometimes called supplementation but term to be avoided as confusing	The term "Repletion" will be used when deficiency or losses are identified or presumed: the administration aims at restoring a normal status.
Supplementation	The act of adding something to something else to improve or complete it	Term used when the aim is to deliver *higher than standard doses* (i.e. superior to DRI or PN recommendation) [41]. The term does not include pharmaco-nutrition but designates doses higher than basal requirements delivered in an attempt to correct depletion or deficiency.	This term is often applied without differentiation of amount whenever a MN is prescribed, which leads to confusion
Pharmacological dose	Connected with the scientific study of drugs and their use in medicine	Treatment with a specific micronutrient to improve host defenses, or any other biological endpoint associated with good clinical evolution and improve the outcome of critically ill patients.	Generally, only one micronutrient is prescribed (typically selenium, vitamin C, vitamin E). Administration route is not determinant. This is not a nutritional effect, but is a pharmacological action

Between 2nd − 30th July 2021, a first online voting on the trace element chapters as well as the chapters on carnitine, choline, and coenzyme Q10 (CoQ10) was performed using a platform provided by the Clinical Guideline Services GmbH (guideline-services.com). A second online voting on the vitamin chapters, as well as a chapter on cobalt followed between 1st September − 30th September 2021. In the first online voting, 49 recommendations reached an agreement >90%, 39 recommendations reached an agreement of >75–90% and four recommendations an agreement ≤75%. In the second online voting, 73 recommendations reached an agreement >90%, 14 recommendations reached an agreement of >75–90% and two recommendations an agreement ≤75%. In parallel to the second online voting, an additional voting round on 22 modified recommendations of the trace element chapters was conducted; the participants of this online voting were selected experts in the field of the trace elements. A total of 103 votes were submitted in all three online votings.

On 30th November 2021, an online consensus conference took place, where a total of 21 recommendations as well as two tables (Table 15) were voted on. One recommendation was deleted, all the other recommendations reached an agreement >90% (see Table 2).

Finally, it is important to note that the guideline process was funded exclusively by the ESPEN society.

2.1. Language standardization

During the process, it became obvious that a glossary was required because many words have been used imprecisely, resulting in confusion. This is particularly true for the words 'deficiency', and 'supplementation'. The "Oxford English dictionary online" definitions served as reference, and Table 3, Table 4, and Table 5 aim at standardising the language.

2.2. Micronutrients status

Defining precisely suboptimal or deficient status is the basis for a therapeutic intervention. The term "deficiency" has been used too broadly: it has often been applied as soon as the laboratory returns blood values below the local or international reference range. Table 3 provides the definitions that will be used hereafter to qualify the status and the therapeutic actions. Particular attention is drawn to the definitions of "deficiency" and "depletion".

2.3. Requirements, dosage and treatment considerations

In 1940, the National Academy of Sciences received the mandate to study nutrition problems in the United States [1]. This

Table 6A
Disease-specific risks of depletion or deficiency in trace elements and vitamins. Note: the below list of diseases associated with known alterations of MNs is non-exhaustive (alphabetic order) and may in some cases be less fully supported by the evidence. These and other diseases may have further or still unknown associations with various MN inadequacies.

Disease	Deficiency favouring disease development	Inadequacy or deficit worsening the condition	Deficiency as a result of disease	References
Alcoholism		B1, Fe	A, D, E, K, B1, B2, B6, B7, B9, B12, C, Zn	[4, 44]
Alcoholic hepatitis	B6, Zn	Se, Zn		[45]
Anaemia	B1, B6, B9, B12, Fe, Cu, Co			[4]
Cancer cachexia	D, Zn			[46, 47]
Cardiomyopathies/ Heart failure	B1, B6, D, Se, Fe	Se		[4, 48, 49]
Chronic obstructive pulmonary disease	D, Cu, Se, Mn, Zn			[50, 51]
Chronic intestinal failure			B2, B7, B9, B12, A, D, E, K, Cu, Fe, Zn	[52–54]
Chronic (atrophic) gastritis			B9, B12, C, D, Fe	[55, 56]
Diabetes mellitus	B9, Cr			[57-61]
Inflammatory bowel diseases		Zn	B1, B6, B12, A, D, E, K, Fe, Se, Zn	[4, 62]
Non-alcoholic fatty liver disease	Cu			[63]
Liver diseases		Zn	B12, A, D, E, Se, Zn	[4, 64, 65]
Multiple Sclerosis	B7			[66]
Obesity	β-carotene, E, Se, Zn	B1, B9, D, Fe, Se, Zn		[55,67–69]
Obesity Post Bariatric surgery			A, D, E, K, B1, B9, B12, C, Cu, Fe, Zn	[69–71]
Osteoporosis	B12, D, K, Cu, Fe, Zn, Mn, F, Bo			[72, 73]
Renal failure (chronic)			B1, B6, B9, K, D, Cu, Se, Zn	[74, 75]
Sarcopenia	B1, B12, D, carnitine, Zn	D, Se, Zn		[55, 76]
Critical illness		B1, C, D, Cu, Fe, Se, Zn	B1, B12, C, D, Fe, Se, Zn	[76–79]

resulted in the first Recommended Daily Allowances (Daily being subsequently renamed Dietary, and abbreviated RDA). The goal was to recommend "allowances sufficiently liberal to be suitable for maintenance of good nutritional status" in the general population. In the subsequent decades, a different nutritional health challenge began to emerge for an increasing proportion of the population, that of overweight and obesity and risk of diet-related chronic disease. In part, as a response to this challenge, the RDA process was revised and the DRIs were developed [23]. In the 1990s, the risk of chronic disease was integrated in the DRI concept. Indeed, chronic diseases are multifactorial in nature and not directly nutrient-specific; the body of evidence supporting nutrients and other food substances as modifiers of risk of chronic disease is generally limited; and there is a lack of consistency in findings across study types.

The DRIs are fundamental to inform national nutrition policies and regulations. However, the feasibility of reviewing and updating the 51 nutrients that have DRIs has limitations, and most DRIs have not been reviewed for over 20 years [24]. Nevertheless, we will hereafter use the definitions of the Food and Nutrition Board and the values that are available in clinical settings (Table 3). Whether treating out- or inpatients, there comes a point where MNs need to be prescribed. In the present guideline, the terms proposed in Tables 4 and 5 will be used.

In *enteral nutrition*, all MN are within the specific feeding mixture in a fixed combination, although different commercial preparations may contain different amounts. The European Council has published directives "Food for Special Medical Purposes" (FSMP) that regulate the minimal and maximal content of nutrients in oral and enteral feeding solutions [25]. By choosing a specific enteral product, clinicians can change to some extent the amount of MN provided. Oral single MN may be required in case of specific deficiencies, and IV additional doses may be required. However, it is much more common for malnourished individuals to be depleted in multiple MN, which require the balanced amounts present in enteral products to improve nutritional status.

Parenteral nutrition is different, as the intestine is bypassed, thereby increasing the risk of both insufficiency (absence of MN) and toxicity, if high doses are delivered by the IV route. The first recommendations were proposed by the Nutrition Advisory Group of the Department of Food and Nutrition and by the American Medical Association (AMA) in 1975. The values were extrapolated from RDAs and DRIs based on knowledge about bioavailability. These recommendations have been modified thereafter in ASPEN and ESPEN guidelines/documents and developed in response to deficiencies that were documented in the early years of PN. Follow-up of patients on long term PN generated knowledge about the minimal doses to deliver to stable patients [26]. There is no universal PN recommendation, nor terminology (Table 4). Over the past two decades, ESPEN [16], the American Society for Parenteral and Enteral Nutrition (ASPEN) [5,27], and Australasian Society of Parenteral and Enteral Nutrition (AuSPEN) [6] have published

Table 6B
Treatment associated specific risks of depletion, deficiency, or excess in MNs.

Therapy	MN deficit due to treatment	
Renal replacement therapy	all water-soluble vitamins, Carnitine Cu, Fe, Se,	[80]
Diuretic therapy	Thiamine (B1)	[81,82]
	Se	[49]
Proton Pump Inhibitors	B12	[83]
	Fe	
Metformin	B12	[84–87]
Isoniazid treatment	B6	[88] [89,90]
Antiretroviral HIV therapy	D	[91]
Antiepileptic drugs	B9, B12, D	[92]
Thymoglobulin (immunosuppression)	B6	[93]

guidelines for parenteral trace element provision. The three recommendations differ in some points, and ESPEN is the only society that included from the start the 9 trace elements associated with clinical consequences in case of deficit [28].

The PN patient population is heterogeneous and fixed doses may not fit individual requirements. Patients may require increased zinc and selenium in presence of increased gastrointestinal losses, copper and manganese should be reduced in patients with cholestasis, and manganese and chromium are frequently found in elevated levels in long-term [8].

Another problem is the commercial availability in different countries [8]. There are currently several varieties of pre-mixed multi-TE combinations available for addition to PN solutions. Many of these are incomplete, providing only 4 or 6 trace elements, and still contain doses that are not in keeping with the current knowledge, potentially providing inadequate or excessive amounts of different MNs [5]. Provision of MNs requires active consideration of the amount to be added, while multiple trace elements and vitamins products are formulated in fixed combinations. On some occasions, consideration must be given to provide an extra quantity of individual elements. If smaller amounts of individual elements are required, e.g., of manganese or copper in cholestasis, it may be necessary to add individual trace elements separately.

It is also important to note the problem of shortage of parenteral micronutrients in many countries, that may occur as a result of reprioritisation of the industrial production lines. Occasional transient shortages have occurred during the last decades for both vitamins and trace elements [29], and have recured during the recent years during the COVID-19 pandemic. The scarcity of multivitamin and multi-trace element products for patients receiving PN (both in the hospital and at home) can induce depletion and deficiency through the lack of provision or decrease in frequency of administration [29–31]. Some international societies ASPEN [32], BAPEN [33] and SFNCM [34] have developed recommendations to prioritize the provision of these micronutrients during periods of shortage. They have concluded that closer monitoring of these vitamins and trace elements is required in these situations.

2.4. General comments on provision of micronutrients

Although the current document is focusing primarily on nutritional support, that is patients receiving either EN or PN, it is important to remember that some patients will benefit from “oral nutrition supplements”. Specifically, regarding MNs such supplements could either be single or multiple trace element and/or vitamin preparations.

Recommendations for provision of trace elements and vitamins during nutritional support have come from several sources. As well as using the results of studies on MN provision in both enteral and PN, careful use has also been made of the many recommendations for oral provision, from European and American expert bodies, with extrapolation to parenteral supply.

An important concept for the water-soluble vitamins is that they have very low toxicity and hence most of the recommendations are for a minimal level of supply but increased amounts of all of them would be safe and effective, although possibly wasteful. This is especially relevant to parenteral supply of water-soluble vitamins. As summarized in the ASPEN position paper on MN requirements in PN [5], the rationale for providing higher doses than the minimum calculated to be required IV is that many patients have higher vitamin requirements due to malnutrition, baseline vitamin deficiencies, and metabolic changes secondary to illness, and moreover there is likely to be increased excretion of water-soluble vitamins when provided IV [5]. Hence for some of these vitamins, the parenteral recommendation is higher than the enteral (Table 15). The potential of toxicity of the fat-soluble vitamins (A and D especially) is addressed in the specific texts.

The refeeding syndrome is a well-recognized complication of provision of nutrition to individuals who are malnourished or recently had a low intake. Provision of energy substrates leads to a redistribution of vitamins, trace elements, and other major minerals. Unless recognized and treated, this can lead to life-threatening complications. Treatment includes a combination of MNs and electrolytes. Detailed discussion of this is beyond the scope of this guideline, but is summarized in the following references [35–37].

Recommendation 1

Adequate amounts of all essential trace elements and vitamins shall be supplied to all patients receiving medical nutrition from the beginning of the period of nutritional support.

Grade of recommendation A – Strong consensus 100%

Recommendation 2

Micronutrient supplements shall be provided orally or enterally if this can be done safely and effectively.

Grade of recommendation A – Strong consensus 100%

2.5. Impact of inflammation

The presence of inflammation in the context of surgery, trauma, infection or many acute or chronic diseases, complicates the assessment of the status based on blood levels. Using the surrogate C-reactive protein (CRP) as a marker of its intensity, it has been clearly shown that inflammation induces a redistribution of many MNs from the circulating compartment to other organs, resulting in low levels for most MNs [20]. Low blood levels therefore do not necessarily indicate deficiency or even depletion. Within 24 h of elective surgery in otherwise healthy individuals, plasma concentrations of many trace elements and vitamins have fallen markedly, without any change in whole body MN status [38]. The effects of inflammation in response to acute trauma or infection is usually rapid but may also be prolonged in chronic illness. There are some variations across diseases that will be discussed with every MN.

Although there is no universal adjustment tool for inflammation, the international group BRINDA (Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia) developed an all-in-one R package inflammation adjustment equation for five MN biomarkers: retinol-binding protein, serum retinol, serum

ferritin, soluble transferrin receptor (sTfR), and serum zinc, using the inflammation indicators Alpha(1)-acid glycoprotein (AGP) or CRP for various population groups (https://cran.rproject.org/web/packages/BRINDA/index.html) [777].

Recommendation 3

C-reactive protein should be determined at the same time as any micronutrient analysis.

Grade of recommendation B − Consensus 87%

Single MNs are rarely determined alone, which explains this general recommendation to include CRP. Some vitamins such as cobalamin (B12) are not influenced by inflammation but are rarely determined alone. The impact of inflammation usually appears with CRP levels >20 mg/L: therefore hs-CRP, which aims at detecting mild inflammation, is not appropriate.

Albumin is a carrier protein for many MNs including zinc. Its level may be influenced by dilution, and by inflammation, being a negative acute phase protein [38]. Therefore, albumin determination is desirable whenever a series of MNs is determined.

2.6. Pathologies at risk

Most guidelines are disease oriented and do mention MN problems when they are disease specific. The present guidelines have taken the reverse option, i.e., to deliver MN specific recommendations, indicating in which disease they are at high risk in the direction either of insufficiency or overload.

Nevertheless, to orient the clinicians, the below Table 6A and B presents a non-exhaustive list of diseases, and medical treatments justifying concern and monitoring of a combination of MNs. This table aims at raising awareness about some often-overlooked aspects of the different diseases, and at considering a combination of MNs.

3. Chromium

3.1. Main functions

Chromium (Cr) is a transition element which exists in several valence states. While Cr IV, V and VI are carcinogenic, the trivalent chromium is an essential trace element that is a component of metalloenzymes. Trivalent chromium is stable and is the biologically active form. It is found in foods (mainly high-bran cereals), dietary supplements, and PN [5,94,95]. Chromium enhances insulin action in peripheral tissues, and intervenes in the metabolism of carbohydrates, protein, fat and in the oxidative state. Chromium acts by increasing the number of insulin receptors, modifying the insulin/receptor binding, increasing internalisation of insulin and activating the translocations of the glucose transporters Glut1 and Glut4 [96].

Chromium absorption in the small bowel ranges from 0.4 to 2.5%, depending on the total body chromium concentration [94]. It is transported in the blood bound to transferrin and albumin. Most of the absorbed chromium is excreted rapidly in the urine and ranges from 3 to 50 μg/d, so dose reduction should occur in renal failure. Biliary excretion via the small intestine may be an ancillary route of elimination. Chromium is stored in the liver, spleen, soft tissue, and bone.

3.1.1. Needs

The oral chromium adequate intake (AI) is 35 μg/day and 25 μg/day for young men and women, respectively. Although current parenteral recommendation of chromium in adults is 10–15 μg [5,97], based on oral absorption in healthy individuals, the parenteral requirements may be as low as 0.14–0.87 μg/d [94,98–100]. However, the precise requirements during PN when there is a high intake of glucose provided over a prolonged period are not known. Adult multiple trace element products currently available provide 10–15 μg/d of chromium.

3.2. Biomarkers, and analytical methods

The assessment of the chromium status in humans is difficult, and often indirect:

- *Plasma or serum levels:* May not be good indices of chromium status, because blood chromium does not readily equilibrate with tissue chromium stores [94]. Plasma chromium levels may be reduced in deficiency but are also reduced in acute illness. Serum chromium concentrations range from 1 to 5 μg/L (or even <0.5 μg/L according to some authors, depending on the sampling technique). Total chromium can be measured in whole blood, plasma, serum, or urine preferably by inductively coupled plasma coupled to mass spectrometry (ICP-MS), or by atomic absorption spectroscopy.
- *Response of glucose tolerance test to chromium administration:* chromium supplementation can normalize the glucose tolerance curve from the diabetic-like curve typical of chromium deficiency. This response is a more meaningful indicator of chromium status than serum levels [94,101].
- *Urinary excretion of chromium*: Reflects recent dietary chromium intake, but it is not a useful predictor of chromium tissue status
- *Chromium in tissues:* Reported concentrations of chromium in human tissues may be unreliable due to sampling and analytical problems.

3.2.1. Conversion

Chromium: 1 μg/L = 19.2 nmol/L and 1 nmol/L = 0.052 μg/L

3.2.2. Effect of inflammation

In a recent systematic review of 2344 publications, no reference was found about the effect of systemic inflammation on the levels on chromium [102]. However, low chromium levels have been described since the 1970s during acute illness (burns, trauma, and infected patients) with abnormalities in glucose metabolism [80,103].

Chromium has anti-inflammatory properties that have been highlighted in a meta-analysis of 7 studies showing a significant reduction of hs-CRP associated with supplementation [104].

3.3. Deficiency

Insufficient intakes are frequent in industrial countries, and are associated with alterations of glucose metabolism, especially in older adults [105]. Also, at risk of deficiency are patients with acute illness due to metabolic stress (burns, trauma, infection), or patients with decreased absorption/intake (short bowel syndrome and PN patients without chromium supplementation). While prolonged continuous renal replacement therapy, affects the status of trace elements such as copper, this does not seem to be the case for chromium [106].

Acute infection appears to reduce the availability of circulating chromium, which may contribute to the altered glucose metabolism in this setting. There are only case reports about chromium administration for hyperglycemia and severe insulin resistance in the ICU [107,108]. When used in conditions such as pancreatitis,

cardiac procedures, solid organ transplants, trauma, esophagus and thymus surgery and corticosteroid treatment, insulin requirements decreased with chromium doses of 3–20 μg/h for 10 h up to 4 days [109].

Chromium deficiency has been reported in adults with chronic intestinal failure after massive bowel resection receiving long-term PN without chromium [94,98–100]. The clinical manifestations were glucose intolerance, weight loss, elevated plasma free fatty acids and neuropathy that were reversed by daily chromium supplementation in the PN solution.

A low plasma chromium level is associated with hyperglycemia, insulin resistance, high inflammatory status and increased cardiovascular risk in humans [61,101]. Chromium insufficiency has been hypothesized to be a contributing factor to the development of type 2 diabetes and some studies have revealed a negative relationship between serum chromium and HbA_{1C} levels. However, chromium supplementation trials have not shown consistent benefits. A meta-analysis of 41 RCTs found that chromium supplementation in patients with type 2 diabetes may have a modest beneficial effect on glycemia and dyslipidemia. In contrast, there was no beneficial effect of chromium supplementation in those without diabetes. The authors found large heterogeneity and an overall poor quality across the studies [110]. In a systematic review of 9 RCTs, chromium supplementation was not effective in the treatment of obesity [111]. By contrast the meta-analysis of 11 trials testing chromium supplements shows that chromium significantly reduces both systolic and diastolic pressures [112].

Several factors suggest that the elderly might be more vulnerable to chromium depletion than younger people and improved glucose and lipid control with chromium supplementation in elderly diabetic patients has been described [113].

3.3.1. When and how to treat

Acute deficiency occurring during nutritional support is rare, except if chromium is excluded from the MN formulation. chromium deficiency can be treated by oral or iv supplementation. Oral chromium is poorly absorbed, and it has been used mostly in studies, administered as different formulations (as chromium yeast, chloride, nicotinate, or picolinate the latter being the best absorbed). IV chromium, 200–250 μg/day for a period of 2 weeks or longer, has been given to PN patients suspected to be deficient in chromium [94].

In ICU patients with major insulin resistance (30–50 U/h of insulin required to maintain blood glucose <10 mmol/L), iv chromium (as chromium chloride) with doses ranging from 3 to 20 μg/h for 10 h up to 4 days decreased insulin requirements [107,108,114]. A difficulty in the treatment is the non-availability of single element chromium additives for iv use in many countries, making necessary the use of multi-trace element solutions.

3.4. Toxicity

The toxicity of chromium differs widely depending on the chromium valency. Particularly Cr^{6+} is carcinogenic, nephrotoxic and causes dermatitis by causing DNA damage and genomic instability [95]. Both Cr^{6+} and Cr^{3+} are capable of producing ROS (reactive oxygen species) [95]. Ingested trivalent chromium has a low level of toxicity due partially to its poor absorption. Even though there is no upper level of safe intake, isolated case reports exist of renal and hepatic failure with high-dose oral chromium supplementation (oral chromium picolinate 600 μg/d and 2400 μg/d, respectively) [94].

Parenteral Cr^{3+} may have a higher potential toxicity. The accumulated scientific data points to a need to lower the recommended amount of parenteral chromium. It has been suggested that it is not necessary to give extra chromium in patients on PN, due to the widespread contamination in PN components, especially from the 70% dextrose solution [5,94]. Chromium contaminants in PN solutions can increase the amount delivered by 10–100%. High levels of chromium in serum and urine have been found in adults and children treated with PN, even in short-term. In autopsy tissues of patients on long-term PN, chromium levels were 10-100-fold higher than normal concentrations in heart, skeletal muscle, liver, and kidney [115]. However, in adults there are no reported cases of chromium toxicity in patients on long-term PN or in patients with hip protheses with very high levels of chromium, suggesting that these high chromium concentrations are not toxic.

Chromium toxicity may be more of a concern in pediatric patients, and an inverse correlation between serum chromium levels and glomerular filtration rates in PN-dependent children was found [116]. ESPGHAN/ESPEN do not recommend chromium addition in PN in children, whereas ASPEN recommends a reduced dose compared to previous recommendations and removal of chromium in patients at risk of toxicity [5,117].

Chromium has been shown to accumulate in the bones of patients with end-stage renal disease, and increased serum chromium levels have been found in patients undergoing maintenance hemodialysis [118].

Oral intoxication is rare due to the low absorption. Plasmapheresis has been successfully used in severe dichromate intoxication [119]. Chelators and antioxidants have also been used.

3.5. Recommendations N°4 - chromium

3.5.1. When to measure?

Recommendation 4.1

Regular monitoring of chromium status shall not be performed; however, it can be required when there is clinical suspicion of deficiency or toxicity.

Grade of recommendation GPP – Strong consensus 94%

Recommendation 4.2

In the case of suspected chromium deficiency, response of glucose tolerance test to chromium supplementation can be performed.

Grade of recommendation 0 – Strong consensus 100%

3.5.2. What to measure?

Recommendation 4.3

Serum chromium can be determined, but is rarely required.

Grade of recommendation 0 – Strong consensus 100%

3.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 4.4

Enteral nutrition should provide at least 35 μg/day chromium with 1500 kcal/day.

Grade of recommendation B – Strong consensus 94%

Recommendation 4.5

Parenteral nutrition can provide at least 10 μg per day.

Grade of recommendation 0 – Strong consensus 100%

The requirement in PN is still debated, especially since absorption of chromium is low, and no trials have been performed with lower doses. The above doses have been used safely and effectively in adults for many years. More research on chromium is required.

3.5.4. When to provide additional amounts?

Recommendation 4.6

In cases of proven or suspected clinical deficiency, additional supplementation of chromium can be provided orally or IV as available.

Grade of recommendation GPP – Strong consensus 94%

Recommendation 4.7

Chromium supplementation should not be used to improve glycemia and dyslipidemia control in patients with type 2 diabetes, obesity, and non-diabetic patients.

Grade of recommendation B – Strong consensus 100%

Recommendation 4.8

In case of severe insulin resistance and hyperglycemia in critically ill patients, a therapeutic trial with IV chromium can also be used to reduce insulin requirements.

Grade of recommendation 0 – Strong consensus 100%

This recommendation is not applicable to general diabetic patients, but only for critically ill patients, in case of increasing insulin doses (30–50 U/h of insulin required to maintain blood glucose <10 mmol/L). Such a trial is limited to 4 days [107,108,114].

3.5.5. How to provide additional amounts?

Recommendation 4.9

Chromium (200–250 μg/day for 2 weeks) can be given parenterally in patients on parenteral nutrition suspected to be deficient in chromium based on insulin-resistance: reassess insulin-resistance after 2 weeks.

Grade of recommendation 0 – Strong consensus 91%

Such a chromium trial may be the way to confirm diagnosis in patients on PN requiring high dose insulin for blood glucose control [7].

Recommendation 4.10

In insulin-resistant critically ill patients, chromium with doses ranging from 3 to 20 μg/h IV for 10 h and up to 4 days may be required.

Grade of recommendation 0 – Strong consensus 100%

As for 4.8 this recommendation only applies to critically ill patients requiring very high doses of IV insulin.

4. Cobalt

4.1. Main functions

Cobalt is a rare magnetic element with properties similar to iron and nickel [120]. Cobalt is an essential element necessary for the formation of vitamin B12 (hydroxocobalamin). All the essential functions of cobalt are covered in the chapter about vitamin B12.

The bioavailability of cobalt and its intestinal absorption depend on the solubility of vitamin B12 and the ingested amount [120].

4.1.1. Needs

The main source of cobalt for the population is diet, or an industrial exposure [121]. The average nutritional intake ranges from 5 to 45 μg/day [120], with the higher concentrations being observed in fish and vegetables in Canada.

There is no DRI for cobalt [122,123], but only for vitamin B12.

There is only one product on the market for PN providing Cobalt 1.47 μg as gluconate (Decan®, Laboratoires Aguettant, Lyon, France). Other products do not provide it.

4.2. Biomarkers, and analytical methods

In cases of suspected cobalt toxicity, the assessment of the status is based on determination of serum [121] and urine levels [124,125] using ICP-MS.

4.2.1. Conversion

Cobalt: 1 μg = 16.96 nmol and 1 μmol = 58.9 μg

4.2.2. Effect of inflammation

There is no known effect of inflammation.

4.3. Deficiency

See vitamin B12.

4.4. Toxicity

Human beings can be exposed to cobalt through occupational contact (glass, inks, and paints), in processing plants, hard-metal industry, diamond polishing, and the manufacture of ceramics. As cobalt can stimulate the production of red blood cells (RBC), cobalt has been used for the treatment of refractory anemia and by athletes to increase red blood cell mass and increase exercise performance, as an alternative to blood doping [126].

Cobalt is toxic to the heart muscle. Excessive amounts of cobalt may result in the syndrome of “beer drinker cardiomyopathy” observed in Quebec [120]: it is characterized by pericardial effusion, high hemoglobin concentrations and congestive heart failure. The course of cobalt-related cardiomyopathy may be progressive and fatal [121]. The clinical emergence of cobalt cardiomyopathy requires the coexistence of cofactors such as a low-protein diet, thiamine deficiency, alcoholism, and hypothyroidism [121].

Cobalt alloys have been used in hip arthroplastic procedures since 1938 [127], and increased concentrations of cobalt in blood, hair, and urine have been reported in patients receiving cobalt alloy implants since 1967 [121].

4.5. Recommendations N°5 - cobalt

4.5.1. When to measure?

Recommendation 5.1

Determination of cobalt may be required in case of suspicion of toxicity in the context of cardiomyopathy.

Grade of recommendation GPP – Strong consensus 92%

4.5.2. What to measure?

Recommendation 5.2

Serum and blood determinations may be done in the context of suspected toxicity.

Grade of recommendation 0 – Strong consensus 94%

4.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 5.3

Enteral nutrition should provide cobalt as vitamin B12.

Grade of recommendation B – Strong consensus 95%

Recommendation 5.4

Parenteral nutrition does not need to provide additional cobalt, as long as vitamin B12 is administered.

Grade of recommendation GPP – Strong consensus 92%

5. Copper

5.1. Main functions

The essentiality of copper in mammals was established in 1928 [128,129]. Copper has the specific ability to adopt two different redox states: the oxidized cupric (Cu^{2+}) and reduced cuprous (Cu^{+}) forms.

Copper absorption occurs in the stomach and small intestine, primarily in the duodenum [130]. Most absorbed copper is lost via biliary excretion [131]. Copper absorption is highly regulated, and the gastrointestinal tract has specific adaptations that are responsible for its transport and is a saturable process.

Most of the body's copper is present as Cu^{2+}. It serves as an essential catalytic cofactor in redox chemistry for proteins involved in growth and development [132], and as an essential cofactor for oxidation-reduction reactions involving copper-containing oxidases. Copper enzymes regulate energy production, iron metabolism, connective tissue maturation (elastin and collagen synthesis via lysyl oxidase), and neurotransmission (dopamine synthesis), and peptidyl glycine α-amidating monooxygenase, necessary for the activation and deactivation of different peptide hormones. In addition, copper is essential for cholesterol, thyroid hormone and glucose metabolism, aspects of immune function, blood pressure control and the formation of melanin pigment [133,134].

5.1.1. Needs

The DRI of copper vary between 1.1 and 2 mg/day in adults, but absorption is highly variable ranging between 20 and 50% [129,135]. Sources of copper are cereals, fresh fruits and vegetables, fish and seafood [135,136].

Diets in Western countries usually provide copper in the low range of the estimated safe and adequate daily dietary intake (ESADI) [137] typically in the alluvial areas, but may be more abundant in certain geographic areas [135,138]. Recommendations for dietary intake and total exposure, including that from drinking water, should consider that copper, although essential, has potential toxicity, as obvious in Wilson's disease, a genetic disorder characterized by copper accumulation in several organs [139]. A no-observed-adverse-effect level (NOAEL) of 10 mg/day was identified in a 12-week, double blind study in seven adults testing up to 12 mg/day: liver function tests remained normal [140].

In PN the commercial trace element products provide 0.5–1.2 mg/day [5]. For many years, typical copper provision in PN was around 1 mg/day, but this is now regarded as excessive, and potentially dangerous, especially in patients with abnormal liver function. Balance studies have indicated that 0.3–0.5 mg/day is sufficient to meet the needs of stable patients during PN [5,141]. In long term PN, the plasma concentrations should be checked every 6–12 months, and more frequently if there is evidence of cholestasis [142].

5.2. Biomarkers and analytical methods

Deficiency and toxicity are diagnosed on blood samples. Copper may be determined in serum or heparinized plasma. Several methods are available: the most precise and applicable to biological fluids is ICP-MS, although atomic absorption spectroscopy is also frequently used.

Urine measurements are of limited value for status assessment considering the small proportion of copper that is excreted by the kidney. Nevertheless, it is used for diagnosis and monitoring in Wilson's disease. The urinary copper excretion rate is > 100 μg/d (reference range, < 40 μg/d) in most patients with symptomatic Wilson disease. The rate may also be elevated in other cholestatic liver diseases.

Ceruloplasmin: 98% of circulating copper is bound to ceruloplasmin, an alpha-2-globulin and ferroxidase protein which is synthesized by the liver, and which enables storage of copper, preventing toxicity of the free ions - its determination is part of copper status assessment. Copper also exists in plasma as a diffusible “free” form (2%), loosely bound to albumin and some amino acids.

5.2.1. Conversion

Copper: 1 μg/dL = 0.157 μmol/L and 1 μmol/L = 6.35 μg/dL

5.2.2. Effect of inflammation

Different from most MNs, copper concentrations in plasma increase in the context of an inflammatory response since ceruloplasmin is a positive acute phase reactant [20]. Therefore, the diagnosis of deficiency requires simultaneous determination of plasma CRP and copper. A normal serum copper in the presence of an elevated CRP would suggest copper depletion or deficiency. In case of uncertainty, ceruloplasmin concentrations will assist diagnosis, as low values of the latter provide confirmation of deficiency.

5.3. Deficiency

Copper depletion is observed in some acute conditions such as major burns, after gastric and bariatric surgery and in patients requiring continuous renal replacement therapy, or in prolonged PN or EN without adequate copper [80,131,143–147]. Symptoms of deficiency require some weeks to develop and are not readily recognized [143]. The acute symptoms which are rare include cardiac arrhythmias, myeloneuropathy, and delayed wound healing [131,133]. The chronic symptoms, observed in patients on PN with inadequate copper, include microcytic anemia, neutropenia, osteoporosis, and hair de-pigmentation (copper is essential for melanin synthesis) [133].

5.3.1. When and how to treat?

Copper may be delivered by the oral, enteral or IV routes [148]. With blood concentrations <12 μmol/L and high CRP >20 mg/L, a deficiency is likely and copper administration can be considered. With values < 8 μmol/L with or without elevated CRP, repletion should be indicated. The choice of the route will be determined by the severity of the copper deficit (see figure Appendix B). Treatment of deficiency usually will require provision of copper 4–8 mg/day

IV. (Copper >2 mg/day is phlebogenic on a peripheral line, whatever the dilution).

5.4. Toxicity

Supra-normal levels may be observed in inflammatory conditions, reflecting the increase in ceruloplasmin. Elevated free copper levels exist in Alzheimer's disease [149]. It may also be found in pathologies such as infections, hemopathies, haemochromatosis, hyperthyroidism, liver cirrhosis and hepatitis, and physiologically in pregnancy. Such values only require monitoring.

Intoxication is rare but may occur in an industrial context. It may be caused by dietary supplements or from drinking contaminated water. Cholestasis can also affect the liver's ability to excrete copper, resulting in chronic copper toxicity [150].

Copper toxicity symptoms include: hematemesis, hypotension, melena (black "tarry" feces), coma, headaches, behavioral changes, fever, diarrhea, abdominal cramps, brown ring-shaped markings in eyes (Kayser-Fleischer rings), and jaundice. It is also increased in genetic disorders such as Wilson's disease, and in Menke's syndrome. Long-term toxicity may cause organ failure affecting first the kidney and liver, but also heart and brain [150].

Intoxication treatment: The administration of oral zinc is a validated strategy in Wilson's disease, where blood levels are low, but copper is sequestered in the liver [132]. In case of acute toxicity, treatments with oral D-penicillamine (250–500 mg/day) and then increased by 250 mg increments every four to seven days to a maximum of 1000–1500 mg daily in two to four divided doses) is a validated option. In veterinary medicine molybdenum is a recognized treatment.

5.5. Recommendations N°6 - copper

5.5.1. When to measure?

Recommendation 6.1

Copper levels should be measured:

- **In patients coming for post-bariatric surgery follow up or after other abdominal surgeries that exclude the duodenum**.
- **In patients admitted for neuropathy of unclear etiology**.
- **In major burn patients whether or not receiving complements of copper**.
- **In the context of continuous renal replacement for more than 2 weeks**.
- **In patients on home enteral nutrition fed by jejunostomy tubes**.
- **In patients on long term parenteral nutrition, regularly every 6–12 months**.

Grade of recommendation B – Strong consensus 94%

5.5.2. What to measure?

Recommendation 6.2

Copper status shall be determined by measurement of plasma copper simultaneously with CRP determination.

Grade of recommendation A - Consensus 89%

5.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 6.3

Enteral nutrition shall provide 1–3 mg copper per day with 1500 kcal.

Grade of recommendation A – Strong consensus 97%

Recommendation 6.4

Parenteral nutrition should provide 0.3–0.5 mg copper per day.

Grade of recommendation B – Strong consensus 94%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

5.5.4. When to provide additional amounts?

Recommendation 6.5

With plasma concentrations <12 μmol/L and high CRP >20 mg/L, a deficiency is likely and copper administration can be considered.

Grade of recommendation GPP – Consensus 89%

Recommendation 6.6

With plasma copper values < 8 μmol/L with or without elevated CRP, repletion measures should be taken.

Grade of recommendation GPP – Strong consensus 97%

5.5.5. How to provide additional amounts?

Recommendation 6.7

In chronic conditions, oral administration may be considered first.

Grade of recommendation GPP – Strong consensus 94%

Recommendation 6.8

With severe copper deficiency, the IV route should be preferred with administration of doses of 4–8 mg/day as slow infusion.

Grade of recommendation GPP – Strong consensus 92%

6. Fluoride

6.1. Main functions

Fluoride is the world's 13th most abundant element [151], being widely distributed in the environment, occurring in soils, rocks, and water: it is therefore naturally present in the food and drink we consume. It is considered a normal constituent of the human body, but its status as "essential" is debated.

It is well absorbed by the small intestine. and gets attached to bone and teeth, transforming apatite into fluoro-apatite. Nearly 99% of the body's fluoride is bound strongly to calcified tissues. Fluoride in bone appears to exist in both rapidly and slowly exchangeable pools [152,153]. Blood and bone concentration are in equilibrium [154]. About half the absorbed fluoride is excreted via the kidneys. Fluoride skeletal uptake is also modified by factors such as the activity of bone remodeling and age.

Fluoride inhibits glycolysis enzymes, a capacity which is used in laboratories to assist blood glucose determination [155].

Main sources include foods, fluoridated water, fluoridated toothpastes and some dietary supplements [156]. Fluoride intake from most foods is low, but tea may be an important source of high doses as shown in a well-documented intoxication case report [157]. Fluoride's unique role in mineralization is the reason for its

recognition as a beneficial trace element for dental health of humans [158].

6.1.1. Needs

There is no DRI. Adults typically consume <0.5 mg of fluoride daily in food [157]. Nutritional intakes in adults are safe up to 4 mg/day in men and 3 mg/day in women.

Fluoridation programs were started in numerous western countries in the 1960s [151]. The fluoridation of drinking water aims to bring fluoride levels up to a range preventing or minimizing tooth decay by 26–44% in children, teenagers and adults [153]. Some bottled drinking waters contain up to 8 mg/L. The need for fluoridation remains debated [159].

Fluoride can reasonably be provided in EN at doses of up to about 3 mg/day.

For PN, the need for adding fluoride is debated, especially in USA [160]. However, fluoride has been supplied for over 40 years as part of standard multi-element mixtures in European products at a dose of 0.95 mg without any side effects, and with the potential for beneficial effects on bones and teeth. This dose continues to be recommended for standard PN.

6.2. Biomarkers and analytical methods

Fluoride may be determined in serum, or in 24hr urine collection. Analytical methods use a fluoride-specific electrode (urine), flow injection analysis coupled with a fluoride-specific electrode (serum and urine) (FIA-FE) [161], or by ion chromatography with conductivity detection (IC-CD).

6.2.1. Reference values

Serum <50μg/L (or <2500 nmol/L), and for urine <0,5 mg/24 h or < 25 nmol/24 h. When used in treatment of osteoporosis, the serum values are increased 5–10 times.

In professional or other (hydro-telluric) exposure, the urinary toxicity range is 10 mg/24 hr.

Dental fluorosis is diagnosed by bilateral symmetrical developmental enamel opacities (brown discoloration) [162].

6.2.2. Conversion

Fluoride: 1 μg/L = 52.6 nmol/L and 1 nmol/L = 0.019 μg/L

6.2.3. Effect of inflammation

No human data available. In rats, fluoride seems to induce IL-17 mediated inflammation in cardiac tissues [163].

6.3. Deficiency

Reported unequivocal signs of fluoride deficiency are almost non-existent. Pharmacological doses prevent caries.

6.3.1. When and how to treat?

Fluoride is principally a public health issue. Both inadequate and excessive fluoride intakes can affect dental health [151]. Inadequate intakes are associated with increased tooth decay (dental caries) and excessive intakes with damage to tooth enamel (dental fluorosis).

6.4. Toxicity

Chronic toxicity is most frequent, and may present as gastric complaints, anemia, osteomalacia, teeth problems, and neuromuscular and gastrointestinal symptoms. Chronic renal failure has been described. Chronic toxicity has been observed along with excessive water supplies and industrial exposures (excess of 2 mg/day) resulting in dental fluorosis and mottled enamel. Skeletal fluorosis is a rare toxic osteopathy characterized by massive bone fluoride fixation that occurs with doses 10–25 mg/day for many years. The disease is an endemic problem in some parts of the world and results from prolonged ingestion or rarely by industrial inhalation of high amounts of fluoride [164].

In patients on home PN for chronic intestinal failure (short bowel), high blood fluoride values have been observed in a series of 31 patients who developed osteoporosis and were explained by high fluoride intakes from drinking water [165].

An increased prevalence of cardiac complications has been observed in residents of fluorosis endemic areas chronically exposed to fluoride [163].

Fluoride might also have an impact on children's neurological development as shown by a meta-analysis. Greater exposure to high levels of fluoride in water was significantly associated with reduced levels of intelligence in children [166]. Some authors consider that artificial water fluoridation should be reconsidered globally [151] and studies are ongoing [167].

Poisoning most commonly follows ingestion (accidental or intentional) of fluoride-containing (mostly dental) products. In many parts of the world (e.g., regions of India and China), elevated levels of fluoride in groundwater result in chronic fluoride toxicity (fluorosis). The clinical course of systemic toxicity from ingested fluoride begins with gastric signs and symptoms and can develop with alarming rapidity. Toxicity treatment includes minimizing absorption by administering a solution containing calcium, monitoring and managing plasma calcium and potassium concentrations, acid-base status, and supporting vital functions [154].

There is no established treatment for skeletal fluorosis [157]. Calcium and vitamin D supplementation might mineralize or prevent excessive osteoid production. Oral calcium can diminish bone resorption, perhaps by reducing parathyroid hormone secretion, but may not block gut absorption of fluoride.

6.5. Recommendations N° 7 - fluoride

6.5.1. When to measure?

Recommendation 7.1

In case of suspicion of fluorosis blood determination should be performed.

Grade of recommendation GPP – Consensus 88%

6.5.2. What to measure?

Recommendation 7.2

The fluoride status shall be determined by blood measurements.

Grade of recommendation A – Strong consensus 91%

6.5.3. How much to provide in typical enteral or parenteral nutrition regimens?

Recommendation 7.3

Enteral nutrition may provide up to 3 mg fluoride per day with 1500 kcal.

Grade of recommendation 0 – Strong consensus 100%

Fluoride is not essential in children nor in adults. Small doses of fluoride (0–3 mg per day) may be beneficial [25].

Recommendation 7.4

There is no equivalent recommendation for parenteral nutrition.

Grade of recommendation GPP – Strong consensus 100%

Although not essential in adult PN, 0.95 mg per day has been provided without any complication and may be continued.

6.5.4. When to treat?

Recommendation 7.5

In case of fluoride poisoning, symptomatic treatment should be applied.

Grade of recommendation GPP – Strong consensus 91%

6.5.5. How to treat?

Recommendation 7.6

In case of acute poisoning, support of vital function and electrolyte management should be applied.

Grade of recommendation GPP – Strong consensus 97%

Recommendation 7.7

There is not a specific treatment that can be offered to treat skeletal fluorosis, except to control the source of the excess of fluoride exposure.

Grade of recommendation – GPP Strong consensus 94%

7. Iodine

7.1. Main functions

Iodine is a relatively rare element, ranking 63rd in earth composition. Iodine, in the form of iodide, plays a central role in thyroid physiology, being both a major constituent of thyroid hormones and a regulator of thyroid gland function [168,169]. Thyroid hormones are responsible for the regulation of metabolic rate with effects on various enzymes in fat and carbohydrate metabolism. The thyroid gland concentrates iodide (I-) against an electrochemical gradient by a carrier-mediated mechanism driven by adenosine triphosphate (ATP). A similar I- uptake mechanism is found in other organs, including salivary glands, stomach, choroid plexus, and mammary glands, but only in the thyroid does thyroid stimulating hormone (TSH) regulate the process. All the subsequent steps in the hormonal biosynthesis, from oxidation and organification of iodide to the secretion of thyroxine (T4) and triiodothyronine (T3) into the circulation, are stimulated by TSH, and inhibited by excess iodide. In depth endocrine considerations are beyond the scope of this text and are reviewed elsewhere [170].

Importantly, the healthy thyroid function depends also on an adequate provision of selenium and iron at any age [171]. Iron deficiency impairs thyroid metabolism [172]. Deiodination of T4 to T3 by the liver is dependent upon Type 1 5′-deiodinase, a selenoenyzme [171].

Iodide is well absorbed and may even result in acute toxicity symptoms. Nutritional intakes are dependent on the content of this element in the soil and the fortification strategies in different foods. According to the Iodine Global Network, there are 23 countries in the world currently classified with insufficient intake, while it is classified as excessive in 14 countries [173].

7.1.1. Needs

The DRI for iodine is 150 μg/day in adults, 220 μg/day in pregnant women, and 290 μg/day in breast-feeding women [135]. There is presently no evidence that iodine intakes superior to DRI are beneficial. The Tolerable Upper intake level (UL) for adults is 1.1 mg/day.

Daily iodine requirements in adult patients receiving EN or PN are estimated to be 70–150 μg. Thyroidal iodine stores are suggested to be sufficient to meet the needs of adult patients requiring PN for at least up to 3 months [28]. Furthermore, PN patients are frequently exposed to iodine from various exogenous sources, especially from iodine-based skin antiseptics.

Nutritional sources of iodine are fish, seaweed, shrimps and other seafood, dairy products, and iodized salt [174–176].

Iodide is well absorbed and can be delivered by oral or enteral route, alternatively it may be delivered by IV or IM injection.

7.2. Biomarkers and analytical methods

Because more than 90% of dietary iodine eventually appears in the urine [177], iodine status is best determined by 24 h urine collections [178]. Classically, deficiency diagnosis is based on urinary excretion of iodine <100 μg/24hr. Analytical methods consist in selective electrode (iodide), a chemical method (total iodine) or more recently on ICP-MS. Iodine may also be determined in serum by ICP-MS.

Serum TSH is not a sensitive indicator of iodine status whether in children or adults, as concentrations are usually maintained within a normal range despite frank iodine deficiency [179].

Reference values: serum iodine 40–100 μg/L (or 315–790 nmol/L); urine iodine 100–300 μg/24hr or 790–2360 nmol/24 h.

7.2.1. Conversion

Iodine: 1 μg/L = 7.87 nmol/L and 1 μmol/L = 127 μg/L

7.2.2. Effect of inflammation

There is no known effect of inflammation on status determination.

7.3. Deficiency

Iodine deficiency disorders represent a global health threat to individuals and societies, including affluent countries such as Switzerland where mild deficiency was recently confirmed [178]. The adverse effects of iodine deficiency are diverse and impose a significant burden on public healthcare systems. Severe iodine deficiency causes goiter and hypothyroidism because, despite an increase in thyroid activity to maximize iodine uptake and recycling in this setting, iodine concentrations are still too low to enable production of thyroid hormone [174,180]. Iodine deficiency increases the risk of developing autonomous thyroid nodules that are unresponsive to TSH control [179,181].

Moreover, iodine deficiency during pregnancy and breastfeeding adversely affects the development of the child [182,183]. Even mild or moderate iodine deficiency of the mother affects the synthesis of thyroid hormones and may impair brain development, neurocognitive function and reduces offspring IQ (mental retardation, cretinism and varying degrees of growth and development abnormalities). During pregnancy, women have a sharply increased need for iodine, which is frequently not covered by food sources and iodine supplements. Universal salt iodization is the preferred strategy of iodine deficiency disorder prevention and is

recommended by WHO, UNICEF and the Iodine Global Network (IGN) as the most cost-effective method [184,185].

Patients on long term PN may be at risk of deficiency, as shown by a retrospective study including 31 patients receiving long-term home PN of whom half did not receive iodine with PN [186]. The average urinary excretion of iodine was generally low. In 3% of the patients, the serum TSH was lower than the normal reference range, whereas they were higher than reference ranges in 23% of the patients [187].

Patients on long term EN may also be at risk of iodine deficiency as shown by a study in children [188].

7.3.1. When and how to treat

Iodine deficiency is a worldwide public health problem. The WHO recommends to address and correct population deficit with public health measures, mainly salt iodination/fortification [189]. In pregnant and lactating women living in iodine deficient countries, 150 μg iodine supplements, which shall be the mandatory dose for all prenatal vitamin/mineral preparations, shall be taken daily. There is a critical period during the correction as increasing the iodine intake in an iodine deficient population modifies the thyroid status. Programs providing 150–200 μg/day in iodine-deficient populations have been associated with an increased incidence of iodine-induced hyperthyroidism [174,179,181]. Subclinical hypothyroidism increases, as does hyperthyroidism, and thyroid autoimmunity for an unpredictable time. Iodine intake increase does not affect Graves' disease or thyroid cancers [174].

Iodide is well absorbed and can be delivered by oral or enteral route [190]. In acute severe deficiency, iodide can be given iv by sodium iodide solutions, that are available for PN in some countries (distinct from multi-trace element vials). Iodine may also be given IM or IV [191].

During PN Iodide has been delivered as part of multi-element mixtures at a standard dose of 131 μg/24hr without any problems for many years [192].

7.4. Toxicity

Excess consumption of iodine is uncommon, even if fortification of food is used, such as salt iodination, as the content is very low 60 μg/g of salt. However, excess may occur in different clinical contexts, the most common being the use of iodinated contrast agents used for radiologic studies [193], topical iodine disinfectants or the chronic intake of amiodarone, a frequently prescribed antiarrhythmic drug [193]. Non-nutritional sources of excessive iodine are numerous [188], including in addition to the previous substances, chemicals (catalysts) for photography and engraving dyes and inks, Lugol's solution potassium iodide, and radioactive iodine used for certain medical tests or the treatment of thyroid disease.

Chronic exposure to excess iodine intake induces autoimmune thyroiditis, partly because highly iodinated thyroglobulin is more immunogenic.

In iodine-sufficient individuals, excess iodine intake is most associated with elevated blood concentrations of TSH lower levels of thyroid hormones and increased thryroid autoimmunity, leading to hypothyroidism and goiter [193].

Clinical signs of toxicity include abdominal pain, loss of appetite, metallic taste in mouth coughing, fever, delirium, diarrhea, gum and tooth soreness, and vomiting [194].

In patients exposed to prolonged povidone iodine (PVP–I) disinfection or topical application as cream such as used in e.g. major burns [195], fasciotomies [196], or in treatment of mediastinitis [197], thyroid function and urinary Iodine excretion measurement should be considered, independent of the size of the wounds. Indeed, the excess iodine will induce hypothyroidism and may also alter renal function. A high degree of suspicion should be raised in patients with reduced kidney function, and an unexplained acidosis. Iodine excess may also establish a status of excessive thyroid hormone synthesis and release, thus inducing autonomic thyroid function in iodopenic areas or can contribute to the development of iodine-induced hyperthyroidism in iodine abundant areas.

7.4.1. Toxicity treatment

There is no specific antidote to iodine poisoning. Activated charcoal and symptomatic treatment [194], and in some cases renal replacement therapy [197] have been used.

7.5. Recommendations N°8 - iodine

7.5.1. When to measure?

Recommendation 8.1

In populations with high prevalence of thyroid disorders, iodine status should be assessed.

Grade of recommendation B – Strong consensus 94%

Recommendation 8.2

In patients presenting with thyroid disorders in countries with high incidence of iodine deficiency, iodine status shall be assessed.

Grade of recommendation A – Strong consensus 97%

Recommendation 8.3

In patients exposed to prolonged povidone iodine (PVP–I) disinfection or topical application as cream, thyroid function and, if available, urinary Iodine excretion measurement should be considered.

Grade of recommendation GPP - Consensus 88%

Urinary iodine excretion measurement is not usually available in hospitals. It is related to recent iodine intake. Iodine status evaluation can be considered in patients with hyperthyroidism or hypothyroidism and prolonged topical iodine exposure after having excluded other etiological factors.

7.5.2. What to measure?

Recommendation 8.4

Iodine status shall be assessed by urinary 24 h excretion, combined with assessment of thyroid function and size.

Grade of recommendation A – Strong consensus 94%

7.5.3. How much to provide in typical enteral or parenteral nutrition regimens?

Recommendation 8.5

Enteral nutrition shall provide at least 150 μg iodine per day, with an upper level of 300 μg, in 1500 kcal.

Grade of recommendation A – Strong consensus 91%

Recommendation 8.6

Parenteral nutrition should provide the standard dose of 130 μg/day.

Grade of recommendation B – Strong consensus 91%

7.5.4. How to provide additional amounts?

Recommendation 8.7

In case of deficiency, Iodine should be delivered by oral or enteral route as it is well absorbed (about 300–600 μg/day), or alternatively by IM injection.

Grade of recommendation B – Strong consensus 94%

Recommendation 8.8

In acute severe deficiency, iodide can be given IV by sodium iodide solution, that is available for parenteral nutrition in some countries (distinct from multi-trace element vials which usually contain 130 μg per dose).

Grade of recommendation GPP – Consensus 79%

8. Iron

8.1. Main functions

Iron (Fe) is the most abundant trace element in the human body and is required in small amounts to maintain normal physiological processes, being necessary for most, if not all, pathways for energy and substrate metabolism [198]. The two most common iron states are the divalent ferrous (Fe^{2+}) and the trivalent ferric (Fe^{3+}) [198,199]. The main function of iron is as a functional component of heme, participating in oxygen binding and transport (hemoglobin, myoglobin), oxygen metabolism (catalases, peroxidases), cellular respiration and electron transport (cytochromes) [198–200]. Proteins containing non-heme iron play an important role in fundamental cellular processes such as DNA synthesis, cell proliferation and differentiation (ribonucleotide reductase), gene regulation, drug metabolism, and steroid synthesis [201]. The redox cycling of ferrous and ferric iron in the presence of H_2O_2 and $O_2\cdot-$, which physiologically results from aerobic respiration and enzymatic reactions, can contribute to the production of hydroxyl radicals (Fenton chemistry) that in turn readily bind and damage cellular macromolecules [202]. Iron is also essential for both innate and adaptive immunity [203].

Iron is stored in the form of ferritin or hemosiderin in the liver, spleen, and bone marrow or in myoglobin in muscle tissue. Circulating iron is bound by transferrin, which transports iron throughout the body. Humans typically lose only small amounts of iron in urine, feces, the gastrointestinal tract, uterus (menstruating women) and skin. Both iron absorption and distribution throughout the body are regulated by hepcidin, a circulating peptide hormone [204], which is upregulated by high-intensity exercise, iron supplementation and inflammation, and is defective in hereditary hemochromatosis.

8.1.1. Needs

The DRI for iron varies according to stages of life and gender [135]. Maintenance of iron status requires the absorption of 1–3 mg/day of iron, to compensate for the losses from desquamated cells. In absence of regulated excretion mechanisms, absorption is tightly regulated [205]. For adult men and post-menopausal females, the current DRI is 8 mg/day, of which 1 mg/day is absorbed, whereas pre-menopausal females may require 1.5 mg/day or more to maintain iron balance, the DRI being 18 mg/day.

Principal nutritional sources are lean meat, liver, black pudding and seafood. Non-heme iron is mostly present in nuts, beans, vegetables, and fortified grain products. Non-heme iron absorption depends on solubilization in the stomach of ferric iron in food, followed by its reduction to ferrous iron. This can be enhanced by ascorbic acid, and other food components.

For patients on PN, the estimated requirement for maintenance of iron status is 1 mg/day for adult men and post-menopausal women, and about 2 mg per day for pre-menopausal women [206].

In Europe the trace element products used in PN have been providing 1.0–1.2 mg/day for many years and have elemental iron either as ferric chloride or ferrous gluconate [8]. These products have been extensively used without complications. However, the preparations available in the US do not contain iron because of concerns, not felt in Europe, regarding emulsion stability [5], or infection enhancing risk [203]. Since iron deficiency is one of the most common complications of long term PN, it is strongly recommended that regular iron provision is maintained by use of a preparation that provides iron as part of the PN regimen [206].

8.2. Biomarkers and analytical methods

There are many traditional and newer methods of assessing iron status (Table 7). The methods available in most laboratories for diagnosis of the iron status require the simultaneous determination of hemoglobin, ferritin (storage form of iron), transferrin saturation and total Iron-binding capacity, and occasionally bone marrow iron staining [207]. The most recent methods include the determinations of hepcidin, zinc protoporphyrin, and soluble transferrin receptor.

Iron is determined in serum using a colorimetric reaction with a chromagen such as ferrozine to form a color complex with iron. Variability in methods means that no generic reference ranges are agreed. If a commercial method is used for iron concentration determination, the laboratory should indicate its own reference ranges.

Transferrin saturation is the ratio of the serum iron concentration and the TIBC expressed as a percentage.

Ferritin: There are various immunological methods of determining serum ferritin. Reference intervals in adults are 20–250 μg/L in men and 20–200 μg/L in women.

Hepcidin: not yet widely available but may prove to be the most precise way to diagnose deficiency in inflammatory conditions. It is analyzed using ultra-high-pressure chromatography coupled to triple quadripole [208].

The additional assessment of the reticulocyte hemoglobin content which reflects the iron available in the bone marrow for erythropoiesis, may be helpful [208]. Alternatively, soluble transferrin receptor can be measured. When results are unclear and it is important to diagnose iron deficiency, the lack of stainable iron in a bone marrow biopsy remains the gold standard for diagnosis [209].

8.2.1. Conversion

Iron: 1 μg/dL = 0.179 μmol/L and 1 μmol/L = 55.8 μg/L

8.2.2. Effect of inflammation

Most common indices of iron status are affected by inflammation (Table 7), to start with serum iron. The ferritin level may be

Table 7
Impact of deficiency and inflammation on the most frequent biomarkers of iron status (adapted from [16]) (M = men, F = women).

	Reference ranges	What is measured?	Change in deficiency	Influenced by inflammation?
Plasma iron	50–175 μg/dL	Amount of circulating iron	↓	**yes**
Haemoglobin	**M:** 13.5–17.5 g/dL **F** 12.0–15.5 g/dL	Haemoglobin concentration	↓	**no**
Mean red cell volume (MCV)	80-100 fL	Average size of red blood cells	↓ microcytosis	**yes**
Transferrin	200–400 mg/dL	Indicator of status: ↑ proportional to iron needs	↑	**yes**
Transferrin saturation	20–50%	(Iron μg/dL/Transferrin) x 71.24 as %	↓	**yes**
Total iron binding capacity (TIBC)	250–370 μg/dL (45–66 μmol/L)	capacity of transferrin to bind with iron	↑	**yes**
Hepcidin	6.7–10.4 ng/mL	Regulation of iron efflux from the enterocyte	↓	**yes**
Ferritin	M: 24–336 mg/L F: 11–307 mg/L	Size of iron stores	↓	**yes**
Bone marrow iron	Not visible or small iron particles	Hemosiderin: iron stores	↓	**no**
Soluble transferrin receptor	0.76–1.76 mg/L	Amount of receptors – functional status	↓	**no**

misleading and falsely normal when there is the coexistence of acute or chronic inflammation. Algorithms have been proposed which relate the extent of the changes to the CRP level in the blood, but these still require further validation in the clinical setting [210]. In a large cohort of 1161 critically ill patients with variable degrees of inflammation [211], hepcidin has proven to be a more reliable indicator of iron deficiency than transferrin saturation [211,212].

Although there is no universal adjustment tool for inflammation, the international group BRINDA developed an all-in-one R package inflammation adjustment equation for, among others, serum ferritin and soluble transferrin receptor using the inflammation indicators AGP or CRP for various population groups (https://cran.rproject.org/web/packages/BRINDA/index.html) [777].

8.3. Deficiency

Worldwide, iron deficiency is the most common nutritional deficiency, affecting hundreds of millions of people [213,214]. Iron deficiency leads to impaired physical and cognitive functions, and to a high risk of morbidity for mother and child in pregnancy. Iron deficiency is often overlooked, especially when the complete blood count is abnormal. However, iron deficiency anemia is less common than iron deficiency without anemia. Iron deficiency has economic consequences as it reduces working capacity, increasing sick-leave, and being often incorrectly treated with antidepressants [215].

Iron depletion and deficiency progresses through several stages [216,217]:

Storage iron depletion: Serum ferritin concentrations and levels of iron in bone marrow decrease.

Marginal deficiency, mild functional deficiency, or iron-deficient erythropoiesis (erythrocyte production): Iron stores are depleted, iron supply to erythropoietic cells and transferrin saturation are reduced, but hemoglobin parameters are usually within the normal range.

Iron Deficiency Anemia: Iron stores are exhausted; hematocrit and levels of hemoglobin decline; and the resulting microcytic, hypochromic anemia is characterized by small RBC with low hemoglobin concentrations [215].

The greatest risk of deficiency is observed in older infants and toddlers, teenage girls, patients with chronic diseases and frequent blood sampling (e.g. dialysis patients), patients after major surgery, especially after bariatric surgery, women of childbearing age, pregnant women, and breastfeeding. Men and postmenopausal women are considered at low risk.

Iron deficiency is of particular concern when it results from bleeding in gastrointestinal, urological and gynecological disorders. In addition, common causes of iron deficiency are deficient iron intake or reduced iron absorption.

8.3.1. When and how to treat?

Iron deficiency should be treated when it is associated with anemia and/or low ferritin levels. Iron supplementation in the presence of normal or even high ferritin values is, however, not recommended and is potentially harmful [205]. Both oral and parenteral routes are possible.

After exclusion of medical causes of deficiency, dietary advice is important. Integrating heme and free iron regularly into the diet and avoiding inhibitors of iron uptake provides an additional benefit. Typical doses of oral iron supplements are 100–200 mg/day, in divided doses. Gastrointestinal side effects of iron therapy are not rare (most often constipation, diarrhea, nausea). Recent data suggest better iron resorption and possibly fewer adverse effects with alternate day dosing [218].

IV iron administration is used to replace iron losses rapidly in patients not reaching target therapeutic goal with oral supplementation, those requiring a fast supplementation, e.g. before elective surgery (patient blood management) [6,206], or in case of repeated failure of first-step oral therapy.

When IV iron is required, risk minimization should be addressed: reactions during iron infusions are very infrequent (<1:250,000 administrations with recent formulation) but may be life threatening [219,220]. There are many forms of iron suitable for IV use. Iron sucrose and ferric gluconate are widely used but may require multiple administration. As iron is strongly bound to carbohydrates (carboxymaltose, ferumoxytol, isomaltoside, gluconate, sucrose, low molecular weight iron dextran), the amount of labile iron is low, allowing the rapid administration of large single doses [220–224]. The risk is highest with high molecular weight iron dextran. The best studied example is ferric carboxymaltose, infused over 15 min [225,226].

In inflammatory anemic critically ill patients, Lasocki et al. have shown that after diagnosis of deficiency with hepcidin, a 1 g element iron dose as ferric carboxymaltose was associated with a reduction of length of hospital stay and of 90-day mortality [211,227].

To measure the success of treatment, the basic blood tests should be repeated after 8–10 weeks [16,17], and not earlier after iron infusion as ferritin levels are falsely high.

8.4. Toxicity - iron overload

The most common causes of iron overload are hereditary hemochromatosis *(HFE*-associated), other rare genetic disorders, and secondary to repeated transfusion (as may occur in thalassemia and other).

The signs and symptoms of iron overload are non-specific [205], and include chronic fatigue, joint pain, and diabetes: the disorder evolves towards end-organ failure, involving particularly the pancreas and liver [6].

The treatment of systemic iron overload is iron removal by blood donation/phlebotomy in the absence of anemia (applicable in most forms of hereditary hemochromatosis) and chelation in transfusion associated overload in hematologic diseases [205,206,228,229].

8.5. Recommendations N°9 - iron

8.5.1. When to measure?

Recommendation 9.1

Full investigation of iron status shall be performed in case of anemia, and in case of persistent major fatigue.

Grade of recommendation A – Strong consensus 94%

8.5.2. What to measure?

Recommendation 9.2

Investigation of both suspected deficiency and overload shall include a combination of tests: plasma iron, transferrin, transferrin saturation, ferritin, CRP, hepcidin, and evaluation of red blood cell morphology.

Grade of recommendation A – Strong consensus 97%

8.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 9.3

Enteral nutrition shall provide 18–30 mg iron per day with 1500 kcal.

Grade of recommendation A – Strong consensus 94%

This recommendation is high for men and post-menopausal women, but the modestly higher doses provided are likely to be beneficial and not harmful considering the high prevalence of iron deficiency.

Recommendation 9.4

Parenteral nutrition shall provide at least 1 mg/day of elemental iron, or an equivalent amount at periodic intervals by separate infusion.

Grade of recommendation A – Strong consensus 92%

While nutritional doses shall be provided, additional iron supplementation during infections and hemato-oncologic disease shall be balanced against the consequences of deficiency as supplements may have adverse effects on the course of the disease.

If countries do not have iron containing multi-trace element products the above alternative should apply.

In patients with low body weight (<40 kg), the 1 mg per day dose should be adapted.

8.5.4. When and how to provide additional amounts?

Recommendation 9.5

If more than basic amounts are required to correct iron deficiency, a single IV dose of whole-body iron replacement should be given, as 1 g of iron provided as a large single dose over 15 min using one of the recent carbohydrate products.

Grade of recommendation B – Strong consensus 100%

Recommendation 9.6

In anemic critically ill patients, with iron deficiency confirmed by low hepcidin levels, 1 g of iron provided as one of the recent carbohydrate products should be delivered.

Grade of recommendation B – Strong consensus 100%

8.5.5. When to provide reduced amounts?

Recommendation 9.7

In hemochromatosis, and in iron overload conditions, iron stores should be reduced by repeated venesection.

Grade of recommendation B – Strong consensus 94%

9. Manganese

9.1. Main functions

Manganese (Mn) is one of the most common metals in the human body with a range of 0.3–2.9 μg manganese per gram wet tissue weight, mainly present in the bone, liver, kidney, pancreas, and adrenal and pituitary glands [230].

Manganese is important for many physiological processes such as regulation of blood sugar and cellular energy, reproduction, digestion, bone growth, blood coagulation, and hemostasis, antioxidant defense, and proper immune function, although many of these have only been characterized in animals [231]. The biological effects of manganese are due to the incorporation of the metal into metalloproteins including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases [232]. Additionally, high levels of manganese occur normally in the brain as a component of arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, pyruvate carboxylase, and Mn superoxide dismutase enzymes [231]. Manganese exists in two oxidized states: Mn^{2+} and Mn^{3+}. Mn^{2+} is more stable and is the main form in tissues and in blood, where it is bound mainly by albumin and transferrin [231].

In humans, the daily turnover is estimated to be 20 μg, with approximately 2–22 mg/day as the intake, and approximately 2%–10% absorbed [233]. The main route of manganese absorption is through the gastrointestinal tract (GI), but lung inhalation and IV infusion provide additional routes of exposure.

9.1.1. Needs

The Institute of Medicine's DRI for manganese cites approximately 2 mg/day as an AI for adults (2.3 mg for men and 1.8 mg for women). Manganese is available in a variety of foods including whole grains, clams, oysters, mussels, nuts, soybeans and other

legumes, rice, leafy vegetables, coffee, tea, and many spices, such as black pepper.

Manganese has been provided in PN formulations both as an essential element in a range from 1 to 10 μmol (55–550 μg)/day, but also as a contaminant. It is now clear that IV intakes of 2 μmol (110 μg)/day are excessive during PN [234]. Evidence currently is therefore for a provision of 1 μmol (55 μg)/day in patients receiving PN [5,234]. Manganese contamination should be limited to less than 40 μg/d total in a typical adult PN formula [235].

9.2. Biomarkers and analytical methods

Total manganese can be measured in whole blood, RBC, plasma, serum, or urine preferably by ICP-MS, or by atomic absorption spectroscopy.

Manganism (toxicity) is diagnosed on whole blood manganese, and by neuroimaging [236].

Plasma manganese can be analyzed by spectrophotometry, mass spectrometry, neutron activation analysis, and x-ray fluorimetry.

RBC/whole blood manganese is important as the majority of circulating manganese is within erythrocytes.

9.2.1. Conversion

Manganese: 1 μg/L = 18.2 nmol/L and 1 nmol/L = 0.055 μg/L

9.3. Deficiency

Manganese deficiency is exceptional in humans, but well described in animals and plants. In critically ill patients, the proportion of patients with decreased values is low (2.1%) compared with other trace elements such as Zinc or Copper [237]: in addition, high or low values do not seem to be related to outcome. In animal studies, inadequate dietary intake of Manganese resulted in impaired growth, poor bone formation and skeletal defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism [238]. In experimental human settings, Manganese -depleted diets caused transient skin rash, decreased serum cholesterol concentrations and elevated alkaline phosphatase, calcium and phosphorus blood concentrations [239]. Low consumption of manganese in women led to altered mood and increased pain during the premenstrual phase of the estrous cycle [240]. Low plasma concentrations of manganese in mothers were associated with decreased birth weight in neonates. Low neuronal manganese levels have recently been associated with Huntington's disease [231].

9.4. Toxicity

Manganese toxicity is a greater concern than deficiency. The most common somatic effects of manganese toxicity are hypertension and increased heart rate due to blocking of calcium channels by manganese, and elevated cholesterol levels because of the reduced conversion of cholesterol to bile acids. Other symptoms are decreased fertility in men as well as increased fetal abnormalities [241]. Nevertheless, the brain is the main target organ of manganese toxicity. Manganese overexposure results in compromised mitochondrial function, oxidative stress, protein misfolding and trafficking, and neuro-inflammation [242]. Neurological damage might be irreversible. In patients exposed to manganese, elevated whole blood manganese has been shown to correlate with MRI signal intensity in globus pallidus [243]. Manganese overload initially induces non-specific symptoms such as headache, asthenia, irritability, fatigue, and muscular pains, but later, a neurodegenerative syndrome with psychiatric symptoms, known as manganism. This condition is like the cognitive, motor, and emotional defects seen in Parkinson's disease.

Dietary intake does not lead to toxicity, because absorption is tightly regulated in the gut [230]. The UL for manganese in the diet is 11 mg/d for adults (established by the U.S. FNB/IOM [2001]). Manganese toxicity has been associated with environmental and occupational exposure [244]. Furthermore, manganese toxicity may also arise from certain medical conditions [245] and from PN [5].

Toxicity has been observed in adults receiving IV > 500 μg/d and in pediatric patients receiving >40 μg/kg/d [242], but even as little as 110 μg/d to adults causes an elevation in whole blood manganese concentration [234]. Furthermore, the duration of PN treatment is associated with increased blood and brain concentrations of manganese. Also, patients suffering from cholestasis, liver failure or hepatic encephalopathy can develop manganese toxicity, as manganese is excreted in the bile [246,247].

Manganese toxicity can be caused by Iron deficiency, because competing for similar transport proteins with decreased iron levels leads to an accumulation of manganese to toxic levels over time [245,248]. Due to neuronal cell death in basal ganglia structures, functional recovery and effective treatment for manganism is currently limited [246].

9.4.1. Treating toxicity

The first step in treatment of toxicity is removing any manganese containing additives.

The treatment strategy thereafter includes chelation agents, and delivery of iron. Pharmacological treatment attempts of chronic manganism include para-aminosalicylic acid (variable success) [249] and possibly in the future, stem cell therapy [246].

9.5. Recommendations N°10 - manganese

9.5.1. When to measure?

Recommendation 10.1

Measurements should be made when manganese excess or toxicity is suspected, especially in long term parenteral nutrition (>30 days, manganese intake >55 μg/day) with impairment of liver function or iron deficiency.

Grade of recommendation B – Strong consensus 94%

Recommendation 10.2

Monitoring should not be more frequent than at 40 day-intervals (biological half-life).

Grade of recommendation GPP – Consensus 88%

9.5.2. What to measure?

Recommendation 10.3

In patients at-risk of manganese toxicity, whole blood, or RBC concentrations should be measured.

Grade of recommendation B – Strong consensus 94%

Recommendation 10.4

Brain MRI may contribute to confirming the diagnosis, showing high intensity signals in globus pallidus being correlated with elevated manganese levels.

Grade of recommendation 0 – Strong consensus 97%

9.5.3. How much to provide in typical enteral and parenteral nutrition regimen?

Recommendation 10.5

Enteral nutrition should provide 2–3 mg manganese per day, but doses up to 6 mg/day have been safely provided in 1500 kcal.

Grade of recommendation B - Strong consensus 91%

Recommendation 10.6

Parenteral nutrition shall provide 55 μg manganese per day.

Grade of recommendation A – Strong consensus 91%

9.5.4. When to treat?

Recommendation 10.7

Whole blood or serum manganese values greater than twice the upper limit of normal laboratory reference ranges should be treated.

Grade of recommendation GPP - Consensus 88%

9.5.5. How to treat?

Recommendation 10.8

Manganese toxicity can be treated by exclusion of manganese from parenteral nutrition admixture, chelation therapies (EDTA, PAS) or iron supplementation in case of iron deficiency.

Grade of recommendation GPP – Strong consensus 94%

10. Molybdenum

10.1. Main functions

Molybdenum (Mo) is an essential trace element for enzymes of microorganisms, plants and animals. It is used in bacterial organisms mainly for catalysing the process of biological nitrogen fixation and in plants and mammals in amino acid and purine metabolism [250,251].

There are 4 enzymes identified in human metabolism requiring Molybdenum: Sulfite oxidase is an enzyme in mitochondria catalyzing oxidation of sulfite to sulfate in metabolism of sulfur containing amino acids cysteine and methionine; in adenine metabolism, xanthine oxidase converts hypoxanthine to xanthine, and further converts xanthine to uric acid, preventing hypoxanthine induced DNA mutations: the activity of xanthine oxidase is proportional to the amount of Molybdenum in the body [252]. Aldehyde oxidase is found mainly in the liver and is an important enzyme in drug metabolism; and mitochondrial amidoxime reducing component (mARC) helps reduce a variety of N-hydroxylated substrates for which physiological significance is still unclear [250–252].

Molybdenum absorption is a highly efficient passive process. The human body's ability to absorb molybdenum across the intestinal tract is around 90% of intake, but may be lower depending on the food source [135,251]. The ability to absorb Molybdenum across the intestinal tract is dependent on its form and binding to food [251].

10.1.1. Needs

Balance studies over a broad intake range (22 μg/d to 1.5 mg/d) have been used as the basis for an Estimated Average Requirement (EAR) for molybdenum for adults which has been set to 34 μg/d and the RDA to 45 μg/d [135].

For EN, according to the European Community Directive, Molybdenum should be provided in dosage of 70–270 ug/day [25].

The average minimum molybdenum requirement has been estimated to be 22 μg/d plus an additional 3 μg/d to allow for miscellaneous losses, such as perspiration, resulting in a minimum requirement of 25 μg/d [135,253]. There have been no reports of biochemical or clinical problems with this dose. Intakes are probably greater due to contaminants in the regimen. The UL has been set by the Food and Nutrition Board of the National Academy of Sciences' Institute of Medicine at 2 mg/d [135].

Soil content of molybdenum varies and can result in a wide range of molybdenum content for a given food. The best sources are estimated to be legumes, grains, and nuts [251], and dairy products in younger people [254].

10.2. Biomarkers and analytical methods

Molybdenum can be determined in blood, urine or hair by ICP-MS [255], and also by neutron activation analysis (NAA) (7). Molybdenum in whole blood varies widely but averages 5 nmol/L.

Urine molybdenum concentrations are influenced by recent dietary intake [256].

Metabolic biomarkers: see below (10.3)

10.2.1. Conversion

Molybdenum: 1 μg//L = 10.4 nmol/L and 1 nmol/L = 0.096 μg/L.

10.2.2. Effect of inflammation

No effect documented.

10.3. Deficiency

Clinically apparent nutritional deficiency induced by low dietary molybdenum has not been reported in humans. Even at intakes as low as 22 μg/d, urinary molybdenum excretion has balanced intake over several months [250].

Molybdenum deficiency during PN has been reported to lead to biochemically detectable high plasma methionine, low serum uric acid, and high urinary thiosulfate, xanthine and hypoxanthine [251]. However, these observations have not been associated with molybdenum intakes in healthy people and cannot be used as indicators for estimating molybdenum requirement [135].

Molybdenum deficiency may occur in long-term PN without added molybdenum. There has been one well documented case report with the above mentioned biochemical changes consistent with molybdenum deficiency, and with clinical signs of nausea, rapid breathing and heart rate, vision problems, and ultimately coma, symptoms which were relieved by administration of IV molybdenum [257].

Failed functionality of molybdenum can occur as a result of genetic defects in enzymes that produce molybdenum cofactors. The latter is synthesized through a multistep process, and mutations in any of the MoCo synthesis enzymes result in inadequate activity of all molybdenum enzymes. These defects are very rare, occurring in an estimated 1 in 100,000–200,000 live births. Symptoms include feeding difficulties, seizures, and severe developmental delays. The clinical result is severe neurodegeneration and ultimately death during childhood [250].

Molybdenum as tetrathiomolybdate is used to treat copper overload in Wilson's disease (120 mg/day, in 6 divided doses, 20 mg

3 times daily with meals, and 20 mg 3 times daily between meals, for 8 weeks): it forms a strong complex with copper and protein and reduces copper absorption [258].

There is no other indication.

10.4. Toxicity

There are no reports of acute toxicity of dietary molybdenum in humans. Acute toxicity has been reported in one case study in which molybdenum supplements were taken at 300–800 μg/d over an 18-d period, resulting in hallucinations and seizures [251].

However, a controlled study in four, healthy young men found that molybdenum intakes, ranging from 22 μg/day to 1490 μg/day (almost 1.5 mg/day), elicited no serious adverse effects when molybdenum was given for 24 days [255].

A high concentration of molybdenum may act as an inhibitor in purine catabolism [259], and has been shown to cause copper deficiency in animals. In areas with extreme high soil contents as in Armenia, intakes of 10–15 mg/d have been associated with aching joints, gout like symptoms, hyperuricosuria, and elevated blood molybdenum [250].

10.5. Recommendations N°11 - molybdenum

10.5.1. When to measure?

Recommendation 11.1

Molybdenum measurement is rarely required, and it should only be assessed in case of suspected molybdenum deficiency.

Grade of recommendation GPP – Strong consensus 91%

10.5.2. What to measure?

Recommendation 11.2

In a case of suspected molybdenum deficiency, urine concentration of sulphite, hypoxanthine, xanthine and plasma uric acid, in addition to blood molybdenum should be measured.

Grade of recommendation B – Strong consensus 97%

10.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 11.3

Enteral nutrition should provide 50–250 μg Molybdenum per day in 1500 kcal.

Grade of recommendation B – Strong consensus 100%

Recommendation 11.4

Parenteral nutrition should provide 19–25 μg molybdenum per day.

Grade of recommendation B – Strong consensus 100%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

10.5.4. When to provide additional amounts?

Recommendation 11.5

Molybdenum may be used to treat copper overload in Wilson's disease as tetrathiomolybdate.

Grade of recommendation GPP – Strong consensus 94%

11. Selenium

11.1. Main functions

Selenium (Se) is an essential MN in mammals, being required for synthesis of the amino acid selenocysteine, an essential component of at least 25 selenoproteins in human tissues [260]. The biochemical functions of these selenoproteins include antioxidant and redox activity, control of thyroid hormone metabolism, together with several proteins of uncertain function [261]. Only a portion of the sequenced selenoproteins has been functionally characterized [262]. The selenoproteins are widespread across all tissues and organs, are involved in control of cell proliferation and apoptosis, and reduction of retroviral virulence, but with many non-specific effects due to the antioxidant activity. There is growing interest in the role selenium may have in protecting vascular endothelium [263].

The antioxidant activity of selenium in enzymes is related to its six electrons in the outermost shell which give selenium a variety of valence numbers and makes Se an optimal electron donor and receiver [264]]. The glutathione peroxidases (GPX) enzyme family (also often abbreviated GSHPx) is the first line of enzymes involved in antioxidant activity both in the extra- and intracellular milieu [262]: the most analyzed are GPX-1 for red cells, and GPX-3 for plasma.

Selenium is well absorbed with low inter-individual variation during isotopic studies (56–81%): the form of selenium and food constituents appear to be key determinants of post-absorptive metabolism [265].

11.1.1. Needs

The recommendations for dietary selenium intake range from 20 μg/day to 90 μg/day [23]. The lowest values are those needed to prevent Keshan disease [23], whereas the highest are those to maximize plasma and whole blood GPX [266]. Most recommendations regarding daily oral intakes of selenium range between 50 and 70 μg/day [23]. As selenium is well absorbed from the digestive tract, the basic requirements for PN are very similar.

In PN, an IV supply of 60–100 μg per day is sufficient to bring the plasma concentration into the target range [16,260].

Optimization of selenium status is reflected by the saturation of plasma GPX-3. This occurs at a plasma selenium concentration of about 1.20 μmol/L [266]. However, levels of selenium intake sufficient to saturate plasma GPX-3 activity may be insufficient to optimize the immune response and reduce cancer risk [267]. A plasma concentration of 1.5–1.9 μmol/L may be closer to optimal in reducing mortality in free-living individuals [261].

11.2. Biomarkers and analytical methods

Selenium matrices: Whole blood or plasma/serum selenium concentration is the main indicator of actual selenium status and can be obtained after acid digestion using a fluorometric method [268]. The use of carbon furnace atomic absorption spectrometry (CFAAS) assay is widely encountered [269], although today ICP-MS is probably the procedure of choice [270].

Plasma Selenium: The most widely used test of selenium status is total plasma selenium, the main components of which are Selenoprotein P and extracellular GPX. GPX is then generally measured by enzymatic methods using peroxide substrates [271]. In absence of inflammation, a plasma concentration of 0.75 μmol/L (60 μg/L) is considered adequate since it corresponds to the level that plasma GPX begins to plateau [272]. Of note, the level required to optimize GPX function is around 90 μg/L [266].

Whole blood Selenium: Since there is substantial selenium in both plasma and erythrocytes, measurement of whole blood selenium may provide a composite result [266].

Urine selenium is considered as an indicator of recent dietary input but remains rarely used. Urine selenium is measured with similar methods as blood samples.

Hair selenium analysis has been proposed as a marker of long-term dietary selenium intake. It can be measured using CFAAS or ICP-MS after hair digestion. However, due to the risk of hair contamination by other sources of selenium than dietary intake, such as cosmetics, results must be carefully evaluated. It is of limited value in acute medicine.

11.2.1. Conversion

Selenium: 1 μg/L = 0.01266 μmol/L and 1 μmol/L = 79 μg/L

11.2.2. Reference intervals

Because selenium concentrations in whole blood, plasma/serum, urine, or hair are affected by dietary selenium intake, reference intervals for selenium should be locally established, considering local diet. However, in absence of local reference intervals, published data can be used with caution.

Plasma Glutathione Peroxidase: Extracellular GPX-3 is readily measured in plasma and is used to assess short term changes in selenium input. It correlates closely with plasma selenium although only accounting for some 20% of the total plasma concentration [266].

Red cell glutathione peroxidase: GPX-1 is used to assess selenium intake over a longer time period, relating to the lifespan of the erythrocyte [272].

Selenoprotein P: This is the major selenoprotein in plasma accounting for over 50% of the selenium. Assays are available but are not yet widely used and tend to give different results [273]. With a molecular weight like that of albumin, inflammation will cause a similar fall in the plasma concentration [274].

11.2.3. Effect of inflammation

In many patients there is an element of inflammation. This leads to a reduction in plasma selenium [20], related to redistribution out of the circulating compartment since plasma selenium returns to normal in many cases without supplementation [275]. The reduction is proportional to inflammation: requiring its simultaneous assessment using e.g. CRP measurement.

Depending on the severity of the inflammatory response, a "correction" of the value is required: CRP concentrations of 10–40, 41–80, and greater than 80 mg/L would be expected to produce falls in plasma selenium of 15–25%, about 35% and about 50% respectively [20].

11.3. Deficiency

Insufficient selenium intake is the most common cause of selenium deficiency, and is largely geography dependent, some areas of the world being characterized by low soil content, that leads to population deficiency and specific chronic pathologies: two examples are the Keshan cardiomyopathy, and Kashin-Beck osteochondropathy in China [276]. Selenium deficiency is associated with increased incidence and virulence of viral infections [277,278]. Milder selenium depletion over a prolonged period will cause effects on metabolism and tissue function, the poorer the status, the greater the risk of diseases such as cancer and type2 diabetes [261].

Deficiency has been recognized during PN as cardiac and skeletal muscle myopathy, and as skin and nail effects [260]. These must be regarded as the end points of severe deficiency. In PN, the main objective is to prevent the clinical consequences of Se deficiency, and to minimize the more non-specific consequences of selenium depletion, such as impaired immune function. Most studies suggest that a plasma selenium concentration of 0.75 μmol/L would achieve these objectives [266,272,279]. On this basis, in a patient without an inflammatory response, selenium supplements should be provided if the plasma concentration is < 0.75 μmol/L.

A nationwide shortage of IV selenium occurred in April 2011 in the U.S. and resulted in adults, children and infants receiving very low Se supply. A study in 49 infants showed that the reduction of the daily 6 μg/kg/d dose of selenium resulted in biochemical deficiency and also in increased costs ($13 per patient for laboratory draws alone, plus pharmacist and nursing time) [280].

In case of specific losses, balance studies can be used to orient the required additional selenium dose, which may be as high as an additional 300 μg/day [281].

A value of plasma selenium <0.4 μmol/L (<32 μg/L), should always trigger supplements provision, and other actions should be tailored to the combined data from plasma selenium and CRP [282]. Long term EN in adults requires monitoring and may result in selenium deficiency depending on the product used for feeding [283].

11.3.1. When and how to treat?

The decision on when to provide an increased amount of selenium is complex. It depends on the objective, and the risk of not taking any active measures. It also depends on the availability of the gastrointestinal tract, and on the demonstration of selenium-containing biological fluids losses (e.g. in burns or during continuous renal replacement therapy).

The basic requirement to normalize plasma selenium during home PN in patients without inflammation is about 60–100 μg/d [284–290].

Certain groups may have higher requirements:

- Patients who are depleted because of a recent reduced intake may require twice the normal daily amount (up to 200 μg/day), with monitoring of plasma selenium level. If the gastrointestinal tract is available, this can be given orally.
- Burns patients who have high losses of selenium, benefit from large IV supplies of around 375 μg/day, with more rapid healing and fewer infections [145].
- Patients with other major trauma, and cardiac surgery may similarly benefit from a supplement of 275 μg/day [291].
- Patients receiving renal replacement therapy have increased losses and oxidative stress and will require increased amounts [143].
- Genetic polymorphisms in many of the selenoproteins, which increase the risk of a variety of diseases including cancer and diabetes [2] is a specific issue under research.

Deficiency may also occur during prolonged EN [292,293] due to low selenium concentrations in feeding products. Considering the high absorption of selenium, enteral supplements may be considered, but the IV route will be more rapid: 100 μg/d of selenium for 2 weeks should restore blood levels and reduce the symptoms [294].

11.4. Toxicity

Upper limits for plasma selenium before toxicity symptoms occur are not clear-these range from about 6 μmol/L [295] to about 12 μmol/L [23]. Selenium toxicity outbreaks have occurred due to misformulation of dietary supplements resulting in clinical signs of selenosis [296]. The concern comes from recent awareness that selenium overexposure is positively associated with type 2 diabetes and high-grade prostate cancer. In addition, a natural experiment has suggested an association between overexposure to inorganic

hexavalent selenium and two neurodegenerative diseases, amyotrophic lateral sclerosis and Parkinson's disease [272]. Further a Danish RCT has shown that selenium in doses 100–300 μg/day delivered for 5 years in the form of enriched yeast decreased TSH and FT4 concentrations in euthyroid subjects with marginal selenium deficiency. Similar results were observed in Venezuela [36]. However, these effects on thyroid function contrasted with those in a UK population receiving the same dose [297].

There has been much speculation whether ICU patients might benefit from a massively increased supply of 1000–4000 μg per day. Meta-analysis of all such studies shows no consistent benefit [298], and therefore is now advised against [16].

11.5. Recommendations N°12 - selenium

11.5.1. When to measure?

Recommendation 12.1

All patients likely to receive parenteral nutrition for more than two weeks or about to commence home parenteral nutrition should have plasma selenium and CRP measured on commencing parenteral nutrition. Tests should be repeated as required depending on the results, and at least once every 3–6 months.

Grade of recommendation B – Strong consensus 92%

11.5.2. What to measure?

Recommendation 12.2

Blood selenium is required to determine status, but ideally the plasma GPX-3 shall be determined to reflect functional status. Simultaneous determination of CRP and albumin is required for interpretation.

Grade of recommendation A – Strong consensus 91%

11.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 12.3

Enteral nutrition should provide 50–150 μg selenium per day in 1500 kcal.

Grade of recommendation B – Strong consensus 94%

Recommendation 12.4

Parenteral nutrition should provide 60–100 μg selenium per day.

Grade of recommendation B – Strong consensus 91%

11.5.4. When to provide additional amounts?

Recommendation 12.5

A value of plasma selenium <0.4 μmol/L (<32 μg/L) should prompt selenium administration, starting with 100 μg/day (enteral or IV): the duration of administration will depend on response.

Grade of recommendation GPP – Strong consensus 100%

Recommendation 12.6

In a patient without an inflammatory response (e.g., CRP <20 mg/L), a plasma selenium concentration of <0.75 μmol/L should trigger selenium supplements.

Grade of recommendation GPP – Strong consensus 100%

11.5.5. How to provide additional amounts?

Recommendation 12.7

Considering the good enteral absorption, and in absence of contraindication, the enteral route can be used with doses starting at 100 μg/day. In case of plasma selenium <0.4 μmol/L (30 μg/L) the IV route may be used for rapid correction: up to 400 μg/day may be required for at least 7–10 days, and status then be rechecked.

Grade of recommendation 0 – Strong consensus 100%

12. Zinc

12.1. Main functions

More than 300 zinc metalloenzymes are present in biology, and they play essential roles in virtually all metabolic pathways [299–301]. Some examples in man include carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases and alcohol dehydrogenase. Zinc-finger proteins are central to the control of transcription of DNA into RNA. The key roles of zinc in protein and nucleic acid synthesis explain the failure of growth and impaired wound healing observed in individuals with zinc deficiency. Zinc is also part of several aspects of the antioxidant defense system, as a structural component of cytoplasmic superoxide dismutase, by stabilizing cell membranes, and by inducing metallothionein synthesis which removes reactive oxygen species.

The role of Zinc in the body can be grouped into three general functional classes: structural, catalytic, and regulatory. As a structural component in proteins; as a catalytic factor it exists in six main enzyme classes: oxidoreductases (dehydrogenases), transferases, hydrolases, lyases; isomerases, and ligases; and in addition, zinc is functional as a signaling mediator in endocrine, paracrine, and autocrine systems. For example, zinc reduces insulin secretion and suppresses hepatic insulin clearance.

Over 85% of body zinc is found in skeletal muscle and bone, with only a very small amount (0.1% of total) in the plasma, where most (70%) is bound to albumin, the concentration being fairly tightly controlled at around 10–17 μmol/L.

12.1.1. Needs

Zinc is widely distributed throughout foods and after digestion of foodstuffs, it is absorbed primarily in the jejunum. Zinc is efficiently excreted into bile. Some of the secreted zinc is reabsorbed, undergoing an enterohepatic circulation, the net gastrointestinal (GI) loss being 2–4 mg/d. Urinary zinc excretion in adults is about 0.5 mg/d. Other physiologic losses occur in skin and hair.

The RDA/DRI of three major organizations for adults over 18 years old vary and are as follows: WHO- for females 3.0–9.8 mg/d, and for males 4.2–14.0 mg/d with bioavailability 50% and15% respectively; the Institute of Medicine recommends 8 mg/d for females receiving a mixed diet and 11 mg/d for males; and the European Food Safety Agency 7.5–12.7 mg/d for females and 9.4–16.3 mg/d for males, the amount depending on the phytate in the diet [302]. Gibson et al. also discuss the dietary recommendations for infants, children and during pregnancy and lactation [302]. For simplification the adult DRI of zinc is 8–15 mg. The EC Directive for FSMP suggests 7.5–22.5 mg Zinc in 1500 Kcal of feed [25] which is present in current nutritionally complete EN standard formulae [303].

Since PN bypasses the gut, the IV requirements in patients without abnormal losses are lower. The long-standing balance studies of Wolman et al. [304] showed that in the absence of significant diarrhea, positive zinc balance could be achieved with

3 mg/d of infused zinc: in their patients, 6 mg/d was sufficient in virtually all other patients, a small number with increased gastrointestinal losses requiring as much as 12 mg/day [305]. Industrial products supply daily vials containing between 3 and 10 mg zinc as inorganic (i.e. zinc chloride and zinc sulfate) and organic salts (gluconate). Most recently, ASPEN has recommended that such products should provide 3–5 mg/day [5]. This would be the basic provision, to be supplemented depending upon the amount of zinc lost in gastrointestinal secretions [115,304,306].

In special situations such as major burns which are characterized by increased loss of body fluids (exudates) through the damaged area, larger amounts of zinc (30–40 mg per day) are needed to maintain zinc balance, reducing infection rates and improving healing [145,307]. Continuous renal replacement therapy increases zinc losses, but non-specific contamination of replacement solutions results in a balance close to zero [308,309].

12.2. Biomarkers and analytical method

Total zinc can be measured in whole blood, plasma, serum, urine, or hair preferably by ICP-MS, or by atomic absorption spectroscopy.

Plasma zinc: This determination remains the most widely used test to confirm clinical zinc deficiency and to monitor adequacy of provision. In subjects on oral food, serum zinc concentrations fluctuate by as much as 20% during a 24-h period, largely because of food ingestion. It is essential that results are interpreted together with changes in serum albumin and the magnitude of the inflammatory response (CRP) [20].

Blood cell zinc: The zinc content of neutrophils, lymphocytes and platelets declines more rapidly than plasma zinc in experimental studies of zinc depletion. However, technical aspects make this determination difficult in hospital.

Of note, in studies in which zinc deficiency was induced in healthy individuals, erythrocyte concentrations showed little or no change whereas plasma zinc fell significantly [310].

Hair zinc: There is some evidence for low hair zinc in children with poor growth, but difficulties in collection (multiple contaminants) and interpretation mean that this is not used in hospital practice.

12.2.1. Conversion

Zinc: 1 μg/L = 0.0153 μmol/L and 1 μmol/L = 65.4 μg/L

12.2.2. Effect of inflammation

Serum zinc concentrations are reduced during the inflammation associated acute phase response: the change occurs very quickly as shown by perioperative studies [311]. Low zinc values resulting from inflammation are observed in patients admitted for respiratory failure [312] and in general ICU patients [237]: the values normalize with clinical improvement. This results largely from the redistribution of zinc from plasma albumin to the liver, where it binds to the increased amount of metallothionein. Moreover, reduced circulating zinc correlates with increased IL-6, IL-8 and TNF-α levels [313,314].

The amplitude of the inflammatory response should always be checked by simultaneous determinations of CRP (or other markers of the acute phase response). Plasma zinc decreases significantly whenever CRP exceeds 20 mg/L [20], complicating the interpretation of the results.

Zinc is part of the antioxidant defense, stabilizing cytosolic Zn/Cu superoxide dismutase, inhibiting the enzyme NADPH oxidase and inducing production of cysteine-rich metallothionein [315,316].

Although there is no universal adjustment tool for inflammation, the international group BRINDA developed an all-in-one R package inflammation adjustment equation for, among others, serum zinc using the inflammation indicators AGP or CRP for various population groups (https://cran.rproject.org/web/packages/BRINDA/index.html).

12.3. Deficiency

The clinical features of severe deficiency include alopecia, skin rash of face, groins, hands, and feet, growth retardation, delayed sexual development and bone maturation, impaired wound healing and immune function, diarrhea, and blunting of taste and smell [301,317]. The only clearly demonstrated signs of mild zinc deficiency are reduced growth rate and impairment of immune defense. Other signs, such as impaired taste and wound healing, resulting from a low zinc intake, are less consistently observed.

Zinc deficiency affects cells involved in both innate and adaptive immunity at the survival, proliferation and maturation levels [318]. Monocytes, polymorphonuclear-, natural killer-, T-, and B-cells are all affected. T cell functions and the balance between the different T helper cell subsets are particularly susceptible to changes in zinc status. While acute zinc deficiency causes a decrease in innate and adaptive immunity, chronic deficiency increases inflammation [318].

Genetic disorder: Acrodermatitis enteropathica, a congenital zinc malabsorption due to an autosomal recessive mutation in the gene coding for the ZIP4 transporter leads to zinc deficiency that presents in childhood [319].

Acquired zinc deficiency is a potentially underdiagnosed disorder. The general causes of zinc deficiency include inadequate intake, increased requirements, malabsorption, increased losses and impaired utilization. Infants, children, adolescents, pregnant and lactating women have increased requirements for zinc and thus are at increased risk of depletion [320]. Zinc deficiency can develop in eating disorders such as anorexia nervosa and bulimia as well as alternative eating habits (vegetarianism, veganism) or heavy reliance on foods with little, or poorly absorbable zinc.

Zinc malabsorption may occur in patients with short bowel syndrome, bariatric surgery, cystic fibrosis, chronic pancreatitis, inflammatory bowel disease or consuming a diet rich in phytate. Increased gastrointestinal losses of zinc are observed in patients with an enterostomy, or enterocutaneous fistula chyle leaks. Increased urinary losses may be present in any hypercatabolic condition, such as burns, trauma and sepsis, in renal disease, alcoholism, dialysate and many drugs. Prolonged renal replacement therapy may cause deficiency [75,321].

Chronic PN without or with low zinc supplementation [322], starvation, alcoholic cirrhosis, and diabetes mellitus are other conditions at risk for zinc deficiency. Zinc deficiency has also been reported in patients receiving prolonged complete EN [306,323].

12.4. Toxicity

The clinical feature of zinc toxicity relates to the route and the dose of exposure and differs between acute and chronic exposure. Symptoms appear when ingestion exceeds 1–2 g of zinc. Toxic

exposures can occur through the gastrointestinal (ingestion of nutritional supplements and other zinc containing substances), dermal (overuse of makeup, sunscreen, and ointments), respiratory (occupational exposure to inhalation of zinc chloride), and parenteral routes through erroneously prepared PN [324].

Acute toxic ingestions primarily cause gastrointestinal symptoms, including hematemesis, due to their direct caustic effects. Interstitial nephritis or acute tubular necrosis. Acute respiratory distress syndrome (ARDS) and liver necrosis may also occur.

Chronic zinc toxicity manifests primarily as copper deficiency with bone marrow (sideroblastic anemia, granulocytopenia, myelodysplastic syndrome) and neurologic effects (ascending, sensorimotor polyneuropathy syndrome). The body's response to hyperzincemia is to produce more metallothionein to decrease free zinc concentrations: of note this mechanism is used to treat Wilson's disease, a copper overload disease [132]. As copper is the metal with the highest affinity to metallothionein, high levels of zinc lower the level of copper.

12.4.1. When and how to treat intoxication

Acute toxicity secondary to oral ingestion is treated by antiemetics, fluids, as well as proton pump inhibitors or H_2-blockers [324]. Whole bowel irrigation may be required. Chelation with calcium disodium edetate ($CaNa_2EDTA$) or DTPA has also been shown to decrease zinc levels in patients with toxicity.

Chronic zinc toxicity is primarily treated with copper sulfate. Chelation may be required in severe cases.

12.5. Recommendations N°13 - zinc

12.5.1. When to measure?

Recommendation 13.1

Zinc measurement should be done:

- **In patients with increased gastrointestinal and/or skin losses**
- **on commencing long term parenteral nutrition, and repeated as required depending on the presence of conditions associated with risk of deficiency**.
- **in patients on long-term parenteral nutrition, every 6–12 months**

Grade of recommendation GPP – Consensus 88%

12.5.2. What to measure?

Recommendation 13.2

Plasma zinc shall be used to confirm clinical zinc deficiency and to monitor adequacy of provision. Simultaneous determination of CRP and albumin is required for interpretation.

Grade of recommendation A – Strong consensus 91%

12.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 13.3

Enteral nutrition shall provide at least 10 mg per day in 1500 kcal.

Grade of recommendation A – Strong consensus 97%

Recommendation 13.4

Parenteral nutrition should provide 3–5 mg zinc IV per day in patients without abnormal losses.

Grade of recommendation B - Consensus 88%

12.5.4. When to provide additional amounts?

Recommendation 13.5

In patients on parenteral nutrition who have gastrointestinal losses (fistulae, stomas, and diarrhea), while nil per mouth, IV doses up to 12 mg per day can be used and are usually sufficient to maintain the status: this addition will be required for as long as gastrointestinal losses persist.

Grade of recommendation 0 – Strong consensus 100%

Recommendation 13.6

Patients with major burns >20% BSA have increased requirements due to exudative losses: 30–35 mg/day IV for 2–3 weeks should be provided.

Grade of recommendation B – Strong consensus 91%

Recommendation 13.7

In acquired zinc deficiency, 0.5–1 mg/kg per day of elemental zinc (Zn^{2+}), can be given orally for 3–4 months. Organic compounds such as zinc histidinate, zinc gluconate and zinc orotate show a comparatively better tolerability than inorganic zinc sulfate and zinc chloride.

Grade of recommendation GPP – Consensus 82%

Recommendation 13.8

In acrodermatitis enteropathica, a life-long oral intake of 3 mg/kg per day of elemental zinc (Zn^{2+}) may be provided, with the dosage adjusted accordingly to plasma or serum zinc levels.

Grade of recommendation 0 – Strong consensus 94%

12.5.5. How to provide additional amounts?

Recommendation 13.9

Oral, enteral and parenteral routes of administration can be used, route depending on gastrointestinal function. Supplementation can be combined with nutritional support or provided separately.

Grade of recommendation GPP – Strong consensus 97%

13. Thiamine (vitamin B1)

13.1. Main functions

Thiamine is a water-soluble vitamin essential for carbohydrate metabolism and energy metabolism [325]. It is an indispensable cofactor for four enzymes involved in the production of energy as ATP and the synthesis of essential cellular molecules, synthesis of various neurotransmitters and nucleic acids, and control of oxidative stress. In humans, body stores of thiamine are limited and dependent on dietary thiamine intake.

There are five known natural thiamine phosphate derivatives: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP) [i.e. the active form also called thiamine pyrophosphate (TPP)], thiamine triphosphate (ThTP), and the recently discovered adenosine thiamine triphosphate (AThTP), and adenosine thiamine diphosphate (AThDP). About 80% of the approximately 25–30 mg of thiamine in the adult human body is in the form of thiamine [326]. ThDP is a coenzyme for several enzymes that catalyze the transfer of two-carbon units and for pyruvate dehydrogenase activity. Hence it is key to the production of energy (ATP).

Thiamine is rapidly absorbed in the jejunum and ileum by an active, carrier-mediated, and rate-limited process, but at higher concentrations, the uptake is by passive diffusion [327]. Absorption

can be inhibited by alcohol consumption, or by folate deficiency [328].

Complex thiamine biosynthesis occurs in bacteria, particularly in the intestinal microbiota.

13.1.1. Needs

In adults the EAR for women and men are 0.9–1.0 mg/day, with RDAs being 1.1–1.2 mg/day [327]. Of note these values have not been revised since 1998 [24].

In PN, doses 2–6 mg/day are usually recommended and included in industrial multivitamin preparations for PN. The preparations most widely used in Europe contain 2.5–3.5 mg thiamine, and there have been no reports of deficiency in patients receiving PN with this regular supply. ASPEN recommend 6 mg, to allow for very high requirements that may exist in a small number of patients receiving high dose glucose as part of their PN. In EN suggested doses are 1.2–10 mg/day [329]. In children and teenagers, the estimated adequate requirements (EAR) vary between 0.7 and 1.2 mg/day, the RDA being a little higher (0.9–1.2 mg/day).

The main nutritional sources are represented by whole grains, legumes, meats, nuts, and fortified foods. The half-life of the active forms of thiamine is relatively short. Therefore, insufficient dietary intake, especially in combination with increased metabolic needs due to oxidative stress and systemic inflammation in critical illness (trauma, sepsis, cardiac arrest, and after cardiac surgery), can quickly generate a state of thiamine deficiency.

13.2. Biomarkers and analytical methods

Thiamine status can be determined using indirect and direct methods [330–332]. Erythrocyte transketolase activity is an indirect and functional assay related to thiamine status that measures the degree of ThDP-saturation of the thiamine-dependent enzyme, via ThDP-dependent pentose phosphate pathway. Nevertheless, this assay might not be readily available, and its outputs can be difficult to interpret in clinical conditions where transketolase synthesis is impaired [330,332]. Direct methods include the quantification of ThDP, the coenzyme form of thiamine, in whole blood, or RBC. Plasma measurement is not used since virtually all circulating ThDP is in the erythrocytes. Particular sample collection procedures and pre-analytics have to be respected (protection from light, temperature storage) to ensure reliable results [332]. Different analytical methodologies employing high pressure liquid chromatography coupled to optical or mass spectrometry detection are available to determine ThDP [331,333]. Thiamine status determination in erythrocytes was suggested to more reliable in the presence of a systemic inflammatory response [334].

To confirm diagnosis of deficiency, a thiamine supplementation trial should be performed to assess clinical benefit since treatment should not be delayed by waiting for the laboratory result. Other biomarkers of deficiency such as lactate, pyruvate, alpha-ketoglutarate, and glyoxylate concentrations can be performed.

13.2.1. Effect of inflammation

Although inflammation causes a fall in the plasma concentration of many vitamins, this is not relevant for thiamine since there is little thiamine in the plasma in normal individuals. Studies on inflammation due to elective surgery, or to a range of conditions causing an inflammatory response in individuals at risk of nutritional deficiency, have shown that red cell ThDP is not affected and is therefore a good marker in assessment of such patients [334].

13.3. Deficiency

Thiamine deficiency is a major public health concern in several low- and middle-income countries [325]. Clinical thiamine deficiency may present as a range of clinical signs and symptoms involving the neurological, psychiatric, and cardiovascular systems [325,335]. The neurological symptoms range from mental changes such as apathy, decrease in short-term memory, confusion, and irritability to cognitive deficits and the Wernicke-Korsakoff encephalopathy, optic neuropathy, Leigh's disease, African Seasonal Ataxia, and central pontine myelinolysis [336]. The involvement of other organs manifests as in beriberi, congestive heart failure, or unexplained metabolic lactic acidosis [329]. Among the thiamine disorders, the refeeding syndrome is of particular concern in inpatients and is associated with increased mortality [35,337–339].

Early suspicion and recognition of thiamine deficiency are needed to enable immediate initiation of therapy, as thiamine reserves are depleted as early as 20 days of inadequate oral intake [329].

13.3.1. When and how to treat?

Patients at risk are numerous, and include malnutrition, poor oral intake and chronic alcohol consumption, malignancies, and increased metabolic requirements (pregnancy) [329]. Often, multiple factors coexist. Reduced gastrointestinal absorption due to disease or surgery (resections), increased gastrointestinal or renal losses (chronic diuretic therapy or continuous renal replacement therapy [308]) should also be considered. Obesity pre-bariatric surgery and post-surgery also frequently present with deficiency [55]: thiamine is among the MNs at highest risk for deficiency [4,340]. The inadvertent non-administration of thiamine during PN is a preventable condition. In ambulatory patients with heart failure, deficiency is found in 6% of patients [341]. Critical illness (sepsis, major trauma, etc.) is a risk condition with its multiple metabolic challenges, and deficiency or depletion may be found in >90% of patients [82,342].

Due to poor absorption particularly in patients with chronic alcohol ingestion, IV thiamine 250 mg is required to manage encephalopathy [343].

Thiamine is available as a generic medication for oral and IV use and is cheap all over the world. There is a wide range of suggested dosages [329,344,345]. The strategy will depend on the clinical situation, and prevention of encephalopathy with thiamine is supported by guidelines [346] (Table 8).

Table 8
Recommended thiamine treatment doses (adapted from [329,346,347]).

Clinical situation	Dose
Mild deficiency – outpatients	10 mg/day thiamin for a week, followed by 3–5 mg/daily for at least 6 weeks [348]
Chronic diuretic therapy	Suggestion: 50 mg a day, by mouth
At risk for deficiency	100 mg, 3 times a day, IV
High suspicion or proven deficiency	200 mg, 3 times a day, IV
Encephalopathy of uncertain etiology including Wernicke encephalopathy	500 mg, 3 times a day, IV [347]
Maintenance dose in proven deficiency	50–100 mg/day, orally
Refeeding syndrome	300 mg IV before initiating nutrition therapy, 200–300 mg IV daily for at least 3 more days
Continuous renal replacement therapy	100 mg/day
Hospitalized patients-critical illness	100–300 mg/day [345,349–351]

In case of suspicion of chronic deficiency without any acute disease, the oral route is adequate. In case of acute disease, the suspicion of inadequate intake, even short term, should prompt the use of the IV route.

The laboratory measurement of blood ThDP should be done, although treatment is not based on it: results become available later, and confirm the diagnosis.

13.4. Toxicity

None, with no UL [327]. The only effect of doses in excess of needs is increased urinary excretion [352,353].

High IV dose has rarely led to anaphylaxis [354], whilst doses of more than 400 mg may induce nausea, anorexia, and mild ataxia.

13.5. Recommendations N°14 – thiamine (vitamin B1)

13.5.1. When to measure?

Recommendation 14.1

RBC or whole blood thiamine may be determined in.

- **patients suspected of deficiency in the context of cardiomyopathy and prolonged diuretic treatment**
- **patients undergoing a nutritional assessment in the context of prolonged medical nutrition, and post-bariatric surgery**
- **refeeding syndrome**
- **encephalopathy**

Grade of recommendation 0 – Consensus 90%

13.5.2. What to measure?

Recommendation 14.2

Thiamine status shall be determined by measuring RBC or whole blood thiamine diphosphate (ThDP).

Grade of recommendation A – Consensus 90%

If RBC or whole blood ThDP determination is not available, measurement of red cell transketolase and its activation by thiamine may be considered.

13.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 14.3

Enteral nutrition shall provide 1.5–3 mg per day of vitamin B1 in patients receiving 1500 kcal per day.

Grade of recommendation A – Strong consensus 92%

In "mild deficiency" or depletion, identified by low dietary intakes and low blood ThDP, but no clinical symptoms, an intake of 10 mg per day for one week should be prescribed [348].

Recommendation 14.4

Parenteral nutrition should provide at least 2.5 mg per day.

Grade of recommendation B – Strong consensus 92%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

13.5.4. When to provide additional amounts?

Recommendation 14.5

In patients admitted to emergency or intensive care, the administration of thiamine (100–300 mg/day IV) should be prescribed without hesitation from admission for 3–4 days.

Grade of recommendation B – Consensus 80%

Recommendation 14.6

In patients admitted on the ward with any suspicion of reduced food intake during the previous days or high alcohol consumption, thiamine 100–300 mg/day should be administered by either oral or IV route.

Grade of recommendation B – Strong consensus 92%

13.5.5. How to provide additional amounts?

Recommendation 14.7

As thiamine is well absorbed (except in alcohol related gastritis), thiamine can be administered orally, enterally, or IV. Nevertheless, considering the severity of acute deficiency symptoms, using the IV route is the most efficient, providing 3 x 100–300 mg per day.

Grade of recommendation 0 – Consensus 88%

14. Riboflavin (vitamin B2)

14.1. Main functions

Riboflavin (vitamin B2) is involved in redox reactions and antioxidant functions, metabolism of other B vitamins (niacin, B6, B12, and folate) and energy production. Riboflavin is also required for normal antibody production and has several immunomodulatory effects [121]. Intracellular metabolism involves phosphorylation of riboflavin to form the cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which account for most of riboflavin in plasma and tissues. These cofactors function in many flavo-enzymes, among them xanthine oxidase, succinic dehydrogenase, glutathione reductase, methylene-tetrahydrofolate reductase (MTHFR), pyridoxine phosphate oxidase, and in the conversion of tryptophan to niacin [5,355]. Steps in the cyclical β oxidation of fatty acids are also dependent on flavins as electron acceptors. This explains why the inborn error of the metabolism that affects MADD (multiple acyl-CoA dehydrogenase deficiency) is improved with riboflavin supplementation [355].

Absorption takes place predominantly in the proximal small intestine through an active, carrier-mediated, saturable transport process [355]. Riboflavin is also produced by the microflora of the large intestine. It is excreted in the urine, and is not stored in the body in ample amounts, making a constant dietary supply a necessity. All flavins are light-sensitive and decompose after irradiation.

14.1.1. Needs

The RDA of riboflavin in males is 1.3 mg, in females 1.1 mg, and 1.4 mg and 1.6 mg during pregnancy and lactation, respectively. The dose recommended in PN is 3.6–5 mg [5,8,355].

The main sources of riboflavin are enriched and fortified grains, cereals, and bakery products; meats, dairy products, fatty fish, eggs, and dark-green vegetables [5,355].

Daily dose: 1 mg/day = 3.324 mmol/day.

14.2. Biomarkers and analytical methods

Riboflavin status can be assessed by different methods [356]. Whereas stability of FAD is acceptable for several days at room temperature, it is recommended to store samples at −20 °C and to ensure protection against light to prevent photodegradation before and during analysis. The erythrocyte glutathione reductase activity test is a riboflavin functional assay that remains a well-accepted methodology because it is considered more indicative of tissue saturation and long-term status. It is used more often in population

studies than in the clinical setting. The measurement is done before and after the addition of FAD. The increase in activity by this addition is an index of deficiency. Less than 20% (ratio <1.2) is acceptable, 1.2–1.4 is subnormal, and >1.4 is a deficient state. Red cell FAD is increasingly used in a hospital setting, where it is likely to be a reliable measure of status in the critically ill patient [357] and also in those with inflammation resulting from various conditions [334].

Microbiological assay applied to serum or plasma samples that uses the growth of *Lactobacillus casei* as an indicator of endogenous riboflavin can also be used but requires a laboratory microbiology experience that might not always be available. Alternative and convenient high-pressure liquid chromatography (HPLC) methodologies such as its coupling with fluorescence detection have been developed to simultaneously quantify riboflavin, FAD, flavin mononucleotide in whole blood, serum or plasma samples.

14.2.1. Conversion

Riboflavin: 1 μg/dL = 26.57 nmol/L and 1 nmol/L = 0.0376 μg/dL

14.2.2. Effect of inflammation

In a recent systematic review including 2344 publications, plasma riboflavin was consistently decreased in the context of inflammation [102]. There was one study in surgical patients and 2 in patients with chronic diseases in which the plasma levels of this vitamin decreased from minor to moderate to major inflammation by 30–40% [102]. However, in another study erythrocyte concentration of vitamin B_2 did not decrease with inflammatory response, confirming that erythrocyte assays are more reliable in the context of inflammation [334].

14.3. Deficiency

Deficiency is manifested with oral-buccal lesions (cheilosis, glossitis, and angular stomatitis), seborrheic dermatitis of the face, trunk, and scrotum. Other manifestations are ocular (itching, burning, dryness, corneal inflammation, and photophobia), and normochromic, normocytic anemia and marrow aplasia [5,355]. There is reasonably good evidence that poor riboflavin status interferes with iron handling (iron absorption and mobilization of ferritin from tissues) and contributes to the etiology of anemia when iron intakes are low [355].

Riboflavin deficiency is frequently associated with pyridoxine, folate and niacin deficiencies with their associated symptoms [5,355].

Patients at risk of deficiency are those with malabsorption (short bowel syndrome, celiac disease), thyroid dysfunction, diabetes, renal disease (pre-dialysis and during hemodialysis and peritoneal dialysis), alcoholism, and in pregnancy, lactation, and in the elderly [5]. Also, patients with surgery, trauma, burns, or fractures, and patients on psychotropic drugs, tricyclic antidepressants, or barbiturates [5]. Patients with anorexia nervosa who avoid dairy products area can be at risk for deficiency [5].

Riboflavin deficiency has been implicated as a risk factor for cancer, although this has not been satisfactorily established in humans [355].

In one patient on HPN receiving riboflavin 3 times a week instead of daily doses, a low plasma level with no clinical symptoms has been reported [358].

In old adult patients, low levels, and at-risk levels of riboflavin have been described probably due to decreased intake of dairy products and alteration in absorption and metabolism. It has been hypothesized that this might influence the CVD risk and cognitive impairment [359]. In a RCT in elderly patients, supplementation with 1.6 mg/day of riboflavin did not prove to be an effective homocysteine-lowering agent, even in the face of sub-optimal riboflavin status [360]; meanwhile in another RCT 10 mg/day of riboflavin was effective [361].

14.3.1. When and how to treat?

Acute deficiency occurring during nutritional support is rare, except if riboflavin is excluded from the MN formulation, or during the treatment of patients at risk, previously listed in the text. It is important to consider that riboflavin deficiency is frequently associated with pyridoxine, folate and niacin deficiencies.

In acute deficiency, riboflavin 5–10 mg/day orally is given until recovery. In a severe case of clinical riboflavin deficiency IV administration of 160 mg of riboflavin for 4 days led to clinical cure in 10 days [362].

Current interest is focused on the role that riboflavin plays in determining circulating concentrations of homocysteine, especially in patients with polymorphisms in MTHFR gene as a risk factor for hypertension and cardiovascular disease [355,363]. Randomized trials conducted in hypertensive patients (with and without overt CVD) homozygous for MTHFR 677 TT genotype show that targeted riboflavin supplementation (1.6 mg/day) lowers systolic blood pressure, independently of the effect of antihypertensive drugs [364–367].

Unrelated to nutrition, potential benefits of high dose riboflavin supplementation (400 mg) may be obtained in the prophylaxis of migraine [368].

14.4. Toxicity

Riboflavin consumed orally from the diet or from most multivitamin supplements rarely causes side effects (eventually yellow-colored urine). Repeatedly consumed pharmacologic doses (>100 mg) have the potential to react with light, resulting in formation of isoalloxazine ring and potentially toxic peroxides and/or forming an atypical tryptophan metabolite: the tryptophan-riboflavin adduct has been shown to exhibit hepato- and cytotoxic effects [369].

14.5. Recommendations N°15 – riboflavin (vitamin B2)

14.5.1. When to measure?

Recommendation 15.1

Assessment of riboflavin status can be required when there is clinical suspicion of deficiency.

Grade of recommendation GPP – Strong consensus 96%

Regular monitoring of riboflavin status is not required.

14.5.2. What to measure?

Recommendation 15.2

The riboflavin status can be assessed by the glutathione reductase activity in RBC.

Grade of recommendation 0 – Strong consensus 96%

Red blood cell FAD is another validated method of assessment, especially in the context of inflammation.

14.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 15.3

Enteral nutrition shall provide at least 1.2 mg per day of riboflavin in 1500 kcal.

Grade of recommendation A – Strong consensus 98%

Recommendation 15.4

Parenteral nutrition should provide 3.6–5 mg riboflavin per day.

Grade of recommendation B – Strong consensus 96%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

14.5.4. When to provide additional amounts?

Recommendation 15.5

Additional amounts of riboflavin can be given in the following cases:

- **Suspected or proven clinical deficiency**
- **Patients at risk of deficiency**
- **In patients with deficiencies of other group-B vitamins, provided as multivitamin pills**
- **In patients with MADD as some of them are sensitive to this cofactor**

Grade of recommendation GPP – Strong consensus 100%

14.5.5. How to provide additional amounts?

Recommendation 15.6

Riboflavin 5–10 mg/day can be used orally in case of deficiency.

Grade of recommendation GPP – Strong consensus 96%

Recommendation 15.7

In cases of clinical riboflavin deficiency, IV administration of 160 mg of riboflavin for four days may be necessary.

Grade of recommendation GPP – Strong consensus 94%

Recommendation 15.8

In multiple acyl-CoA dehydrogenase deficiency (MADD) patients, riboflavin can be given at doses of 50–200 mg/day.

Grade of recommendation GPP – Consensus 87%

15. Niacin (Vitamin B3)

15.1. Main functions

Niacin is a collective term for nicotinic acid and nicotinamide. All tissues in the body convert absorbed niacin into its main metabolically active form, the coenzyme NAD. More than 400 enzymes require NAD to catalyze reactions in the body, which is more than for any other vitamin-derived coenzyme. Niacin helps to convert nutrients into energy, create cholesterol and fats, create and repair DNA, and exert antioxidant effects [327,370].

Niacin is obtained in the diet from a variety of whole and processed foods, with highest contents in fortified packaged foods, meat, poultry, red fish such as tuna and salmon, lesser amounts in nuts, legumes and seeds. Further, niacin can be synthesized from the amino acid tryptophan in the liver (1 mg nicotinamide is produced from 67 mg tryptophan), a pathway which also requires thiamine, riboflavin and pyridoxine [371].

15.1.1. Needs

The DRI of niacin intake for humans differs at different points in the life span: Adolescents and adults; males >14 years:16 mg/day, females>14 years:14 mg/day, pregnant 18 mg/day, lactating 16 mg/day [372,373]. One mg of nicotinamide = 1 NE (so called niacin equivalents). PN doses are 40 mg/day [374].

The European Food Safety Authority (EFSA) refers to the collective niacin in the DRI [375]: the compounds nicotinic acid and nicotinamide have slightly different metabolic activity, and separate ULs, due to adverse effects (see below).

15.2. Upper intake levels (ULs)

Nicotinic acid: The UL is set at 10 mg/d for free nicotinic acid. This value is derived from occasional flushing seen at clinical doses in young subjects at 30 mg per day, using an uncertainty factor of 3. Such effects might cause transient hypotensive episodes in the elderly. This upper level is 300-fold below the dose frequently used clinically for the treatment of hypercholesterolaemia (3 g/d). Nicotinic acid is not used for food or supplement fortification. Therefore, it is present at negligible levels in foods [376].

Nicotinamide: The UL for nicotinamide is 12.5 mg/kg body weight/d or approximately 900 mg/d for adults: there is no effect such as flushing. No adverse effect was observed at doses up to 25 mg/kg body weight/d in prolonged studies in diabetic subjects. An uncertainty factor of 2 was applied. There are no concerns regarding intakes of nicotinamide (preformed niacin) within the range currently consumed in foods [376].

15.3. Biomarkers and analytical methods

Determination of niacin status remains a challenge due to limitations of currently used biomarkers in terms of representativeness of niacin body stores and responsiveness to intake under clinical conditions. The urinary determination of the two major niacin metabolites N-methyl-nicotinamide (NMN), N-methyl-2-pyridone-carboxamide (2-Pyr) is used to determine niacin biomarker status [375]. In a controlled niacin intake study carried out in healthy young men fed 6.1 to 32 NE per day for 11 weeks, NMN and 2-Pyr 24 h urinary levels were reported reliable indices of niacin status with NMN being most sensitive to marginal niacin intake [377]. Quantitation of urinary metabolites is thus considered a marker of niacin status with recognized caveats [375]. Niacin urine metabolites can be simultaneously quantified employing HPLC with ultraviolet detection [378]. More recently, the development of methods based on HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) provides a convenient solution for improved throughput, selectivity, and sensitivity in the quantitation of urinary metabolites [379].

Measurement of pyridine nucleotide levels (NAD and NADP) in erythrocytes or whole blood has been used to assess niacin status. Erythrocyte NAD or the ratio of NAD to NADP (niacin index) have been proposed as indicators of niacin status [380]. In a controlled niacin intake study in seven healthy men, erythrocyte NAD levels responded to both depletion and repletion of NE intake. Because NADP levels were shown relatively stable during the experiment, the niacin index might be informative about niacin status and representative of tissue niacin stores [380]. Quantitation of niacin urinary metabolites was recommended over measures of whole blood niacin index in subjects with clinical pellagra due to an unexpected lack of depletion of pyridine nucleotides in the studied cohort [381]. As more than 98% of the total pyridine nucleotide pool is in erythrocyte fraction, determinations of NAD and NADP can be

made from a few microliters of whole blood [382]. Total NAD and NADP can be measured by enzymatic cycling assays performed using microtiter plates with absorbance determination [382].

15.3.1. Effect of inflammation

While there are no data regarding impact of inflammation on niacin, some trials have shown that niacin exerts anti-inflammatory actions, especially in acute coronary syndrome [383–385], sepsis and lung fibrosis (preclinical data) [386].

15.4. Deficiency

Primary causes of pellagra include dependence on a corn-based diet, or general malnutrition (associated with poverty, neglect, abuse, and famine, as well as anorexia nervosa). Some secondary causes include chronic alcoholism and general malabsorptive states such as prolonged diarrhea [387].

Severe niacin and/or tryptophan deficiency leads to a variety of clinical symptoms, including diarrhea, dermatitis and dementia, collectively known as “pellagra” or “the three D disease” and even death (four D) if not recognized and treated promptly [388,389]. In addition to a diet deficient in niacin or its precursor tryptophan, the common causes of pellagra, other mechanisms can contribute to suboptimal niacin status.

Causes of niacin deficiency include inadequate oral intake, poor bioavailability from grains, defective tryptophan absorption, carcinoid tumors, metabolic disorders, and the long-term use of chemotherapeutic treatments [390].

The major risk factor for niacin deficiency is the ongoing consumption of a diet that relies mostly on non-fortified refined grains and grain products [391]. Certain populations are at risk of niacin deficiency even in the context of supplemented food products, i.e., secondary niacin deficiency. This may occur with either increased niacin demand and increased metabolic NAD consumption (elderly people and pregnant women), or cancer patients undergoing treatments that induce DNA damage (radiation therapy or exposure to DNA-damaging drugs). When supplemented at physiological amounts, nicotinic acid (15–20 mg/day) and nicotinamide (300 mg/day) are effective in treating traditional pellagra [388,392]. Nonetheless, at higher concentrations, they display separate additional pharmacological activities, ranging from anti-dyslipidemic to anti-inflammatory action [393,394]. The oral/enteral route should be used whenever the gastrointestinal tract is functional.

Dietary niacin may protect against Alzheimer Disease and age-related cognitive decline, as suggested by a prospective population-based study: the Chicago Health and Aging Project (CHAP) study, considering a geographically defined community of 6158 residents aged 65 years and older, found an inverse association between AD and niacin intakes, after correction for several dietary (antioxidant nutrients, fats, folate, and pyridoxine, vitamin B12, thiamine and riboflavin) and non-dietary (age, education, race, ApoE"4) risk factors for dementia [395]. More recent studies have not confirmed this finding.

15.5. Toxicity

The well-known side effect of niacin is flushing, most commonly in the face, arms, and chest, which typically occurs within 30 min of ingestion and abates after 60 min [396]. Niacin can also cause serious hepatotoxicity that ranges from a mild elevation of liver enzymes to acute liver failure leading to liver transplantation and multiple organ failure. Niacin associated hepatotoxicity is generally related to ingestion of around 3 g per day. In contrast, the more common symptom of flushing can occur at doses as low as 30 mg per day [397]. Energy drinks can contain large quantities of vitamins, including niacin. There have been isolated reports of acute hepatitis involving ingestion, which appear to be the result of high amounts of niacin from energy drinks [398], or high dose ingestion to mask an upcoming drug screen [399].

15.6. Recommendations N°16 – niacin (vitamin B3)

15.6.1. When to measure?

Recommendation 16.1

In case of clinical symptoms, including diarrhoea, dermatitis, and dementia (Pellagra disease), blood or tissue NAD levels may be measured.

Grade of recommendation GPP – Consensus 89%

Since measurement may be difficult to organize, storing a blood sample and awaiting the effects of niacin supplements on symptoms may be a pragmatic alternative.

15.6.2. What to measure?

Recommendation 16.2

Blood or tissue NAD shall be used as a measure of niacin status.

Grade of recommendation A – Strong consensus 91%

15.6.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 16.3

Enteral nutrition shall provide 18–40 mg per day of niacin in 1500 kcal.

Grade of recommendation A – Strong consensus 98%

Recommendation 16.4

Parenteral nutrition should provide at least 40 mg of niacin per day.

Grade of recommendation B – Strong consensus 95%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

15.6.4. When to provide additional amounts?

Recommendation 16.5

When there is suspicion of niacin deficiency from at risk clinical history and/or presence of signs or symptoms, higher doses may be required.

Grade of recommendation GPP – Strong consensus 95%

15.6.5. How to provide additional amounts?

Recommendation 16.6

The oral/enteral route should be used whenever the gastrointestinal tract is functional. In malabsorption and short bowel, the parenteral route can be used.

Grade of recommendation GPP – Strong consensus 93%

16. Pantothenic acid (vitamin B5)

16.1. Main functions

Pantothenic acid is a constituent of the coenzyme A (CoA) and acyl carrier protein (ACP) and therefore is involved in numerous biochemical processes in oxidative respiration, lipid metabolism, synthesis of steroids, acetylated molecules (amino acids, carbohydrates) as well as prostaglandins [400].

The biochemical functions arise from its occurrence as an essential component of CoA and ACP, which cannot be synthesized *de novo* in mammals from precursors. It is therefore essential in the degradation and synthesis of fatty acids, sterols and other compounds synthesized form isoprenoid precursors. It is also involved in protein acylation with some long-chain fatty acids.

A considerable proportion (85%) of pantothenic acid ingested by humans exists as derivatives such as CoA phosphopantetheine [400] and ACP, which are converted to pantothenic acid by pancreatic enzymes (nucleosidases, peptidases, and phosphorylases). This is largely released as free pantothenic acid and is then absorbed along the entire small intestine by a combination of active transport and passive diffusion (355).

16.1.1. Needs

The DRI for ages 14 to over 70 years in both sexes is 5 mg/day [327]. The needs increase to 6–7 mg/day in pregnant and lactating women.

In PN, pantothenic acid needs are 15 mg/day, and this is usually provided along with other B-vitamins [401,402].

The food sources are varied and include fortified cereals, organ meats (liver, kidney), beef, chicken, mushrooms, avocado, nuts, seeds, and dairy milk products.

16.2. Biomarkers and analytical methods

Circulating levels of pantothenic acid are considered acceptable to determine status. Whole blood and urine (24 h collection) are the sample matrices that have proven most informative [403]. Pantothenic acid can be quantified using microbiological assay with a microorganism such as *Lactobacillus plantarum*, this historical method remaining the gold standard. Alternatively, pantothenic acid can be quantified in biological fluids using liquid chromatography coupled to optical (ultraviolet, fluorescence with a preliminary derivatization) and mass spectrometric detection. Pantothenic acid can also be measured using enzyme-linked immunosorbent assay but, as the molecule is not immunogenic, this methodology requires purification and derivatization steps [403,404].

16.2.1. Effect of inflammation

There is no known impact of inflammation on the circulating levels.

A long-term study was conducted to evaluate the relationship of pantothenic acid intake to CRP concentration in 908 (349 men, 559 women) healthy adults aged ≥40 years living in a rural area of South Korea [405]: The dietary intake was inversely related to subsequent CRP concentration in both men and women.

16.3. Deficiency & depletion

Naturally occurring pantothenic acid deficiency is very rare and observed only in conditions of severe malnutrition. In an experimentally induced pantothenic acid deficiency in 3 healthy subjects, a fall in the diastolic and lability of systolic blood pressure, with postural hypotension and vertigo developed, accompanied by tachycardia after slight exertion. The men complained of easy fatigability, occasional bouts of epigastric distress, frequent upper respiratory infections, especially acute pharyngitis [406]. Severe deficiency can cause numbness and burning of the hands and feet, headache, extreme tiredness, irritability, restlessness, sleeping problems, stomach pain, heartburn, diarrhea, nausea, vomiting, and loss of appetite.

Reduced skeletal muscle free CoA availability may decrease the contribution of fat oxidation to ATP production during high-intensity, submaximal exercise or, alternatively, limit pyruvate dehydrogenase complex (PDC) flux and thereby carbohydrate oxidation. The administration of 3 g/day for several weeks may restore the levels [407].

Because of the role of pantothenic acid in triglyceride synthesis and lipoprotein metabolism, experts have hypothesized that pantothenic acid supplementation might reduce lipid levels in patients with hyperlipidemia, and have trialed a form of pantothenic acid known as pantethine to reduce lipid levels using large doses (900 mg/day for 12 weeks): modest but significant declines in triglyceride, total cholesterol and non-HDL cholesterol were observed [408,409].

According to Patassini et al., cerebral pantothenate deficiency might be a newly identified metabolic defect in human Huntington and Alzheimer diseases [410,411]. The mechanisms explaining neurological dysfunction include a) impaired neuronal CoA biosynthesis; b) impaired glycolysis and tricarboxylic acid cycle activity; and c) modified brain-urea metabolism [410]. This opens intervention perspectives, as some authors consider the DRI to likely be insufficient [412].

16.3.1. When and how to treat

The indications for a separate treatment are extremely rare. The derivative of pantothenic acid, pantothenol (panthenol), is a more stable form of the vitamin and is often used as a source of the vitamin in multivitamin supplements. Another common supplemental form of the vitamin is calcium pantothenate. Calcium pantothenate is often used in dietary supplements because, as a salt, it is more stable than pantothenic acid.

In candidates for statin therapy, pantethine in doses 600–900 mg/day may be attempted along with nutritional counselling: different dosages are available (100, 200 and 500 mg tablets) and is usually provided in combination with other B vitamins.

16.4. Toxicity

Toxicity of pantothenic acid is also rare. In fact, no Tolerable Upper-Level Intake (UL) has been established. Large doses of the vitamin, when ingested, have no reported side effects and massive doses (e.g., 10 g/day) may only yield mild diarrhea and muscle pain.

16.5. Recommendations N° 17 – pantothenic acid (vitamin B5)

16.5.1. When to measure?

Recommendation 17.1

Pantothenic acid blood determination should be performed in the context of neurological symptom investigations.

Grade of recommendation GPP – Consensus 86%

16.5.2. What to measure?

Recommendation 17.2

Pantothenic acid shall be determined in blood.

Grade of recommendation A – Strong consensus 93%

16.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 17.3

Enteral nutrition should deliver at least 5 mg pantothenic acid per day when providing 1500 kcal.

Grade of recommendation B – Strong consensus 95%

Recommendation 17.4

Parenteral nutrition should deliver at least 15 mg pantothenic acid per day.

Grade of recommendation B– Strong consensus 98%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

16.5.4. When to provide additional amounts?

Recommendation 17.5

In the context of atypical neurological symptoms additional pantothenic acid may be delivered along with other B vitamins.

Grade of recommendation GPP – Strong consensus 91%

17. Pyridoxine (vitamin B6)

17.1. Main functions

The name Vitamin B6 refers to a group of six water-soluble pyridine compounds (B6 vitamers) comprising pyridoxine, pyridoxamine , pyridoxal and their respective phosphorylated forms [413]. The biologically active form of vitamin B6 is pyridoxal phosphate (PLP), which serves as coenzyme for more than 160 enzymatic reactions. These reactions include transaminations, racemizations, decarboxylations and aldol cleavage [413], affecting carbohydrate, protein, and lipid metabolism. The most important function of active, phosphorylated vitamin B6 in the cell is related to the biosynthesis as well as the degradation of amino acids, is central to transamination reactions [414]. Further functions include gluconeogenesis (through glycogen phosphorylase), steroid receptor binding, neurotransmitter synthesis, and heme biosynthesis.

Absorption occurs in the small bowel. Before absorption, a phosphate group must be removed from the dietary vitamin, allowing B6 to be a free molecule. Then, the vitamin is absorbed from the intestine into the blood by passive diffusion. After absorption in the liver of mammals, PLP becomes tightly bound to serum albumin, being secreted into the circulatory blood system for delivery to the different tissues and organs [414]. This step is considered as a protective step against early de-phosphorylation of the vitamin.

17.1.1. Needs

The RDA and DRI for ages 14–70 in both sexes is 1.3–1.7 mg/day [327]. The needs can reach 2 mg/day in pregnant women. The UL for pyridoxine is: for 14–18 years: 80 mg/day, for 19–70 years:100 mg/day, and >70 years 100 mg/day [327].

As there are no stores of pyridoxine, diet is the only source of vitamers. Pyridoxine is found in meat, whole grains and fortified cereals, as well as potatoes [414].

In PN, pyridoxine requirements are 4–6 mg per day [372].

17.2. Biomarkers and analytical methods

Plasma levels of pyridoxal 5-phosphate (PLP) correlate with pyridoxine intake and body stores and is recognized as a status biomarker [415,416]. Direct plasma PLP levels respond to intake and reflect liver stores and plateau in 6–10 days [414]. PLP is relatively stable at 4 °C or −40 °C but degrades at room temperature and/or when exposed to light. Reliable PLP quantitation therefore requires rapid plasma separation after blood sample collection and frozen storage. PLP can be determined in plasma (EDTA), serum and erythrocytes. Typically, PLP can be measured by HPLC with pre-column derivatization and fluorescence detection. Modern methodologies employing HPLC coupled to tandem mass spectrometry minimizing sample preparation have also been developed with high throughput capabilities [416]. Of note, standard reference materials with certified values for PLP were developed by the United States National Institute of Standards and an assurance program for PLP is made available by the EQA provider Instand (http://www.instandev.de). Other potential direct or functional biomarkers include plasma total vitamin B6 derivatives, urine 4-pyridoxic and total pyridoxine, urinary tryptophan metabolites after tryptophan load (xanthurenic and kynurenic acids), transsulfuration pathway metabolites in urine (cystathionine) or plasma (homocysteine, cystathionine) after a methionine load, erythrocyte aminotransferase stimulated activities [415]. Immune markers (lymphocyte proliferation, number of T-helper cells, immunoglobulin D concentration and interleukin-2 production) have been proposed although exhibiting various limitations and thus are not retained [415]. Measurement of erythrocyte aspartate aminotransferase (EAST) and erythrocyte alanine aminotransferase (EALT) baseline levels and after *in vitro* addition of pyridoxine has been used to assess long-term vitamin B6 status because they are related to erythrocyte lifespan. While transaminases are not related to albumin and alkaline phosphatase, these measurements can be confounded by impaired renal function and require fresh whole blood sampling that might not be practical [416].

Normal values of plasma pyridoxal 5-phosphate are 5–50 μg/L (20–200 nmol/L).

17.2.1. Effect of inflammation

Inflammation due to a variety of conditions is well recognized to lead to a fall in plasma PLP, but minimally affects red blood cell concentrations [334]. Plasma albumin concentration, and to a lesser extent alkaline phosphatase activity, influences PLP concentration in plasma. In conditions associated with low albumin (e.g. inflammation) or altered alkaline phosphatase activity, red cell PLP measurements are more likely to be reliable than plasma measurements in differentiating true from apparent vitamin B6 deficiency and to guide vitamin B6 supplementation [416,417]. Additionally, intracellular measurements appear to obviate the need for plasma adjustment for albumin to assess MN status [102].

17.3. Deficiency

Deficiency or lack of pyridoxine can cause a variety of diseases [418], including seborrheic dermatitis with cheilosis and glossitis, microcytic anemia, epileptiform convulsions, confusion, and/or depression and angular stomatitis.

Populations with the greatest risk for deficiency include alcoholics, renal dialysis patients (especially continuous renal replacement therapy) [419,420], the elderly, post-operative (surgical process) [421], infections [422], critical illness [423], pregnancy,

and people receiving medical therapies that inhibit vitamin activity (i.e., isoniazid, penicillamine, anti-cancer, corticosteroids, and/or anticonvulsants) [89].

Deficiency has been observed during isoniazid therapy [88], HIV infection therapy and treatment [89,90], severe alcoholic hepatitis [45], neurological impairment [424,425], postoperative delirium [426], migraine attacks [427], and thymoglobulin immunosuppression for organ transplantation [93].

Not a real deficiency, but a pathology with higher needs, pyridoxine-dependent epilepsy is a rare autosomal recessive epileptic encephalopathy caused by antiquitin deficiency [428]: Pyridoxine supplementation for optimal seizure control and developmental outcomes belongs to the treatment and may require very high doses.

17.3.1. When and how to treat

Deficiency resulting from chronic poor dietary intake will respond to oral supplements. No specific dose is recommended but oral doses of 50–100 mg for one to two weeks are safe and widely available.

Acute deficiency as may occur during isoniazid overdosing-induced seizures may need 5 g (1 g of pyridoxine for each gram of isoniazid ingested, then 1 g IM or IV every 30 min up to a maximum of 5 g) [429].

When treating deficiency PLP levels respond to intake: they reflect liver stores and plateau in 6–10 days.

In treatment of ethylene glycol poisoning (used as antifreeze agent), pyridoxine is recommended at 50 mg IV every 6 h [430].

17.4. Toxicity

Clinical signs observed in case of excess pyridoxine are sensory neuropathy with ataxia or areflexia, impaired cutaneous and deep sensations, and dermatologic lesions.

There have been no adverse effects due to high food intakes of pyridoxine reported. However, large oral supplemental doses (>500 mg/day) have resulted in a variety of side effects. Negative effects have been related to prolonged intakes of 300 mg/d as well. Long-term doses as low as 100 mg/d have been associated with Lhermitte signs, which suggests an effect on the spinal cord [431]. However, the NOAEL reported by the Institute of Medicine is 100 mg/day [327].

17.5. Recommendations N°18 – pyridoxine (vitamin B6)

17.5.1. When to measure?

Recommendation 18.1

Measurement should be done in presence of signs of pyridoxine (B6) deficiency.

Grade of recommendation GPP – Strong consensus 95%

17.5.2. What to measure?

Recommendation 18.2

Vitamin B6 status shall be determined by measuring plasma pyridoxal phosphate (PLP) levels.

In seriously ill patients or in presence of inflammation, red cell PLP shall be measured.

Grade of recommendation A – Strong consensus 95%

17.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 18.3

Enteral nutrition shall deliver at least 1.5 mg pyridoxine per day in 1500 kcal.

Grade of recommendation A – Strong consensus 98%

Recommendation 18.4

Parenteral nutrition should deliver 4–6 mg pyridoxine per day.

Grade of recommendation B – Strong consensus 98%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

17.5.4. When to provide additional amounts?

Recommendation 18.5

In the context of isoniazide overdose or glycol poisoning, high dose of pyridoxine should be part of the therapy.

Grade of recommendation GPP – Strong consensus 95%

18. Biotin (vitamin B7)

18.1. Main functions

Biotin can be found in all cells of the human body. It plays an important role in the metabolism of fatty acids, glucose, and amino acids as it is a cofactor for five carboxylases that are critical for their metabolism [432]: propionyl-CoA carboxylase, pyruvate carboxylase, methylcrotonyl-CoA carboxylase (MCC), acetyl-CoA carboxylase 1, and acetyl-CoA carboxylase 2 [433]. Biotin is also a regulator of gene expression and affects the functions of adaptive immune T and NK cells [432]. Biotin sufficiency is essential for normal fetal development.

18.1.1. Needs

The EFSA recommends an AI of 40 μg/day for healthy adults (45 μg/day for lactating women) [434]. The estimated biotin intake is 35–70 μg/day following a non-vegetarian, non-vegan Western diet [435]. However the Institute of Medicine recommends 30 μg/day plus an additional 5 μg/day for lactating women [327].

The main biotin sources in Western countries are egg yolks, milk, yeast, some organ meats, and multi-vitamin supplements. Avidin, a protein found in raw egg white, combines with biotin and hinders its absorption. For vegans the main sources are peanuts, avocados, sweet potatoes, onions, and tomatoes.

Since the deficiency cases of the 1980s, biotin has been included as a standard in PN vitamin solutions, at doses of 60 μg per day [5,6,436,437].

18.2. Biomarkers and analytical methods

Various direct or indirect biomarkers have been proposed although there is a lack of consensus on one or a set of status biomarkers for biotin that would apply to different population groups. Some methods are widely available while others require advanced methods available in some university hospitals.

Direct analysis of biotin in blood, serum/plasma and urine can be performed by microbiological assays with sensitivity characteristics that might not be adequate for clinical purpose [438]. Biotin and biotin-containing peptides and metabolites can be measured using competitive binding or ELISA assays but with sometimes relatively demanding analytical steps. Recently, high pressure liquid chromatography coupled to mass spectrometry has enabled biotin analysis with high specificity and sensitivity.

Urinary biotin and related metabolites can be assayed for avidin-binding substances after separation by HPLC [439]. The urinary excretion (24-h urine) of biotin and of metabolites produced by biotin-dependent carboxylases and related metabolic pathways (3-hydroxyisovaleric acid, 3-hydroxyisovalerylcarnitine), are sensitive to biotin depletion [440]. 3-hydroxyisovaleric acid and 3-hydroxyisovalerylcarnitine can be measured traditionally by stable isotope dilution gas chromatography coupled to mass spectrometry. More recently, HPLC-tandem mass spectrometry provided simpler methods with sensitivity and precision [438]. Another advantage of HPLC-MS/MS is the possibility to simultaneously measure several acylcarnitine species that enables the analysis of metabolites associated with a broader range of biotin-dependent carboxylases. Another indirect method is the measure of biotinidase activity that can be used with different synthetic substrates with detection by absorbance at 546 nm, HPLC-ultraviolet or MS/MS [438]. Analysis of propionyl-CoA carboxylase (PCC) activity and abundance of biotinylated β-methylcrotonyl-CoA carboxylase and PCC in lymphocytes have also been used [434].

Based on an outpatient feeding protocol to create states of biotin deficiency, sufficiency and supplementation in healthy men and women, Eng et al. have reported that amongst a total of 20 possible biotin status markers, only the abundance of biotinylated carboxylases (3-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase) in lymphocytes enabled the distinction of biotin-deficient and sufficient states [441]. However, validation of these biomarkers requires more supporting evidence [434].

18.2.1. Effect of inflammation

There is no evidence on the effect of inflammation on markers of biotin status. Biotin is involved in the function of the adaptive immunity (lymphocytes T and NK) [432,442]: deficiency enhances inflammatory responses, favoring the secretion after lipolysacharide stimulation of the proinflammatory cytokines TNF-α, IL-12p40, IL-23, and IL-1β [432].

18.3. Deficiency

Biotin deficiency is rare in the general population due to its wide availability. Biotin deficiency leads to dermal (i. e. dermatitis, alopecia) as well as neurological complications such as ataxia [443,444].

Conditions at risk of developing deficiency include chronic alcohol consumption, malabsorption in the context of Crohn's disease and colitis, short bowel syndrome, celiac disease, severe malnutrition, smoking and pregnancy. Long-term antibiotic use may destroy bacteria that produce biotin.

In the 1980s, there were several case reports on biotin deficiency in unsupplemented PN [445–450], including hair loss [445]. An inherited biotinidase deficiency causes biotin deficiencies [451,452].

Anticonvulsant therapy: while case reports from the 1980s suggested that long-term anticonvulsant therapy might interfere with intestinal absorption and increase needs, more recent data suggest there is no concern at least with valproate and carbamazepine [453].

Some data suggest that a marginal biotin deficiency may occur spontaneously in normal human gestation, and may be teratogenic as biotin transport by the human placenta is weak [454].

18.3.1. When and how to treat

For a rapid replenishment, biotin may be given orally. In malabsorption and short bowel, increasing to doses of 10 mg/day may overcome the deficiency [447].

In deficient PN dependent patients, IV doses up to 200 μg/day for 2–3 weeks may be required.

Two small clinical trials hint that biotin needs in pregnancy might be higher than the current EFSA guidance [434]. In 26 pregnant women, a 300 μg biotin supplementation was compared to placebo for 14 days. The formerly increased urinary 3-hydroxyisovaleric acid excretion decreased only in the test group while increasing further with placebo [454]. In another clinical trial, 26 pregnant (third trimester), 28 lactating and 21 controls received a standardized diet with 57 μg/d biotin for 10–12 weeks. The urinary 3-hydroxyisovaleric acid excretion was higher in pregnant women than in the control group suggesting that requirements for biotin may be higher in pregnancy [455].

Biotin has been studied as a drug in progressive multiple sclerosis (biotin 3 x 100 mg/d) which is beyond the scope of nutrition [66].

18.4. Toxicity

Toxicity of biotin is unlikely: no UL has been established. No adverse effects have been shown for both an oral and IV administration of pharmacological doses of biotin up to 5 mg/day for prolonged periods [456].

18.5. Recommendations N°19 – biotin (vitamin B7)

18.5.1. When to measure?

Recommendation 19.1

Biotin status may be assessed in presence of clinical symptoms suggesting biotin deficiency (i. e. dermatitis, alopecia, or neurological symptoms) and a history suggestive of inadequate intake.

Grade of recommendation GPP – Strong consensus 95%

18.5.2. What to measure?

Recommendation 19.2

Biotin status shall be determined by the direct measure of blood and urine biotin, and should be completed by the determination of biotinidinase activity.

Grade of recommendation A – Strong consensus 95%

18.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 19.3

In enteral nutrition at least 30 μg of biotin per day should be provided, in 1500 kcal.

Grade of recommendation B – Strong consensus 100%

Recommendation 19.4

In parenteral nutrition, vitamin additives should provide 60 μg biotin per day.

Grade of recommendation B – Strong consensus 98%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

18.5.4. When to provide additional amounts?

Recommendation 19.5

Breast-feeding mothers should receive an intake of at least 35 μg biotin per day orally.

Additional amounts may also be needed in patients on renal replacement therapy.

Grade of recommendation GPP/0 − Strong consensus 100%

18.5.5. How to provide additional amounts?

Recommendation 19.6

Additional amounts of biotin can be administered either orally, enterally or IV depending on the intestinal function.

Grade of recommendation GPP − Strong consensus 95%

19. Folate and folic acid (vitamin B9)

19.1. Main functions

Folate is a generic term referring to a family of molecules that vary as a function of their oxidation state, the chemical nature of one-carbon substitution groups (methyl, methylene, methenyl, formyl, formimino), and the length of the glutamate side-chain [457]. This family includes both the naturally occurring MN folates and synthetic forms (folic acid) such as folinic and levomefolic acids. Biologically active folate forms include folinic acid and 5-methyltetrahydrofolate (5-MTHF) [457].

Folate, as tetrahydrofolate, is required for the body to make DNA and RNA and metabolise amino acids. In the cytoplasm, one-carbon metabolism is required for the synthesis of purines and thymidylate and the remethylation of homocysteine to methionine. One-carbon metabolism in the mitochondria is required for the synthesis of formylated methionyl-tRNA [458]. Folate therefore plays a critical role as cofactors in the metabolism of nucleic acid precursors and several amino acids, as well as in methylation reactions. The transfer of one-carbon units appears to be the only function of folate coenzymes in the body [459].

Folates are absorbed in the duodenum and jejunum in a pH-dependent carrier-mediated process [460]. Vitamin C improves folate bioavailability by limiting the degradation of natural folate coenzymes and folic acid supplements in the stomach [461]. Folic acid is manufactured synthetically and is available in supplements or fortified foods: it is converted in the body into folate.

19.1.1. Needs

Nutritional sources of folate are pulses (edible seeds from legumes: 200–300 g cover the RDA/DRI) and leafy green vegetables (400 g), but also egg, nuts, and, to some extent, whole grain products. Food folates have a lower bioavailability than synthetic folic acid, therefore, the dietary folate equivalent (DFE) is defined as 1 μg DFE = 1 μg food folate = 0.6 μg folic acid from fortified food or a supplement consumed with food = 0.5 μg of a folic acid supplement taken on an empty stomach or provided IV.

For the general population, the DRI of DFE varies from 250 to 400 μg/d [462,463], corresponding to 125–200 μg/d of folic acid if given IV [327]. The authoritative EFSA report suggests a Population Reference Intake of 330 μg DFE [7]. For pregnant and lactating women, the needs are about twice as high [462,463].

The PN recommended doses are 400–600 μg folic acid per day [5,372]. The relatively high PN doses have been used extensively for many years and no toxicity issues have been described.

19.2. Biomarkers and analytical methods

Folate status is conventionally assessed by measuring levels of folate in serum/plasma or RBC. Serum/plasma concentrations are the earliest indicator of altered folate exposure and reflect recent dietary folate intake. Red blood cell folate level is a sensitive marker of long-term folate status as it informs on folate accumulation during red cell erythropoiesis, thereby reflecting folate status during the preceding 3 months as well as tissue folate stores. Because remethylation of homocysteine to methionine is a folate-mediated process in the one-carbon metabolism, plasma homocysteine concentrations are also measured as a functional marker of folate status. However, plasma homocysteine is also related to the status of vitamins B2, B6, and B12 and can be affected by renal impairment. As both folate and cobalamin deficiencies can result in elevated homocysteine levels, isolated folate deficiency can be differentiated from the latter by normal cobalamin and methylmalonic acid (MMA) levels.

Plasma/serum and whole blood folate concentrations can routinely be measured by a microbiological assay (MBA) using *Lactobacillus rhamnosus*, whose growth is proportional to the amount of total folate present in the sample [464]. Because *L. rhamnosus* can respond to all active monoglutamate forms of folate, this assay is viewed as the gold standard technique for folate status assessment. The folate levels are determined by measuring the turbidity of the inoculated medium using a microplate reader. As most folate forms are susceptible to degradation by light, temperature, pH, and oxygen, several pre-analytical precautions are required from specimen collection to sample preparation to ensure reliable results [465]. Ascorbic acid is commonly used to protect folate from oxidation and maintain it as reduced forms. Determination of RBC folate requires a preliminary hemolysis of whole blood by dilution with an ascorbic acid solution after sample collection. Dilutions of serum or hemolysed whole blood samples in ascorbic acid solutions are also necessary in the MBA folate protocol [466]. MBA folate analysis may be affected by the presence in the specimen of antibiotics affecting *L. rhamnosus* [467].

Folate status protein binding assays are also available but with different affinities for different folate forms, limited linear range, and analytical variabilities. The development of methods using Liquid chromatography-mass spectrometry (LC-MS) has enabled quantification of different folate forms with good sensitivity and precision but requires expensive instrumentation, experienced staff, and careful sample preanalytical conditions such as the conversion of erythrocyte polyglutamates to monoglutamates. Plasma homocysteine can be measured by immuno- and enzymatic assays but when possible, use of LC-MS/MS provides high selectivity, specificity and simultaneously measures other molecules to help interpretation [468,469]. Serum folate levels should be $\geq$ 10 nmol/L and red blood cell folate $\geq$340 nmol/L [463].

19.2.1. Effect of inflammation

Little evidence is available of the effects of inflammation on the assays of plasma or red blood cell folate, and hence of their validity when there is a substantial inflammatory response.

There is, however, some evidence that folic acid may modify some aspects of the inflammatory response [469,470].

19.3. Deficiency

Most symptoms of folate deficiency overlap with cobalamin deficiency, i.e. megaloblastic anemia, and pancytopenia, glossitis, angular stomatitis, oral ulcers [463], neuropsychiatric manifestations, including depression, irritability, insomnia, cognitive impairment, psychosis, anorexia, and fatigue. Patients being evaluated and treated for folic acid deficiency should therefore also be evaluated for cobalamin deficiency. Deficiency in one or both vitamins cause megaloblastic anemia. If the latter is concomitant and ignored during folic acid supplementation, the blood picture may improve but neurological manifestations may worsen [470].

Cases of isolated clinical folate deficiency are extremely rare in Western countries and a diagnosis of deficiency should be made with consideration of a circumstance listed in Table 9 [471]. Historical cases of acute folate deficiency were describe in the 70s when folate administration had not yet become routine during PN [472].

Table 9
Causes of Folate deficiency [471,473].

Cause	Example
Inadequate dietary intake	Alcohol abuse Poverty poor nutrition
Intestinal malabsorption	Inflammatory bowel disease Celiac disease Post-bariatric operations, post-gastrectomy Chronic intestinal failure
Increased needs	Physiological or pathophysiological states with high cell turnover: Pregnancy and lactation Inflammatory and neoplastic diseases Renal dialysis Increased hematopoiesis (e.g. chronic hemolytic anemia) Exfoliative dermatological conditions
Antifolate drug intake	Sulfasalazine Methotrexate Anticonvulsants Metformin Chemotherapy drugs

In patients with chronic kidney disease and/or on hemodialysis, folic acid and vitamin B12 metabolism are impaired, and it has long been know that their folic acid requirements are significantly higher than standard DRI, with 1–5 mg per day having already been proposed in the late 1970s [474]. Some patients, especially those with diabetes, may require as much as 15 mg per day [22]. Hyperhomocysteinemia is common, and folic acid together with vitamin B12 is critical for the conversion of homocysteine to methionine [475]. Folic acid has also been shown to improve endothelial function in chronic kidney disease patients [476].

19.3.1. When and how to treat

All patients with folate deficiency or at risk of it should be offered supplemental folic acid for the correction or prevention of the deficiency and concomitantly advised to eat a balanced diet rich in green leafy vegetables and fruits (if tolerated by gastrointestinal tract).

The need for treatment can either be induced by a reduced intake or by increased needs (Table 1). A reduced intake is either caused by reduced intake via food, by gastrointestinal disorders (e.g. due to malabsorption or inflammation), or by taking drugs that are folate antagonists, thus reducing the available folate. Of the states of increased needs, pregnancy should be especially highlighted. Folate supplementation is protective [477–480], lowers the risk for the child being small for gestational age [481–483], and decreases congenital neural tube and heart defects [484,485].

Combinations of the above-mentioned conditions, e. g. pregnant IBD patients, require special attention.

19.4. Toxicity

Due to the proliferative effects, folic acid might increase cancer risk and progression. Moreover, it is said to cause insulin resistance in children, interact with epilepsy medication, mask a vitamin B12 deficiency, and be hepatotoxic [486]. Nevertheless, oral administration of folic acid in recommended dosage is considered non-toxic. Excess folic acid is excreted in the urine. There was not sufficient evidence to establish a UL based on a NOAEL but rather based on an LOAE, which is set at 5 mg/day. The UL for folic acid was established of 1 mg/day of folate to avoid a delayed diagnosis of vitamin B12 deficiency, as assessed by hematological indices, and thereby minimize the risk of neurological complications in vitamin B_{12}-deficient individuals. Additional research is needed to assess the health effects of folic acid supplement use when the current UL for folic acid is exceeded [487].

19.5. Recommendations N°20 – folate and folic acid (vitamin B9)

19.5.1. When to measure?

Recommendation 20.1

In patients with macrocytic anemia or at risk of malnutrition, folic acid status should be measured at least once at first assessment and repeated within 3 months after supplementation to verify normalization.

Grade of recommendation GPP – Strong consensus 97%

Folic acid and B12 are usually both measured during investigation of anemia.

Recommendation 20.2

In diseases known to increase the needs for folate, folate status can be measured every 3 months until stabilization, and then once a year.

Grade of recommendation GPP – Strong consensus 96%

19.5.2. What to measure?

Recommendation 20.3

Folate status shall be assessed in plasma or serum (short-term status), or RBC (long-term status) using a method validated against the microbiological assay.

Grade of recommendation A – Strong consensus 96%

The gold standard method of measuring folate is microbiological assay with *L. rhamnosus.* Analysis of homocysteine at the same time improves the interpretation of laboratory measurements. Level A is supported by biochemical evidence.

19.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 20.4

Enteral nutrition shall provide 330–400 μg DFE per day in 1500 kcal.

Grade of recommendation A – Strong consensus 98%

Recommendation 20.5

Parenteral nutrition should provide 400–600 μg per day folic acid.

Grade of recommendation B – Strong consensus 100%

19.5.4. When to provide additional amounts?

Recommendation 20.6

In case of dietary deficiency or chronic hemodialysis, 1–5 mg folic acid per day may be given orally.

Grade of recommendation 0 – Strong consensus 100%

In case of deficiency, the oral administration should last four months, or until the reason for the deficiency is corrected. When clinical symptoms have subsided and the blood picture has become normal, a maintenance level should be used, i.e., about 330 μg DFE for adults and 600 μg DFE for pregnant and lactating women, per day.

In patients on chronic hemodialysis with hyperhomocysteinemia, increased amounts may be required for prolonged periods: 5 mg or more per day of folic acid orally to non-diabetic patients and 15 mg per day to diabetic patients [475,476].

19.5.5. Prevention of neural tube defects

Recommendation 20.7

For the prevention of neural tube defects, women who desire to have children or women not taking oral contraceptives and living in countries without folic acid fortification of staple foods shall take folic acid supplements (400 μg/day) periconceptionally/whilst of childbearing age.

Grade of recommendation A – Strong consensus 98%

19.5.6. How to provide additional amounts?

Recommendation 20.8

Additional amounts of folic acid should be administered orally. In case of ineffective oral treatment or intolerance, folic acid can be given (0.1 mg/day), subcutaneously, IV, or IM.

Grade of recommendation GPP – Strong consensus 95%

20. Cobalamin (vitamin B12)

20.1. Main functions

Vitamin B12 (cobalamin) is an essential water-soluble MN synthesized by fungi and microorganisms, and in the stomach of ruminant animals dependent on soil cobalt content [488,489]. Edible plants and mushrooms rarely contain a considerable amount of cobalamin, so humans are totally dependent upon animal sources or fortification [488,489].

Cobalamin absorption consists of several steps: after its release from proteins under the action of gastric acid and pepsin, followed by binding to R-protein produced by the salivary glands, it binds to the gastric intrinsic factor. These steps are followed by the absorption of intrinsic factor–cobalamin complexes through receptor-mediated endocytosis in the terminal ileum [490]. Degradation of R protein in the small intestine allows binding to gastric produced intrinsic factor and uptake by the ileal mucosa [491].

Cobalamin is a cofactor for two enzymes in humans: methionine synthase in methyl transfer from methyl tetrahydrofolate to form methionine from homocysteine; and methyl malonyl-CoA mutase in synthesis of the citric acid cycle intermediate succinyl CoA [489]. These pathways are essential for mitochondrial metabolism, immune response, preservation of DNA integrity, and of the myelin sheath around neurones, and synthesis of neurotransmitters-participating in normal blood cell formation as well as neurological functions [327,490].

20.1.1. Needs

The DRI for healthy adults, based on maintenance of hematological status and serum cobalamin values, is 2.4 μg/day [489] and the AI estimated by EFSA based on a combination of biomarkers is 5 μg/d in pregnancy, 4,5 μg/d in lactation and 4 μg/d for other healthy adults [489].

Physiological needs probably increase with age, with oxidative stress of prolonged illness, or use of certain medication [492,493] (Table 11).

In adults the cobalamin reserves, which are stored mainly in the liver, are estimated to be around 2500 μg [489] and will last for approximately 12–36 months without sufficient intake.

Dietary sources of cobalamin are ruminant meat, organs, milk, fish, shellfish, fortified cereals, and nutritional yeast [492,493].

20.2. Biomarkers and analytical methods

Although lacking a so-called gold standard methodology, cobalamin status is best performed by the quantification of both direct and functional markers [494], although there is not absolute gold standard [471,490]. A combination of at least two biomarkers (holo-transcobalamin [holo-TC], and methylmalonic acid [MMA]) is optimal, with serum cobalamin as a replacement for holo-TC when measurement of this latter is unavailable [495]. When assessing serum cobalamin levels, sampling should be timed prior to blood transfusion or IM administration of cobalamin.

Direct markers include either cobalamin levels, i.e. sum of haptocorrin-bound cobalamin and holo-TC, or holo-TC alone, this latter representing the B12 fraction taken up by cells and referred thus as “active B12”. As cobalamin generates two coenzymes in man, adenosylcobalamin and methylcobalamin, blood levels of MMA and total homocysteine (tHcy) are considered functional markers related to cobalamin status. Adenosylcobalamin is the coenzyme of methylmalonyl-CoA mutase that converts methylmalonyl-CoA to succinyl-CoA, and in situations of cobalamin deficiency methylmalonyl-CoA accumulates and is hydolyzed to MMA. Methylcobalamin is required for methionine synthase that remethylatres homocysteine into methionine via 5′-methyltetrahydrofolate (related to folic acid) as methyl donor. Blood concentrations of tHcy might be related to vitamin cobalamin deficiency but are also determined by 5′-methyltetrahydrofolate levels and thus folic acid status. Cobalamin, holo-TC and MMA are measured in serum and plasma samples depending on the requirements of the chosen analytical technique.

Temperature storage of 2–8 °C for up to 7 days can be used for serum B12, holo-TC and MMA. Serum storage at −20 °C (up to 30 days) or colder (for longer storage) is required. Cobalamin can be measured using microbiological assay with *Lactobacillus Leichmanii*, competitive-binding luminescence-based assays, electrochemiluminescence immunoassay, chemiluminescence or enzyme-linked fluorescence. The holoTC concentrations can be quantified by ELISA, radio-immunoassay or immunoassay. Various automated commercial solutions exist depending on the measurement techniques. Circulating levels of MMA can be quantified using HPLC with fluorescence detection, gas chromatography coupled to mass spectrometry (GC–MS) and more recently by liquid chromatography tandem mass spectrometry (LC-MS/MS). Various methods have been

Table 10
Clinical symptoms of vitamin B12 deficiency [492,493,498,502–504].

	Clinical manifestation
Hematolgical	Macrocytosis, Reticulocytosis
	Anemia- pallor, fatigue, weakness, shortness of breath, tachycardia
	Thrombocytopenia, Leucopenia, Pancytopenia
Neurological	Peripheral neuropathy, paresthesia
	Sensory loss of extremities, Tingling, Numbness, Vertigo
	Demyelisation of corticospinal tract and dorsal columns- ataxia
Neuropsychiatric	Irritability, Mood-disorders/mood-swings, Psychosis, Depression
Cognitive	Confusion, Memory impairment, Cognitive decline, Dementia
non-specific	Glossitis, Malaise, Fatigue, Weakness

proposed for tHcy analysis including enzymatic assays, HPLC assays, immunoassays, capillary electrophoresis, GC–MS and LC-MS/MS [467]. Various options using the combination of four, three or two variables have been proposed for the determination of cobalamin status [494]. Among these, an example laboratory assessment algorithm that combines holo-TC (first-line assay) with MMA (second-line assay) has been proposed [494,495].

20.2.1. Effect of inflammation

Inflammation seems to be associated with increased cobalamin concentrations [496]. Corcoran et al. showed a positive correlation between CRP and elevated cobalamin levels in the first two days of ICU admission [497].

20.3. Deficiency

The prevalence of cobalamin deficiency is estimated to be around 10–26% in the general population in Western countries (US, Europe), with the most susceptible group being the elderly [498]. Deficiency is largely underdiagnosed in the socio-economical vulnerable pregnant populations. According to some authors, deficiency could reach 75%–90% in the population of vegetarian or vegan diet communities without fortification or supplementation [499,500].

Inadequate intake is the main cause of low serum cobalamin in younger adults and likely the main cause in poor populations worldwide [498]: it is caused by low consumption of animal-source foods. Intestinal malabsorption explains numerous cases of cobalamin deficiency. Absorption of cobalamin from food requires normal stomach, pancreas, and small intestine function: intestinal resection or reconstruction are therefore at high risk of causing deficiency [501]. The most prevalent causes of deficiency are (1) an autoimmune condition known as pernicious anemia, resulting from lack of intrinsic factor and, (2) food-bound cobalamin malabsorption. Both conditions are also common with chronic atrophic gastritis, which affects around 10–30% of people over 60 years [499]. Long term treatment of diabetes with metformin exposes numerous patients to deficiency risk [84,85].

As cobalamin is involved primarily in the metabolism of phospholipids and neurotransmitters, the manifestations are primarily haematological or neuropsychiatric deterioration [499], with a variety of non-specific symptoms. Signs and symptoms of deficiency are summarized in Table 10. The conditions at risk of deficiency are indicated in Table 11.

Cobalamin deficiency has become a common problem in bariatric surgery patients that may manifest after a few months without adequate complementation: standard maintenance regimens after this type of surgery include cobalamin at doses far superior to DRI.

Table 11
Risk groups for B12 deficiency in adults.

Ethiopathogenesis		References
Dietary restriction:	Veganism	[505–507]
	Gluten-free diet	[508]
Decreased absorption:	Age over 50	[475,509]
	Age over 60	
GI operations:	Gastrectomy, Biliopancreatic deviation, pancreatoduodenectomy, Ileal resection over 20 cm, cystectomy with urinary diversion, High output ileostoma or enteric fistulae	[501]
Bariatric/metabolic surgery:	Roux-Y-Bypass; Sleeve gastrectomy	[510,511]
Malabsorptive disease:	Chronic atrophic gastritis	[86]
	Coeliac disease	[508]
	Inflammatory bowel disease	
Prolonged drug use (6–12 month)	Biguanides (Metformin)	[84,85,87]
	Proton pump inhibitors/H2 blockers (no RCTs), Possible effect from underlying disease	[512–514]
	Oestrogen contraceptive pill	
	Folic acid treatment	[515]
	Folic acid fortification or supplementation	[516]
	Nitric oxide administration for analgesia	[517,518]
	Heavy alcohol consumption	
Metabolic	*Phenylketonuria*	[519]
	Diabetes mellitus type I/II	[471]
	Multiple Endocrine Neoplasia (MEN) syndrome	
Autoimmune disease:	Thyroiditis	[520]

20.4. Toxicity

There is no upper toxicity limit for cobalamin and no reports of acute toxicity in oral or parenteral cobalamin supplementation or treatment. Excessive provision of cobalamin might be harmful for some populations as combined supplementation of folic acid, vitamin pyridoxine, and cobalamin in patients with diabetic nephropathy resulted in more rapid decline of renal function and an increase in occurrence of vascular events [521].

Cobalamin excess with high blood levels has been observed in a variety of diseases, including alcoholism, liver disease and cancer [496]. Higher cobalamin values have also been observed in critically ill patients, with highest values in non-survivors [496].

20.5. Recommendations N°21 – cobalamin (vitamin B12)

20.5.1. When to measure?

Recommendation 21.1

Cobalamin deficiency should be excluded in all patients who present with anemia, or isolated macrocytosis, established diagnosis of polyneuropathies, neurodegenerative diseases or psychosis.

Grade of recommendation GPP – Strong consensus 98%

20.5.2. When to monitor?

Recommendation 21.2

In all patients at risk, or on treatment with cobalamin, replenishment adequacy should be assessed at least annually by resolution of clinical symptoms and available laboratory markers.

Grade of recommendation GPP – Strong consensus 98%

20.5.3. What to measure?

Recommendation 21.3

Adult patients at risk or suspected of cobalamin deficiency should be screened with the combination of at least two biomarkers (holo-TC, MMA), with serum cobalamin as a replacement.

Grade of recommendation B – Strong consensus 92%

Recommendation 21.4

Patients with autoimmune diseases or with glossitis, anemia and neuropathy should be screened for pernicious anemia with the presence of anti-intrinsic factor antibodies (anti-IFAB) regardless of cobalamin levels.

Grade of recommendation GPP – Strong consensus 100%

20.5.4. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 21.5

Enteral nutrition shall provide at least 2.5 μg cyanocobalamin per day in 1500 kcal.

Grade of recommendation A – Strong consensus 97%

Recommendation 21.6

Parenteral nutrition should provide at least 5 μg cyanocobalamin per day.

Grade of recommendation GPP – Strong consensus 97%

20.5.5. When to provide additional amounts?

Recommendation 21.7

Breast-feeding mothers shall receive an intake of at least 2.8 μg cyanocobalamin per day orally.

Grade of recommendation A – Strong consensus 100%

Recommendation 21.8

Patients with compromised cobalamin absorption should receive life-long supplements either as a daily dose of 350 μg cobalamin, or IM injections of 1000–2000 μg of cobalamin every 1–3 months.

Grade of recommendation GPP – Strong consensus 100%

Intranasal and sublingual administration are alternative routes [510].

The conditions above include, but are not limited to, short bowel syndrome, bariatric surgery, Crohn's diseases, gastrectomy, atrophic gastritis, and ileal resection. See Table 10 for therapies at risk.

20.5.6. How to provide additional amounts?

Recommendation 21.9

In presence of acute clinical symptoms of deficiency, anti-intrinsic factor antibodies, a history of total gastrectomy or continuous malabsorptive diseases, the IM route should be used. Starting with high doses of 1000 μg cobalamin every second day for 2 weeks (or daily for 5 days).

Grade of recommendation GPP – Strong consensus 100%

Treatment should be continued at least twice monthly until resolution of all clinical signs and/or etiopathogenetic factors (including resolution of macrocytosis).

Monitoring blood potassium should be part of repletion therapy.

21. Vitamin A (retinol)

21.1. Main functions

From the precursor Retinol two different active metabolites are formed: retinoic acid and retinal. Retinol and retinal are responsible for vision and reproductive function. Retinoic acid controls cellular growth and differentiation, in particular in mucous membranes [522].

Vitamin A is a prohormone. The active metabolites, all trans- and 9-cis retinoic acid, are ligands for nuclear receptors (RAR, RXR, PPARs), which activate gene expression in more than 500 target genes. The nuclear receptors form heterodimers within the RAR/RXR family and with the nuclear vitamin D receptors or steroid/thyroid hormone receptors [522].

Vitamin A plays an important role in the immune system. In most cases, the effect is achieved by hetero-dimerisation of the two nuclear receptors for vitamin A and vitamin D [523]. Retinol binding protein (RBP) is a negative acute phase protein, which leads to a fall in serum retinol [524,525]. Inflammation also reduces absorption of vitamin A and increases requirement and urinary loss which together may contribute to the development of vitamin A deficiency [526].

While it was long believed that absorption occurred via passive diffusion, it is now known that several proteins are involved in its absorption at the intestinal mucosal level [527]. Fat-soluble MNs including vitamin A and carotenoids are assumed to follow the lipids in the gastrointestinal tract [528,529], and their absorption presumably occurs in the upper half of the small intestine. Retinol uptake occurs by a saturable carrier-mediated process at physiological doses, whereas it occurs by passive diffusion at pharmacological doses. Retinoids are well absorbed (75%–100% absorption), whereas carotenoid absorption varies greatly depending on the food matrix and type of carotenoid [530]. Vitamin A circulates in plasma bound to its specific carrier protein RBP, which is part of a larger complex together with prealbumin (transthyretin).

21.1.1. Needs

The average demand is expressed either in retinol activity equivalents (RAE) or in retinol equivalents (RE). RAEs are based on the amount retinol and of carotenoids actually absorbed from food (with a bioconversion factor of 12 mg β-carotene to 1 mg retinol) [531], whereas REs are the amount of retinol and carotenoids present in food (with a bioconversion factor of 6 mg β-carotene to 1 mg retinol) [532]. The recommendation for adults are 700 μg RAE (women) and 900 μg RAE (men) per day. The UL is based on preformed retinol alone.

The older system of International Units (IU) is still occasionally used 1IU was equal to 0.3 μg of retinol.

Principal nutritional sources are liver, meat, fish, and dairy such as cheese and butter; vegetarian sources are sweet potatoes, carrots, beans, spinach, broccoli and mango.

In case of vitamin A intake below recommendation, the liver stores are sufficient to maintain functions for about 6 months.

In PN, the recommended doses are 800–1100 μg per day [533,534].

21.2. Biomarker and analytical methods

The concentration of vitamin A in the liver, or total body retinol are seen as the best measurements of vitamin A status with up to 90% or more of whole-body vitamin A being stored in the liver, mainly in the form of retinyl esters [535]. However, liver tissue analysis is invasive and whole body measurements require stable isotope dilution methods that might not be available [536].

Methodologies for the analysis of serum retinol have been reported using high pressure liquid chromatography with ultraviolet detection [535,537]. Such measurements however are limited by providing an index of vitamin A stores only when body stores are severely depleted or at excessive levels and can also be confounded by protein and zinc deficiencies and by inflammation [536]. The relative dose–response methodology, which is an indirect measurement of hepatic retinol stores using serum analysis before and after an oral retinol dose, is considered valid to determine inadequate vitamin A status [536].

Retinoids (retinol, retinal, retinoic acid, and related compounds) in high concentrations are teratogenic and must then be handled with caution [535]. Furthermore, both retinoids and carotenoids are susceptible to chemical modification by oxygen, radicals and light exposure. During sample collection, preparation and analysis, it is important to use antioxidants, light protection measures and handling/storage temperatures [535]. This has been compiled in a laboratory medicine best practice guideline [538].

A relatively reliable biomarker of overdose is the concentration of retinyl esters in serum. If this exceeds 250 nmol/L a hypervitaminosis may be present.

Retinol Binding Protein in urine (uRBP), is a biomarker of proximal renal tubular disease [539]. Serum RBP4 levels have been found to be 4-fold higher in chronic kidney disease patients (102 mg/L) compared to healthy controls (28 mg/L) [540].

Urinary RBP might be a marker for kidney function and might be used to monitor vitamin A status. In 454 patients with chronic kidney disease (stages 3 and 4) it was observed that the amount of RBP in urine correlated inversely with the glomerular filtration rate. This inverse relationship was found for all forms of albuminuria [541]. Also in patients (n = 90) with ascites in liver cirrhosis a significantly higher RBP excretion was described compared to healthy controls [542].

21.2.1. Conversion

Vitamin A (retinol) = 1 μg/dL = 0.0357 μmol/L and 1 μmol/L = 28 μg/dL

Retinol: 1 RAE = 1 μg = 12 μg β-carotene = 3.33 IU

21.2.2. Effect of inflammation

Serum/plasma retinol concentrations decrease with increasing inflammation [20]. The concentrations demonstrate high specificity after correcting for inflammation, but questionable sensitivity compared with liver stores calculated from isotope dilution [525]. During inflammation, the release of RBP from the liver is reduced. In addition, there is a fall in the plasma concentration of the prealbumin-RBP complex due to redistribution from plasma as part of the systemic inflammatory response. It was therefore suggested to adjust for serum levels of inflammatory biomarkers (CRP, AGP) [525].

Although there is no universal adjustment tool for inflammation, the international group BRINDA developed an all-in-one R package inflammation adjustment equation for, among others, retinol-bindingprotein and serum retinol using the inflammation indicators AGP or CRP for various population groups (https://cran.rproject.org/web/packages/BRINDA/index.html) [777].

21.3. Deficiency

Vitamin A deficiency is a public health problem in most developing countries due to malnutrition, especially in children and pregnant women [527]. Before the well-known signs of vitamin A deficiency (night blindness due to insufficient rhodopsin synthesis, Bitot spots-grey/white, foamy appearance on the conjunctiva, xerophthalmia), there is an increased susceptibility to infections, especially of the respiratory tract as the main symptom [543,544]. There is also impairment of the intestinal immune and barrier function [528].

The worst development is keratomalacia (keratin deposits in which centres of inflammation are spreading), with expansion to iris and lens area leading to Xerophthalmia (maceration of the cornea), secondary infection, and finally blindness.

Deficiency should be sought in:

- *Liver disease:* Patients with chronic liver disease show a high prevalence of vitamin A deficiency. The more severe the disease the more the decline of serum retinol [545].
- *Chronic alcohol* consumption results in a depletion of vitamin A liver stores [546]. Nevertheless, serum retinol and RBP levels may still be in the normal range, but the availability of vitamin A is limited with a risk of to deficit.
- In *liver transplantation candidates*: a vitamin A deficiency (defined only by a low plasma level of retinol) occurs in 69.8% [547].
- *Chronic kidney disease:* High retinol serum levels are often observed in patients with chronic kidney disease and therefore supplementation is not recommended [548]. This increase is only temporary if the liver stores become depleted and can lead to a vitamin A deficiency [549]. Monitoring on a regular basis is recommended.
- *Short bowel syndrome:* due to the risk of reduced fat absorption, these patients may develop deficiency [534]. Further situations are cystic fibrosis, coeliac disease, and chronic diarrhea.
- *Obesity:* RBP is produced in the liver, and in other cells. Thus, adipocytes can form RBP, also called RBP4, and RBP4 has an adipokine function [550,551]. RBP shows a positive correlation with BMI, visceral fat tissue and insulin resistance. Since the apoRBP is also increased, this results in a low retinol-RBP ratio [552].

In cases with very low serum retinol levels, the IV administration of retinol palmitate has been proposed, but the data are not sufficient to make it a recommendation.

In PN, deficiency may occur in prolonged hypermetabolic conditions. Vitamin A is light sensitive [553] and may undergo photodegradation so light protecting material should be used during administration. It may also be lost by adsorption to infusion bags. In long-term PN these losses call for periodic monitoring.

21.4. Toxicity

Acute toxicity develops when quantities of natural vitamin A above 300,000 IU (adults) or > 60,000 IU (children) are ingested within a few hours or days [554]. Symptoms include increased intracranial pressure, nausea, headaches, pain in joints and bones.

Chronic toxicity results from the ingestion of daily amounts of >25,000 IU for more than 6 years or >100,000 IU for more than 6 months, with a high inter-individual variability [555]. Above 14,000 μg/d for longer time periods may cause hepatotoxic effects.

The IOM has set the UL for vitamin A at 3000 μg/d (10,000 IU) for women of childbearing age [556].

21.4.1. How to treat deficiency and toxicity

A number of different oral and IM preparations are available. Mild deficiency can also be treated with the doses proposed in Table 12 [8].

There is no recognized treatment of vitamin A toxicity. If signs of toxicity occur, any supplementation with the vitamin should be stopped.

Table 12
Comparison of recommendations for daily vitamin A administration [8].

Society	Proposed doses
2012 ASPEN consensus Statement North America	Vitamin A/retinol 3300 IU (990 μg RE)
2016 AuSPEN enteral and parenteral nutrition vitamin guidelines [534]	3500 IU (1050 μg RE)
2016 ESPEN Chronic Intestinal failure guidelines	No recommendation
EC direct for FSMP	525–2700 μg RE for 1500 kcal

21.5. Recommendations N°22 – vitamin A (retinol)

21.5.1. When to measure?

Recommendation 22.1

Serum retinol and retinyl esters (if available) measurements should be considered in patients being investigated for malabsorption.

Grade of recommendation B – Strong consensus 96%

Malabsorption/reduced binding protein/reduced storage may occur in the context of several diseases including persistent critical illness.

21.5.2. What to measure?

Recommendation 22.2

Vitamin A status shall be determined by measuring serum retinol.

Grade of recommendation A – Strong consensus 95%

Interpretation can be improved by also measuring CRP and retinol binding protein.

21.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 22.3

Enteral nutrition shall provide 900–1500 μg RE per day, when providing 1500 kcal per day.

Grade of recommendation A – Strong consensus 97%

Recommendation 22.4

Parenteral nutrition should provide 800–1100 μg RE per day.

Grade of recommendation B – Strong consensus 97%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

21.5.4. When to provide additional amounts?

Recommendation 22.5

In conditions causing fat malabsorption, prevention of deficiency with oral supplements may be considered.

Grade of recommendation GPP – Strong consensus 97%

22. Vitamin C (ascorbic acid)

22.1. Main functions

Vitamin C has numerous functions, which are all based on electron donation [557,558]. It is the most potent water-soluble antioxidant, which directly scavenges radicals, mitigates the production of oxygen radicals, and recycles other antioxidants. Furthermore, vitamin C is an important cofactor/cosubstrate for the biosynthesis of neurotransmitters (noradrenaline, serotonin), cortisol, peptide hormones (vasopressin), and collagen. In addition, it protects the endothelium by promoting collagen synthesis, and maintaining endothelial vasodilation and barrier function [559]. Vitamin C can limit the inflammatory response and ischemia-reperfusion injury, improve host defence [560], wound healing [561] and mood [562], and has a role in pain reduction [562,563]. Importantly, vitamin C has an epigenetic role by suppressing response element-controlled genes through enhanced HIF-1 degradation thereby mitigating chronic inflammation and dysoxia [564].

22.1.1. Needs

Humans are unable to synthesize vitamin C, so status strictly depends on dietary intake of fruits and vegetables. The recommended DRI of vitamin C in healthy individuals is 90–100 mg per day [23,565], which results in plasma vitamin C levels between 60 and 100 μmol/L [565,566]. Numerous studies suggest that higher dosages are needed in patients at risk of depletion or deficiency (Table 13).

Table 13
Vitamin C deficiency: pathophysiology, mechanisms, and clinical conditions leading to higher needs.

Pathophysiological conditions	Mechanisms	Clinical conditions
Inflammation Ischemia reperfusion Trauma	Oxidative stress → • metabolic consumption ↑ • recycling ↓	Sepsis Cardiac arrest Trauma, Burns Transplantation Diabetes mellitus Severe COPD
Malnutrition Malabsorption Renal replacement therapy (acute and chronic)	Intake ↓ Enteral absorption ↓ Losses ↑ • glomerular hyperfiltration tubular reabsorption ↓	Bariatric surgery Alcoholism Renal failure

COPD, Chronic obstructive pulmonary disease.

PN doses of Vitamin C are 100–200 mg per day [5,534].

The optimal dose during critical illness remains unknown. Some facts are established. First, during critical illness, IV administration is crucial, as enteral uptake is unpredictable: it is limited because the enteral transporter is satiable [557]) and gut function is often impaired. Second, high vitamin C doses (2–3 g/day) must be administered to restore plasma concentrations to normal [567,568] and sustained therapy is needed to prevent reoccurrence of hypovitaminosis [568]. Third, very high doses (100–200 mg/kg/day) are required to obtain supernormal plasma concentrations [569]. Despite the observation of an association between low plasma values and poor outcome [570], it is not known whether correcting the plasma concentration during severe inflammation improves outcome - further research is needed.

22.2. Biomarker and analytical methods

Assessment of vitamin C status can be determined from its concentration in either plasma or leukocytes [571,572]. Vitamin C includes L-ascorbic acid (AA) and its oxidation product dehydroascorbic acid (DHAA). While intracellular leukocyte vitamin C level is supposed to be more indicative of tissue vitamin C stores, this analysis requires more sample volume and preanalytical steps than plasma and has some caveats related to variability of vitamin C in different cell types [572]. Strict sample collection and preanalytical procedures (stabilization) are required whenever a sampling is done. For these reasons, plasma vitamin C analysis is the preferred option for status assessment. Serum determination should be avoided.

The determination of plasma ascorbic acid necessitates considerable logistical and analytical effort [573]. The high susceptibility of vitamin C to degradation related to temperature, light, pH, dissolved oxygen, and the presence of oxidizing/reducing agents, requires specific pre-analytical precautions. Plasma samples should be immediately separated (centrifugation at 4 °C) after blood drawing and stored at ultra-low-temperature (−70° and −80 °C). Protection against light exposure is recommended along the entire sample collection/preparation/analysis workflow. Following plasma separation, AA is rapidly converted to DHAA (a reversible reaction), and if oxidation is not prevented it irreversibly leads to 2,3-diketogulonic acid. Lithium heparin is broadly employed when total vitamin C (sum of AA and DHAA) is determined [571,574,575]. Efficient analytical methods exist for AA, DHAA or total vitamin C using HPLC with ultraviolet (UV), fluorescence or electrochemical detection [571,574]. A typical sample stabilization preanalytic step before HPLC includes acidification followed by immediate cold storage at ultra-low temperature. Total vitamin C analysis is conventionally performed by HPLC with the addition of a reducing agent such as dithiothreitol (DTT) or dithioerythritol (DTE) to convert DHAA into AA [571,572,575,576]. DHAA can also be determined by substracting the amount of AA before reduction from total vitamin C [577].

Knowing the above technical problems, an alternative may be to estimate plasma ascorbic acid using a recently developed point-of-care device that mesures the blood static oxidation-reduction potential (sORP): the latter is strongly related to the plasma vitamin C concentration. sORP is measured in non-acidifed, non-reduced plasma within 20 min, directly after centrifugation [573].

22.2.1. Reference intervals

Normal plasma vitamin C levels are defined as $\geq$ 23 μmol/L. Hypovitaminosis C has been defined as plasma levels less than 23 μmol/L and vitamin C deficiency as less than 11 μmol/L, although solely on the basis of the plasma levels and without clinical signs [578].

22.2.2. Conversion

Ascorbic acid: 1 mg/dL = 56.78 μmol/L and 1 μmol/L = 0.0176 mg/dL

22.2.3. Effect of inflammation

Vitamin C plasma levels decline rapidly with progressive inflammation, making interpretation difficult [20]. Blood levels decrease as soon as CRP >10 mg/L: normal values are not detected if CRP>40 mg/L.

22.3. Deficiency

Clinical conditions with increased inflammation and oxidative stress, such as sepsis, trauma, cardiac arrest, major surgery, and burns are associated with high risk of depletion. True clinically visible deficiency has only rarely been observed in hospital patients. Plasma vitamin C concentrations decline rapidly and very low values are observed in a substantial proportion of patients [567,569,570,578–587] due to enhanced metabolic demands for vitamin C owing to inflammatory and/or infectious processes. Furthermore, decreased intake, reduced recycling and increased losses play a role. In critically ill patients, low plasma vitamin C concentrations are associated with severity of oxidative stress [588], organ failure and mortality [570].

The requirements in critical care are particularly uncertain. Adding doses > DRI of vitamins C and E to EN has been associated with reduction of markers of oxidative stress [589]. Not to be considered as nutrition but addressing ascorbic acid depletion, numerous studies have trialed the combination of high dose vitamin C with thiamine and hydrocortisone in sepsis after an initial retrospective study [590,591]. But only minor beneficial effects have been observed [591]. Fowler et al. [592] tested “pure” vitamin C, delivering 200 mg/kg/day of vitamin C for 4 days in severe sepsis and acute respiratory failure. First considered “negative” the study finally shows an organ function benefit after recalculation of the SOFA with integration of all cases [593].

Chronic depletion is encountered in patients after bariatric surgery (incidence of about 35%) due to poor intake and malabsorption [594]), those with alcohol abuse (poor intake) [595], chronic dialysis (increased loss, chronic inflammation, poor intake), smoking (oxidative stress) [596], chronic inflammation [597], or chronic oxidative stress (diabetes mellitus [598,599], smoking, heart failure [600], alcoholism, severe chronic obstructive pulmonary disease (COPD) and chronic dialysis).

In patients with malabsorption due to previous bariatric surgery or Crohn's disease, a repletion dose ranging between 200 and 500 mg/day may be required [601,602].

Symptoms of classical scurvy include lassitude; anemia; poor wound healing; myalgia and bone pain; edema, petechiae and easy bruising; spongy and purplish gums that are prone to bleeding; loose teeth; bulging eyes; scaly, dry, and brownish skin with typical corkscrew hairs; dry hair that breaks off close to the skin; shortness of breath. During critical illness, vitamin C deficiency may go unnoticed because the assay is complex and not available, and symptoms mimic critical illness.

22.3.1. When and how to treat

Aims of additional administration are either to achieve plasma concentrations in the high normal range (repletion) in patients without inflammation or to achieve supranormal plasma concentrations to interfere with an overwhelming oxidative stress (pharmacological dosing).

Usually administered orally, vitamin C may be administered IM, IV, or subcutaneously when malabsorption is suspected. For IV injection, the drug should be diluted with normal saline or glucose to minimize adverse reactions [603].

Although cells accumulate vitamin C against a concentration gradient, intracellular concentrations seem to respond to plasma concentrations (summarized in [564,604]).

Vitamin C is unstable in PN, being rapidly oxidized according to first-order kinetics [605]. The degradation is based directly on the amount of oxygen present in the mixture, becoming relevant to the packing material, stimulated principally by high temperatures, and catalyzed by trace elements such as copper. Multilayered bags should be used, and light protection is mandatory.

Numerous studies have tested ascorbic acid as a drug in doses far higher than RDI which beyond the scope of clinical nutrition Table 14 proposes doses in different conditions.

Table 14
Summary of proposed doses of ascorbic acid (please see also Table 15).

	Dose	Level of evidence	Grade of recommendation
Daily recommended dose in healthy	50–100 mg/day	4	Physiology – RDA [565]
Daily dose in PN	100–200 mg/day	3	GPP
Daily recommended dose	500 mg/day	4	GPP [598–600,606,607]
In patients with chronic oxidative stress [a]			
Daily recommended dose	200–500 mg/day	4	GPP
In patients with chronic malabsorption			
Repletion dose during critical illness	2–3 g/day IV	1+	A [291,589,608–611]
Repletion dose during CRRT	2–3 g/day IV	4	GPP
Periprocedural repletion dose cardiac surgery	1-2 g-day for 5–7 days IV	1+	A [612–614]

Abbreviation: CRRT, continuous renal replacement therapy; IV, intravenously.
[a] Diabetes mellitus, heart failure, smoking, alcoholism, severe COPD, intermittent hemodialysis.

22.4. Toxicity

Vitamin C supplementation is contraindicated in blood disorders like thalassemia, G6PD deficiency, sickle cell disease, and hemochromatosis [603]. Reported adverse events include urinary calcium oxalate crystallization, renal stone formation and nephropathy due to increase oxalate excretion in susceptible patients receiving higher than repletion doses for longer periods of time; factitious hyperglycemia in patients with high plasma concentrations when using bedside glucose monitors [615]; hemolysis in patients with G6PD deficiency when using very high (>60 g) dosages. Of note, up to 6 g/day may protect against hemolysis [616]. No adverse events were reported in current high dose clinical trials or meta-analyses [617], nor in a prospective case series exploring high-dose vitamin C (up to 100 g IV thrice weekly) [618].

22.5. Recommendations N°23 – Vitamin C (ascorbic acid)

22.5.1. When to measure?

Recommendation 23.1

Plasma vitamin C concentrations may be measured in all patients with clinical suspicion of scurvy or chronic low intake.

Grade of recommendation GPP – Consensus 86.84%

A clinical trial of vitamin C of about 1 g/day for at least one week, should not be delayed in the presence of clinical symptoms.

Recommendation 23.2

Measurement of plasma vitamin C is not recommended in critical illness or severe inflammation, due to the difficulty in interpretation of results.

Grade of recommendation GPP – Strong consensus 92%

22.5.2. What to measure?

Recommendation 23.3

Vitamin C status should be assessed by a measure of total plasma vitamin C (sum of AA and DHAA) or AA.

Grade of recommendation B – Strong consensus 100%

22.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 23.4

Enteral nutrition shall provide at least 100 mg of vitamin C per day in 1500 kcal.

Grade of recommendation A – Strong consensus 97%

Recommendation 23.5

Parenteral nutrition should provide 100–200 mg vitamin C per day.

Grade of recommendation GPP – Strong consensus 97%

22.5.4. When to provide additional amounts?

Recommendation 23.6

In patients with chronic oxidative stress (diabetes mellitus, smoking, heart failure, alcoholism, severe COPD, and chronic dialysis) or malabsorption, a dose of 200–500 mg/day may be provided.

Grade of recommendation GPP - Strong consensus 92%

Recommendation 23.7

During critical illness, a higher vitamin C repletion dose of 2–3 g per day should be given IV during the acute phase of inflammation.

Grade of recommendation B – Consensus 84%

23. Vitamin D (25-hydroxyvitamin D)

23.1. Main functions

Vitamin D is not a classic vitamin but a steroid hormone precursor. Rickets, as the classic vitamin D deficiency disease, was first described in the 17th century, and the Nobel prize for chemistry was awarded in 1928 to Adolf Windaus for his studies on vitamin D. Cutaneous endogenous production is possible from cholesterol with UV-B exposure, explaining the strong seasonal variation in vitamin D levels. Vitamin D supply is also possible by nutrition (e.g. fatty fish, eggs, some types of mushrooms), but usually does not cover the needs. The vitamin D receptor is expressed in many body tissues including muscle (skeletal and cardiac), bone, immune system, skin, and endocrine organs - this is the other important difference from other vitamins. The classic vitamin D deficiency syndrome is rickets in children, osteomalacia in adults, well known for its visible skeletal changes and still a public health concern today. Vitamin D has classic effects regulating bone and mineral (calcium, phosphorus) metabolism as a key player in the target organs bone, intestine and kidneys. Vitamin D also has non-classic effects on many organs including the immune system, muscle, heart, and nervous system/brain [619]. It is now recognized that vitamin D is involved in the regulation of hundreds of genes.

23.1.1. Vitamin A and D interactions

Various studies suggest that the combined application of both vitamin A and D can selectively improve the function of the vitamin D receptor, especially in the immune response [620]. Patel et al. have demonstrated the beneficial effect of vitamin A and D (20,000

IU Vitamin A, 2000 IU Vitamin D) administration on the effectiveness of influenza vaccination in children [621]. Inflammation of human monocytes has revealed that many genes are regulated differently compared to the non-inflamed state-vitamin A and vitamin D modulate many of these genes and thus modulate the immune response [622].

23.1.2. Needs

The recommended daily oral intakes of vitamin D vary between 600 and 800 IU in adults [623], or 1500–4000 IU in patients "at risk for vitamin D deficiency" [624,625]. Vitamin D deficiency, as defined by a plasma concentration of <50 nmol/L affects about 40% of Europeans, severe deficiency (plasma concentration <30 nmol/L) being present in 13% [626] (see comment below on definition of deficiency).

The general upper daily limit for vitamin D intake is 4000 IU [625], but the Endocrine Society has recommended an upper limit of 10,000 IU for patients „at risk" for vitamin D deficiency [624]. In EN products, usually 400 IU per day are currently provided. Patients requiring nutritional therapy will frequently be depleted/deficient in vitamin D because of low intake and lack of UV light: their need may therefore be significantly higher. Parenteral multivitamin preparations typically include about 200 IU per ampoule. For stable patients the most recent AusPEN recommendations propose 5.5 μg (200 IU) per day in PN [534].

23.2. Biomarkers and analytical methods

Serum/plasma concentrations of total 25-hydroxyvitamin D (25-OHD), the sum of 25-OHD3 and 25-OHD2, is recognized as a valid biomarker for vitamin D status. LC-MS/MS has become the gold standard methodology with several techniques accepted as reference method procedures by the Joint Committee for Traceability in Laboratory Medicine and that can benefit from a standardization and external quality assessment program [627]. LC-MS/MS methods have the technical possibility to quantify separately 25-OHD2 and 25-OHD3 as well as to exclude 3-epi-25-OHD, as this latter should not be included in measurements of total 25-OHD. Besides LC-MS/MS, various commercial automated immunoassays are available with different technical specifics and performance [627].

There is no ideal time to measure 25(OH)D. There is no circadian rhythm, but a strong seasonal rhythm with the lowest levels after winter (March for the Northern Hemisphere) and the highest after

Table 15
ESPEN Recommendations for daily trace element and vitamin intakes – 2022 (all values are per day).

	PN Home & long-term A	PN high requirements[a] B	EN in 1500 kcal[b] C	EN high requirements in 1500 kcal[c]	DRI per day Age 31->70 yrs	EC directive[d]: Min-max per 1500 kcal [25]
Trace elements						
Chromium	10–15 μg	15 μg	35–150 μg	200 μg	20–35 μg	18.75–225 μg
Copper	0.3–0.5 mg	0.5–1.0 mg	1–3 mg	Same as C	0.9 mg	0.9–7.5 mg
Fluoride	0–1 mg	Same as A	0–3 mg	3–4 mg	3–5 mg (AI)	0–3 mg
Iodine	130 μg	Same as A	150–300 μg	Same as C	150 μg	97.5–525 μg
Iron	1 mg	Same as A	18–30 mg	30 mg	8 mg (18 mg F 19–50yrs)	7.5–30 mg
Manganese	55 μg	Same as A	2–3 mg	Same as C	1.8–2.3 mg	0.75–7.5 mg
Molybdenum	19–25 μg	Same as A	50–250 μg	250 μg	45 μg	52.5–270 μg
Selenium	60–100 μg	150–200 μg	50–150 μg	200 μg	55 μg	37.5–150 μg
Zinc	3–5 mg	6–12 mg	10–20 mg	20 mg	8–11 mg	7.5–22.5 mg
Lipo-soluble vitamins						
A Retinol[e]	800–1100 μg	1100 μg	900–1500 μg	1500 μg	700–900 μg	525–2700 μg
D3 Cholecalciferol	200 IU/5 μg	800–1000 IU/ 20–25 μg	25 μg	30 μg	15–20 μg	7,5–37,5 μg
E α-tocopherol	≥9 mg	20 mg	15 mg	40 mg	15 mg	7.5–45 mg
K1 phylloquinone	150 μg[f]	1–10 mg[g]	120 μg	Same as C	90–120 μg	52.5–300 μg
Water-soluble vitamins	Provide at least[h]		Provide at least[h]:			
B1 Thiamine	2.5 mg	100–200 mg	1.5 mg	100 mg	1.1–1.2 mg	0.9–7.5 mg
B2 Riboflavin	3.6 mg	10 mg	1.2 mg	10 mg	1.1–1.3 mg	1.2–7.5 mg
B3 Niacin	40 mg	Same as A	18 mg	40 mg	11–16 mg	13.5–45 mg
B5 Pantothenic acid	15 mg	Same as A	5 mg	7.5 mg	5 mg	2.25–22.5 mg
B6 Pyridoxine	4 mg	6 mg	1.5 mg	7.5 mg	1.5–1.7 mg	1.2–7.5 mg
B7 Biotin	60 μg	Same as A	30 μg	75 μg	30 μg (AI)	11.25–112.5 μg
B9 Folic acid	400 μg	600–1000 μg	330–400 μg DFE	500 μg	400 μg DFE	150–750 μg
B12 Cyancobalamin	5 μg	Same as A	>2.5 μg	7.5 μg	2.4 μg	1.05–10.5 μg
C Ascorbic acid	100–200 mg	200–500 mg	100 mg	200 mg	75–90 mg	33.75–330 mg

Abbreviations: EN = enteral nutrition, FSMP = Foods for Special Medical Purposes, PN = parenteral nutrition, AI = Adequate Intake, DFE = dietary folate equivalent.
Note 1: Major burns and some gastrointestinal conditions (fistulae) may have losses that are not covered by the above "increased doses.
Note 2: Cobalt is provided as vitamin B12: please see text chapters.
Note 3: As there are no DRI for carnitine, choline and CoQ10, they do not appear in the Table: please see text.

[a] Increased requirements may occur in patients with on-going increased losses such as gastrointestinal losses, continuous renal replacement therapy, those who are hypermetabolic or who are depleted before commencing PN, and in pregnancy.

[b] The 1500 kcal value has been chosen based on numerous studies confirming that this value seems to be a very common objective. In case of higher nutrient delivery (e.g. 2000 kcal per day or more), exceeding this recommendation is not exposing the patient to any risk considering upper tolerable levels.

[c] Increased requirements during critical illness and in patients with acute admission with malnutrition (NRS ≥5): intended for max 15 days as repletion, to avoid requiring IV supply.

[d] The EC directive [25] regulates the contents of FSMP. Amounts are indicated per 100 kcal in the EC document. This column indicates the minimal and maximal trace element contents of such FSMP for 1500 kcal/day.

[e] Retinol includes retinol and retinyl ester.

[f] During PN, vitamin K requirements are usually provided by the lipid emulsions.

[g] High dose administered in case of coagulopathy (not nutrition-related).

[h] For water-soluble vitamins, amounts recommended are minimum amounts, and more can usually be safely delivered.

summer (August/September for the Northern Hemisphere) [628]. This should be considered when interpreting an individual serum level.

23.2.1. Conversion

25(OH)D: 1 ng/mL = 2.5 nmol/L and 1 nmol/L = 0.4 ng/mL
Vitamin D3: 40 IU = 1 μg

23.2.2. Effect of inflammation

Plasma levels of vitamin D are significantly reduced in the context of inflammation: in presence of CRP>40 mg/L nearly all values are below reference ranges [20], complicating interpretation.

23.3. Deficiency

The definition and relevance of vitamin D deficiency, particularly in acute illness, remains debated. Also, data on timing of the sample, the influence of fluid loading in early resuscitation, the inflammatory response, and the use of dialysis/ECMO is scarce [629,630].

The terminology generally used for vitamin D deficiency is inconsistent with the terminology in most of the present guidelines where to be deficient requires both a low plasma level and clinical signs or symptoms. Most vitamin D recommendations in the literature link plasma levels to the risk of clinical consequences, but do not require evidence of them.

A level below 50–75 nmol/L (or 20–30 ng/ml) of serum/plasma 25(OH)D concentration is considered to define vitamin D deficiency by most [625,631]. A cut-off <25 or <30 nmol/L (or 10/12 ng/mL) increases the risk for osteomalacia and nutritional rickets dramatically and therefore is considered to determine severe vitamin D deficiency [625]. There are many large and relevant risk groups for vitamin D deficiency, including patients with severe kidney or liver dysfunction, bed ridden and chronically ill patients [626]. Importantly, benefit from vitamin D supplementation can only be expected in deficiency, not in the general population [632].

Vitamin D3 or vitamin D2 may be given by the oral, enteral, IV, or IM route [633,634]. Standard doses within the recommended daily allowance take many weeks to improve/normalize low vitamin D levels. When time is of concern (as for acutely ill patients or after a fragility fracture when aiming to initiate potent antiresorptive or anabolic treatment), a loading dose is necessary. Many regimens from single loading doses of up to 600,000 IU to multiple daily or weekly 50,000 IU doses to weight-adjusted doses have been proposed, but there are only few studies comparing different regimens. As the individual response to a given dose is largely unpredictable and dependent on genetic variations in vitamin D metabolism, a follow-up vitamin D level should be measured at least once after 3–6 months to ensure adequate dosing. While bolus doses are useful in many circumstances, maintenance doses are still needed and longer dosing intervals than a week may be inefficient or even harmful [635,636].

23.3.1. When to treat?

There are two different strategies for vitamin D treatment in acute illness: 1) physiological complementation, providing at least the DRIs determined for healthy subjects, and 2) high dose supraphysiological supplementation aiming to correct/improve vitamin D levels rapidly. The usual vitamin D complements should be provided to all patients during the hospital stay, but currently standard commercial enteral/parenteral products contain less than the minimum recommended 600–800 IU for healthy adults and even more so the 1500–2000 IU for patients at risk [637]. The second option has been considered in critically ill patients who display a high prevalence of low vitamin D levels: a clear association with greater illness severity, morbidity, and mortality has been observed in both medical or surgical adult and pediatric ICU patients [79]. The most important question remains unanswered: whether low vitamin D status is caused by the acute illness itself, simply reflecting greater disease severity, or if it represents an independent and modifiable risk factor amenable to rapid normalization through loading dose supplementation.

23.3.2. High-dose bolus

A parenteral high dose native vitamin D (cholecalciferol) preparation (50,000 IU) is needed for patients with vitamin D deficiency not responsive to oral vitamin D supplementation [638]. Such a dose is currently available only as IM injection. However, IM is more complicated and may be contraindicated in many patients due to anticoagulation or infection risk. In some countries, oral calcifediol [25(OH)D] is available and may be a good alternative as it has a higher rate of intestinal absorption, and this may have important advantages in case of decreased intestinal absorption capacity [639].

Three meta-analyses on vitamin D in critical care have been published, each one with important limitations and differing results [640–642]. The VIOLET trial including 1078 adult ICU patients with low vitamin D (25(OH)D < 20 ng/ml [50 nmol/]) did not show a difference between the placebo group and the vitamin D group [643]: a one-time ultra-high loading dose (540,000 IU) was given without a maintenance dose. This approach has been shown to be inefficient in a large meta-analysis for the prevention of respiratory infections, while daily or weekly vitamin D showed a strong protective effect, especially in severe vitamin D deficiency [644].

23.4. Toxicity

Intoxication is rare, but has been described with 1) true overdoses, deliberate or accidental (typically single doses of millions IU or daily doses of >10,000 or even 100,000 IU), 2) manufacturing errors and 3) increased vitamin D sensitivity (i.e. CYP24A1 loss of function mutations, or idiopathic infantile hypercalcemia) [645]. Vitamin D toxicity symptoms are mediated by high calcium levels and include hypercalcemia, hypercalciuria, dizziness and renal failure [646].

23.5. Recommendations N°24 – Vitamin D (25-hydroxyvitamin D)

23.5.1. When to measure?

Recommendation 24.1

Vitamin D status may be determined in all patients considered at risk of vitamin D depletion or deficiency.

Grade of recommendation 0 – Strong consensus 92%

23.5.2. What to measure?

Recommendation 24.2

Status shall be determined by serum 25-hydroxyvitamin D (25(OH)D).

Grade of recommendation A – Strong consensus 95%

23.5.3. How much to provide in typical enteral and parenteral nutrition regimen?

Recommendation 24.3

Enteral nutrition shall provide at least 1000 IU (25 μg) per day of vitamin D in 1500 kcal.

Grade of recommendation A – Strong consensus 95%

Patients on EN frequently receive 400–800 IU/day. Although this may be adequate in some patients, the above dose is higher because patients receiving EN are likely to have higher requirements because of poor status resulting from prior illness.

Recommendation 24.4

Parenteral nutrition should provide at least 200 IU (5 μg) of vitamin D per day.

Grade of recommendation B – Strong consensus 95%

Some patients will have higher requirements, which should be checked by a blood determination. The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

23.5.4. When to provide additional amounts?

Recommendation 24.5

Vitamin D in doses 4000–5000 IU (100–125 μg) per day should be administered for 2 months in patients with recurrent deficiency to achieve blood levels of 25(OH)D between 40 and 60 ng/ml. Substantially higher doses might be required. Severity of deficiency and dose required for treatment will determine the frequency of blood determination for efficacy and safety.

Grade of recommendation B - Strong consensus 100%

Studies have suggested that these doses are required in patients who have recurrent deficiency with extremely low 25(OH)D levels [647]. Populations at risk include inflammatory bowel disease, obese adults, bariatric surgery, chronic liver disease, pancreatic insufficiency, chronic intestinal failure, pregnant women, and older adults.

Patients with advanced and chronic kidney disease are a group requiring specialized care.

24. Vitamin E (α-tocopherol)

24.1. Main functions

Vitamin E, a fat-soluble antioxidant, plays an essential role in normal metabolism. α-Tocopherol, the natural vitamin E with the highest biological activity, is a component of all biological membranes and is the most important lipid-soluble antioxidant. Its most important function is to protect membrane lipids, lipoproteins and depot fats from lipid peroxidation [648]. Vitamin E can protect the polyunsaturated fatty acids in the membrane from oxidation (so called chain breaking antioxidant), regulate the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which arise during metabolic processes and inflammation, and modulate signal transduction [649]. Oxygen radicals are produced especially during hypermetabolism with pronounced ATP hydrolysis, so a good supply of vitamin E is important. Oxidative stress is involved in acute and/or chronic postoperative complications, such as acute respiratory distress syndrome (ARDS), inadequate wound healing, sepsis, and multiple organ failure [650]. Oxidative stress is defined as an imbalance between the extent of reactive oxygen species and a comparable low antioxidant capacity of a biological system [651].

Vitamin E inhibits protein kinase C (PKC) activity by increasing PKC-dephosphorylation through the activation of protein phosphatase 2 A. This inhibition has been reported in various cells, with the inhibition of platelet aggregation; reduced proliferation of monocytes, macrophages, neutrophils, and vascular smooth muscle cells; and decreased superoxide production in neutrophils and macrophages [649,652]. Vitamin E has been shown to exert immunostimulatory effects: intervention studies have reported increased lymphocyte proliferation in response to mitogenic stimulation, enhanced delayed type hypersensitivity response, increased IL-2 production, and decreased IL-6 production with vitamin E supplementation above the recommended levels [649].

The activity of vitamin E is limited to the naturally occurring form, α-tocopherol, and the synthetic forms. As they are not converted to α-tocopherol by humans, the other naturally occurring forms of Vitamin E (β–, γ– and δ-tocopherol and tocotrienols) do not contribute toward meeting Vitamin E requirements [23,648].

The mechanism of vitamin E absorption by the enterocyte following its solubilization in a micellar form, and the mechanisms for traversing the cell and secretion in lipoproteins have not been entirely clarified [653]: absorption is enhanced if vitamin E supplements are consumed with fat, and inhibited by disorders causing impaired bile secretion.

24.1.1. Needs

The DRI for vitamin E for adult men and women (and individuals 14–18 years) is set at a daily EAR of 12 mg α-tocopherol and a RDA of 15 mg for both men and women [23]. EFSA recommends for adults, an AI for α-tocopherol of 13 mg/day for men and 11 mg/day for women [654]. The RDA is 15 mg in pregnancy, and 19 mg/day in lactation. The aging process does not reduce the absorption or tissue concentrations of vitamin E [23], but intakes as high as 200 mg/day may be needed for optimal immune function in older people [655].

Estimates of adequate vitamin E intakes depend on the intake of polyunsaturated fatty acids [656,657]: 0.5 mg RRR-α-tocopherol should be consumed for every gram of diene fatty acids. Several plant oils do not contain these amounts (e.g., in soy oil, approximately 0.3 mg). An intake of 24 mg of diene equivalents (18 g linolenic acid) would therefore mean a calculated requirement of 12 mg α-tocopherol per day.

In PN, the intakes vary with the type of lipid emulsion used [658]. The α-tocopherol content can be quite high with the w-9 and w-3 fatty acids and contributes to liver protection [659,660], compared to the previous w-6 soybean solutions. There is a variable amount of the different isomers of vitamin E (α, β, γ, δ-tocopherol) in fat emulsions depending on the lipid base (Olive-, Fish-, Soybean-oil), but alpha tocopherol should always be added to ensure an AI.

Recommendations for vitamin E in adult parenteral solutions are in the range of 9–10 mg/day for adults [661,662], the most recent recommendations come from AuSPEN [534]. Whether this dose is sufficient to ensure adequate body stores and sufficient antioxidant activity is controversial. In adult patients receiving home PN, high breath pentane (indicator of lipid peroxidation) was associated with low vitamin E plasma levels [662]. Vitamin E is available in parenteral vitamin additives in doses between 9.1 and 10.2 mg.

24.2. Biomarker and analytical methods

Vitamin E status is determined by the quantification of α-tocopherol in blood plasma or serum [661] collected into plain, gel separator, heparin or EDTA tubes followed by HPLC coupled with UV or fluorescence detection. Because the circulating levels of α-tocopherol, lipoproteins and cholesterol are positively correlated, it is recommended to express vitamin E levels as a ratio to lipids (cholesterol and triglycerides) [663]. Like many antioxidant molecules, sample processing and storage requires special attention to ensure the validity of the results: it is recommended to chill samples to 4 °C during the transport to the laboratory. The sample storage temperature depends on the delay from collection/processing to analysis with recommended temperatures of 4 °C (between 24 and 96 h), ≤-20 °C (up to 6 weeks), and ≤-70 °C (longer term storage) [538]. Depending on the sample preparation strategy, the use of an antioxidant such as ascorbic acid may be required to prevent artefactual degradation of α-tocopherol [661]. Other biomarkers such as the vitamin E urinary catabolites (2′-carboxyethyl-6-hydroxychroman products) have interesting perspectives as alternative vitamin E status indicators but still require clinical validation [661].

The major part of vitamin E is transported in LDL and from these it is released to endothelial cells. A maximum load is approximately 9 mol vitamin E/mol LDL. As the plasma vitamin E level is regulated via the activity of the tocopherol binding protein, a normal level may exist despite low tissue levels. Plasma vitamin E is age dependent, and in children and young people significantly lower than for adults. Concentrations of α tocopherol <12 μmol/L are defined as inadequate in healthy adults [23], while values > 12 μmol/L do not necessarily indicate a sufficient vitamin E supply in patients on home PN.

The vitamin E plasma value should therefore be related to LDL to exclude the effects of hyperlipidemia. The normal value of α-tocopherol is about 7 mol/mol LDL, for γ-tocopherol 1.5 mol/mol LDL. If only the vitamin E level is determined, then the normal value is around 20–30 μmol/L. In fat malabsorption, low plasma lipid levels can affect plasma vitamin E levels, thus a low ratio of plasma α-tocopherol to plasma lipids (<0.8 mg/g total lipid) is the most accurate indicator of inadequacy in adults.

The relation of vitamin E to the lipoproteins is valid in absence of malnutrition. However, malnutrition often leads to changes in lipoproteins, especially LDL. If both plasma lipids and α-tocopherol are abnormally low, then correction of circulating α-tocopherol concentrations for plasma lipids will yield a value indicating a normal α-tocopherol:lipid ratio. The assumption of adequate vitamin E status would then be invalid because the low lipids reflect the inadequacy of the plasma carriers for delivery of vitamin E to tissues [664].

24.2.1. Conversion

α-tocopherol: 1 mg/dL = 23.22 μmol/L and 1 μmol/L = 0.043 mg/dL

d-α-tocopherol (natural) 1 IU = 0.67 mg

Or dl-α-tocopherol (synthetic) 1 IU = 0.9 mg

24.2.2. Effect of inflammation

Blood levels of vitamin E are little affected by inflammation [20]: nevertheless the blood concentrations become less interpretable with CRP values > 80 mg/L.

Frey et al. showed that in 13 patients who had undergone cardiac surgery and developed post-operative systemic inflammation and multiple organ failure, the ratio of γ−tocoquinone (degradation product of vitamin E) to γ-tocopherol in plasma increased significantly compared to a control group without complications [664]. These data suggest α-tocopherol is degraded in inflammatory and organ failure conditions due to increased lipid peroxidation.

24.3. Deficiency

Deficiency is rare and may appear in context of severe malnutrition. Patients with fat malabsorption due to either inflammatory diseases or cystic fibrosis are at risk of inadequate supply of fat-soluble MNs [665]. Genetic causes include abetalipoproteinemia with disturbance of absorption [666], or the absence of the α-tocopherol transfer protein (α-TTP), with distribution restrictions.

In adults with fat malabsorption, early vitamin E inadequacy is generally asymptomatic [667]. Neurological symptoms are associated with balance and coordination disorders, peripheral neuropathy, and muscle weakness. Instructions to reduce fat intake as part of weight management results in a 50% reduction in vitamin E intake [668,669]. In cases of bariatric surgery this problem may increase.

When to treat: In cases of long-standing fat malabsorption (e.g. short bowel syndrome), Vitamin E supplementation (200 mg/day) improves neurological symptoms after a few months, following normalization of vitamin E status [667]. Rarely IV supplements may be required.

24.4. Toxicity

Toxic effects from high doses of vitamin E are rare even after a high intake for several years [670]. The UL for adults is set at 1000 mg (2325 μmol) [23]. From numerous studies on the prophylactic and therapeutic use of vitamin E, it has been concluded that vitamin E, even in large supplemental oral doses of up to 3200 IU per day, causes no consistent adverse effects. Data regarding toxicity of parenteral vitamin E do not exist.

Vitamin E supplements may induce bleeding risks. One study in adults with normal coagulation (clotting) status found that daily supplementation with 1000 IU (670 mg) of RRR-α-tocopherol for 12 weeks decreased γ-carboxylation of prothrombin, a vitamin K-dependent factor in the coagulation cascade [671]. Individuals taking anticoagulant drugs like warfarin and those who are vitamin K deficient should not take vitamin E supplements without medical supervision because of the increased risk of bleeding [672].

24.5. Recommendations N°25 – Vitamin E (α-tocopherol)

24.5.1. When to measure?

Recommendation 25.1

Vitamin E should be determined when there is clinical suspicion of Vitamin E deficiency. These would include cystic fibrosis, a-beta lipoproteinemia, and thrombotic thrombocytopenic purpura (TTP). In the absence of clinical signs of deficiency, there is no indication to measure vitamin E status during PN.

Grade of recommendation B – Consensus 89%

24.5.2. What to measure?

Recommendation 25.2

To detect vitamin E deficiency, plasma α-tocopherol should be measured.

Grade of recommendation B – Strong consensus 95%

24.5.3. How much to provide in typical enteral and parenteral nutrition regimens?

Recommendation 25.3

Enteral nutrition shall provide at least 15 mg α-tocopherol per day with 1500 kcal.

Grade of recommendation A – Strong consensus 100%

Recommendation 25.4

Parenteral nutrition should provide at least 9 mg α-tocopherol per day.

Grade of recommendation B - Strong consensus 97%

The literature would only justify a grade 0. However, since these doses have been extensively and safely used for many years, together with knowledge of physiology and nutritional requirements, the working group decided to upgrade it to grade B.

24.5.4. When to provide additional amounts?

Recommendation 25.5

Vitamin E should be supplemented if plasma α-tocopherol levels are below <12 μmol/L, starting with 100 mg per day depending on the cause of depletion/deficiency.

Grade of recommendation GPP – Strong consensus 92%

25. Vitamin K (phylloquinone)

25.1. Main functions

Vitamin K includes a group of lipid-soluble molecules that possess carboxylase enzyme cofactor activity necessary for the activation of vitamin K dependent-proteins (VKDPs) [673]. These include the coagulation factor proteins C, S, M, Z, factors VII, IX, X and prothrombin. VKDPs also include Bone Gla (gamma-carboxyglutamic acid) Protein (BGP, or osteocalcin), Matrix Gla Protein, Growth Arrest-Specific 6 Protein (Gas6), Gla Rich Protein (GRP) and Periostin, which are important for bone and vascular health, metabolism as well as reproduction [674,675]. VKDPs that play important roles in anti-tumour responses have been investigated [675,676].

Vitamin K includes vitamers known as vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). While phylloquinone is produced by plants, menaquinones are synthetized by human intestinal microbiota: it also occurs in fermented foods and animal products. Menaquinones are designated as menaquinone-n (MK-n) where n represents the number of 5-carbon units in the side chain of the molecules (MK-2 to MK-14). Vitamin K3 (menadione) is a synthetic provitamin K that requires conversion to menaquinone-4 (MK-4) to be active [677].

Vitamin K status has been associated with lower concentrations of inflammatory markers in vivo [677], and has been proposed to exert an anti-inflammatory role by suppressing nuclear factor κB (NF-κB) signal transduction [678]. A protective effect against oxidative stress has been suggested through blockage of ROS generation [678,679].

25.1.1. Needs

The AI for vitamin K is 1 μg/kg body weight per day according to EFSA and 120 μg for male adults and 90 μg for female adults as recommended by IOM [135]. The present AI is performed for vitamin K1 only, due to lack of data for vitamin K2 [677].

The most abundant nutritional sources are leafy greens, cruciferous vegetables, asparagus, prunes, peas, and parsley.

Many intestinal bacteria, including E. coli synthesize vitamin K2, but not K1, and contribute to meet vitamin K requirements [680].

Enteral Nutrition: Vitamin K content in EN formula may range from a minimum of 3,5 μg up to 20 μg/100 kcal, but generally provide the recommended daily dose [681]. Nevertheless, it is important to consider the significant impact of EN on anticoagulation response in patients on vitamin K antagonists and an adjustment in the drug administration with a 1-h interruption of EN before, and after anticoagulant administration.

Parenteral Nutrition: The natural source of vitamin K in PN is phylloquinone contained in the lipid emulsion. Depending on the lipid source, the vitamin K content may range from a minimum of 6 μg up to 300 μg/100 g [682]. Weekly IV supply of 250–500 μg phylloquinone from lipids is sufficient to both restore and maintain plasma phylloquinone within the normal range. Nevertheless, adult multivitamin preparations with vitamin K provide additionally 150 μg which not only cover the requirements of all patients, but also is more effective in maintaining the carboxylation status of noncoagulation Gla proteins [683–686].

Anticoagulant control in patients taking vitamin K antagonists may be improved by a regular vitamin K intake at recommended dose, but the amount from lipids needs to be factored into requirement calculations, because higher doses than 150 μg could cause K antagonist resistance [682].

25.2. Analytical methods and biomarkers

Although several methods looking either at direct/indirect or functional measurements are available, there is no single biomarker established to determine Vitamin K status. The quantification of circulating phylloquinone (vitamin K1) in blood plasma or serum remains the most used marker of vitamin K status, although being mainly a biomarker of short term phylloquinone intake.

Different analytical approaches are available including high pressure liquid chromatography coupled to fluorescence, electrochemical or even more recently mass spectrometry [687]. Lack of vitamin K availability results in the circulation of biologically inactive forms (i.e. undercarboxylated) of vitamin K-dependent proteins. The measurement of PIVA-II (protein induced by vitamin K absence or antagonism-II, or undercaboxylated factor II) is a quantification of circulating levels of undercaboxylated prothrombin species. This is recognized as a sensitive homeostatic biomarker of hepatic subclinical vitamin K deficiency [677,687]. Automated immunoassay of PIVKA-II species using chemiluminescent reaction is available for routine application [677,687]. Other vitamin K functional markers such as prothrombin or partial prothrombin time, circulating concentration/activity of blood coagulation factors, undercarboxylated osteocalcin and matrix g-carboxyglutamic acid protein, urinary concentration of Gla (g-carboxyglutamic acid) residues or of vitamin K metabolites have also been used. These have limitations such as evidence of a dose–response relationship with phylloquinone intake, insufficient sensitivity and specificity, lack of cut-off values of adequate vitamin K status or of being representative of whole-body vitamin K status [677]. On a general note and whatever the chosen analytical method, special preanalytical, analytical and storage conditions must be applied to prevent vitamin K degradation by light exposure, alkaline conditions and temperature [677,687].

Concentrations <0.15 μg/L are indicative of vitamin K1 depletion/deficiency.

25.2.1. Effect of inflammation

Vitamin K status has been associated with lower concentrations of inflammatory markers in vivo and has been proposed to exert an anti-inflammatory role by suppressing NF-κB signal transduction [678,679].

25.3. Deficiency

The most common causes of vitamin K deficiency are conditions with fat malabsorption (celiac disease, cystic fibrosis, short bowel, etc), malnutrition, antibiotic and anticoagulant (warfarin) treatments.

Vitamin K deficiency can contribute to significant bleeding, poor bone development, osteoporosis, and increased cardiovascular disease. In normal healthy adults, 8–31% have vitamin K deficiency based on undercorboxylated protein analysis. Classically, the deficiency results in the prolongation of prothrombin time with impaired clotting or bleeding, is confirmed by response to vitamin K. However, clinically significant bleeding has mainly been reported in new-borns [688] and extremely inadequate intake or malabsorption syndromes.

25.4. Toxicity

Vitamin K1 and vitamin K2 are not associated with toxicity. Rare anaphylactoid reactions with bronchospasm and cardiac arrest after IV vitamin K1 (phytonadione) administration for anticoagulation reversal have been reported [689].

The synthetic vitamin K3 is very toxic and could cause jaundice, hyperbilirubinemia, hemolytic anemia, and kernicterus in infants. The mechanism is associated with its water-soluble properties and increases oxygen uptake in the liver, leading to a significant increase in lipid peroxidation, which in turn causes cell damage and death. For this reason, vitamin K3 is no longer available [673].

25.4.1. When and how to treat?

The maximum effect for IV administration is 6–12 h, while oral supplementation will take about 24 h [673]. Alterations in the intake of vitamin K can affect the response to anticoagulant agents. However, through dose titration and patient counselling, the maintenance of stable anticoagulation control is possible, if the patient's vitamin K intake is known [690].

Administration of vitamin K may result in interactions [691]:

Patients using anti-vitamin K drugs should be monitored (blood clotting tests) and should avoid making major sudden changes in their vitamin K intake.

Continuous EN should be withheld for 1 h before and after anticoagulant drugs administration to prevent interactions.

Additional amounts from lipid emulsions during PN should be included in requirement calculations. These treatments are summarized in Appendix C.

25.5. Recommendations N°26 – Vitamin K (phylloquinone)

25.5.1. When to measure?

Recommendation 26.1

The vitamin K status may be measured in at risk patients, including pathologies causing steatorrhea, prolonged use of broad-spectrum antibiotics, and chronic kidney disease.

Grade of recommendation GPP – Consensus 89%

25.5.2. What to measure?

Recommendation 26.2

Vitamin K status shall be determined by a combination of biomarkers in combination with dietary intake, as there is no agreed standard.

Grade of recommendation A – Strong consensus 95%

25.5.3. How much to provide in typical enteral and parenteral nutrition regimen?

Recommendation 26.3

Enteral nutrition in adults should provide at least 120 μg vitamin K per day with 1500 kcal.

Grade of recommendation B – Strong consensus 97%

Recommendation 26.4

Parenteral nutrition may provide 150 μg of vitamin K1 per day.

Grade of recommendation 0 – Strong consensus 95%

26. L-carnitine

26.1. Main functions

Carnitine is not classified as a vitamin but being essential in energy metabolism [5], it has been included as "assimilated vitamin" in the present guidelines. Carnitine is a quaternary ammonium compound, an amino acid derivative found in high energy demanding tissues (skeletal muscles, myocardium, liver and adrenal glands) [692]. Its primary role is in fatty acid metabolism, but it is also involved in glucose metabolism [693].

Carnitine is the carrier molecule which transports long-chain fatty acid from the cytosol across the outer and inner membranes of the mitochondrial matrix for β-oxidation, i.e. for energy generation [693]. Fatty acid oxidation is controlled by the carnitine palmitoyltransferase system, which consists of three enzymes: carnitine palmitoyltransferase I (CPT I), carnitine palmitoyltransferase II (CPT II), and carnitine:acylcarnitine translocase (CACT) [694].

A summary of metabolic pathways is reviewed in Appendix D.

Carnitine can be biosynthesized within the human body (kidney, liver) using amino acids (L-lysine and L-methionine) as substrates [695]. Healthy individuals (children and adults), including strict vegetarians, synthesize enough L-carnitine in vivo not to require supplementation [696].

Carnitine is absorbed in the small intestine via more than one transporter, and the type of transport varies with the dose of ingested carnitine [697].

26.1.1. Needs

Carnitine is not considered essential by the Food and Nutrition Board of the National Academies hence there are no RDA or DRIs [135]. The typical carnitine intake of omnivore humans is 2–5 mg/kg/day, which averages to about 250 mg/day for a 70-kg human. Nutritional supplementation of carnitine should be in this range. L-Carnitine is found in animal products with red meats, such as beef and lamb, being the best choices for adding carnitine into the diet. Good carnitine sources also include fish, poultry, and milk. Milk is the main source of carnitine for infants. The concentration of carnitine increases as the proportion of type I muscle fibers increases. Thus, as the redness of the meat increases, the carnitine concentration increases.

26.2. Biomarkers and analytical methods

The concentrations of total carnitine, free carnitine, carnitine esters and the carnitine precursors are required to assess carnitine status. Free and total carnitine can be measured by tandem mass spectrometry (MS/MS) stable isotope dilution analysis. Hydrolysis enables measurement of total carnitine, and esterified carnitine (acylcarnitine) is calculated as the difference between the total and

free carnitine. From these values the acyl-to-free carnitine ratio can be calculated: if the latter ratio = 0.25, the status is normal, if the ratio >0.4, carnitine deficiency is present [693]. These determinations require access to specialist laboratory facilities.

Investigation of a suspected deficiency requires the simultaneous determination of blood triglycerides, liver function tests (AST; ALT), glucose, lactate, ammonium, and urine ketones.

26.2.1. Effect of inflammation

Inflammation does not directly affect blood levels of carnitine. On the contrary, carnitine seems to be a strong anti-inflammatory agent in several trials.

Chronic renal failure with uremia is associated with an enhanced inflammatory response and an increased oxidant load. Regular L-carnitine supplementation is used in hemodialysis patients to improve cellular defense against chronic inflammation and oxidative stress, most likely by modulating the specific signal transduction cascade activated in this condition [698]. A meta-analysis including 13 trials in hemodialysis patients indicated that L-carnitine supplementation was significantly associated with lower levels of CRP compared to controls [699]. L-carnitine reduced inflammatory mediators, especially in studies with a duration of more than 12 weeks.

In patients undergoing gastric or colorectal cancer surgery, a small RCT showed that L-carnitine supplementation increased serum concentrations and decreased CRP between postoperative days 3 and 7 significantly more than the placebo (P = 0.011) [700].

In coronary artery disease patients, a RCT showed that L-carnitine supplementation (1 g) attenuated inflammation [701].

26.3. Deficiency & depletion

Carnitine deficiency is a condition that prevents the body from using various types of fats. There are two types of carnitine deficiency [702].

1) Primary carnitine deficiency is a genetic disorder of the cellular carnitine-transporter system that usually manifests itself by five years of age with symptoms of cardiomyopathy, skeletal muscle weakness, and hypoglycemia.
2) Secondary carnitine deficiencies may occur in chronic renal failure, or under particular conditions (e.g., use of certain antibiotics, organic acidemias and other inborn errors of the metabolism) that reduce carnitine absorption or increase its excretion.

The first case report of carnitine deficiency was in 1973 [697]. In adults, carnitine deficiency is most commonly iatrogenic [703]. Biologic effects of low carnitine levels may not be clinically significant until they reach less than 10–20% of normal. Profound carnitine deficiency causes hypoketotic hypoglycemia due to FA oxidation impairment, but also muscle weakness, rhabdomyolysis, cardiomyopathy, arrhythmia and sudden death. Acute deficiency includes increased plasma triglycerides and lactate associated with a rapid loss of lean body mass resulting in amyotrophy and hepatomegaly with fatty liver changes [703].

Dialyzed patients: For years, nephrologists have been using carnitine therapy (20 mg/kg) in dialyzed patients with cardiomyopathy, muscle weakness and erythropoietin unresponsive anemia [704]. The rationale for carnitine supplementation is that this small molecule is highly dialyzable and low plasma carnitine levels are prevalent in dialysis patients, many of whom have hypertriglyceridemia. Prospective trials are sparse [705,706].

Patients with HIV have been recognized as being at risk for carnitine deficiency and mitochondrial dysfunction, due to the specific toxicity of the pharmacological treatments controlling the disease [707]. HIV treatment with antiretroviral therapy [703], and drugs such as valproate interfere with mitochondrial metabolism and carnitine intracellular pool [708,709].

Critically ill are at high risk of deficiency in presence of preexisting energy protein malnutrition, acute renal failure with continuous renal replacement therapy, prolonged PN without supplementation, prolonged status epilepticus treated with valproate, and antiretroviral therapy [703]. The risk for deficiency increases by the end of the first month. Carnitine deficiency probably occurs in chronic critically ill patients more often than is known due to difficulty in getting the analyses. Deficiency causes major alterations of mitochondrial energy metabolism, resulting in organ dysfunction, as shown by a Japanese study of the dynamic evolution of carnitine [710]. Carnitine deficiency (free-carnitine <36 nmol/mL) was observed in 23.4% of their patients at ICU admission [710].

In *septic shock* a pilot study of early high dose carnitine supplementation in 31 patients was inconclusive but generated interesting hypotheses on potential responders and non-responders [711,712]. A subsequent dose finding study showed no beneficial effect on the evolution of early organ failure scores [713]. Of note, both RCT's focused on application of carnitine as a drug rather than treatment of carnitine deficiency. In addition, carnitine deficiency might play a role in the poorly understood yet potentially lethal propofol infusion syndrome [714].

Intestinal failure patients, and particularly those with intestinal resection are at risk of deficiency, as endogenous carnitine synthesis from lysine and methionine may become insufficient [715,716]. Approximately one-third of patients with gut failure on long-term home PN have low total and free plasma carnitine concentrations [717].

Parenteral nutrition: There is no carnitine in PN solutions nor in the vitamin solutions, therefore these patients are dependent on in vivo carnitine synthesis and have the possibility of developing carnitine deficiency. Carnitine is currently added to neonatal PN, and only in selected cases adult PN [5].

Enteral nutrition: The carnitine content of the diets depends on the protein source. Products whose main protein source is soy protein isolate, casein, or egg white contain sufficiently to cover the needs [718]. Products that contain proteins of animal origin contain about 60 mg/500 ml. Deficiency may arise in case enteral products originating from peas are used.

26.3.1. When and how to treat

In patients on prolonged PN and prolonged continuous renal replacement therapy, carnitine deficiency or depletion should be considered: alternatively, prevention of deficit is simple [719]. L-Carnitine can easily be administered, although guidelines are currently missing [703]. In the patients at risk, a systematic supplementation of 0.5–1 g/day should be considered.

Pharmacologic supplementation of carnitine is usually dosed at one order of magnitude higher (50–100 mg/kg/day), with adults often receiving 3 g/day of carnitine [697].

26.4. Toxicity

At doses of approximately 3 g/day, carnitine supplements can cause nausea, vomiting, abdominal cramps, diarrhea, and a “fishy” body odor. Rarer side effects include muscle weakness in uremic patients and seizures in those with seizure disorders [720].

A case report that occurred in the context of a metabolic study, found that acute infusion of 100 mg carnitine over 4 h resulted in an increased rate of protein oxidation and a reduced rate of fat oxidation on both PN-regimens that were tested [721]. These may

be due to an impairment of fat oxidation by excess amounts of carnitine.

26.5. Recommendations N°27 – L-Carnitine

26.5.1. When to measure?

Recommendation 27.1

Carnitine determination is not a routine requirement. In critically ill patients, carnitine status should be explored in presence of an unexpected loss of lean body mass, with the concomitant presence of hypertriglyceridemia and hyperlactatemia, particularly in case of prolonged parenteral nutrition or continuous renal replacement therapy.

Grade of recommendation GPP – Strong consensus 91%

26.5.2. What to measure?

Recommendation 27.2

The simultaneous concentrations of total carnitine, free carnitine, carnitine esters and the carnitine precursors should be measured, to enable the calculation of the acyl-to-free carnitine ratio. This should only be used to confirm a clinical diagnosis and should not delay commencing supplements.

Grade of recommendation GPP – Strong consensus 91%

26.5.3. How much to provide in typical enteral or parenteral nutrition regimens?

Recommendation 27.3

Carnitine is not an essential nutrient: at this time there is insufficient evidence to support its routine addition in enteral nutrition or parenteral nutrition.

Grade of recommendation 0 – Strong consensus 100%

26.5.4. When and how to provide additional amounts?

Recommendation 27.4

In proven deficiency situations, the administration of L-carnitine supplementation of 2–5 mg/kg/day has been suggested via the route used for administration of macronutrients, until carnitine and acyl-to-free ratio revert to normal values.

Grade of recommendation GPP – Strong consensus 91%

Availability of suitable supplements may be limited.

Recommendation 27.5

In case of antiretroviral drug toxicity, pharmacologic doses (50–100 mg/kg/day) may be administered.

Grade of recommendation 0 – Strong consensus 100%

27. Choline

27.1. Main functions

Choline, a quaternary amine, appeared on the list of essential nutrients of the Institute of Medicine in 1998 [722]. Endogenous biosynthesis is insufficient even when availability of vitamin B12 and folate is abundant. Choline availability depends moreover on the intestinal microbiota [723].

Choline and its derivatives serve as components of structural lipoproteins, blood and membrane lipids, and as a precursor of the neurotransmitter acetylcholine [724]. Consequently, choline availability impacts central and peripheral neurotransmission, quality of cell membranes, and production of very low-density lipoproteins in the liver. Choline is oxidized to betaine that serves as an osmoregulator and is a substrate in the betaine–homocysteine methyltransferase reaction, which links choline and betaine together with vitamin B12- to folate-dependent one-carbon metabolism [327,724].

Pancreatic exocrine function is required for liberation of choline from phosphatidylcholine (PhC), phosphocholine (PC) and glycerophosphocholine. Free choline follows the portal circulation while phosphatidylcholine requires integration in chylomicrons to be absorbed via the lymphatic vessels [327].

The endogenous production of choline via phosphatidylcholine is catalysed by phosphatidyl-ethanolamine-N-methyltransferase (PEMT) which is oestrogen dependent: this explains differences in endogenous production and in appearance of clinical signs of choline depletion before and after the menopause and during pregnancy [725,726].

27.1.1. Needs

The AI of choline preventing liver damage is set at 550 mg/day for male adults and 425 mg/day for women [327,722,727]: in absence of data, no EAR could be defined. More recently the European Food Safety (EFSA) Panel defined AI for adults at 400 mg/day based on the average observed choline intake in the healthy population [728].

Choline is abundantly available in eggplants and has been evaluated as eggplant powder [729]. Ingestion of choline in bitartrate form or as krill oil both result in increased free choline levels but the bitartrate form generated higher free choline peaks and enhanced conversion into the active metabolites betaine and dimethylglycine [730]. Also liver, milk, eggs and peanuts are good choline sources [327]. Breast milk contains choline in the form of phosphocholine, glycerophosphocholine, free choline, phosphatidylcholine, sphingomyelin and lyso-phosphatidylcholine.

27.2. Biomarkers and analytical methods

Choline is hydrophilic and the active transport into different types of tissue –presynaptic cholinergic nerve terminals, blood–brain barrier, erythrocytes, mammary glands, etc.- is determined by specific active transporters. As a consequence, plasma choline levels are not a good reflection of tissue levels [728].

The Institute of Medicine used plasma free choline concentration as a status biomarker [327], whilst recognising this was of limited value, whereas EFSA did not consider it a useful [728]. The EFSA panel also concluded that erythrocyte PC concentration, urinary excretion of betaine and total trimethylamine cannot be used to set choline dietary reference values [728]. Despite the absence of consensus on the relevance of circulating levels of choline and related metabolites for choline status assessment, such measurements are still reported. Circulating free choline appeared to be more stable in plasma than in serum and its quantitation together with its metabolites can be achieved using a series of techniques including high pressure liquid chromatography coupled with mass spectrometry [731]. A non-invasive method is under assessment: Magnetic Resonance Spectroscopy scanning of the calf captured the impact of choline-rich structured-lipid on muscle choline content in a pilot RCT in children suffering from cystic fibrosis [732]. Choline and betaine status might also be quantified indirectly through their role in one-carbon metabolism [728].

27.2.1. Effect of inflammation

Lower choline serum levels, observed in man during perioperative stress and inflammation, might be clinically relevant since

choline has been shown to have anti-inflammatory properties in animal and *in vitro* experiments [733].

27.3. Deficiency

In healthy volunteers exposed to a complete and well-balanced experimental diet providing normal amounts (550 mg/d for 70 kg body weight about AI) of dietary choline for 10 days and subsequently up to six weeks of very low amounts (50 mg/d choline for 70 kg body weight), the clinical consequences that prompted choline repletion were 1) non-alcoholic liver steatosis (confirmed by computer tomography or MRI or an increase in gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT) or lactate dehydrogenase (LH)) and/or 2) subclinical muscle damage, reflected by increased creatine phosphokinase levels [725]. Occurrence of such signs was highly variable, partially explained by differences in age and gender. During total fasting, more choline might be released from membrane phospholipids explaining a slower decrease of plasma levels [734].

Whether age-related macular degeneration should be considered as a hallmark of choline and betaine deficiency, remains to be established [735].

27.3.1. When and how to treat?

Preventive administration of supplemental choline is not supported by evidence in healthy adults. A systematic review including 50 studies published between 1978 and 2014 could not identify clear benefit from preventive choline administration for any outcome studied in 61,709 healthy volunteers ranging between birth and very old age, including pregnant women [736].

Pregnancy and early life: Experimental data suggest a potential role for choline among other maternal dietary supplements in preventing neurological dysfunction at birth [737,738]. Particularly in mothers consuming large amounts of alcohol, choline supplementation favored visual recognition and physical growth in two RCTs [739,740]. But the largest (N = 367), 3-armed RCT, revealed no additional benefit of choline with MNs as compared to MNs alone in alcohol drinking and non-drinking mothers [741].

Liver steatosis: A 3 month treatment with a nutrient mixture rich in −among others-choline had no impact on markers of non-alcoholic fatty liver steatosis in 113 subjects [742]. In 40 children however, a combined intervention of lifestyle changes and Docosahexaenoic acid (DHA) + choline + vitamin E appeared to have a beneficial effect on biopsy proven non-alcoholic steatohepatitis [743].

Choline depletion might contribute to steatosis and myopathy observed in cystic fibrosis patients. Enteral administration of choline rich structured lipids increased the plasma levels of choline, betaine and their metabolites in 110 cystic fibrosis patients and improved muscular choline stores in a subgroup (N = 26) [732]. In the latter subgroup, no effect on liver steatosis could be observed. Also the potential of choline supplementation together with moderate nutrient restriction in preserving hepatic function in a fatty liver post transplantation remains hypothetical [744].

Long-term PN: Choline deficiency appeared to contribute significantly to liver steatosis in an RCT evaluating 15 patients on long term total PN (≥12 weeks) [745]: 2 g of IV choline chloride provided over 10–12 h was added to the home PN preparation in 11 intervention-group patients and was well tolerated. In the intervention group, changes in liver attenuation as assessed by CT-scan and relative liver to spleen attenuation showed resolving hepatic steatosis: these changes correlated with the choline levels. Liver function parameters − particularly alkaline phosphatase - improved likewise. Also verbal and visual memory improved after 24 weeks of IV choline chloride 2 g together with PN [746]. No other neurological testing was affected.

A protective role for IV choline supplementation against intestinal atrophy during total PN was only shown in animal experiments [747].

Cystic fibrosis: choline depletion is common in cystic fibrosis patients despite enzyme treatment and may result in liver, fatty acid, and muscle abnormalities [732]: a randomized trial showed clinical benefit of administering additional choline.

Enteral administration: A crossover study in 8 healthy men showed that choline with or without betaine administration attenuated the rise in homocysteine concentrations normally observed after methionine loading, proving that choline is readily converted when administered and effectively impacts the methionine conversion [748]. Citicoline a choline precursor is administered in doses up to 2 g daily in one or more administrations [749]. Two grams choline daily, provided as grape flavored drink was well tolerated and provoked a minor increase in nausea and dyspepsia in 70 pregnant heavy-drinking patients [750].

27.4. Toxicity

Acute ingestion of a high dose of choline may provoke hypotension and, more often, a fishy smell has been reported [327]. The UL for adults was defined at 3.5 g/day. A concern with enteral administration of choline (and also L-carnitine and betaine) is conversion into methylamine-N-oxide (MAO) by the gut microflora and subsequent metabolism by the liver into the uremic toxin trimethylamine-N-oxide (TMAO), with a potential negative long-term impact on cardiovascular health [751]. A RCT evaluating probiotic supplementation for 3 months in 21 chronic kidney disease patients revealed no increase in plasma TMAO levels while betaine levels were increased, confirming adequate administration and absorption of choline in the intervention arm [751].

27.5. Recommendations N° 28 - choline

27.5.1. When to measure?

Recommendation 28.1

Plasma free choline may be determined in patients on home parenteral nutrition who develop unexplained liver steatosis/ steatohepatitis or subclinical muscle damage with high creatine kinase levels.

Grade of recommendation GPP − Strong consensus 100%

Recommendation 28.2

Plasma free choline can be integrated in long-term follow-up of cystic fibrosis patients.

Grade of recommendation GPP − Strong consensus 91%

27.5.2. What to measure?

Recommendation 28.3

There is no routinely accessible biomarker in blood, although choline and its metabolites can be measured.

Grade of recommendation GPP − Strong consensus 100%

27.5.3. How much to provide in typical enteral or parenteral nutrition regimen?

Recommendation 28.4

Choline is not an essential nutrient. Although there is limited evidence, a dose of 400–550 mg per day has been suggested to support lipid metabolism.

Grade of recommendation 0 − Strong consensus 100%

27.5.4. When to provide additional amounts?

Recommendation 28.5

In patients on home parenteral nutrition and patients presenting with unexplained liver steatosis or steatohepatitis with suspected or proven deficiency, the administration of 550 mg to 2 g/day may be considered.

Grade of recommendation 0 – Strong consensus 100%

27.5.5. How to provide additional amounts

Recommendation 28.6

In the treatment of patients with probable choline deficiency, and tolerating enteral nutrition, choline rich feeds or enteral choline preparations can be safely provided in equivalents of 500 mg–1500 mg per day for adults.

Grade of recommendation GPP – Consensus 90%

28. Coenzyme Q10

28.1. Main functions

Coenzyme Q10 (CoQ10) is a fat-soluble compound, synthesized in the mitochondrial inner membrane. It is not strictly a vitamin, but considering the growing metabolic research on this molecule, the group decided to include it in the guideline.

The Q refers to the quinone chemical groups and the 10 to the number of isoprenyl subunits in its tail [752]. CoQ10 is also named "ubiquinone", because of its quinone structure and its ubiquitous presence in most animals and bacteria and in virtually all cells in the human body. CoQ10 has 2 main functions: First, it plays a fundamental role in mitochondrial bioenergetics as electron and proton carrier (electron transport mediator from complex I or II to complex III), facilitating cellular energy (ATP) production. CoQ10 is therefore crucial in tissues with a high energy requirement, such as the heart, skeletal muscles, kidneys, liver, and brain. Second, it is the only endogenously synthesized lipid-soluble antioxidant. It is present in all cellular membranes, both high- and low-density lipoproteins and mitochondria, protecting them against the toxic effect of free radicals generated during normal cellular metabolism. It also helps to regenerate vitamin E to its antioxidant form. Other functions of CoQ10 are gene regulation of overall tissue metabolism, neuroprotection by inhibition of glutamate release and calcium influx, and possibly immunomodulation [753]. Taken altogether, CoQ10 is essential for the health of all tissues and organs [754].

CoQ10 is a vitamin-like compound, which is predominantly synthesized *de novo* in the human body at an estimated rate of 500 mg/day [755]: endogenous biosynthesis tends to decline with age [752]. CoQ10 is synthesized from several components, among which mevalonate, tyrosine, riboflavin, folate, B12 and C [756]. It is transported in plasma by low-density lipoproteins (LDL).

The intestinal absorption of CoQ10 is low due to its hydrophobicity and large molecular weight. It is slow with peak plasma levels occurring 5–10 h after ingestion [757,758].

28.1.1. Needs

Average daily nutritional intake is 3–5 mg/day. Nutritional sources are mostly from heart, chicken leg, herring, and trout. The endogenous levels are determined by the rate of production, amount of intake and the rate of consumption.

No DRI or RDA have been established. Studies have mostly used doses of CoQ10 ranging from 50 to 1200 mg in adults (up to 3000 mg/day), and up to 10 mg/kg/day for children.

28.2. Biomarker and analytical methods

Because CoQ10 levels are dependent on lipoprotein status (as the major carriers of CoQ10 in the circulation), it has been suggested that plasma CoQ10 levels should be expressed as a ratio with respect to total plasma cholesterol [759]. Probably, plasma CoQ10 is more clinically relevant, since plasma CoQ10, but not CoQ10/total plasma cholesterol correlates with CoQ10 content in thrombocytes and leukocytes [760].

28.2.1. Analytical methods

CoQ10 levels are usually determined in blood samples, but it is unclear whether serum levels adequately reflect CoQ10 availability in mitochondria [761]. Measurement of total CoQ10 represents the sum of the reduced form (Ubiquinol-10) and the oxidized form (Ubiqinone-10). In human plasma, CoQ10 is predominantly found in the reduced form. The hydrophobicity and easy oxidation make measurement of CoQ10 difficult. Ubiquinol-10 can be oxidized into ubiqinone-10 at room temperature so blood samples should be collected in heparinized tubes and immediately placed on ice. Subsequently, the sample should be centrifuged to extract the plasma and stored at −80°. CoQ10 measurements in most publications have used reverse-phase HPLC with electrochemical detection [762].

28.2.2. Reference intervals

Most published adult reference intervals for plasma CoQ10 ranged from 0.40 to 1.91 μmol/L (0.34–1.65 μg/mL) [763–765].

28.2.3. Conversion

CoQ10: 1 mg/L = 1.19 μmol/L and 1 μmol/L = 0.84 μg/dL

28.2.4. Effect of inflammation

In several inflammatory conditions, plasma CoQ10 levels are inversely associated with inflammatory markers. In patients with sepsis, CoQ10 plasma levels were negatively correlated with vascular cell adhesion molecule-1 (VCAM-1) and after adjustment for LDL levels also with interleukin-10 (IL-10) plasma levels [756] and at admission with IL-8 and TNF-α [761]. In post-cardiac arrest patients, there was an inverse relationship between IL-6 and IL-8 and CoQ10 levels, although when accounting for multiple comparisons these associations did not remain significant [766]. In a systematic review and meta-analysis, CoQ10 supplementation had a significant lowering effect on inflammatory markers including CRP, IL-6 and TNF-α [767].

28.3. Deficiency

CoQ10 deficiency can result from impaired CoQ10 synthesis due to nutritional deficiencies (pyridoxine deficiency, an essential cofactor for CoQ10 synthesis) or a genetic or acquired defect in CoQ10 synthesis [768]. Furthermore, plasma CoQ10 levels can be decreased in clinical conditions due to increased loss via body fluids, increased endothelial permeability, redistribution, altered protein binding, inadequate intake or utilization or increased needs.

Plasma CoQ10 levels may not reflect tissue storages and actual availability in mitochondria. They could just represent an epiphenomenon indicating severity of the disease or reflect an adaptive response. Decreased plasma CoQ10 levels are reported in a number of disease states, such as primary CoQ10 deficiencies, mitochondrial diseases, cardiovascular disease (chronic heart failure), cancer, diabetes mellitus, neurodegenerative disorders, sepsis [761], and post-cardiac arrest [766,769] severity of signs and symptoms vary widely and depend on cause and age at onset of deficiency. Reported symptoms of CoQ10 deficiency may include sore, aching muscles, muscle weakness, fatigue, mental confusion, gingivitis,

elevated blood pressure, high cholesterol levels, seizures, vision and/or hearing loss and kidney damage.

28.3.1. When and how to treat?

Supplementation CoQ10 is available commercially as either ubiquinol (reduced form) or ubiquinone (oxidized form). There is currently no IV formulation [762].

Supplementation studies have been carried out in a variety of conditions but generally with little benefit [761,770–773].

Primary deficiencies and mitochondrial diseases are beyond the scope of this guideline.

28.4. Toxicity

Supplementation with CoQ10 appears to be safe with only few observed side effects. Some gastrointestinal effects were reported, like nausea, vomiting, diarrhea, and anorexia. The safety of escalated doses of CoQ10 in an RCT of 80 patients with Parkinson's disease using doses varying from 300 to 1200 mg/day did not show a difference in drug-related toxicities (including gastro-intestinal symptoms) compared with placebo. Doses of 3000 mg/day for 8 months have been tolerated well in patients with Parkinson or ALS [757].

28.4.1. Drug interaction

The most significant drug interaction occurs with warfarin. CoQ10 shares some structural similarity to vitamin K and may increase the metabolism of warfarin through selective interaction with the cytochrome p450 enzymes. Multiple reports have demonstrated difficulties in achieving adequate anticoagulation targets in patients taking CoQ10 and warfarin. This may be of concern in a population with heart failure where a significant proportion of patients have atrial fibrillation and may be anticoagulated [762]. However, a RCT showed CoQ10 supplementation at 100 mg/day had no effect on the clinical action of warfarin [774].

28.5. Recommendations N°29 – coenzyme Q10

28.5.1. When to measure?

Recommendation 29.1

There is no clinical indication to measure plasma CoQ10 levels. Measurements are largely for research studies.

Grade of recommendation GPP - Strong consensus 100%

28.5.2. What to measure

Recommendation 29.2

For the assessment of CoQ10 status for research purposes, the plasma CoQ10 concentration may be measured.

Grade of recommendation GPP – Strong consensus 100%

29. Conclusion

The above texts focus separately on all essential MNs, emphasizing their individual specificities and potential importance in acute and chronic disease: this may give the wrong perception that they can be addressed separately. But they work as a web, each being responsible, often in combination, for various steps of metabolic, antioxidant, endocrine and immune reactions. This was well shown for immunity in a comprehensive review [775], showing how the different vitamins and trace elements were interacting at different levels to ensure barrier, innate, and acquired immunity. And the same is true for virtually all functions. In nutrition and metabolism, it is therefore essential to consider the MNs globally. Hence, the complete range of MNs needs to be provided together [303,776].

Some MN deficiencies or inadequacies may lead to or worsen certain diseases, whereas others may be the consequence of some conditions. Clinicians should be aware of these combinations, and suitable consideration given to the assessment, provision, and monitoring of a group of MNs. On the other hand, some therapies may directly impact MN status. Providing targeted amounts of MN and monitoring status should be included in routine management.

The data on MNs remain limited, and this guideline should encourage research in this area. However, as we have discussed, there is sufficient evidence to recommend safe levels of intake of all MNs to prevent deficiency and meet most requirements. We have taken the opportunity to review these levels and provide tables of recommended intake in patients receiving most of their nutrition either from enteral or PN. Such amounts should be provided from the beginning of any period of nutritional support. Further research is required to identify the amounts needed to optimize tissue function. Based on the above, such research should explore not only the amounts of individual MNs but also suitable combinations.

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Conflict of Interest

The expert members of the working group were accredited by the ESPEN Guidelines Group, the ESPEN Education and Clinical Practice Committee, and the ESPEN executive. All expert members have declared their individual conflicts of interest according to the rules of the International Committee of Medical Journal Editors (ICMJE). If potential conflicts were indicated, they were reviewed by the ESPEN guideline officers and, in cases of doubts, by the ESPEN executive. None of the expert panel had to be excluded from the working group or from co-authorship because of serious conflicts. The conflict-of-interest forms are stored at the ESPEN guideline office and can be reviewed by ESPEN members with legitimate interest upon request to the ESPEN executive.

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Appendix A. Supplementary data

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