

Project Start Date	01/01/03
Project End Date	30/06/03
Project Code	ZQ0203

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JHCI Ref: JHCI/76/03

FINAL TECHNICAL REPORT

PART 1:

A Process to Define and Identify Well-Established Health Statements

PART 2:

A list of Well-Established Nutrient Function Statements

A report by the Joint Health Claims Initiative to the Food Standards Agency

Prepared by
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17th Dec 2003

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EXECUTIVE SUMMARY

Background

In recognition of the growing need to protect and promote public health, the European Commission has recently adopted a draft proposal for a Regulation on nutrition and health claims, which will require that all health claims for food be approved before they are released on the market. This includes the adoption of a list of health claims based on well-established science although at this stage the Commission has not yet defined a process for identifying well-established health claims, or addressed the issue of how to handle existing health claims on the European market.

The Commission welcomes contributions and suggestions from Member States, and as such, the Food Standards Agency commissioned the Joint Health Claims Initiative to develop a framework and produce an initial list of well-established health statements on which claims could be based.

In the absence of specific controls on health claims, the Food Standards Agency supports the work of the UK Joint Health Claims Initiative (JHCI), a democratic group that represents the concerned interests of the consumer movement, the food industry and food law enforcement officers. A Code of Practice for Health Claims for Food has been developed by JHCI and an Expert Committee of independent, respected scientists has been created to assess the scientific validity of claims (further details about the work of the JHCI can be found at www.jhci.co.uk).

Purpose

The objectives of this project were to deliver:

- (i) a process to define and identify well-established health statements, and
- (ii) a list of well-established nutrient-function statements for twenty-eight vitamins and minerals.

This work was undertaken primarily to help inform the UK position during negotiations with the European Commission on its proposed legislation for nutrition and health claims. In addition to presenting a ‘tried and tested’ process and real examples of well-established nutrient function statements during negotiations, it is also expected that the results of this report will form the UK’s contribution to a European-wide list “of permitted claims describing the role of a nutrient or other substance in growth, development and normal physiological functions of the body”, which are “based on long-established and non-controversial science” (Brussels, 16.7.2003, COM (2003) 424 final).

It is also envisaged that this report could:

- assist in the development of a mechanism for handling health claims that are already on the market when the health claims legislation comes into force. This will help ensure that suitable products carrying well-established statements are not withdrawn from the market unnecessarily;
- provide a framework for identifying well-established health statements that are based on generally accepted scientific data, which could, over time, be used as a tool to add well-established claims to the European-wide list described above;
- provide a transparent process for fast-track approvals of well-established health claims in the UK, until such time as the European legislation has been implemented; and
- provide a list of well-established nutrient function statements for 28 vitamins and minerals for regulators wishing to determine the scientific accuracy of related health claims on products being sold to consumers.

The process

Before the list of well-established health statements could be produced it was necessary, firstly, to define ‘well-established’, given that this can mean different things to different people. For example, it could mean that a health claim is in common usage or has been on the market for many years. But it might also, more legitimately, mean that a health claim is well attested by a vast body of knowledge and is, for example, to be found in textbooks of nutrition. For the purposes of this project, well-established was defined as ‘Consistent reporting in the majority of source documents of relevant functions’.

Secondly, it was necessary to set out a clear process for deciding whether or not statements were in fact ‘well-established’. Therefore the process, developed in the first part of the project, explains how the list of well-established nutrient function statements was produced. Full details of this process have been documented in the Final Technical Report.

Essentially, the process involved selecting and reviewing reports of respected scientific committees to assess the consistency in reporting about nutrients and their functions. Information provided by the USA’s Institute of Medicine’s (IOM) publications on Dietary Reference Intakes was used as a starting point for drawing up a list of possible functions, as these documents are internationally recognised and based on objective studies in humans.

Reports by reputable expert groups from the UK and Europe were also reviewed, both to cross-check and demonstrate consistency in the functions reported by the IOM and to anglicise the health statements for the UK population.

The list

The process was designed to define and identify well-established health statements for any dietary component, from vitamins to whole foods, and any type of health benefit, including those related to enhanced functions and functions that reduce the risk of developing disease.

However, the second part of the project was limited to identifying well-established statements for 28 vitamins and minerals listed in Annex 1 to the Food Supplements Directive (2002).

Statements were also limited to those linked to a health effect in the body that were based on normal physiological functions and were related to quantities of nutrients that can be obtained from a normal diet. To ensure that the statements were related to normal structures and functions only, they had to correspond with the following structured phraseology: 'x is necessary for / contributes to the normal structure / function of y'.

It must be noted that the statements included on the final list were not considered in terms of their legal acceptability or meaningfulness to consumers, nor were they considered in terms of their application to food products, and, as such, are not approved health claims for food.

These limitations were adopted to ensure the timely provision of information to the Food Standards Agency during its negotiations with the European Commission on the forthcoming nutrition and health claims legislation.

The method

Monographs were prepared for each nutrient by reviewing the agreed source documents and quoting relevant functions that met specified inclusion criteria (see Final Technical Report for details). The monographs were presented to an independent committee of scientific experts (the JHCI Expert Committee), which made a recommendation about the inclusion of the statements to final list. A second independent party (the JHCI Council) ratified the experts' recommendations, ensuring a consistent and transparent approach has been applied throughout the process to generate the statements.

This project successfully developed a robust and transparent process to define and identify well-established health statements. The application of which has resulted in 82 well-established nutrient function statements, which were presented in two groups:

- a) Well-established nutrient function statements common to all vitamins and minerals.
- b) Well-established nutrient function statements specific to certain vitamins and minerals.

Statements that did not meet the criteria for inclusion on the final list were also noted, together with their reasons for rejection. This was usually, for example, because the function was already captured by, or the result of, functions encapsulated by another statement; they did not relate to a normal structure or function role in the body; the data was insufficient or inconsistent; or there was not a plausible mechanism to support the supposed function.

The statements have been limited to quantities of nutrients that can be obtained from a normal diet and therefore functions based on pharmacological levels of intake have not been considered. Although the remit of this project did not include quantification of the statements, it is recommended that such work be carried out in the future, so that consumers are aware of the quantities of nutrients required from their diet in order to maintain these normal physiological functions.

This research demonstrates that a model framework for adopting well-established health statements is workable and effective and, as such, could be adapted for use by other European Member States during the development of the Community-wide list of well-established health claims. By doing so, it is hoped that a smooth transition period will follow the implementation of the proposed nutrition and health claims Regulation and minimise the unnecessary withdrawal and subsequent return of legitimate, well-established health claims that have already been on the market for some time.

The Joint Health Claims Initiative, in due course, intends to publish an expanded list of approved generic health claims. This will include those well-established nutrient function statements, which, after consultation with members of the JHCI Council with expertise in current UK food law and the consumer perception of health claims, are considered to be legally acceptable and are likely to be meaningful and not misleading to consumers.

Until such time however, the statements are not approved for use as health claims on food products.

INTRODUCTION

In recent years the European Community has seen rapid growth in consumer demand for healthier food choices. Together with the growing body of evidence for the beneficial effects of certain foods and nutrients on health, this has resulted in the increased use of health claims on food products. It is important that information on labels, in advertising or in other promotional contexts about the health benefits of the food is scientifically accurate, meaningful to consumers and honest.

Current UK legislation on claims includes the Food Safety Act 1990 and the Trade Descriptions Act 1968, which prohibit false and misleading claims. In addition, the Food Labelling Regulations 1996 (as amended) lay down the labelling requirements of foods generally and prohibits medicinal claims, that is, claims that a food will prevent, treat or cure a disease. In the absence of specific controls on health claims, the Food Standards Agency supports the work of the UK Joint Health Claims Initiative (JHCI), a democratic group that represents the concerned interests of the consumer movement, the food industry and food law enforcement officers. A Code of Practice has been developed by JHCI and an Expert Committee of independent, respected scientists has been created to assess the scientific validity of claims (further details about the work of the JHCI and the members of its Council and Expert Committee have been included as Annex 1).

The JHCI Code of Practice defines a health claim as: ‘A direct, indirect or implied claim in food labelling, advertising and promotion that consumption of a food carries a specific health benefit or avoids a specific health detriment’. The JHCI process for approving a health claim for food is rigorous, as it not only considers the validity of health statements, but also the legitimacy of their application to food products. Due to the reliance on submissions from interested parties, and the comprehensive nature of the validation process, expansion of the list of JHCI validated health claims has been slower than expected.

The European Commission has adopted a Proposal for a Regulation of the European Parliament and of the Council on nutrition and health claims made on foods. This proposal is likely to institute a pre-market approval system for health claims, which includes the development of a positive list, or ‘Register’, of health claims based on “generally accepted data”. However, the Commission has not yet proposed a definition of “generally accepted data”, nor has it addressed the issue of how to handle existing health claims on the European market.

There are few data available about the range of existing health claims in the UK market. The Food Standards Agency is addressing this information gap by commissioning a market audit of health claims, due for publication in late 2003. In the meantime, the JHCI operates to provide, to those who seek it, an opinion on the validity and application of health claims. To date the JHCI has considered six generic health claim applications, which then raises questions about the authenticity of health claims that do not appear on the list of JHCI approved claims, or have not been publicly vetted by independent experts.

Such claims may be considered to be well-established, because of their common usage or because they have been in use for many years, on the assumption that they are supported by “generally accepted data”. But in the absence of a positive list of validated well-established claims, the sometimes inconsistent and exaggerated use of the facts have led to consumer confusion and scepticism about the truthfulness of some health claims.

The Food Standards Agency is committed to help ensure that consumers are not misled as to the safety or nature of foodstuffs. As such, it commissioned the JHCI to develop a process to define and identify claims that are well-established because they are substantiated by scientific statements of fact that are found in a range of credible reference documents.

To demonstrate the workability of the process to identify well-established health statements, a list of ‘well-established nutrient function statements’ has been generated. The process is applicable to any type of health claim, or any dietary component. To begin with its application has been limited to the identification of well-established normal functions for the 28 vitamins and minerals, which may be found in food products, listed in Annex 1 to the Food Supplements Directive (2002/46/EC).

The process, together with the list of well-established nutrient function statements, has been developed primarily to assist the Food Standards Agency in negotiations with the European Commission on its forthcoming nutrition and health claims legislation.

The JHCI intends, in due course, to use the well-established nutrient function statements as a basis to expand its list of approved generic health claims by including statements that are deemed suitable for use on food products, in terms of legal and consumer acceptability.

OBJECTIVES AND AIMS

OBJECTIVE 1:

To develop a process for defining and identifying well-established health statements

Aim 1.1

To help inform the UK position during negotiations with the European Commission on its forthcoming nutrition and health claims legislation.

Aim 1.2

To assist in the development of a mechanism for handling existing health claims during the implementation of the forthcoming nutrition and health claims legislation.

Aim 1.3

To provide a framework for identifying claims that are based on generally accepted scientific data, which can be added to a positive list of well-established health claims for use by European member states.

Aim 1.4

To provide a transparent process for fast-track approvals of well-established health claims in the UK, in the absence of forthcoming nutrition and health claims legislation.

OBJECTIVE 2:

To use the process at objective 1 to produce a list of well-established nutrient-function statements

Aim 2.1

To demonstrate the workability and rigour of the process developed at objective 1.

Aim 2.2

To help further inform the UK position during negotiations with the European Commission on its forthcoming nutrition and health claims legislation.

Aim 2.3

To help form the UK's contribution to a European-wide positive list of well-established health statements.

Aim 2.4

To provide a list of well-established nutrient function statements for 28 vitamins and minerals listed in Annex 1 to the Food Supplements Directive (2002/46/EC) for regulators wishing to determine the scientific accuracy of related health claims.

Aim 2.5

To provide a basis for comparative analyses of health claims currently on the market in the UK.

SCOPE OF WORK

Scope of Work

Objective 2 (to produce a list of well-established nutrient-function statements) has been limited to the generation of statements that:

- i) are based on well-established scientific evidence - not emerging scientific evidence;
- ii) are linked to a health effect in the body which is based on a normal physiological function - not an enhanced function; function that reduces the risk of developing a disease; or, function that can be attributed to the prevention, treatment or cure of a disease;
- iii) are restricted to the vitamins and minerals listed in the Annex 1 to the Food Supplements Directive (2002/46/EC);
- iv) are related to quantities of nutrients that can be obtained from a normal diet - not pharmacological quantities which may be present in foods or supplements;
- v) have not been considered in terms of their legal acceptability or meaningfulness to consumers; and
- vi) have not been considered in terms of their application to food products and as such **are not approved health claims for food.**

These limitations were adopted to ensure the timely provision of information to the Food Standards Agency during its negotiations with the European Commission on the forthcoming nutrition and health claims legislation.

The Agency envisages that, in the future and by using the process presented in Part 1, the list of well-established health statements could be expanded to include statements that relate to all relevant dietary components, and to enhanced functions or functions that reduce the risk of developing disease.

PART 1

A Process to Identify and Define Well-Established Health Statements

This process has been designed to define and identify well-established health statements for any dietary component, from micronutrients to whole foods, and any type of health benefit, including those related to enhanced functions and functions that reduce the risk of developing disease.

This process does not involve consideration of the potential application of the statements to food products, in terms of their legal acceptability or meaningfulness to consumers, and therefore does not result in a list of approved health claims. It does however provide a sound scientific basis for generating health claims from well-established scientific statements of fact.

Steps to define and identify well-established health statements:

- Step 1.** Clearly define 'well-established'.
- Step 2.** Determine priority order for groups of nutrients, dietary components and types of potential health claims to be considered.
- Step 3.** Establish working definitions as necessary.
- Step 4.** Agree credible source documents, to draw up a list of possible functions, effects or benefits for nutrients and dietary components, and to identify which of these functions are 'well established'.
- Step 5.** Develop phraseology as necessary to provide a guide for wording of statements.
- Step 6.** Draw up list of well-established health statements.

PART 2

A list of Well-Established Nutrient Function Statements

Adoption of the Process to Define and Identify Well-established Health Statements, developed in Part 1

The Process to Define and Identify Well-Established Health Statements was adopted and approved for use following a pilot study using vitamin C to test the workability of Steps 1 – 6. An evaluation, including refinements to the process following the pilot study, has been included as Annex 2.

This process has been employed to produce a list of well-established nutrient function statements for the vitamins and minerals listed in Annex 1 to the Food Supplements Directive (2002/46/EC). For the purposes of this project, consideration has been given only to statements linked to health effects in the body which are based on normal physiological functions - not enhanced functions, functions that reduce the risk of developing a disease, or, functions that that can be attributed to the prevention, treatment or cure of a disease.

METHODOLOGY

Overview of methodology

The following is an overview of the methodology employed by the JHCI to develop a list of well-established nutrient function statements, using the process developed at Part 1 to produce the list resulting from Part 2. A detailed description of this methodology is presented in the next section.

I. Completion of Steps 1 – 5 of the Process to Define and Identify Well-established Health Statements:

Step 1: Clearly define 'well-established'.

Step 2: Determine priority order for groups of nutrients, dietary components and types of potential health claims to be considered.

Step 3: Establish working definitions as necessary.

Step 4: Agree credible source documents, to draw up a list of possible functions, effects or benefits for nutrients and dietary components, and to identify which of these functions are 'well established'.

Step 5: Develop phraseology as necessary to provide a guide for wording of statements.

II. Preparation of monographs for each nutrient, by reviewing the agreed source documents and quoting relevant functions that meet inclusion criteria.

III. Generation of statements using phraseology as a guide.

IV. Recommendation by independent experts (in this case, the JHCI Expert Committee), about the inclusion of statements to final list.

V. Revision of the list of well-established nutrient function statements, in accordance with recommendations by the independent experts.

VI. Ratification, by a second independent party (in this case, the JHCI Council), of the experts' recommendations, ensuring a consistent and transparent approach has been applied throughout the process to generate the statements.

VII. Completion of Step 6 of the Process to Define and Identify Well-established Health Statements:

Step 6: Draw up list of well-established health statements.

Detailed methodology

I. Completion of Steps 1 – 5 of the Process to Define and Identify Well-established Health Statements

Step 1:

Clearly define ‘well-established’.

For the purposes of this process, ‘well-established’ has been defined as:

‘Consistent reporting in the majority of source documents of relevant functions’.

It was considered unrealistic for all source documents to be consistent in their reporting of relevant functions, due to variations in the date of publication and with emerging evidence contained in the more recent documents.

Step 2:

Determine priority order for groups of nutrients, dietary components and types of potential health claims to be considered.

For the purposes of this project the following list of nutrients, Annex 1 to the Food Supplements Directive (2002/46/EC), has been selected. It is considered likely that reference to these nutrients will be made in the forthcoming European nutrition and health claims legislation:

Vitamin A	Calcium
Vitamin D	Magnesium
Vitamin E	Iron
Vitamin K	Copper
Thiamin (B ₁)	Iodine
Riboflavin (B ₂)	Zinc
Niacin	Manganese
Pantothenic Acid	Sodium
Vitamin B ₆	Potassium
Folate	Selenium
Vitamin B ₁₂	Chromium
Biotin	Molybdenum
Vitamin C	Fluoride
	Chloride
	Phosphorus

Alternative nomenclature for these nutrients has been included as Annex 3, as listed in the British Journal of Nutrition’s ‘Directions to Contributors’.

In order to ensure the timely provision of information to the Food Standards Agency during its negotiations with the European Commission on the forthcoming nutrition and health claims legislation, consideration has been given only to nutrient function statements linked to health effects in the body which are based on a normal physiological functions.

Step 3:

Establish working definitions as necessary.

i) Well-established

‘Consistent reporting in the majority of source documents of relevant functions’
(As agreed in Step 1)

ii) Nutrient function

‘A claim that describes the physiological role of the nutrient in growth, development and normal functions of the body’
(Codex Guidelines for the Use of Nutrition Claims, CAC/GL 23-1997).

iii) Health statement

‘A statement related to a health benefit, or the avoidance of a health detriment’
(adapted from JHCI Code of Practice, 2000)

Step 4:

Agree credible source documents, to draw up a list of possible functions, effects or benefits for nutrients and dietary components and to identify which of these functions are ‘well-established’.

Information provided by the USA’s Institute of Medicine’s (IOM) publications on Dietary Reference Intakes has been used as a starting point for drawing up a list of possible functions, as these documents are internationally recognised and based on systematic reviews of in vivo evidence.

Reports by reputable expert groups from the UK and Europe have also been reviewed, both to cross-check and demonstrate consistency in the functions reported by the IOM and to anglicise the health statements for the UK population. The UK Expert Group on Vitamins and Minerals (EVM) has recently published its findings on Safe Upper Levels for Vitamins and Minerals (May 2003). As this final report was not published at the time that JHCI undertook its reviews of vitamins and minerals, draft reports of the EVM on Safe Upper Levels for Vitamins and Minerals were used instead (see Source document Reference List, page 264, for details).

Reference Groups

The complete list of source documents is presented in Annex 5. References have been grouped according to the expert group that produced the report, to demonstrate consistency used in the range of source documents reviewed, as follows:

Reference Group 1:

US Institute of Medicine - Dietary reference intakes for vitamins and minerals (1997 – 2001).

Reference Group 2:

'Encyclopedia of Human Nutrition 2E'.

Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego: Academic Press, c1999.

Reference Group 3:

Reports of the European Scientific Committee on Food (1992 - 2003).

Reference Group 4:

Draft Reports of the UK Expert Group on Vitamins and Minerals (2002).

Reference Group 5:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference Group 6:

Reports of the UK Committee on Medical Aspects of Food and Nutrition Policy (1991 – 2000).

Reference Group 7:

Introduction to Human Nutrition. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.

Reference Group 8:

Reports of the British Nutrition Foundation Task Force (1989 – 1995).

Reference Group 9:

International Life Sciences Institute (Europe) Concise Monograph Series (1999).

Step 5:

Develop phraseology as necessary to provide a guide for wording of statements

To provide a framework to develop standardised health statements the nutrient function statements have been phrased according to the following structured phraseology:

a) 'x is necessary for / contributes to the normal structure / function of y'

or

b) 'x is necessary for / contributes to normal z'

Where:

- **x** is a vitamin or mineral listed in Annex 1 of the Food Supplements Directive (2002)
- **x** is 'necessary for' 'y' or 'z' if the structure / function cannot occur without them
- **y** is the whole body; a bodily system (e.g. cardiovascular system); organ (e.g. heart,); a tissue (e.g. blood); or a component of a tissue (e.g. red blood cells)
- **z** is a normal function of the body (e.g. metabolism) or a specific function (e.g. oxidative processes)

II. Preparation of a monograph for each nutrient, by reviewing the agreed source documents and quoting relevant functions

Selection of source documents for each nutrient

- Source documents from reference groups 1 – 4 have been reviewed for all nutrients, except for iron, sodium, potassium, fluoride and chloride, for which alternative source documents were used in the absence of reports from groups 1 – 4 (refer to Annexes 4.16, 4.21, 4.22, 4.26 and 4.27 respectively for detailed reference lists for these nutrients).
- Additional documents were reviewed for many nutrients following the advice of independent experts about reputable source documents for specific nutrients. This was necessary in cases where an expert group had not yet undertaken a review of a specific nutrient, or when additional clarity about the role of the nutrient was required.
- The actual source documents reviewed for each nutrient are listed together with quotes presented in Annex 4, in addition to the full Reference List found in Annex 5.

Criteria for the inclusion of quotes

Quotes were selected for inclusion if they:

- i) stated the role of the nutrient in normal physiological functions in humans; and
- ii) related to quantities of nutrients that can be obtained from a normal diet; and
- iii) were supported by a corresponding quote in at least one other source document; and
- iv) illustrated the range of consistency in the reporting of the role of the nutrient in normal physiological functions; and
- v) represented the totality of evidence in relation to the functions cited in the source documents; and
- vi) provided supporting information about:
 - classical symptoms of clinical deficiency; and
 - plausible mechanisms of the role of the nutrient in normal physiological functions; and
 - possible influences of the role of the nutrient in normal physiological functions on disease endpoints.

Criteria for the exclusion of quotes

Quotes were not selected for inclusion if they:

- i) related to enhanced functions, functions that reduce the risk of developing a disease, or, functions that that can be attributed to the prevention, treatment or cure of a disease; or
- ii) related to quantities of the nutrient over and above that which can be obtained from the normal diet; or
- iii) were not supported by a corresponding quote in at least one other source document.

III. Generation of statements using phraseology as a guide

Statements were generated for well-established nutrient functions by categorising the information to reflect the phraseology described in Step 5, as follows:

Nutrient	Effect on	Necessary for	Contributes to	Normal structure	Normal function
Vitamin A	Vision	✓			✓

Resulting in the nutrient function statement:

Vitamin A is necessary for normal vision.

Well-established nutrient function statements common to all vitamins and minerals

All of the nutrients reviewed are essential and necessary for health and normal body functions including: reproduction; conception; development; growth and body maintenance. Such statements considered to be well-established have been presented in Table 1a and are phrased, for example, as follows: *'Zinc contributes to normal development'*, whereby the word 'contributes' indicates that all nutrients have an equally essential role in these general functions.

Well-established nutrient function statements specific to certain vitamins and minerals

Table 1b focuses on specific, notable functions for individual nutrients. Some nutrients have a particularly significant role in the general functions mentioned above and have therefore warranted an additional statement in Table 1b. Such statements have been phrased, for example, as follows, *'Zinc contributes to normal reproductive development'*, to indicate that the nutrient has an essential role over and above that which is listed in the table of statements common to all vitamins and minerals (Table 1a). The word 'necessary' has been used in Table 1b when the structure / function cannot occur without the relevant nutrient.

Results of the review of nutrients have therefore been summarised in three tables:

Table 1a: Well-established nutrient function statements common to all vitamins and minerals.

Table 1b: Well-established nutrient function statements specific to certain vitamins and minerals.

Table 2: Rejected nutrient function statements.

IV. Recommendation by independent experts about the inclusion of statements to final list

A list of the names of the independent experts (in this case, the JHCI Expert Committee) involved in this project has been included as Annex 1.

Criteria for the inclusion of statements

Statements were recommended for inclusion on the final list when the independent experts agreed that:

- i) they complied with the definitions for ‘well-established’, ‘nutrient function’ and ‘health statement’, as set out in Step 3; and
- ii) the wording of the statement was based on correct interpretation of the evidence; and
- iii) the functions allowed for a scientifically accurate health statement; and
- iv) there was sufficient data to substantiate the health statement; and
- v) there was consistent data to substantiate the health statement; and
- vi) there was a plausible mechanism.

Reasons for the exclusion of statements

Statements that did not meet all of the above criteria were recommended for rejection from the final list. The independent experts assigned one of the following reasons for exclusion, which correspond with the inclusion criteria, to each rejected statement:

- i) No (not a health statement)
- ii) No (inaccurate interpretation of evidence)
- iii) No (too imprecise)
- iv) No (data insufficient)
- v) No (data inconsistent)
- vi) No (no plausible mechanism)

Additionally, statements were rejected if the experts considered that the related function was captured by, or the result of, functions encapsulated in another statement. The reason for exclusion was recorded as follows, together with the code of the relevant included statement, as follows, as in Table 2, page 38:

- vii) No (see [*e.g.*] VA1b)

The independent experts also considered that a number of statements related to functions that were common to all the vitamins and minerals reviewed. Such statements were moved to Table 1a and appear in Table 2 with the following reason for rejection, as in Table 2, page 39:

- viii) No (see Table 1a)

Exceptions

The independent experts recommended that two statements that did not meet the above criteria were also included in the final list. The first was in relation to beta-carotene, but because it was considered to provide a valuable source of vitamin A for vegetarians it was recommended for inclusion (refer Table 1b, page 27).

The second was a statement in relation to fluoride, which did not strictly fit the criteria for either a ‘normal structure’ or ‘normal function’ statement for teeth, but because of its significant role in the maintenance of healthy teeth it was recommended for inclusion (refer F11a, Table 1b, page 37).

V. Revision of the list of well-established nutrient function statements, in accordance with recommendations by the independent experts

Where necessary, this part of the process involved refinements to the wording of the statements, for scientific accuracy, or a further review of the evidence in cases where the experts sought clarification about particular nutrient functions. Following these amendments, the list was prepared for ratification by a second independent party.

VI. Ratification, by a second independent party, of the experts’ recommendations

After consideration of the experts’ recommendations, a second independent party (in this case, the JHCI Council) endorsed the list of well-established nutrient function statements and agreed that a consistent and transparent approach had been applied throughout the process to generate the statements. A list of the names of the second independent party involved in this project has been included as Annex 1.

VII. Completion of Step 6 of the Process to Define and Identify Well-established Health Statements

The final list of well-established nutrient function statements, for the nutrients listed in Annex 1 to the Food Supplements Directive (2002/46/EC), is presented in Tables 1a and 1b.

DISCUSSION AND RECOMMENDATIONS

This project has successfully developed, tested and adopted a robust and transparent process to define and identify well-established health statements. The application of the process has resulted in 82 well-established nutrient function statements and demonstrated that the process is workable. It is recommended that this process should now be used to identify well-established health statements for other dietary components and other types of health benefit, including those related to enhanced functions and functions that reduce the risk of developing disease. The process is likely to be useful not only in the development of a European-wide positive list of well-established health claims, but also in the identification of health statements that are not supported by a body of consistent, well-established data.

Points to Note

It should be noted that during the review of nutrients some anomalies were encountered in the application of the process. The manner in which these were addressed is presented below:

- i) **Exceptions to the definitions and standard phraseology.** It was essential to clearly define the scope of the review (in this case to normal structural and functional roles for pre-specified nutrients), to facilitate the development of standardised statements and demonstrate the workability and reliability of the process. Two exceptions were made however, in relation to fluoride and tooth enamel and beta-carotene as a source of vitamin A (refer page 20 for details).
- ii) **Inconsistent use of reference material.** Whilst the process pre-determined the source documents to be used for reviewing all nutrients, this was not always possible. Many of the reports focused primarily on toxicity and safety data than on nutrient functions, therefore additional sources were required for some nutrients. On such occasions, guidance was sought from the independent scientists for additional reputable source documents.
- iii) **Identification of the primary role of the nutrient.** It was necessary to present statements in terms of the nutrient's direct function (for example vitamin C as a co-factor in collagen formation), rather than an indirect, or secondary function (for example, collagen's role in the structure of skin).
- iv) **Determination of a tangible effect in humans.** On a number of occasions it was not possible to determine the health outcome that resulted from the nutrient function. Such functions have been rejected on the basis that they did not meet the definition of a health statement; 'a statement related to a health benefit, or the avoidance of a health detriment' (refer to Table 2 for rejected statements).

Issues requiring further consideration

Quantification of statements. The second part of this project was limited to identifying well-established health statements that related to quantities of nutrients obtainable from a normal diet, rather than functions based on pharmacological levels of intake. The remit of this project did not include quantification of the statements, and as such, it is recommended that work to quantify these, and other statements, be carried out in the future.

Extrapolation from deficiency data. A number of nutrient function statements in this report have been based on deficiency state data and therefore the assumption that the absence of clinical deficiency symptoms equates to a 'normal' function. However, further consideration ought to be given to whether these statements are true only in very specific circumstances and apply only during the disease or deficiency state.

Risk assessment framework. Application of the process developed under Objective 1 has demonstrated that it is relatively simple to identify scientifically valid statements of fact. What is more complicated is the determination of whether the statements can be applied to foods as a health claim, particularly when they relate to nutrients that are considered to be harmful when consumed at certain levels (for example sodium and vitamin A). A risk assessment framework should be developed to identify health statements that are likely to prove contentious in relation to particular nutrients and areas of concern to public health.

From health statements to health claims. This review has resulted in 82 well-established nutrient function statements, however not all of these statements will be applied to foods as health claims. The JHCI intends to consider each statement for its legal acceptability, to help ensure that the resulting claims are meaningful and easily understood by consumers and to produce guidelines for the application of the resulting claims. The context of the statements will need to be carefully considered, for example it should not be implied that 'more is better' in cases where evidence for the physiological function relates only to consumption of the Recommended Daily Amount (RDA). It is recommended that the Food Standards Agency undertake research into consumer understanding of the possible health claims resulting from the list.

Summary of Recommendations

- i) That the process is now applied to other dietary components and other types of health benefits to develop a comprehensive list of well-established health statements.
- ii) That further work be carried out to quantify the well-established nutrient function statements.
- iii) That consideration be given to the accuracy in extrapolating deficiency state data to normal physiological functions.
- iv) That a risk assessment framework be developed in relation to particular nutrients and areas of concern to public health.
- v) That research be undertaken to ascertain likely consumer understanding of potential claims that may be developed from the list.

GENERAL REFERENCES

Section 15, Food Safety Act 1990 (UK).

Section 1, Trade Descriptions Act 1968 (UK).

Food Labelling Regulations 1996 (as amended) (UK).

Joint Health Claims Initiative Code of Practice on Health Claims on Foods (December 2000).

Joint Health Claims Initiative Guidelines for Preparing Dossiers to Substantiate Health Claims (February 2002, version 1.0).

Proposal for a Regulation of the European Parliament and of the Council on nutrition and health claims made on foods (Brussels, 16.7.2003, COM(2003) 424 final).

JHCI Generic Claims Considered (www.jhci.co.uk).

Directive 2002/46/EC of the European Parliament and of the Council of 10th June 2002 on the approximation of the laws of the Member States relating to food supplements, Annex 1.

British Journal of Nutrition's 'Directions to Contributors'.

Guidelines for the Use of Nutrition Claims (CAC/GL 23-1997). Codex Alimentarius, Food Labelling, Complete Texts, Revised 2001; Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome.

RESULTS

Presentation of the results

Each health statement presented in Table 1b or Table 2 has been assigned a code, which relates to the supporting quotes presented in Annex 4. Nutrients are listed in the order that they appear in Annex 1 of the Food Supplement Directive (2002/46/EC).

Criteria for the inclusion of statements (check previous page and bring across)

Statements were recommended for inclusion on the final list when the independent experts agreed that:

- i) they complied with the definitions for ‘well-established’, ‘nutrient function’ and ‘health statement’, as set out in Step 3; and
- ii) the wording of the statements was based on correct interpretation of the evidence; and
- iii) the functions allowed for a scientifically accurate health statement; and
- iv) there was sufficient data to substantiate the health statement; and
- v) there was consistent data to substantiate the health statement; and
- vi) there was a plausible mechanism.

Table 1a: Well-established nutrient function statements (common to all vitamins and minerals)

Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
1. Reproduction		✓		✓	‘X’ contributes to normal reproduction.	Yes	Yes
2. Conception		✓		✓	‘X’ contributes to normal conception.	Yes	Yes
3. Development		✓	✓		‘X’ contributes to normal development.	Yes	Yes
4. Growth		✓	✓		‘X’ contributes to normal growth.	Yes	Yes
5. Body maintenance		✓		✓	‘X’ contributes to normal body maintenance.	Yes	Yes

Table 1b: Approved well-established nutrient function statements (specific to certain vitamins and minerals)

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VITAMIN A								
NB:								
a) Epidemiological studies have indicated that high levels of Vitamin A (retinol and retinoic acid) during pregnancy might increase the risk of birth defects [#]								
b) Beta-carotene can be converted to Vitamin A. Where beta-carotene is the main source the following statements could be preceded by: "Beta-carotene can be converted to Vitamin A. Vitamin A is necessary for / contributes to..." [*]								
VA1b	1b. Vision	✓			✓	Vitamin A is necessary for normal vision.	Yes	Yes
VA2	2. Skin and mucous membranes	✓		✓	✓	Vitamin A is necessary for the normal structure and function of the skin and mucous membranes (such as in the lung, intestines, nose, eyes and female reproductive tract).	Yes	Yes
VA4	4. Cell differentiation	✓			✓	Vitamin A is necessary for normal cell differentiation (such as in the immune system).	Yes	Yes
VITAMIN D (NB: Sufficient vitamin D can be synthesised in the body with adequate exposure to sunlight).								
VD1	1. Calcium and phosphorus absorption and utilisation	✓			✓	Vitamin D is necessary for the normal absorption and utilisation of calcium & phosphorus.	Yes	Yes

[#] *Safe Upper Levels for Vitamins and Minerals*. Expert Group on Vitamins and Minerals, May 2003 (<http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf>)

^{*} Exception; see page 21

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VD2a	2a. Cell division		✓		✓	Vitamin D contributes to normal cell division.	Yes	Yes
VD3	3. Bone	✓		✓		Vitamin D is necessary for the normal structure of bone.	Yes	Yes
VITAMIN E								
VE1	1. Antioxidant activity	✓			✓	Vitamin E is necessary for cell protection from the damage caused by free radicals (such as the oxidation of polyunsaturated fatty acids in red blood cell membranes).	Yes	Yes
VITAMIN K								
VK1	1. Coagulation	✓			✓	Vitamin K is necessary for normal coagulation (blood clotting).	Yes	Yes
VK2	2. Bone		✓	✓		Vitamin K contributes to the normal structure of bone.	Yes	Yes
THIAMIN								
Th1	1. Carbohydrate metabolism	✓			✓	Thiamin is necessary for the normal metabolism of carbohydrates.	Yes	Yes
Th2	2. Neurological and cardiac systems	✓			✓	Thiamin is necessary for normal neurological and cardiac function.	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
RIBOFLAVIN								
Ri1	1. Release of energy from food		✓		✓	Riboflavin contributes to the normal release of energy from food.	Yes	Yes
Ri2	2. Transport and metabolism of iron		✓		✓	Riboflavin contributes to the normal transport and metabolism of iron in the body.	Yes	Yes
Ri3	3. Mucous membranes		✓	✓		Riboflavin contributes to the normal structure of mucous membranes (such as the surface of the tongue, the mouth, eyes and intestines).	Yes	Yes
NIACIN (NB: Sufficient niacin can be synthesised in the body with an adequate dietary intake of protein or tryptophan.)								
Ni1	1. Release of energy from food	✓			✓	Niacin is necessary for the normal release of energy from food.	Yes	Yes
Ni4	4. Skin and mucous membranes	✓		✓	✓	Niacin is necessary for the normal structure and function of skin and mucous membranes (such as in the intestines).	Yes	Yes
Ni5	5. Neurological system	✓			✓	Niacin is necessary for normal neurological function.	Yes	Yes
PANTOTHENIC ACID								
Pa1	1. Fat metabolism	✓			✓	Pantothenic acid is necessary for the normal metabolism of fat.	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VITAMIN B₆								
VB ₆ 1	1. Protein metabolism	✓			✓	Vitamin B ₆ is necessary for the normal metabolism of protein.	Yes	Yes
VB ₆ 2	2. Transport and metabolism of iron	✓			✓	Vitamin B ₆ is necessary for the normal transport and metabolism of iron in the body.	Yes	Yes
VB ₆ 4	4. Homocysteine metabolism		✓		✓	Vitamin B ₆ contributes to the maintenance of normal blood homocysteine levels.	Yes	Yes
FOLATE								
Fo1	1. Cell division	✓			✓	Folate is necessary for normal cell division (such as in the gastrointestinal tract).	Yes	Yes
Fo2	2. Developing neural tube	✓		✓		Folate is necessary for the normal structure of the neural tube in developing embryos.	Yes	Yes
Fo4	4. Blood formation	✓			✓	Folate is necessary for normal blood formation.	Yes	Yes
Fo5	5. Homocysteine metabolism		✓		✓	Folate contributes to the maintenance of normal blood homocysteine levels.	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VITAMIN B₁₂								
VB ₁₂ 1a	1a. Cell division	✓			✓	Vitamin B ₂ is necessary for normal cell division (such as in the gastrointestinal tract).	Yes	Yes
VB ₁₂ 1b	1b. Blood formation		✓		✓	Vitamin B ₂ contributes to normal blood formation.	Yes	Yes
VB ₁₂ 2	2. Neurological system	✓		✓	✓	Vitamin B ₂ is necessary for the normal structure and function of the neurological system.	Yes	Yes
VB ₁₂ 4	4. Homocysteine metabolism		✓		✓	Vitamin B ₂ contributes to the maintenance of normal blood homocysteine levels.	Yes	Yes
BIOTIN								
Bi1	1. Fat metabolism and energy production		✓		✓	Biotin contributes to normal fat metabolism and energy production.	Yes	Yes
VITAMIN C								
VC1	1. Connective tissue	✓		✓	✓	Vitamin C is necessary for the normal structure and function of connective tissue (such as that required for normal gums, skin, healing processes, bone and cartilage).	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VC5	5. Blood vessels	✓		✓	✓	Vitamin C is necessary for the normal structure and function of blood vessels.	Yes	Yes
VC9	9. Iron absorption		✓		✓	Vitamin C contributes to the absorption of iron from food.	Yes	Yes
VC10	10. Antioxidant activity		✓		✓	Vitamin C contributes to cell protection from the damage caused by free radicals (such as epithelial cell integrity).	Yes	Yes
VC12	12. Neurological system	✓			✓	Vitamin C is necessary for normal neurological function.	Yes	Yes
CALCIUM								
Ca1	1. Bones and teeth	✓		✓		Calcium is necessary for the normal structure of bones and teeth.	Yes	Yes
Ca2	2. Nerves and muscle	✓			✓	Calcium is necessary for normal nerve and muscle function.	Yes	Yes
Ca3	3. Coagulation	✓			✓	Calcium is necessary for normal coagulation (blood clotting).	Yes	Yes
MAGNESIUM								
Mg1	1. Energy metabolism	✓			✓	Magnesium is necessary for normal energy metabolism.	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
Mg3	3. Electrolyte balance	✓			✓	Magnesium is necessary for normal electrolyte balance.	Yes	Yes
Mg4	4. Nerve and muscle	✓			✓	Magnesium is necessary for normal nerve and muscle function.	Yes	Yes
Mg6	6. Bone and teeth	✓		✓		Magnesium is necessary for the normal structure of bone and teeth.	Yes	Yes
IRON								
Fe1	1. Oxygen transport	✓			✓	Iron is necessary for the normal transport of oxygen in the body.	Yes	Yes
Fe2	2. Energy production		✓		✓	Iron contributes to normal energy production.	Yes	Yes
Fe3	3. Metabolism of foreign substances		✓		✓	Iron contributes to the body's ability to metabolise drugs and other substances.	Yes	Yes
Fe5	5. Immune system	✓			✓	Iron is necessary for the normal function of the immune system.	Yes	Yes
Fe7	7. Blood formation		✓	✓		Iron contributes to normal blood formation.	Yes	Yes
Fe8	8. Neurological development in embryos	✓			✓	Iron is necessary for normal neurological development in embryos.	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
COPPER								
Cu1	1. Connective tissues		✓	✓		Copper contributes to the normal structure of connective tissues (such as in bone, lungs and the vascular system).	Yes	Yes
Cu2	2. Transport and metabolism of iron		✓		✓	Copper contributes to the normal transport and metabolism of iron in the body.	Yes	Yes
Cu5	5. Antioxidant activity		✓		✓	Copper contributes to cell protection from the damage caused by free radicals (for example, as a constituent of superoxide dismutase).	Yes	Yes
Cu4	4. Energy production	✓			✓	Copper is necessary for normal energy production.	Yes	Yes
Cu6	6. Neurological system	✓			✓	Copper is necessary for normal neurological function.	Yes	Yes
Cu7	7. Immune system	✓			✓	Copper is necessary for the normal function of the immune system.	Yes	Yes
Cu12	12. Skin and hair pigment	✓		✓		Copper is necessary for normal colouration of skin and hair.	Yes	Yes
IODINE								
I1	1. Production of thyroid hormones	✓			✓	Iodine is necessary for the normal production of thyroid hormones.	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
I3	3. Neurological development	✓		✓		Iodine is necessary for normal neurological development.	Yes	Yes
I4	4. Energy metabolism	✓			✓	Iodine is necessary for normal energy metabolism.	Yes	Yes
ZINC								
Zn1	1. Immune system	✓			✓	Zinc is necessary for the normal function of the immune system.	Yes	Yes
Zn2	2. Cell division	✓			✓	Zinc is necessary for normal cell division.	Yes	Yes
Zn7	7. Reproductive development		✓	✓		Zinc contributes to normal reproductive development.	Yes	Yes
Zn8	8. Skin and wound healing		✓	✓	✓	Zinc contributes to the normal structure of skin and normal wound healing.	Yes	Yes
MANGANESE								
Mn1	1. Bone formation		✓	✓		Manganese contributes to normal bone formation.	Yes	Yes
Mn2	2. Energy metabolism		✓		✓	Manganese contributes to normal energy metabolism.	Yes	Yes
Mn3	3. Antioxidant activity		✓		✓	Manganese contributes to cell protection from the damage caused by free radicals (such as the superoxide free radical).	Yes	Yes

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
SODIUM (NB: It is essential that consumers continue to be encouraged to reduce sodium intake)								
Na1	1. Water and electrolyte balance	✓			✓	Sodium is necessary for normal water and electrolyte balance throughout the body.	Yes	Yes
Na4	4. Nutrient absorption	✓			✓	Sodium is necessary for the normal absorption of nutrients during digestion (such as the active transport of nutrients and water from the gut).	Yes	Yes
POTASSIUM (NB: It is essential that consumers continue to be encouraged to increase potassium intake)								
K1	1. Water and electrolyte balance	✓			✓	Potassium is necessary for normal water and electrolyte balance throughout the body.	Yes	Yes
SELENIUM								
Se1	1. Antioxidant activity	✓			✓	Selenium is necessary for cell protection from some types of damage caused by free radicals.	Yes	Yes
Se2	2. Utilization of iodine in the production of thyroid hormones	✓			✓	Selenium is necessary for the normal utilization of iodine in the production of thyroid hormones.	Yes	Yes
Se7	7. Immune system	✓			✓	Selenium is necessary for the normal function of the immune system.	Yes	Yes
CHROMIUM								

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
MOLYBDENUM								
FLUORIDE (NB: The following statement relates to an enhanced function rather than an essential function)								
Fl1a	1a. Teeth		✓			Fluoride contributes to the maintenance of healthy teeth.*	Yes	Yes
CHLORIDE								
Cl1	1. Water and electrolyte balance	✓			✓	Chloride is necessary for normal water and electrolyte balance throughout the body.	Yes	Yes
Cl2	2. Stomach acid and digestion	✓			✓	Chloride is necessary for the normal production of hydrochloric acid in the stomach, which is required for digestion.	Yes	Yes
PHOSPHORUS								
P1	1. Bone and teeth	✓		✓		Phosphorus is necessary for the normal structure of bone and teeth.	Yes	Yes
P2	2. Cell membranes	✓		✓		Phosphorus is necessary for the normal structure of cell membranes, in the form of phospholipids.	Yes	Yes
P4	4. Energy metabolism	✓			✓	Phosphorus is necessary for normal energy metabolism.	Yes	Yes

* Exception; see page 22

Table 2: Rejected nutrient function statements

Reasons for the exclusion of statements

Statements that did not meet all of the inclusion criteria were recommended for rejection from the final list. The independent experts assigned one of the following reasons for exclusion, which correspond with the inclusion criteria, to each rejected statement:

- i) No (not a health statement)
- ii) No (inaccurate interpretation of evidence)
- iii) No (too imprecise)
- iv) No (data insufficient)
- v) No (data inconsistent)
- vi) No (no plausible mechanism)

Additionally, statements were rejected if the experts considered that the related function was captured by, or the result of, functions encapsulated in another statement. The reason for exclusion was recorded as follows, together with the code of the relevant included statement, as follows:

- vii) No (see (e.g.) VA1b)

The independent experts also considered that a number of statements related to functions that were common to all the vitamins and minerals reviewed. Such statements were moved to Table 1a and appear in Table 2 with the following reason for rejection:

- viii) No (see Table 1a)

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VITAMIN A								
VA1a	1a. Eyes	✓			✓	Vitamin A is necessary for the normal function of the eye.	No (see VA1b)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VA3	3. Embryonic development		✓	✓		Vitamin A contributes to normal embryonic development.	No (see Table 1a)	No
VA5	5. Growth	✓		✓		Vitamin A is necessary for normal growth.	No (see Table 1a)	No
VITAMIN D								
VD2b	2b. Skin		✓	✓		Vitamin D contributes to the normal structure of skin.	No (data inconsistent)	No
VD2c	2c. Immune system		✓		✓	Vitamin D contributes to the normal function of the immune system.	No (data inconsistent)	No
VITAMIN E								
VE2	2. Cell proliferation & differentiation		✓	✓		Vitamin E contributes to cell growth and multiplication.	No (see Table 1a)	No
VE3	3. Immune system		✓		✓	Vitamin E contributes to the normal function of the immune system.	No (data inconsistent; see VE1)	No
VE4	4. Vasodilation/circulation		✓		✓	Vitamin E contributes to the normal function of arteries.	No (data inconsistent)	No
VITAMIN K								
VK3	3. Arteries		✓		✓	Vitamin K contributes to the normal function of arteries.	No (data inconsistent)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VK4	4. Embryonic development		✓	✓		Vitamin K contributes to normal embryonic development.	No (see Table 1a)	No
THIAMIN								
RIBOFLAVIN								
Ri4	4. Fetal growth		✓	✓		Riboflavin contributes to normal fetal growth.	No (see Table 1a)	No
Ri5	5. Eyes		✓	✓		Riboflavin contributes to the normal structure of eyes.	No (see R13)	No
Ri6	6. Red blood cells		✓	✓		Riboflavin contributes to the normal structure of red blood cells.	No (see Ri2)	No
NIACIN								
Ni2a	2a. DNA replication	✓			✓	Niacin is necessary for the normal repair and replication of DNA.	No (inaccurate interpretation of evidence)	No
Ni2b	2b. Growth		✓	✓		Niacin contributes to normal growth in the developing fetus.	No (see Table 1a)	No
Ni3	3. Fatty acid and steroid synthesis		✓	✓		Niacin contributes to the normal structure of some steroids, which are required to make hormones.	No (too imprecise)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
PANTOTHENIC ACID								
Pa2	2. Molecule structure		✓	✓		Pantothenic acid contributes to the normal structure of numerous essential molecules in the body.	No (too imprecise)	No
VITAMIN B₆								
VB ₆ 3	3. Hormones	✓			✓	Vitamin B ₆ is necessary for the normal function of some hormones.	No (too imprecise)	No
FOLATE								
Fo3	3. Neurotransmitters	✓		✓		Folate is necessary for the normal structure of some neurotransmitters.	No (data inconsistent)	No
VITAMIN B₁₂								
VB ₁₂ 3	3. Energy production		✓		✓	Vitamin B ₁₂ contributes to normal energy production.	No (too imprecise)	No
BIOTIN								
Bi2	2. Fatty acids	✓			✓	Biotin is necessary for the synthesis of fatty acids, which are important for the normal structure of cell membranes.	No (see Bi1)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
Bi3a	3a. Cell proliferation	✓			✓	Biotin is necessary for normal cell proliferation.	No (see Table 1a)	No
Bi3b	3b. Growth		✓	✓		Biotin is contributes to normal growth in the developing embryo and infant.	No (see Table 1a)	No
VITAMIN C								
VC2	2. Wound healing	✓		✓		Vitamin C is necessary for the normal structure of wounds.	No (see VC1)	No
VC3	3. Scar tissue	✓		✓		Vitamin C is necessary for the normal structure of scar tissue.	No (see VC1)	No
VC4	4. Gums	✓		✓		Vitamin C is necessary for the normal structure of gums.	No (see VC1)	No
VC6	6. Skin	✓		✓		Vitamin C is necessary for the normal structure of skin.	No (see VC1)	No
VC7	7. Bone	✓		✓		Vitamin C is necessary for the normal structure of connective tissue in bone.	No (see VC1)	No
VC8	8. Joints		✓	✓		Vitamin C contributes to the normal structure of joints.	No (see VC1)	No
VC11	11. Carnitine	✓		✓		Vitamin C is necessary for the normal structure of carnitine.	No (see VC1)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
VC13	13. Metabolism of foreign compounds		✓		✓	Vitamin C contributes to the breakdown of undesirable chemicals.	No (see VC10)	No
VC14	14. Muscle function	✓			✓	Vitamin C is necessary for the normal function of muscles.	No (see VC1)	No
CALCIUM								
Ca4	4. Nerve transmission	✓			✓	Calcium is necessary for normal nerve signals and messages.	No (see Ca2)	No
Ca5	5. Cell wall permeability	✓			✓	Calcium is necessary for the normal permeability of cell membranes.	No (see Ca2)	No
Ca6	6. Hormone secretion		✓		✓	Calcium contributes to the normal release of hormones, such as insulin	No (see Ca2)	No
Ca7	7. Blood pressure		✓		✓	Calcium contributes to maintaining normal blood pressure.	No (data inconsistent)	No
Ca8	8. Digestion	✓			✓	Calcium is necessary for the normal function of enzymes, such as those required for digestion.	No (too imprecise)	No
MAGNESIUM								
Mg2	2. Cell replication		✓		✓	Magnesium contributes to normal cell replication.	No (see Table 1a)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
Mg5	5. Vitamin D metabolism	✓			✓	Magnesium is necessary for the normal activation of vitamin D in the body.	No (see Mg6)	No
IRON								
Fe4	4. DNA synthesis, growth		✓		✓	Iron contributes to normal DNA synthesis, required for growth.	No (see Table 1a)	No
Fe6	6. Taste		✓		✓	Iron contributes to normal taste function.	No (data insufficient)	No
COPPER								
Cu3	3. Red blood cells		✓	✓		Copper contributes to the normal structure of red blood cells.	No (see Cu2)	No
Cu8	8. Fetal development		✓	✓		Copper contributes to the normal development of the fetus, including the brain.	No (see Table 1a)	No
Cu9	9. Allergic reaction		✓		✓	Copper contributes to the normal control of an allergic reaction.	No (data inconsistent)	No
Cu10	10. Cholesterol and glucose metabolism		✓		✓	Copper contributes to the normal metabolism of glucose and cholesterol.	No (data inconsistent)	No
Cu11	11. Blood clots		✓	✓		Copper contributes to the normal structure of blood clots.	No (data inconsistent)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
IODINE								
I2	2. Growth	✓		✓		Iodine is necessary for normal growth.	No (see Table 1a)	No
ZINC								
Zn3	3. Enzyme function	✓			✓	Zinc is necessary for the normal function of numerous enzymes.	No (see Table 1a)	No
Zn4	4. General growth		✓	✓		Zinc contributes to normal growth.	No (see Table 1a)	No
Zn5	5. Neurological function		✓		✓	Zinc contributes to normal brain function	No (data inconsistent)	No
Zn6	6. Insulin action	✓			✓	Zinc is necessary for the normal synthesis and action of insulin.	No (data inconsistent)	No
Zn9	9. Wound healing		✓		✓	Zinc contributes to normal wound healing.	No (see Zn8)	No
Zn10	10. Antioxidant activity		✓		✓	Zinc contributes to cell protection from the damage caused by free radicals.	No (data inconsistent)	No
MANGANESE								
Mn4	4. pH regulation		✓		✓	Manganese contributes to the normal regulation of pH levels in the body	No (data insufficient)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
Mn5	5. Insulin action		✓		✓	Manganese contributes to the normal action of insulin, required for energy metabolism	No (data inconsistent)	No
SODIUM								
Na2	2. Blood pressure		✓		✓	Sodium contributes to normal blood pressure.	No (see Na1)	No
Na3	3. Nerves and muscle	✓			✓	Sodium is necessary for the normal function of nerves and muscle.	No (see Na1)	No
Na5	5. Metabolic rate		✓		✓	Sodium contributes to the body's normal metabolic rate.	No (see Na1)	No
POTASSIUM								
K2	2. Blood pressure		✓		✓	Potassium contributes to normal blood pressure.	No (see K1)	No
K3	3. Nerves and muscle		✓		✓	Potassium contributes to normal nerve and muscle function, including those involved in digestion.	No (see K1)	No
K4	4. Energy metabolism		✓		✓	Potassium contributes to normal energy metabolism, required for cell activity.	No (see K1)	No
K5	5. Secretion of insulin	✓			✓	Potassium is necessary for the normal secretion of insulin by the pancreas.	No (see K1)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
K6	6. Growth	✓		✓		Potassium is necessary for normal growth.	No (see Table 1a)	No
K7	7. pH regulation		✓		✓	Potassium contributes to normal pH regulation (acid-base balance).	No (see K1)	No
K8	8. Nutrient transfer	✓			✓	Potassium is necessary for the normal transfer of nutrients in and out of cells.	No (see K1)	No
SELENIUM								
Se3	3. Regeneration of molecules		✓		✓	Selenium contributes to the body's normal ability to re-use some molecules such as vitamin C.	No (not a health statement)	No
Se4	4. Muscle		✓		✓	Selenium contributes to normal muscle function.	No (data inconsistent)	No
Se5	5. Embryonic development		✓	✓		Selenium contributes to normal embryonic development.	No (see Table 1a)	No
Se6a	6a. Sperm development		✓	✓		Selenium contributes to the normal development of sperm.	No (data inconsistent)	No
Se6b	6b. Reproduction		✓		✓	Selenium contributes to normal reproduction.	No (data inconsistent)	No
CHROMIUM								
Cr1	1. Insulin regulation	✓			✓	Chromium is necessary for the normal regulation of insulin.	No (no plausible mechanism)	No

Reference #	Effect on	Necessary for	Contributes to	Normal structure	Normal function	Nutrient function statement	Recommended by Expert Committee (yes / no*)	Recommended by Council (yes / no*)
Cr2	2. Lipid metabolism		✓		✓	Chromium contributes to the normal metabolism of lipids.	No (see Table 1a)	No
Cr3	3. DNA synthesis		✓		✓	Chromium contributes to normal DNA synthesis and the expression of some genes.	No (see Table 1a)	No
MOLYBDENUM								
Mo1	1. Enzyme activity	✓			✓	Molybdenum is necessary for the normal activity of some enzymes in the body.	No (see Table 1a)	No
FLUORIDE								
F11b	1b. Tooth enamel	✓		✓	✓	Fluoride is necessary for the normal structure and function of enamel in teeth.	No (see F11a)	No
CHLORIDE								
PHOSPHORUS								
P3	3. pH regulation		✓		✓	Phosphorus contributes to the normal regulation of pH levels in the body.	No (too imprecise)	No
P5	5. Tissue growth	✓		✓		Phosphorus is necessary for the normal tissue growth, such as muscle.	No (too imprecise)	No
P6	6. Breast milk	✓			✓	Phosphorus is necessary for normal breast milk.	No (too imprecise)	No

ANNEX 1

The Joint Health Claims Initiative

The JHCI is a unique collaboration between the food industry, consumer groups and enforcement authorities to establish a self-regulatory approach to the use of health claims on foods in the light of growing interest in the links between diet and health. The JHCI Code of Practice was launched in December 2000 in the absence of specific EU legislation for health claims and was developed to:

- define a health claim;
- outline the legal framework within which a claim can be made;
- set criteria and general nutrition principles for making a claim;
- identify the ways in which new and existing claims must be scientifically substantiated; and
- set out requirements for labelling and consumer information about the health benefits of a product.

JHCI Expert Committee Members

(The “independent experts” for the purposes of this project)

Carol Stevens, Chairman	(Worcester Scientific Services)
Dr Judy Buttriss	(British Nutrition Foundation, London)
Dr Susan Jebb	(MRC Human Nutrition Research, Cambridge)
Prof Michael Lean	(Queen Elizabeth University, Glasgow)
Prof Tom Sanders	(Kings College London)
Prof Sean Strain	(University of Ulster at Coleraine, Northern Ireland)
Prof Martin Wiseman	(Independent Nutrition Consultant, London)

The Terms of Reference of the Expert Committee are as follows:

‘The Expert Committee exists to underpin the Joint Health Claims Initiative by providing an objective and credible expert opinion on the scientific validity of a health claim under the JHCI Code.’

JHCI Council Members

(The “second independent party” for the purposes of this project)

<u>Member:</u>	<u>Representative of:</u>
Roger Manley, OBE	(JHCI Chairman, ex-Chief Trading Standards Officer)
Sheila Kelly	(Proprietary Association of Great Britain)
Mike Buchanan	(British Retail Consortium)
Valerie Saint	(Food and Drink Federation)
Mike O’Neill	(National Consumer Council)
Dr Mike Rayner	(Sustain, The National Alliance for Better Food and Farming)
Kate Lees	(British Dietetic Association)

David Walker (Local Authorities Coordinators of Regulatory Services)
Kevin Wardle (Local Authorities Coordinators of Regulatory Services)
David Pickering (Local Authorities Coordinators of Regulatory Services)

The Council is the primary policy and control forum for the Joint Health Claims Initiative and, together with the Executive Director, it directs and manages the affairs of the Joint Health Claims Initiative.

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ANNEX 2

Evaluation report of a pilot study

The following evaluation report of a pilot study was a working document in the early stages of this project. References to JHCI documents (denoted (JHCI/xx/xx)) are not included in this report but are publicly available on request to the JHCI Secretariat.

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JHCI/23/03

EVALUATION REPORT

**of a pilot study to test the JHCI process for
identifying well-established health statements.**

Prepared by
JHCI Executive Director

13th March 2003

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INTRODUCTION

The Joint Health Claims Initiative has been sponsored by the UK Food Standards Agency to develop:

- i) A process that defines and identifies well-established health statements
- ii) A list of well-established nutrient-function statements for the nutrients listed in the Annex 1 to the Food Supplements Directive (2002/46/EC).

A proposed process to identify and define well-established health claims was originally presented in paper JHCI/108d/02. Since this draft was prepared it has been agreed that the process will identify and define well-established health 'statements', rather than claims. The rationale for this change is presented in the main body of this document, along with other recommended amendments to the process.

JHCI will use the draft final process to produce an initial list of well-established nutrient function statements, however it is intended that the process itself is also applicable to enhanced function health statements and disease risk reduction health statements for food. This could also provide the European Commission with a useful mechanism for identifying well-established claims currently on the market that could be added to its Register of generally accepted health claims.

A pilot study using vitamin C was undertaken by the JHCI Secretariat to ensure that the proposed process was workable and to identify if any further changes were necessary. Members of the JHCI Council and Expert Committee have considered the results of the pilot study (refer JHCI/11/03, draft 2). Their recommendations have been reported in the following paper and will be incorporated in the draft Final Process that will be used to produce the list of well-established nutrient-function statements, described in (ii) above.

Therefore the purpose of this paper is to report the:

- 1) Proposed process to identify and define well-established health statements
- 2) Pilot study of the proposed process to identify well-established nutrient function statements for vitamin C
- 3) Refinements to the proposed process as recommended by the JHCI Expert Committee and Council.

THE PROPOSED PROCESS

The proposed process, as set out in JHCI/108d/02, is reported below and is split into two stages:

Stage 1 - The proposed process for approving well-established health claims

Stage 2 - Using the proposed process to identify well-established nutrient function claims for vitamin C.

STAGE 1:

Proposed process for approving well-established health claims

Step 1. Clearly define ‘well-established’. Well-established implies ‘agreed by authoritative sources’. For the purpose of this project and to provide a transparent process there needs to be a clear definition of well-established.

Step 2. Determine priority order for groups of nutrients, food components or claims to be considered.

Step 3. Establish working definitions as necessary, such as types of health claims to be considered.

Step 4. Agree credible source documents to draw up a list of possible functions for nutrients and food components and to identify which of the possible functions are ‘well established’.

Step 5. Develop phraseology as necessary to provide a model for acceptable wording for claims.

Step 6. Draw up comprehensive list of functional claims. This step will involve consideration of claims in relation to current legislation and consumer perception principles.

STAGE 2:

Using the proposed process to produce a list of well-established nutrient function claims

1. JHCI agreed definition of ‘well-established’

Functions will be considered to be ‘well-established’ when the source documents are consistent in their reporting of the relevant functions.

2. Priority order for consideration of nutrients

The FSA and JHCI have agreed that the nutrients in the Food Supplements Directive (2002/46/EC) will be considered to produce an initial list of claims (and that vitamin C would be used to test the process).

3. Other definitions

To test the process it has been agreed that JHCI will consider nutrient-function claims at this stage. For these purpose the Codex definition of ‘nutrient function claim’ will be adopted:

‘...a claim that describes the physiological role of the nutrient in growth, development and normal functions of the body’. (Codex Guidelines for the Use of Nutrition Claims 1997).

4. Source documents

The JHCI Expert Committee has suggested that information provided by the USA’s Institute of Medicine (and published on the National Academy of Science/IOM website) should be used as a starting point for drawing up a list of possible functions, as this information is based on systematic reviews of evidence and is internationally recognised. This list will be cross-checked with similar material from the UK and Europe, both to demonstrate consistency in the functions reported (ie, to confirm that the information is well-established) and to anglicise the list for the UK population, as follows:

- Report of the COMA Committee on Dietary Reference Values (1991)
- Draft Report of the Expert Group on Vitamins and Minerals on Safe Upper Levels for Vitamins and Minerals
- Report of the Scientific Committee for Food (Thirty-first series) on ‘Nutrient and energy intakes for the European Community’ (1992).
- ‘Introduction to Human Nutrition’. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.
- The Merck Index

5. Proposed phraseology

It is proposed that, for the purpose of developing a list of well-established nutrient function claims, the claims should be phrased in the following way:

x is essential for / required for / helps in the normal development of y
x is essential for / required for / helps in the normal growth of y
x is essential for / required for / helps in the normal function of y
x is essential for / required for / helps in z

Where:

- **x** is a nutrient where a nutrient is defined as energy, protein, carbohydrate, fat (or sub-fractions thereof); fibre, vitamins and minerals (adapted from the 1990 Nutrition Labelling Directive)

- **y** is the whole body, a bodily system (the cardiovascular system), organ (e.g. the heart.), a tissue (e.g. the blood), or a component of a tissue (e.g. red blood cells)
- **z** is a normal function of the body (e.g. the metabolism) or a specific function (e.g. oxidative processes).

When appearing in food labelling then the wording of the well-established nutrient function claim may be altered, as long as the spirit of the claim remains.

The JHCI Council agreed, at its meeting on 13th December, that the distinctions between ‘essential for / required for / helps in the normal...’ may not be helpful, meaningful or easily understood by consumers. The wording of the claims will be considered during the pilot to determine whether JHCI should recommend to the FSA that consumer research be undertaken.

6. Draw up comprehensive list claims

6a. Pilot the process. The JHCI Council has agreed that vitamin A, vitamin C and a mineral should be used to test that the process works. A report of the pilot will be prepared for consideration by the Expert Committee and Council (and available to the FSA) before the process is approved for use to develop the final list. Refinements to the process will be made if necessary during the pilot process. At this stage, it is envisaged that addition of claims to the list will occur via the following process:

- i. Undertake and audit of functional claims, for the relevant nutrient, currently on the market in major supermarkets and health food shops
- ii. Prepare a brief monograph for each nutrient, presenting functions cited in the source documents and including claims currently on the market as an Annex
- iii. Submit each monograph to the Expert Committee for it to determine whether the functions are well-established
- iv. Generate a ‘model claim’ using the proposed phraseology as a guide and after consideration of how the claim could be presented on food products
- v. Consult with nutrition scientists with expertise in consumer perception to help ensure that the model claims are likely to be understood and meaningful to consumers.
- vi. Add to final list.

6b. The final list. This will be limited to well-established nutrient-function claims for dietary and synthetic forms of nutrients listed in the Annex 1 of the Food Supplements Directive 2002. Members of the JHCI Council, whose expertise includes food law, enforcement and consumer perception of claims, will agree the list before the final version is submitted to the Food Standards Agency.

PILOT STUDY & RECOMMENDATIONS

1. Selection of source documents:

A detailed review of the source documents recommended in Stage 2, (4) above, found that, apart from the Institute of Medicine's Dietary Reference Intakes publication, the documents largely reported information in relation to the determination of safe upper limits, or dietary reference values, rather than a detailed account of the functions of the vitamin. The JHCI Expert Committee therefore recommended the following pool of alternative source documents:

- Reports of the Expert Group on Vitamins and Minerals (EVM)
- British Nutrition Foundation Task Force reports
- CRC Press handbooks
- Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992)
- International Life Sciences Institute documents
- Other papers relevant to each nutrient as identified by the JHCI Expert Committee

It was agreed by the Expert Committee that the following source documents would be used for the review of vitamin C, whereby functions that were reported in Reference 1 would need to be supported by either Reference 2 or Reference 3 in order to meet the requirement of 'well-established':

Reference 1:

Institute of Medicine Dietary Reference Intakes for Vitamin C, E, selenium and carotenoids. Washington D.C. National Academy Press, 2000.

AND

Reference 2 :

Review of Vitamin C. Expert Group on Vitamins and Minerals. November 1999.

AND/OR

Reference 3:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Recommendation:

A tailored mix of source documents will be used to support functions reported in IOM documents, whereby the Expert Committee will provide advice on the most appropriate sources for specific nutrients.

2. Selection of quotes from source documents

The quotes reported for vitamin C were selected to present the broadest range of functions for the nutrient. To minimise repetition, comments that were reported more than once within each source document were quoted only once. Reference 1 provided the most quotes as this document reviewed the functions of vitamin C in more detail than Reference 2 or 3. Quotes about functions were categorised according to the body organ, component or physiological process to which they were linked, with the view to formulating a health statement in accordance with the proposed phraseology.

3. Comparison with information currently available to consumers

An audit of functional claims for vitamin C currently on the market was undertaken to provide a comparison of functions reported in the source documents with actual claims about vitamin C made on foods and dietary supplements. Also, information for consumers about common nutrients published on the UK Food Standards Agency website was also reported.

Recommendation:

Although this information provides an interesting comparison, an audit of this nature should be undertaken for all health claims on the market, not just nutrient function claims. Therefore an audit of nutrient function claims should be removed from Stage 2, 6a (i) of the process.

4. Developing health claims for ‘well-established nutrient-functions’

A summary of the well-established functions for vitamin C were presented in ‘Table 1: Summary of well-established nutrient functions for Vitamin C’. A small section of this table is presented below:

	Essential for	Required for	Helps in	Normal synthesis	Normal development	Normal growth	Normal function	Normal process
1. Collagen	✓			✓				
4. Gums	✓						✓	
9. Iron absorption			✓					✓
10. Antioxidant properties			✓					✓

Model claims were then formulated in accordance with the draft phraseology for nutrient-function claims set out in Stage 2, 5, above. For example:

1. *“Vitamin C is essential for the normal synthesis of collagen”*
4. *“Vitamin C is essential for the normal function of gums”*

9. *“Vitamin C helps the body to absorb iron from food”*
10. *“Vitamin C (a powerful antioxidant) helps to protect cells from the damage caused by free radicals”*

The JHCI Expert Committee’s comments in relation to the overall process have been presented in this paper, rather than the specific details about the functions of vitamin C, which will be reported on separately.

Recommendations:

- (i) **The remit of the project is to identify well-established health ‘statements’ rather than ‘claims’, given that the project will not involve the assessment of health claims on food products. Once the list of well-established nutrient function statements has been published, companies applying these statements to food products, as health claims, should comply with the JHCI Code of Practice for Health Claims for Food (JHCI, 2000)**
- (ii) **The proposed phraseology, presented in section 5 above, should be replaced with:**

‘Nutrient (x) is necessary for/contributes to the normal development/function/process of (y)’

Whereby:

- ***‘Necessary for’* was selected to prevent confusion over the meaning of ‘essential’, given that it has a specific definition with regards to particular dietary components.**
 - ***‘Contributes to’* was selected to replace both ‘required for’ and ‘helps in’ given that the distinction is small between these two terms.**
 - ***‘Normal development’* was retained to replace both ‘normal synthesis’ and ‘normal growth’ given that ‘development’ encompasses development, growth, regeneration and maintenance of tissues and structures.**
 - **The working definition of ‘normal’ will need to be confirmed under Stage 3 of the process when each nutrient is assessed.**
- (iii) **The table of summarised results for each nutrient should be modified to reflect the amended phraseology, as follows:**

	Necessary for	Contributes to	Normal development	Normal function	Normal process
1. Collagen	✓		✓		
4. Gums	✓			✓	
9. Iron absorption		✓			✓
10. Antioxidant properties		✓			✓

- (iii) The scientific accuracy of statements must be maintained if technical jargon is simplified to aid consumer understanding.**
- (iv) British/European terminology should replace American terms whenever possible.**

5. Dosage and applicability of statements to food products

The vitamin C pilot considered whether JHCI should specify a minimum and maximum dosage for products to carry nutrient-function statements. The statements are about normal nutrient functions, not enhanced nutrient functions, therefore the level of the nutrient required to support the function will usually reflect quantities obtainable from natural food sources and a healthy balanced diet.

Recommendations:

- (i) Products must comply with the legally defined minimum nutrient requirements, as set out in the Food Labelling Regulations (refer section 5.1.1 JHCI Code).**
- (ii) The remit of this project does not include quantification of the nutrient function statements, or to recommend a nutrient content range prerequisite in order for products to carry the statements. The Expert Group on Vitamins and Minerals is currently establishing Safe Upper Limits for all nutrients in the Annex 1 to the Food Supplements Directive (2002/46/EC), which the JHCI will make reference to once the EVM has completed its work.**
- (iii) Labelling should not suggest to consumers that pharmacological doses of the nutrient are required to support normal functions, or that consuming high doses will deliver an enhanced function. Products that contain the nutrient in amounts significantly higher than the UK Reference Nutrient Intake for adults should not suggest to consumers that the beneficial effect cannot be obtained from a healthy diet and natural food sources (refer sections 6.2.6 & 6.2.7 JHCI Code of Practice).**

- (iv) **All products carrying the health statements should be made in accordance with the general principles in the JHCI Code of Practice for Health Claims for Food (2000).**

6. Enhanced nutrient functions

The pilot considered whether it would be useful to include information about the nutrient's relationship to enhanced functions and reduced risk of disease.

Recommendation:

The process has been designed to assess any type of claim, in terms of whether or not it is defined as 'well-established', however, only 'normal' functions or processes in the body will be considered during the development of a list of well-established nutrient function statements.

SUMMARY OF RECOMMENDATIONS

- The project will relate to health 'statements' rather than health 'claims'
- A tailored mix of source documents for each nutrient will be reviewed to determine whether statements are well-established
- An audit of nutrient-function claims currently on the market will not be undertaken within this project
- The proposed phraseology has been amended significantly
- The JHCI will not develop maximum upper limits for products to carry the statements or quantify the nutrient function statements (although JHCI is likely to recommend that the latter is carried out at some future date by an authoritative body)
- Enhanced functions and disease risk reduction statements will not be considered during the development of the list of well-established nutrient function statements.

THE DRAFT FINAL PROCESS

Following the pilot study, the recommendations reported in this paper have been incorporated in to the draft final process, presented in paper JHCI/19/01, which will be used to proceed with assessing the nutrients in the Annex 1 to the Food Supplements Directive (2002/46/EC). As other nutrients are assessed, it may become apparent that further refinements to the process are required before it is finalised and submitted to the Food Standards Agency at the completion of the project in late June 2003.

ANNEX 3

Nomenclature of nutrients

1. Nutrients listed in Annex 1 of the Food Supplements Directive (2002/46/EC)

Vitamin A (beta-carotene)	Calcium
Vitamin D	Magnesium
Vitamin E	Iron
Vitamin K	Copper
Thiamin (B ₁)	Iodine
Riboflavin (B ₂)	Zinc
Niacin	Manganese
Pantothenic Acid	Sodium
Vitamin B ₆	Potassium
Folate (folic acid, folacin)	Selenium
Vitamin B ₁₂	Chromium
Biotin	Molybdenum
Vitamin C	Fluoride
	Chloride
	Phosphorus

2. Nomenclature as listed in ‘Directions for Contributors’, British Journal of Nutrition’:

Nomenclature of Vitamins: Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature (see *Nutrition Abstracts and Reviews A* (1978) **48**, 831-835).

<i>Acceptable Name</i>	<i>Other names*</i>
Vitamin A	
Retinol	Vitamin A ₁
Retinaldehyde, retinal	Retinene
Retinoic acid (all- <i>trans</i> or 13- <i>cis</i>)	Vitamin A ₁ acid
3-Dehydroretinol	Vitamin A ₂
Vitamin D	
Ergocalciferol, ercalciol	Vitamin D ₂ califerol
Cholecalciferol, calciol	Vitamin D ₃
Vitamin E	
α-, β- and γ-tocopherols plus tocotrienols	
Vitamin K	
Phylloquinone	Vitamin K ₁
Menaquinone-n (MK-n)+	Vitamin K ₂
Menadione	Vitamin K ₃
	Menaquione
	Menaphthone

<i>Acceptable Name</i>	<i>Other names*</i>
Vitamin B₁	
Thiamin	Aneurin(e), thiamine
Vitamin B₂	
Riboflavin	Vitamin G, riboflavine Lactoflavin
Niacin	
Nicotinamide	Vitamin PP
Nicotinic acid	
Folic Acid	
Pteroyl(mon)glutamic acid	Folacin, vitamin B _c or M
Vitamin B₆	
Pyridoxine	Pyridoxol
Pyridoxal	
Pyridoxamine	
Vitamin B₁₂	
Cyanocobalamin	
Hydroxocobalamin	Vitamin B _{12a} or B _{12b}
Aquocobalamin	
Methylcobalamin	
Adenosylcobalamin	
Inositol	
Myoinositol	Meso-inositol
Choline	
Pantothenic acid	
Biotin	Vitamin H
Vitamin C	
Absorbic acid	
Dehydroascorbic acid	

*Including some names which are still in use elsewhere, but are not used by the British Journal of Nutrition.

+ Details of the nomenclature for these and other naturally occurring quinones should follow the Tentative Rules of the IUPAC-IUB Commission on Biochemical Nomenclature (see European Journal of Biochemistry (1975) 53, 15-18).

Generic descriptors

The terms **vitamin A**, **vitamin C** and **vitamin D** may still be used where appropriate, for example in phrases such as 'vitamin A deficiency', 'vitamin D activity'.

Vitamin E. The term **vitamin E** should be used as the descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of *α*-tocopherol. The term **tocopherols** should be used as the generic descriptor for all methyl tocots. Thus, the term **tocopherol** is not synonymous with the term **vitamin E**.

Vitamin K. The term **vitamin K** should be used as the generic descriptor for 2-methyl-1,4-naphthoquinone (menaphthone) and all derivatives exhibiting qualitatively the biological activity of phylloquinone (phytylmenaquinone).

Niacin. The term **niacin** should be used as the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.

Vitamin B₆. The term **vitamin B₆** should be used as the generic descriptor for all 2-methylpyridine derivatives exhibiting qualitatively the biological activity of pyridoxine.

Folate. Due to the wide range of carbon-substituted, unsubstituted, oxidized, reduced and mono- or polyglutamyl side-chain derivatives of pteroylmonoglutamic acid, which exist in nature, it is not possible to provide a complete list. Authors are encouraged to use either the generic name or the correct scientific name(s) of the derivative(s), as appropriate for each circumstance.

Vitamin B₁₂. The term **vitamin B₁₂** should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. The term **corrinoids** should be used as the generic descriptor for all compounds containing the corrin nucleus and thus chemically related to cyanocobalamin. The term **corrinoid** is not synonymous with the term **vitamin B₁₂**.

Vitamin C. The terms **ascorbic acid** and **dehydroascorbic acid** will normally be taken as referring to the naturally occurring _L-forms. If the subject matter includes other optical isomers, authors are encouraged to include the _L- or _D-prefixes, as appropriate. The same is true for all those vitamins, which can exist in both natural and alternative isomeric forms.

ANNEX 4

Quotes from source documents

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Annex 5 (page 262) provides a detailed reference list for the source documents.

ANNEX 4.1

Vitamin A

Source documents for reviewing vitamin A

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 1.2:

Institute of Medicine Dietary Reference Intakes for Vitamin C, Vitamin E, selenium and carotenoids. Washington D.C. National Academy Press, 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.1:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Preformed Vitamin A (retinol and retinyl esters). September 2002.
(http://europa.eu.int/comm/food/fs/sc/scf/out145_en.pdf).

Reference 3.2:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Beta Carotene. October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80b_en.pdf).

Reference 4.1:

Revised Review of Vitamin A. Expert Group on Vitamins and Minerals. April 2002.
(<http://www.food.gov.uk/multimedia/pdfs/reviewvita.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

1) Eyes and vision

Code	Proposed statement
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<i>VA1a:</i>	<i>Vitamin A is necessary for the normal function of the eye</i>
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<i>VA1b:</i>	<i>Vitamin A is necessary for normal vision</i>
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Reference 1.1:

‘Vitamin A is important for normal vision, ...’ (pg 82)

‘The 11-*cis*-retinaldehyde (retinal) form of vitamin A is required by the eye for the transduction of light into neural signals necessary for vision (Saari, 1994). The retinoic acid form is required to maintain normal differentiation of the cornea and conjunctival membranes, thus preventing xerophthalmia (Sommer and West, 1996), as well as for the photoreceptor rod and cone cells of the retina. Rods contain the visual pigment rhodopsin (opsin protein bound to 11-*cis*-retinal). The absorption of light catalyzes the photoisomerization of rhodopsin’s 11-*cis*-retinal to all-*trans*-retinal in thousands of rods, which triggers the signaling to neuronal cells associated with the brain’s visual cortex. After photoisomerization, all-*trans*-retinal is released, and for vision to continue, 11-*cis*-retinal must be regenerated. Regeneration of 11-*cis*-retinal requires the reduction of all-*trans*-retinol, thereby providing a local storage pool of retinyl esters. When needed, retinyl esters are hydrolyzed and isomerized to form 11-*cis*-retinal, which is oxidized to 11-*cis*-retinal and transported back to the photoreceptor cells for recombination with opsin to begin another photo cycle.’ (pg 84, 85)

‘The most specific clinical effect of inadequate vitamin A intake is xerophthalmia. The World Health Organisation (WHO, 1982) classified various stages of xerophthalmia to include night blindness (impaired dark adaptation due to slowed regeneration of rhodopsin), conjunctival xerosis, Bitot’s spots, corneal xerosis, corneal ulceration, and scarring, all related to vitamin A deficiency. Night blindness is the first ocular symptom to be observed with vitamin A deficiency (Dowling and Gibbons, 1961), and it responds rapidly to treatment with vitamin A (Sommer, 1982).’ (pg 95)

‘The ability of the retina to adapt to dim light depends upon an adequate supply of vitamin A, because 11-*cis*-retinal is an integral part of the rhodopsin molecule of the rods. Without adequate levels of vitamin A in the retina, the function of the rods in dim light situations becomes compromised, resulting in abnormal dark adaptation (night blindness). Before clinically apparent night blindness occurs, abnormal rod function may be detected by dark adaptation testing. In addition to vitamin A deficiency, zinc deficiency and severe protein deficiency also may affect dark adaptation responses (Bankson et al., 1989; Morrision et al., 1978).’ (pg 97)

‘Before the clinical onset of xerophthalmia, mild vitamin A deficiency leads to early keratinizing metaplasia and losses of mucin-secreting goblet cells on the bulbar surface of the conjunctiva of the eye.’ (pg 105)

Reference 2.0:

‘Vitamin A’ is the collective term for compounds that show the biological properties of retinol, including maintenance of epithelial tissue and visual function. This

classification includes retinol, retinyl esters and retinal (vitamin A aldehyde); retinoic acid is included even though it does not sustain visual function. These are isoprenoid compounds, having in common an 11-carbon polyene chain attached to a trimethyl-substituted cyclohexenyl ring. The term 'retinoids' refers to all compounds, natural or synthetic, that show some biological activity typical of vitamin A, such as promoting differentiation of cells in culture; not all retinoids can support all the functions of vitamin A, e.g. some are unable to contribute to vision.' (pg 1708)

'The major roles of vitamin A are in vision, differentiation of epithelial tissues, and in the immune system. Metabolism of vitamin A in the retina of the eye is unique, in keeping with the unusual role of vitamin A in that tissue. Vitamin A is stored in the retinal pigment epithelium as retinyl esters. All-*trans*-retinyl esters are simultaneously hydrolysed and isomerized to 11-*cis*-retinol, a compound unique to the eye. 11-*cis*-Retinol is then oxidized to 11-*cis*-retinal. 11-*cis*-Retinal is transferred from the pigment epithelium to the rod cells by interstitial retinoid binding protein (IRBP), a distinct binding protein (140,000 Da). In the rod cells, 11-*cis*-retinal binds to the protein opsin to form the visual pigment, rhodopsin. When a photon of light is absorbed by a rhodopsin complex, the 11-*cis*-retinal is isomerized to all-*trans*-retinal and released from the protein complex; the resulting conformation change of the protein initiates a cascade of reactions, resulting in a neural signal to the brain.' (pg 1711)

'In contrast to the high turnover rates of vitamin A in other tissues, vitamin A in the eye is highly conserved, with little leakage back to the liver. Prolonged vitamin A deficiency, however, leads to reduced sensitivity to light, usually first noted as impaired vision at night (night blindness). These effects of vitamin A deficiency are generally reversible by subsequent vitamin A supplementation.' (pg 1711)

'In a very different role, the cornea of the eye depends on vitamin A for proper cell differentiation and for secretion of protective glycoproteins. In vitamin A deficiency, these tissues are susceptible to attack by opportunistic bacteria; such attack may not be reversible and, especially on the corneal surface of the eye, may result in permanent scarring and permanent vision loss. These effects of vitamin A deficiency, unlike those in the retina, may not be reversible by subsequent vitamin A supplementation. Such vitamin A-dependent corneal degeneration (given the general name 'xerophthalmia') accounts for an estimated 500,000 new cases of blindness in preschool children in the world each year.' (pg 1711)

'It has been argued, without conclusive proof as yet, that retinoic acid is the active form of vitamin A required for cellular differentiation. Retinoic acid is an endogenous metabolite of vitamin A. Animals maintained on retinoic acid as sole source of vitamin A seem to grow normally and maintain good health, but become blind (because retinoic acid cannot be converted to retinal); some but not all species also show loss of testicular function.' (pg 1711)

Reference 3.1:

'Retinal, the initial oxidised metabolite of retinol, is the chromophore of rhodopsin, a visual pigment of the cone cells of the pigmented epithelium of the retina. The photo-induced isomerisation of 11-*cis*-retinal into all-*trans*-retinal is the initial event of the

phototransduction cascade, which ends by the production of a signal to the ocular nerves.’ (pg 4)

Reference 4.1:

‘Vitamin A is essential to the processes of vision... Lack of vitamin A results in keratinisation of mucus secreting ciliated epithelium and other epithelial changes. The tissues most affected by this include the ... cornea ...’ (pg 17)

‘Vitamin A is required for vision in the dark and for colour perception. The active form of vitamin A in this function is retinal. In the retinal pigment epithelial cells, all-*trans*-retinol undergoes enzymatic isomerisation to 11-*cis*-retinal by retinol (alcohol) dehydrogenase. The 11-*cis*-retinal forms a Schiff’s base with a specific lysyl residue in the membrane bound protein, opsin. The resultant rhodopsin, when exposed to light, isomerises to form a transoid intermediate. Thereafter follow several more protein conformational changes. The intermediate meta-rhodopsin II, interacts with a G protein, transducin, and activates phosphodiesterase resulting in the hydrolysis of GTP to GMP via the formation of cGMP. cGMP maintains the opening of sodium channels in the rod outer segment. As the level of cGMP falls, sodium entry decreases and the rod cell- membrane hyperpolarises. Changes in membrane potential re transmitted to and integrated by the brain. Return to the basal state occurs by the reconversion of meta-rhodopsin II to opsin and all-*trans*-retinal. All-*trans*-retinal is then reduced to the corresponding isomer of retinol, and consequently isomerised back to 11-*cis*-retinol.’ (pg 18)

‘During this cycle, not all vitamin A is conserved and must be replaced from the circulation. A similar sequence of events is involved in the process of colour sensing in cone cells (Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).’ (pg 18)

Reference 5.0:

‘Helps maintain normal vision in dim light – prevents night blindness and xerophthalmia.’ (pg 78)

2) Skin and mucous membranes

Code	Proposed statement
VA2:	<i>Vitamin A is necessary for the normal structure and function of the skin and mucous membranes (such as in the lung, intestines, nose, eyes and female reproductive tract).</i>

Reference 1.1:

‘Vitamin A is required for the integrity of epithelial cells throughout the body (Gudas et al., 1994). Retinoic acid, through the activation of retinoic acid (RAR) and retinoid X (RXR) receptors in the nucleus, regulates the expression of various genes that encode for structural proteins (e.g., skin keratins), enzymes (e.g., alcohol dehydrogenase), extracellular matrix proteins (e.g., laminin), and retinol binding proteins and receptors.’ (pg 85)

‘Because of the role of vitamin A in maintaining the structural integrity of epithelial cells, follicular hyperkeratosis has been observed with inadequate vitamin A intake (Chase et al., 1971; Sauberlich et al., 1974). Men who were made vitamin A deficient under controlled conditions were then supplemented with either retinol or β -carotene, which caused the hyperkeratosis to gradually clear (Sauberlich et al., 1974).’ (pg 96)

Reference 2.0:

‘The action of retinoids in differentiation is manifest in various systems, including maintenance of epithelial tissue (e.g. the lung, intestines and skin, and the cornea of the eye). In the absence of adequate vitamin A, cells of these tissues do not differentiate normally, but change structure (becoming stratified and cornified) and lose the ability to secrete glycoproteins. The common mechanism underlying these roles of retinoids in diverse tissues seem to involve the binding of retinoic acid (and perhaps retinol) to specific proteins associated with nuclear deoxyribonucleic acid (DNA). These nuclear retinoic acid receptor proteins (RARs), which are distinct from the cytoplasmic ‘cellular retinoic acid binding proteins’ (CRABPs), can then bind to specific regions of DNA, either promoting or inhibiting transcription of specific genes.’ (pg 1711)

Reference 3.1:

‘Retinoic acids, both all-*trans*-retinoic acid (TRA) and its 9-*cis* isomer (9CRA) act as regulators of genomic expression.....’ (pg 4)

‘Retinoic acids are able to bind to specific nuclear receptors known as RAR and RXR receptors; RARs can bind either TRA or 9CRA, while RXRs bind only 9CRA. Upon ligand binding these nuclear receptors bind to specific response elements on DNA, and thus regulate gene expression.’ (pg 4)

Reference 4.1:

‘Vitamin A is essential to the processes of ... cellular differentiation... Lack of vitamin A results in keratinisation of mucus secreting ciliated epithelium and other epithelial changes. The tissues most affected by this include the trachea, skin, salivary gland, cornea and testes.’ (pg 17)

‘Retinoic acid is now recognised as an important signalling molecule that, as a ligand to its nuclear receptors, alters gene expression at the level of transcription. Two sets of retinoic acid nuclear receptors have been identified, known as RAR and RXR, each having α , β and γ subgroups. The RARs and RXRs belong to a family of hormonal regulatory proteins, which include those for steroids, thyroid hormones and vitamin D. Each receptor consists of six domains: an amino-terminal activation domain, a DNA-binding domain, a hinge region, a ligand-binding domain, and a carboxy-terminal involved in heterodimerisation. The RAR receptors bind either all *trans*- or 9-*cis*-retinoic acid, whereas the RXR receptors bind only 9-*cis*-retinoic acid. RXR receptors form dimers with themselves as well as with RAR, the vitamin D receptor, the T3 receptor, PXR, PPAR, β CAR etc. Binding of these dimers to specific response elements usually results in increased gene transcription, although inhibitory interactions do occur (Gerster, 1997 and references therein; Evans, 1996; Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).’ (pg 18)

Reference 5.0:

‘Deficiency symptoms: Rough, dry, scaly skin – a condition known as follicular hyperkeratosis (it looks like “gooseflesh”); increased sinus, sore throat, and abscesses in ears, mouth, or salivary glands; increased diarrhoea ...’ (pg 78)

‘Vitamin A is needed for the growth and repair of cells that line both the small and large intestines.’ (pg 1334)

3) Embryonic Development

Code	Proposed statement
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VA3:	<i>Vitamin A contributes to normal embryonic development</i>
------	--

Reference 1.1:

‘Vitamin A is important for normal..., embryonic development, growth, ...’ (pg 82)

‘Retinoic acid plays an important role in embryonic development. Retinoic acid, as well as RAR, RXR, cellular retinol-binding protein (CRBP), and cellular retinoic acid-binding proteins (CRABP-I and CRABP-II), is present in temporally specific patterns in the embryonic regions known to be involved in the development of structures posterior to the hindbrain (e.g., the vertebrae and spinal cord) (Morriss-Kay and Sokolova, 1996). Retinoic acid is also involved in the development of the limbs, heart, eyes, and ears (Dickman and Smith, 1996; Hofmann and Eichele, 1994; McCaffery and Drager, 1995).’ (pg 85)

‘An association of vitamin A deficiency and impaired embryonic development is well documented in animals (Morriss-Kay and Sokolova, 1996; Wilson et al., 1953). In laboratory animals, fetal resorption is common in severe vitamin A deficiency, while fetuses that survive have characteristic malformations of the eye, lungs, urogenital tract, and cardiovascular system. Similar abnormalities are observed in rat embryos lacking nuclear retinoid receptors (Wendling et al., 1999). Morphological abnormalities associated with vitamin A deficiency are not commonly found in humans; however, functional defects of the lungs have been observed (Chytil, 1996).’ (pg 95,96)

Reference 3.1:

‘Of particular importance in the setting of an upper level is the role of retinoic acids during morphogenesis and embryonic development. It has long been recognised that abnormal fetal development is associated with either insufficient or excessive intakes of vitamin A and related compounds.’ (pg 4)

‘Moreover, RARs and RXRs show specific spatio-temporal patterns of expression in all developing systems during embryonic development, which suggests that retinoic acid signalling is involved in most, if not all, morphogenetic and patterning processes (Morriss-Kay and Sokolova, 1996).’ (pg 4)

Reference 4.1:

‘Retinol and retinoic acid are essential for morphogenesis in embryonic development and may be involved in control of *Hox* gene expression, vital for correct sequential development (Marshall et al., 1996).’ (pg 18)

Reference 4.1:

‘Vitamin A is essential to the processes of ..., embryonic development, growth and cellular differentiation...’(pg 17)

4) Cell differentiation

Code Proposed statement

VA4: *Vitamin A is necessary for normal cell differentiation (such as in the immune system).*

Reference 1.1:

‘Vitamin A is important for normal...and immune function.’ (pg 82)

‘Retinoids are necessary for the maintenance of immune function, which depends on cell differentiation and proliferation in response to immune stimuli. Retinoic acid is important in maintaining an adequate level of circulating natural killer cells that have antiviral and anti-tumor activity (Zhao and Ross, 1995). Retinoic acid has been shown to increase phagocytic activity in murine macrophages (Katz et al., 1987) and to increase the production of interleukin 1 and other cytokines, which serve as important mediators of inflammation and stimulators of T and B lymphocyte production (Trechsel et al., 1985). Furthermore, the growth, differentiation, and activation of B lymphocytes requires retinol (Blomhoff et al., 1992).’ (pg 85, 86)

‘Vitamin A deficiency has been associated with a reduction in lymphocyte numbers, natural killer cells, and antigen-specific immunoglobulin responses (Cantorna et al., 1995; Nauss and Newberne, 1985). A decrease in leukocytes and lymphoid organ weights, impaired T cell function, and decreased resistance to immunogenic tumors have been observed with inadequate vitamin A intake (Dawson and Ross, 1999, Wiedermann et al., 1993). A generalized dysfunction of humoral and cell-mediated immunity is common in experimental animals and is likely to exist in humans.’ (pg 96)

‘Epidemiological evidence suggests that host resistance to infection is impaired at lesser stages of vitamin A deficiency, prior to clinical onset of night blindness (Arroyave et al., 1979; Arthur et al., 1992; Barreto et al., 1994; Bloem et al., 1990; Ghana VAST Study Team, 1993; Loyd-Puryear et al., Salazar-Lindo et al., 1993)’ (pg 98)

‘There is sound evidence for a role of vitamin A in the maintenance of both humoral antibody responses and cell-mediated immunity. In experimental animals, both nonspecific immunity (Butera and Krakowka, 1986; Cohen and Elin, 1974) and antigen-specific responses, including delayed-type hypersensitivity (Smith et al., 1987), blastogenesis (Butera and Krakowka, 1986; Friedman and Sklan, 1989), and antibody production (Carman et al., 1989, 1992; Pasatiempo et al., 1990; Ross, 1996; Stephensen et al., 1993), have been shown to be altered by a deficiency of vitamin A or enhanced by vitamin A supplementation. The number and cytotoxic activity of natural killer cells (Dawson et al., 1999; Zhao et al., 1994) is reduced in vitamin A deficiency, although responsiveness to activation is maintained.’ (pg 105)

‘Several human studies have linked impairment in immunity to low plasma or serum vitamin A concentrations (Coutsoudis et al., 1992; Semba et al., 1992, 1996). However, there are no human studies using controlled diets that have evaluated immune function tests as a means to assess the adequacy of different levels of dietary vitamin A.’ (pg 105, 106)

Reference 2.0:

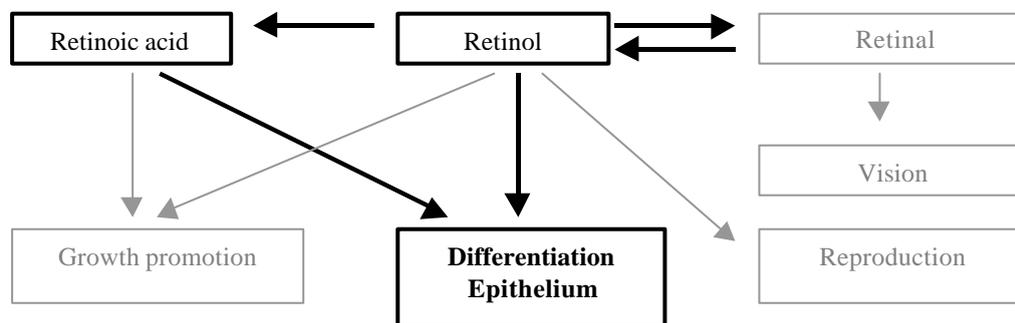
‘The major roles of vitamin A are in vision, differentiation of epithelial tissues, and in the immune system.’ (pg 1711)

‘Although animal studies have long shown a necessity for vitamin A in immune function, the molecular action of retinoids is still unknown. It also seems that some carotenoids function as such in immune function, and perhaps additionally as precursors of vitamin A.’ (pg 1712)

Reference 4.1:

‘...Requirements for vitamin A have also been implicated in the immune response, taste, hearing, and maintenance of appetite. Failure of cell division and differentiation can affect stem cells, and for example, result in impaired haematopoiesis.’ (pg 17)

Figure 4: The functions of retinoic acid, retinal and retinal (adapted from Miller et al., 1998):



Most of the above processes are directly or indirectly dependent upon cellular differentiation and control of gene expression, with the majority of effects of vitamin A explained by the existence of complex signal transduction pathways, retinoid receptors, binding-proteins with bioactive vitamin A metabolites serving as physiological ligands. There is also a hypothesis that vitamin A is involved as a cofactor in the biosynthesis of cell surface glycoproteins that act as antigenic determinants, viral receptors, and markers of cellular identity (Gerster, 1997 and references therein; Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).’ (pg 17)

Reference 5.0:

‘Vitamin A has a wide variety of functions, including specific roles in ...immune status...’(pg 1315)

‘Important for resisting infectious diseases...’(Table 65.1, pg 1334)

5) Growth

Code	Proposed statement
VA5:	<i>Vitamin A is necessary for normal growth</i>

Reference 1.1:

‘Vitamin A is important for normal ... gene expression, ... growth, and ...’ (pg 82)

Reference 4.1:

‘Vitamin A is essential to the processes of ...growth and cellular differentiation ... Failure of cell division and differentiation can affect stem cells, and for example, result in impaired haematopoiesis.’ (pg 17)

6) Reproduction

Code	Proposed statement
VA6:	<i>Vitamin A is necessary for normal reproduction</i>

Reference 1.1:

‘Vitamin A is important for normal ..., reproduction, ...’ (pg 82)

Reference 2.0:

‘It has been argued, without conclusive proof as yet, that retinoic acid is the active form of vitamin A required for cellular differentiation. Retinoic acid is an endogenous metabolite of vitamin A. Animals maintained on retinoic acid as sole source of vitamin A seem to grow normally and maintain good health, ... some but not all species also show loss of testicular function. ’ (pg 1711)

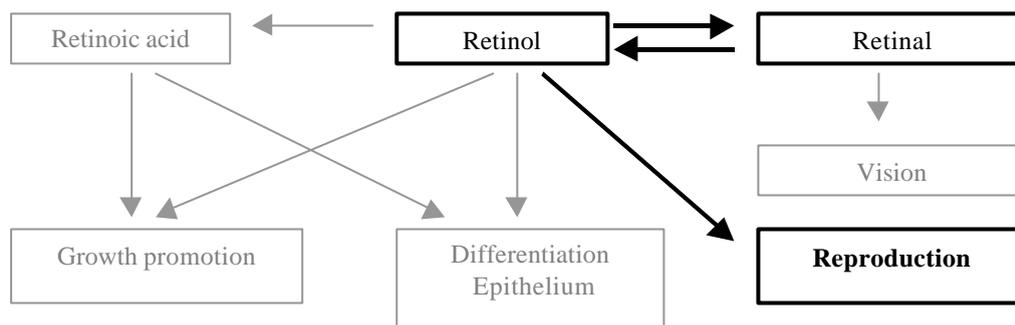
Reference 3.1:

‘Of particular importance in the setting of an upper level is the role of retinoic acids during morphogenesis and embryonic development. It has long been recognised that abnormal fetal development is associated with either insufficient or excessive intakes of vitamin A and related compounds....Moreover, RARs and RXRs show specific spatio-temporal patterns of expression in all developing systems during embryonic development, which suggests that retinoic acid signalling is involved in most, if not all, morphogenetic and patterning processes (Morriss-Kay and Sokolova, 1996).’ (pg 4)

Reference 4.1:

‘Vitamin A is essential to the processes of ... reproduction... and cellular differentiation Lack of vitamin A results in keratinisation of mucus secreting ciliated epithelium and other epithelial changes. The tissues most affected by this include the ... testes.’ (pg 17)

Figure 4: The functions of retinoic acid, retinal and retinal (adapted from Miller et al., 1998):



Most of the above processes are directly or indirectly dependent upon cellular differentiation and control of gene expression, with the majority of effects of vitamin A explained by the existence of complex signal transduction pathways, retinoid receptors, binding-proteins with bioactive vitamin A metabolites serving as physiological ligands. There is also a hypothesis that vitamin A is involved as a cofactor in the biosynthesis of cell surface glycoproteins that act as antigenic determinants, viral receptors, and markers of cellular identity (Gerster, 1997 and references therein; Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).’ (pg 17)

Reference 5.0:

‘Vitamin A has a wide variety of functions, including specific roles in ...growth and reproduction, ...’(pg 1315)

7) Beta Carotene

Code Proposed statement

VA7: *Beta carotene can be converted to vitamin A. Vitamin A is necessary for / contributes to...*

Reference 1.1:

‘There are a number of sources of dietary vitamin A. Preformed vitamin A is abundant in some animal-derived foods, whereas pro-vitamin A carotenoids are abundant in darkly colored fruits and vegetables, as well as oily fruits and red palm oil’ (pg 82)

‘For dietary provitamin A carotenoids- β -carotene, α -carotene, and β -cryptoxanthin-RAEs have been set at 12, 24, and 24 μ g, respectively.’ (pg 82)

‘Although a large body of observational epidemiological evidence suggest that higher blood concentrations of β -carotenes and other carotenoids obtained from foods are associated with a lower risk of several chronic diseases, there is currently not sufficient evidence to support a recommendation that requires a certain percentage of dietary vitamin A to come from provitamin A carotenoids in meeting the vitamin A requirement.’ (pg 83)

Reference 1.2:

‘While there is evidence that β -carotene is an antioxidant in vitro, its importance to health is not known. The one clear function of certain carotenoids that is firmly linked to a health outcome is the provitamin A activity of some dietary carotenoids (α -carotene, β -carotene, and β -cryptoxanthin) and their role in the prevention of vitamin A deficiency.’ (pg 325)

‘Three of these carotenoids, namely α -carotene, β -carotene, and β -cryptoxanthin, can be converted into retinol and are thus referred to as provitamin A carotenoids.’ (pg 326)

‘In humans, the only known function of carotenoids is vitamin A activity (provitamin A carotenoids only). Carotenoids also are thought to have a variety of different actions, including possible antioxidant activity, immunoenhancement, inhibition of mutagenesis and transformation, inhibition of premalignant lesions, quenching of nonphotochemical fluorescence, and activity as a pigment in primate macula (Olson, 1999). Carotenoids have also been associated with various health effects: decreased risk of macular degeneration and cataracts, decreased risk of some cancers, and decreased risk of some cardiovascular events (Olson, 1999). However, as described above, the only known function of carotenoids in humans is to act as a source of vitamin A in the diet.’ (pg 326)

‘The effect of increasing β -carotene intake on several markers of antioxidant activity has been investigated in a series of studies involving humans. These studies have examined antioxidant marker activity in apparently healthy men and women as well as in subjects who were physiologically challenged (i.e., smokers and patients with coronary disease or cystic fibrosis). Studies of the effect of β -carotene intake on measures of antioxidant activity are summarized in Table ... The dietary source of β -carotene ranged from modification of diets with normally consumed foods to giving supplements that provided as much as 120 mg/day of a highly bioavailable preparation. In general, subjects in most studies consumed β -carotene in amounts that would be difficult to achieve from foods alone and, as a result, relate to the pharmacological range of intakes. The findings reported in Table... indicate that β -carotene supplementation did not alter, or inconsistently alter, markers of anti-oxidant activity, which were somewhat dependent on β -carotene intake. In studies in which subjects were fed less than 25 mg/day of β -carotene, either from foods or as a supplement, changes in the markers for antioxidant activity were minimal. Exceptions noted were decreased deoxyribonucleic acid strand breaks observed when 22 mg/day of β -carotene was administered as carrot juice (Pool-Zobel et al., 1997) and lowered copper-induced oxidation of low-density lipoprotein when 12 or 24 mg/day of β -carotene was given along with vitamins C and E (Mosca et al., 1997)... feeding β -carotene in amounts greater than 25 mg/day generally resulted in inconsistent responses of the biological markers monitored.’ (pg 331, 332)

‘Appropriate communication among cells is essential for the coordination of biochemical functions in complex, multicellular organisms. One theory suggest that failure of signaling is one cause of cell overgrowth and eventually cancer. Two research groups have demonstrated that carotenoids stimulate gap junction

communication between cells in vitro (Sies and Stahl, 1997; Zhang et al., 1991).’ (pg 333)

‘It is not known whether the parent carotenoids or their metabolites are the active factors (Hanusch et al., 1995), nor is it known whether carotenoids influence this communication process in vivo. More study is needed to ascertain whether carotenoids play a direct role in cell-cell communication and, if so, what health outcomes are influenced by this action.’ (pg 338)

‘It is important to remember, however, that studies conducted with provitamin A carotenoids may yield results that are attributable to the conversion of carotenoids to vitamin A or other retinoids, not to the effects of the intact carotenoid.’ (pg 338)

‘Santos et al, (1996) showed that long-term β -carotene supplementation enhanced natural killer cell activity in men 65 to 86 years of age, but not in men 51 to 64 years of age; enhancement by β -carotene in this age group was confirmed in a subsequent study (Santos et al., 1998). Hughes et al, (1997) evaluated mechanisms by which β -carotene might enable immune cells to act more efficiently. Subjects were supplemented for 26 days with either 15 mg of β -carotene or a placebo. Subjects receiving the β -carotene treatment had increases in expression of adhesion molecules by monocytes, in ex vivo secretion of tumor necrosis factor- α , and in the percentage of monocytes expressing major histocompatibility complex II, a cell surface molecule responsible for presenting antigen to T-helper cells.’ (pg 338)

‘Other immunological effects that carotenoids are reported to increase are lymphocyte response to mitogens (Kramer and Burri, 1997) and total white blood cells and helper T cells in human immunodeficiency virus-related humans (Coodley et al., 1993). Whether these and the other effects noted are specific to carotenoids and are important in overall immunity is not confirmed.’ (pg 338)

Reference 3.2:

‘Some dietary carotenoids, such as β -carotene, serve as an important source of vitamin A, which is the major known function of carotenoids in humans...Carotenoids containing at least one unsubstituted β -ionone ring and a polyene chain are potential precursors of vitamin A. The preformed vitamin A is only present in animal products (e.g. liver, eggs, milk products), thus, in countries where the intake of animal products is low, carotenoids have to meet (i.e. by 80% or more in Asia and Africa) the vitamin A requirements.’ (pg 2)

‘The best-characterised natural functions of carotenoids are to serve as light-absorbing pigments during photosynthesis and protection of cells against photosensitization. Carotenoids provide considerable coloration and identification for many species, from vegetables to animals. In addition, carotenoids serve several other functions, such as radical quenching, antioxidant and anticarcinogenic activities in different animal sites and are regulators of cell function.’ (pg 2)

‘Carotenoids can act as antioxidants and free radical/reactive species scavengers (Tsuchiya *et al.*, 1993; Everett *et al.*, 1996; IARC, 1998; Omenn, 1998)...The role *in vivo* and in humans is less clear (IARC, 1998; Palozza, 1998; Lambert, 1999). The switch from antioxidant to pro-oxidant behaviour can be, for example, a function of

oxygen concentration (Edge and Truscott, 1997; Palozza, 1998). The pro-oxidant activity of β -carotene has been demonstrated at a high partial pressure of oxygen; because this is highest in the outermost cells of the lung, these cells might be particularly subject to the pro-oxidant effect of β -carotene (cited in Paolini *et al.*, 1999).’ (pg 4)

‘Part of the effects of β -carotene can be mediated by the formation of retinoic acid (RA) that has a key function as a regulator of gene expression, morphogenesis, and growth in vertebrate embryos...RAR β plays an important role in lung development...’ (pg 4)

ANNEX 4.2

Vitamin D

Source documents for reviewing vitamin D

Reference 1.4:

Institute of Medicine Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington D.C. National Academy Press, 1997.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.3:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of vitamin D. December 2002. (http://www.europa.eu.int/comm/food/fs/sc/scf/out157_en.pdf).

Reference 4.2:

Review of Vitamin D – Revised Version. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-00-11r.pdf>).

Reference 6.1:

Nutrition and Bone Health: with particular reference to calcium and vitamin D. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of Food and Nutrition Policy. Report on Health and Social Subjects No. 49. DH: The Stationery Office, 1998.

1) Calcium & phosphorus absorption and utilisation

Code	Proposed statement
VD1:	<i>Vitamin D is necessary for the normal absorption and utilisation of calcium & phosphorus</i>

Reference 1.4:

‘Vitamin D’s major biologic function in humans is to maintain serum calcium and phosphorus concentrations within the normal range by enhancing the efficiency of the small intestine to absorb these minerals from the diet (DeLuca, 1988; Reichel et al., 1989). 1,25 enhances the efficiency of intestinal calcium absorption along the entire small intestine, but primarily in the duodenum and jejunum. 1,25 (OH)₂D₃ also enhances dietary phosphorus absorption along the entire small intestine (Chen et al., 1974), but its major influence is in the jejunum and ileum. When dietary calcium intake is inadequate to satisfy the body’s calcium requirement, 1,25 (OH)₂D, along with parathyroid hormone (PTH), mobilizes monocytic stem cells in the bone marrow to become mature osteoclasts (Holick, 1995; Merke et al., 1986). The osteoclasts, in

turn, are stimulated by a variety of cytokines and other factors to increase the mobilization of calcium stores from the bone. Thus, vitamin D maintains the blood calcium and phosphorus at supersaturating concentrations that are deposited in the bone as calcium hydroxyapatite.’ (pg 253)

‘Although the kidney supplies the body with 1,25 (OH)₂D to regulate calcium and bone metabolism, it is recognized that activated macrophages come lymphoma cells, and cultured skin and bone cells also make 1,25 (OH)₂D (Adams et al., 1990; Holick, 1995; Pillai et al., 1987). Although the physiologic importance of locally produced 1,25 (OH)₂D is not well understood, the excessive unregulated production of 1,25 (OH)₂D by activated macrophages and lymphoma cells is responsible for the hypercalciuria associated with chronic granulomatous disorders and the hypercalcemia seen with lymphoma (Adams, 1989; Davies et al., 1994).’ (pg 254, 255)

Reference 2.0:

‘In the intestine, 1,25(OH)₂D enhances the absorption of dietary calcium and phosphorus across the microvilli of the small intestinal absorptive cells. It also interacts with monocytic stem cells in the bone marrow to initiate their transformation into mature osteoclasts. Thus, 1,25(OH)₂D₃ regulates serum calcium levels by enhancing the efficiency of intestinal calcium absorption and stimulating resorption of calcium from the bone. It remains controversial as to whether 1,25(OH)₂D has any direct action on the renal handling of either calcium or phosphorus.’ (pg 363)

Reference 3.3:

‘The principal physiological function of vitamin D in all vertebrates including humans is to maintain serum calcium and phosphorus concentrations in a range that support cellular processes, neuromuscular function, and bone ossification. Vitamin D accomplishes this goal by enhancing the efficiency of the small intestine to absorb dietary calcium and phosphorus, and by mobilising calcium and phosphorus from the bone (Holick, 1999; Holick et al., 1998).’ (pg 2)

‘The main mechanism of action of vitamin D is the interaction of 1,25(OH)₂D with the nuclear vitamin D receptor (Brown et al., 1999). VDR belongs to the super family of steroid nuclear receptors. Following ligand binding, VDR heterodimerises with retinoid X receptor (RXR) and acts as a ligand-activated transcription factor by binding to genomic vitamin D responsive elements (VDRE) in vitamin D-regulated genes. These include more than 50 other genes important for mineral homeostasis, vitamin D metabolism, energy metabolism, cell differentiation and proliferation, extracellular matrix proteins, oncogenes, growth factors, signal transduction proteins and peptide hormones. Genes can be both up-regulated or down-regulated, but the exact mechanism is unclear. Among genes down-regulated are PTH, osteocalcin, protein-kinase A inhibitors and interleukin-2 genes.’ (pg 6)

‘The most critical role of 1,25(OH)₂D I mineral homeostasis is to enhance the efficiency of the small intestine to absorb dietary calcium. This was clearly demonstrated in the VDR null mouse (Yoshizawa et al., 1997).’ (pg 7)

'1,25(OH)₂D also promotes the intestinal absorption of phosphate. However a significant phosphate absorption also occurs in 1,25(OH)₂D-deficient states (Brown et al., 1999).' (pg 7)

'1,25(OH)₂D enhances the mobilisation of calcium and phosphorus stores from bone at times of calcium deprivation.' (pg 7)

'1,25(OH)₂D regulate calcium homeostasis in close co-operation with PTH, which is the principal hormone regulating extracellular ionised calcium from minute to minute. PTH stimulates 1,25(OH)₂D synthesis and 1,25(OH)₂D suppresses the synthesis and secretion of PTH and controls parathyroid growth through negative gene regulation. Studies in the VDR null mouse suggest that VDR is essential, but works in co-operation with calcium and phosphate (Brown et al., 1999).' (pg 7)

'1,25(OH)₂D increases renal calcium reabsorption and calbinding expression, and it accelerates PTH dependent calcium transport in the distal tubule, which has the highest level of VDR. The enhancing effect of 1,25(OH)₂D on renal phosphate absorption might be an indirect action via PTH suppression (Brown et al., 1999).' (pg 7)

'Synthesis and cellular receptors for 1,25(OH)₂D have been found not only in the intestine, kidney and bone but also in many other tissues, suggesting that 1,25(OH)₂D is fundamental to the regulation of gene expression in many cell types in addition to its probable role in intracellular calcium regulation (Brown et al., 1999; Sehnder et al., 2002a and b).' (pg 7)

Reference 4.2:

'The active form of vitamin D regulates the intestinal absorption of calcium from the diet.' (pg 6)

'Transport is facilitated by two 1,25-(OH)₂D dependent calcium binding proteins, calbindin D9k (CaBP-D_{9k}, the most abundant) and calbindin D28k (CaBP-D_{28k}).' (pg 6)

'Thus, it is recognised that the major response of the intestine to vitamin D is an increase in calbindin synthesis, although the function of calbindin is not yet clear. There is some evidence to suggest that it is related to the intestinal transport of calcium.' (pg 6)

'Magnesium absorption through the gut involves both active and passive mechanisms but is much less tightly regulated by 1,25-(OH)₂D than calcium absorption. Transport of inorganic phosphate across the luminal brush border is dependent on the sodium-phosphate co-transporter; this is the step at which 1,25-(OH)₂D regulated phosphate absorption occurs, but the mechanisms are poorly understood (Sahota and Hosking, 1999).' (pg 6, 7)

'Calcium resorption at the distal nephron is regulated by 1,25-(OH)₂D₂ and PTH. The mechanisms of the active calcium transcellular transport are similar to those in the enterocyte with 1,25-(OH)₂D₂ stimulating the expression of calcium binding protein.'

1,25-(OH)₂D₂ may also stimulate the synthesis of the plasma membrane pump, as well as regulating its activity (Sahota and Hosking, 1999).’ (pg 7)

‘...Reports exist that suggest an effect of 1,25-(OH)₂ vitamin D on stimulation of the renal absorption of calcium (DeLuca and Schnoes, 1976). 1,25-(OH)₂D₃ is able to localise in the nuclei of cells in the distal renal tubules, where it binds to the vitamin D receptor (VDR) (DeLuca and Zierold, 1998). Binding to the VDR is known to be essential to the prevention of rickets and the regulation of calcium and phosphorus.’ (pg 7)

Reference 6.1:

‘The active hormonal form, 1,25(OH)₂vitamin D, controls plasma calcium concentrations by modulating calcium absorption in the small intestine, phosphate resorption in the renal tubules and through calcium release from bone. A specific nuclear receptor for 1,25(OH)₂vitamin D (vitamin D receptor) occurs in tissues involved in calcium homeostasis, such as intestine, kidney and bone. (pg 40)

2) Cell division - skin, immune system

Code Proposed statement

VD2a: *Vitamin D contributes to the normal cell division*

VD2b: *Vitamin D contributes to the normal structure of skin*

VD2c: *Vitamin D contributes to the normal function of the immune system*

Reference 1.4:

‘A multitude of other tissues and cells in the body can recognize 1,25 (OH)₂D (Stumpf et al., 1979). Although the exact physiologic function of 1,25 (OH)₂D in the brain, heart, pancreas, mononuclear cells, activated lymphocytes, and skin remains unknown, its major biologic function has been identified as a potent antiproliferative and prodifferentiation hormone (Abe et al., 1981); Colston et al., 1981; Eisman et al., Smith et al., 1987). There is little evidence that vitamin D deficiency leads to major disorders in these organ and cellular systems.’ (pg 253)

Reference 2.0:

‘There are a variety of other tissues – including the brain, gonads, pancreas, stomach, activated T an dB lymphocytes, monocytes and skin – that have nuclear VDR. Although the exact physiologic function of the ineration 1,25(OH)₂D with these VDRs is not well understood, it is known that *in vivo* and *in vitro* 1,25(OH)₂D₃ can inhibit proliferation and induce terminal differentiation of various normal and tumour cells including normal human keratinocytes. This is the reason why activated vitamin D compounds are now routinely used for the treatment of the hyperproliferative skin disorder, psoriasis.’ (pg 363)

Reference 3.3:

‘In the skin, 1,25(OH)₂D plays an important role by inhibiting proliferation and stimulating differentiation of keratinocytes and vitamin D analogues are used in the treatment of psoriasis. In the immune system, 1,25(OH)₂D modulates synthesis of interleukins and cytokines. Besides stimulating monocytes and macrophages, 1,25(OH)₂D functions as an immunosuppressive agent by decreasing the rate of

proliferation and the activity of both T- and B cells and inducing suppressor T cells (Brown et al., 1999).’ (pg 8)

‘In addition, VDR is expressed in many other tissues, such as muscle and nervous tissue, liver, intestine, reproductive organs, pancreas, pituitary, thyroid gland and lung, where 1,25(OH)₂D apparently has important functions in regulation of cell proliferation and differentiation (Brown et al., 1999; Holick, 1999).’ (pg 8)

Reference 3.3:

‘The last couple of decades it has become increasingly apparent that vitamin D also has other important functions in tissues not primarily related to mineral metabolism (Brown et al., 1999; Holick, 1999). One example is the haematopoietic system, in which vitamin D affects cell differentiation and proliferation including such effects also in cancer cells...’ (pg 2)

Reference 4.2:

‘1,25-(OH)₂D₂ the hormonal form of vitamin D regulates calcium and phosphate metabolism by its action on three target tissues, small intestine, bone and kidney. In addition to these major sites of vitamin D action, many other body tissues and cells have receptors for and responses to 1,25-(OH)₂D vitamin D. These include pancreas, pituitary gland, lymphocytes and monocytes. The precise physiological function of vitamin D in these tissues is uncertain but an anti-proliferative and pro-differentiation action is apparent (FNB, 1997). 1,25-(OH)₂D₃ interacts with a specific receptor protein in its target tissues, the receptor complex is taken up into the nucleus and recycled (DeLuca and Ziorold, 1998). The Vitamin D Receptor (VDR) binds to direct repeat response elements called DR-3 in the promoter regions of target genes to stimulate or suppress transcription. The VDR will only bind to the response elements if the retinoid X receptor is also present.’ (pg 6)

3) Bone

Code Proposed statement

VD3: *Vitamin D is necessary for the normal structure of bone*

Reference 1.4:

‘After vitamin D was recognized as being critically important for the prevention of rickets, the United States, Canada, and many other countries instituted a policy of fortifying some foods with vitamin D (Steenbock and Black, 1924).’ (pg 257)

‘Vitamin D deficiency is characterized by inadequate mineralization or demineralization of the skeleton. In children, vitamin D deficiency results in inadequate mineralization of the skeleton causing rickets, which is characterized by widening at the end of the long bones, rachitic rosary, deformations in the skeleton including frontal bossing, and outward or inward deformities of the lower limbs causing bowed legs and knocked knees, respectively (Goldring et al., 1995). In adults, vitamin D deficiency leads to a mineralization defect in the skeleton causing osteomalacia. In addition, the secondary hyperparathyroidism associated with vitamin D deficiency enhances mobilization of calcium from the skeleton, resulting in porotic bone (Favus and Christakos, 1996)’ (pg 258)

‘Vitamin D deficiency causes a decrease in ionized calcium in blood, which in turn leads to an increase in the production and secretion of PTH (Fraser, 1980; Holick, 1995).’ (pg 258)

‘The elevated PTH leads to an increase in the destruction of the skeletal tissue in order to release calcium into the blood.’ (pg 258)

‘It is well recognized that vitamin D deficiency causes abnormalities in calcium and bone metabolism.’ (pg 258)

‘Little information is available about the level of 25(OH)D that is essential for maintaining normal calcium metabolism and peak bone mass in older children and in young and middle-aged adults. For the elderly, there is mounting scientific evidence to support their increased requirement for dietary vitamin D in order to maintain normal calcium metabolism and maximize bone health (Dawson-Hughes et al., 1991; Krall et al., 1989; Lips et al., 1988).’ (pg 259)

‘The ultimate effect of vitamin D on human health is maintenance of a healthy skeleton. Thus, in reviewing the literature for determining vitamin D status, one of the indicators that has proven to be valuable is an evaluation of skeletal health. In neonates and children, bone development and the prevention of rickets, either in combination with serum 25(OH)D and PTH concentrations, or by itself, are good indicators of vitamin D status (Gultekin et al., 1987; Koo et al., 1995; Kruse et al., 1984; Markested et al., 1986; Meulmeester et al., 1990). For adults, bone mineral content (BMC), bone mineral density (BMD), and fracture risk, in combination with serum 25(OH)D and PTH concentrations, have proven to be the most valuable indicators of vitamin D status (Brazier et al., 1995; Dawson-Hughes et al., 1991, 1995; Lamberg-Allardt et al., 1989, 1993; Sorva et al., 1991; Webb et al., 1990).’ (pg 260, 261)

Reference 2.0:

‘Once 1,25(OH)₂D interacts with the VDR, the complex forms a heterodimer with retinoic acid X receptor (RXR). This new complex site on specific segments of vitamin D responsive genes known as vitamin D responsive elements (VDREs) to either increase or decrease transcriptional activity of the vitamin D sensitive genes such as osteocalcin, calcium binding protein (calbindin), PTH and osteonectin.’ (pg 363)

‘The onset of vitamin D deficiency decreases the efficiency of intestinal calcium absorption. There is a decline in blood ionized calcium which causes the parathyroid glands to produce and secrete more parathyroid hormone. This hormone tries to conserve calcium by enhancing tubular reabsorption of calcium in the kidney. However, in the face of developing hypocalcaemia which could disturb neuromuscular function and a wide variety of metabolic and cellular processes, the body calls upon 1,25(OH)₂D and PTH to mobilize stem cells to become functional osteoclasts, which in turn mobilize calcium from the skeleton. In addition, PTH causes a loss of phosphorus into the urine causing hypophosphataemia. Thus, in early vitamin D deficiency the serum calcium is normal; it is the low serum phosphorus that causes the extracellular Ca XPO₄ to be too low for normal mineralization of bone

matrix. This causes a disruption in the orderly sequence of events in the differentiation of hypertrophied chondrocytes in the epiphyseal plates, resulting in their disorganization and causing a widening of the epiphyseal plates (end of long bones), demineralization of the skeleton, and bony deformities.’ (pg 364)

‘Once the epiphyseal plates are closed later in adolescence, vitamin D deficiency can no longer cause bone deformities. Instead, there is an inability to mineralize newly deposited bone matrix leading to wide osteoid seams within the trabecular and cortical bone, causing the bone disease commonly known as osteomalacia.’ (pg 364)

Reference 3.3:

‘1,25(OH)₂D is essential for development and maintenance of a mineralised skeleton. Deficiency results in rickets during growth and osteomalacia in adults. 1,25(OH)₂D induces bone formation by regulation of matrix proteins important for bone formation, such as osteocalcin, osteopontin, alkaline phosphatase, matrix-gla- protein and collagen, as well as mineral apposition. The bone forming osteoblasts express VDR and it appears that 1,25(OH)₂D inhibits osteoblast proliferation through VDR-dependent signal pathway, and promotes their differentiation (Kveiborg et al., 1999). Vitamin D does not appear to be absolutely essential for the ossification process, but enhances this through increasing serum levels of calcium and phosphate. It has been suggested that not only 1,25(OH)₂D is involved in bone mineralisation, but also 24,25(OH)₂D may be required (Brown et al., 1999).’ (pg 7)

Reference 4.2:

‘The action of vitamin D on bone is not well defined. Fraser (1981) proposes a role for the 1,25-dihydroxy derivative in bone resorption, possibly in the proliferation of macrophages (Sahota and Hosking, 1999). Bone formation begins with the differentiation of mesenchymal stromal cells to form mature osteoblasts. This is regulated by a number of factors including PTH and 1,25-(OH)₂D. It has been proposed that another metabolite, 24,25-(OH)₂D₃, plays a significant role in normal bone formation (Ornoy et al., 1978). 1,25-(OH)₂D and PTH are also involved in the regulation of the migration of the vesicles containing calcium phosphate from the cell processes of the osteoblast to the zone of mineralisation (Sahota and Hosking, 1999). As this process proceeds some of the osteoblasts are incorporated into bone as osteocytes, while others remain as lining cells covering the trabecular surfaces. However, it appears that there is no direct effect of vitamin D or any of its metabolites on bone mineralisation.’ (pg 7)

‘When considering the effects of 1,25-(OH)₂ vitamin D on bone it should be noted that bone contains several cell types, which may respond in different ways to 1,25-(OH)₂D. Osteoblasts have receptors for 1,25-(OH)₂D₃ which appears to increase mineralisation and osteoblast differentiation. In this way, bone formation and hence growth are promoted (Braidman, 1990).’ (pg 7, 8)

‘To maintain a constant plasma calcium concentration, PTH works in conjunction with vitamin D on the osteoblasts in an unknown mechanism to mobilise calcium, and hence phosphate, from bone (DeLuca and Zeorold, 1998).’ (pg 8)

Reference 6.1:

1,25(OH)₂vitamin D also appears to promote calcium deposition in growing ends of bones but the mechanisms are not fully understood but may be mediated via an effect on osteocalcin concentrations. 1,25(OH)₂vitamin D has several other functions not specifically related to calcium; deficiency causes impaired function of nerves and muscles, as well as behavioural changes such as depression.’ (pg 40)

‘Recent data relating plasma levels of PTH to those of 25(OH)vitamin D have led to the suggestion that elevation of PTH might define the level of 25(OH)vitamin D needed for bone health, beyond the avoidance of clinical deficiency.’ (pg 40)

ANNEX 4.3

Vitamin E

Source documents for reviewing vitamin E

Reference 1.2:

Institute of Medicine Dietary Reference Intakes for vitamin C, vitamin E, selenium and carotenoids. Washington D.C. National Academy Press, 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.4:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin E. April 2003. (http://www.europa.eu.int/comm/food/fs/sc/scf/out195_en.pdf)

Reference 4.3:

Review of Vitamin E – Revised Version. Expert Group on Vitamins and Minerals. July 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-00-13r.pdf>).

1) Antioxidant activity in cell membranes

Code	Proposed statement
VE1:	<i>Vitamin E is necessary for protecting cell membranes from some types of damage caused by free radicals (such as the oxidation of polyunsaturated fatty acids).</i>

Reference 1.2:

‘Vitamin E is a chain-breaking antioxidant that prevents the propagation of free-radical reactions (Burton and Ingold, 1986; Burton et al., 1983; Ingold et al., 1987; Kamal-Eldin and Appelqvist, 1996; Packer, 1994; Tappel, 1962). The vitamin is a peroxy radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and in plasma lipoproteins Burton et al., 1983). Peroxy radicals (abbreviated ROO•) react with vitamin E (abbreviated Vit E_{OH}) 1,000 times more rapidly than they do with PUFA (abbreviated RH) (Packer, 1994).’ (pg 195)

‘...α-Tocopherol can be oxidized to the tocopheroxyl radical-one electron oxidation product-which can be reduced back to the unoxidized form by reducing agents such as vitamin C.’ (pg 199)

‘In general, lipid peroxidation markers are elevated during vitamin E depletion and their levels can be normalized upon vitamin E repletion. However, these markers are not necessarily specific to vitamin E, since changes in intake of other antioxidants can

also change the levels of these markers. At present, there is no evidence that lowering lipid peroxidation marker levels is associated with health benefits.’ (pg 203)

‘When vitamin E intercepts a radical, a tocopheroxyl radical is formed (Burton and Ingold, 1981). This radical can be reduced by ascorbic acid or other reducing agents (Doba et al., 1985; Niki et al., 1982), thereby oxidizing the latter and returning vitamin E to its reduced state.’ (pg 224)

Reference 2.0:

‘Vitamin E activity is derived from tocopherols and tocotrienols, which are lipid-soluble compounds originally synthesized by plants. There are four forms (α -, β -, γ - and δ -) of each, all of which have antioxidant properties but differing biological activities. α -Tocopherol is the most effective lipid-soluble antioxidant present in membranes and lipoproteins, and its function in these locations is to protect the unsaturated bonds of phospholipids from damage caused by free radicals.’ (pg 1878)

‘Tocopherols function as lipid-soluble antioxidants which protect membranes and lipoproteins against damage caused by lipid oxidation. However, because the wide array of deficiency symptoms observed in different species is difficult to explain by way of a simple antioxidant hypothesis, this has led to suggestions that tocopherols may also have more specific roles, such as in nucleic acid and mitochondrial metabolism and in the maintenance of membrane integrity. At this time, however, conclusive evidence for non-antioxidant functions is lacking.’ (pg 1880)

‘Lipid oxidation is a process which may occur during normal aerobic cellular metabolism and during the metabolism of drugs. In the process, polyunsaturated fatty acids (PUFA), such as those in cellular and subcellular membranes give up loosely bound hydrogen atoms from an allylic CH_2 group to highly reactive free radicals and are converted to fatty acid radicals. The fatty acid radical usually rearranges to a conjugated diene and takes up molecular oxygen, producing a peroxy radical and this compound attacks a second PUFA, resulting in the formation of a hydroperoxide and a second fatty acid radical. A chain reaction may then ensue. The hydroperoxides which are continuously produced as a part of the process may split in the presence of iron or copper to peroxy or alkoxy radicals, which serve to accelerate the chain reaction, or they may break down to aldehydes, ketones, alkanes and other products. Some of these compounds may bind to and disrupt cellular macromolecules such as DNA and proteins, while others have chemotactic properties which may induce inflammatory reactions.’ (pg 1880)

‘Lipid oxidation may be prevented or retarded in several ways....In the event that a chain reaction does occur, chain-breaking antioxidants can quench the reaction.’ (pg 1880)

‘ α -Tocopherol accounts for most of the lipid-soluble chain-breaking antioxidant activity in mammalian tissues and plasma. It donates its phenolic H atom to peroxy radicals, and in the process becomes an α -tocopheroxyl radical. However, this radical is relatively stable because the unpaired electron on the oxygen atom is delocalized throughout the aromatic ring structure. Under normal conditions it does not react with membrane PUFA, and so propagation of the chain reaction is inhibited.’ (pg 1880)

‘Membranes usually contain less than one ‘ α -tocopherol molecule per 1000-2000 phospholipids, and yet ‘ α -tocopherol is extremely effective in protecting membrane phospholipids against oxidative damage. The hydrophobicity of the isoprenoid side chain helps to fix ‘ α -tocopherol in the most fluid part of the membrane, close to the PUFA at risk of oxidative damage. In addition, ‘ α -tocopherol scavenges peroxy radicals about 10,000 times faster than they can react with PUFA. The length of the side chain also plays a critical role in the overall effectiveness of ‘ α -tocopherol because it positions the phenolic OH group at the membrane surface, allowing any ‘ α -tocopheroxy radicals formed to be converted back to ‘ α -tocopherol.’ (pg 1880,1881)

‘There is also a view that the criterion upon which the RDA for vitamin E ought to be based should be the amount needed for optimal protection of cells and tissues against oxidative damage.’ (pg 1882)

Reference 3.4:

‘The basic mode of action of tocopherols in human tissue is to prevent the oxidation of polyunsaturated fatty acids (PUFA) by trapping free radicals and donating hydrogen. It is effective in protecting the integrity of lipid and phospholipid membranes and thus the requirement for vitamin E and the recommended intake is determined to a large extent by the intake of PUFAs. It has been shown that increasing PUFA content of a diet low in α -tocopherol equivalents has adverse effects on tocopherol status (Horwitt, 1974; SCF, 1993).’ (pg 5)

‘Chronic marginal deficiency can be generally characterised by an enhanced susceptibility to lipid peroxidation and corresponding lipofuscinosis. In rats this first results in weakening of the basement membranes of the muscle capillaries and a breakdown of endothelial cells.’ (pg 6)

Reference 4.3:

‘Information currently available indicates that all its nutritional effects are consistent with its role as a biological antioxidant. In this regard, vitamin E is thought to have basic functional importance in the maintenance of membrane integrity in virtually all cells of the body. The potent antioxidant properties of vitamin E were first demonstrated by Olcott and Matthill in 1931. It was later proposed that the major function of the vitamin was the protection of PUFAs from oxidation *in vivo* to hydroperoxides. Other oxidation reactions prevented are the conversion of free or protein-bound sulphhydryls to disulphides. However, it was not until more recent years that the precise function of vitamin E was elucidated and its central role in protection against free-radical induced cellular damage was recognised (Chow 1985, Basu and Dickerson 1996).

‘Potentially damaging free radicals are produced in cells under normal conditions either by homolytic cleavage of a covalent bond, or by a univalent oxidation or reduction. The PUFA’s of biological membranes are particularly susceptible to attack by free radicals due to their 1,4-pentadiene systems, from which a hydrogen atom is readily removed. The lipoperoxy free radicals thus formed can attack adjacent PUFA residues and thereby initiate a chain of free radical reactions, with widespread harmful consequences to membrane structure. Vitamin E breaks the chain of free radical formation by reacting with the free radicals and converting them to a non-harmful form. This action, termed free radical ‘scavenging’, involves the donation of a

hydrogen atom to a fatty acyl free radical (or superoxide radical) to prevent the attack of that species on other PUFAs (Lucy 1972, Chow 1985). As noted previously, in the course of this process, α -tocopherol is converted to an α -tocopherol radical which is more stable than fatty acid or peroxy radicals and does not react with membrane PUFA. The α -tocopherol radical can then react with another radical to form a non radical product or can be re-converted to α -tocopherol (Bramley et al 2000).’ (pg 5, 6)

‘The clinical manifestations of vitamin E deficiency vary considerably between species. In general, however, the targets are the neuromuscular, vascular and reproductive systems. The various signs of vitamin E deficiency are believed to be manifestations of membrane dysfunction, the result of the oxidative degradation of polyunsaturated membrane phospholipids and/or the disruption of other critical cellular processes (Horwitt 1960). In a wide range of animal species, vitamin E deficiency causes an increase in the tendency for erythrocytes to lyse in a solution of hydrogen peroxide. Of the effects of vitamin E deficiency reported in experimental animals, this is the only feature of deficiency which occurs definitely in man and first suggested the possible role of vitamin E in maintenance of membrane stability.’ (pg 6)

‘There is clear evidence that the requirement for vitamin E increases with the amount of dietary PUFAs.’ (pg 6)

2) Cell proliferation and differentiation

Code	Proposed statement
VE2:	<i>Vitamin E helps cells to grow and multiply</i>

Reference 1.2:

‘... α -Tocopherol inhibits protein kinase C activity, which is involved in cell proliferation and differentiation, in smooth muscle cells (Boscoboinik et al., 1991; Chatelain et al., 1993; Clement et al., 1997; Stauble et al., 1994; Tasinato et al., 1995),...’ (pg 195, 196)

Reference 4.3:

‘Some non-antioxidant functions have been attributed to α but not β -tocopherol (Azzi and Stocker 2000). These include regulation of protein kinase C, modification of cell growth and proliferation, modification of gene transcription, protein phosphatase activation and modifications to gene expression. The authors state that the best evidence for a non-oxidant role is related to the recognition and transfer of a α -tocopherol.’ (pg 6)

3) Immune system

Code	Proposed statement
VE3:	<i>Vitamin E contributes to the normal function of the immune system</i>

Reference 1.2:

‘... α -Tocopherol inhibits protein kinase C activity, which is involved in ...human platelets (Freedman et al., 1996), and monocytes (Cachia et al., 1998; Deveraj et al., 1996).’ (pg 195, 196)

Reference 2.0:

‘Vitamin E deficiency impairs immune responses, while supplementation with higher than recommended dietary levels enhances humoral and cell-mediated immunity and increases the efficiency of phagocytes.’ (pg 1883)

‘The mechanism of the immunostimulatory effect of vitamin E appears to be mainly related to its antioxidant function, although other antioxidants do not produce similar actions. Vitamin E could function either by decreasing concentrations of reactive oxygen species (e.g. hydrogen peroxide), thereby preventing oxidative damage to the stimulated immune and phagocytic cells, or by modulating production of arachidonic acid metabolites, such as prostaglandins.’ (pg 1883)

‘The release of reactive oxygen species is a characteristic feature of inflammation. These compounds (including superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen) may originate via the arachidonic acid cascade or during the respiratory burst which occurs during phagocytosis.’ (pg 1883)

4) Vasodilation/circulation

Code Proposed statement

VE4: *Vitamin E contributes to the normal function of arteries*

Reference 1.2:

‘Vitamin E enrichment of endothelial cells downregulates the expression of intercellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), thereby decreasing the adhesion of blood cell components to the endothelium (Cominacini et al., 1997). Vitamin E also upregulates the expression of cytosolic phospholipase A₂ (Chan et al., 1998a; Tran et al., 1996) and cyclooxygenase-1 (Chan et al., 1998b). The enhanced expression of these two rate-limiting enzymes in the arachidonic acid cascade explains the observation that vitamin E, in a dose-dependent fashion, enhanced the release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation in humans (Szczeklik et al., 1985; Tran and Chan, 1990).’ (pg 196)

‘Vitamin E does inhibit LDL oxidation whether induced by cells in culture (Steinbrecher et al., 1984) or by copper ion in vitro (Dieber-Rotheneder et al., 1991; Jialal et al., Reaven et al., 1993). In addition, vitamin E could affect atherogenesis at a number of steps, based on the following in vitro observations:

- Vitamin E inhibits smooth muscle cell proliferation through the inhibition of protein kinase C (Azzi et al., 1995; Boscoboinik et al., 1991; Chatelain et al., 1993).
- Vitamin E inhibits platelet adhesion, aggregation, and platelet release reactions (Freedman et al., 1996; Higashi and Kikuchi, 1974; Ishizuka et al., 1998; Steiner and Anastasi, 1976).

- Vitamin E inhibits plasma generation of thrombin, a potent endogenous hormone that binds to platelet receptors and induces aggregation (Rota et al., 1998).
- Vitamin E decreases monocyte adhesion to the endothelium by downregulating expression of adhesion molecules (Devaraj et al., 1996; Faruqi et al., 1994; Islam et al., 1998; Martin et al., 1997; Molenaar et al., 1989) and decreasing monocyte superoxide production (Cachia et al., 1998; Islam et al., 1998)
- In human endothelial cells, vitamin E potentiates synthesis of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation (Chan and Leith, 1981; Szczeklik et al., 1985; Thorin et al., 1994; Tran and Chan, 1990)
- Vitamin E mediates upregulation of the expression of cytosolic phospholipase A₂ and cyclo-oxygenase (Chan et al., 1998a,b; Tran et al., 1996)
- Vitamin E enrichment of endothelial cells in culture inhibits the expression of intracellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) induced by exposure to oxLDL (Cominacini et al., 1997).’ (Pg 211, 212)

‘Among the effects of vitamin E intakes from supplements are inhibition of LDL oxidation both in vitro and in vivo; inhibition of smooth muscle cell proliferation through inhibition of protein kinase C; inhibition of platelet adhesion, aggregation, and platelet release reactions; and inhibition of plasma generation of thrombin. In addition, supplemental intakes of vitamin E decrease monocyte adhesion to endothelium, decrease monocyte superoxide production, potentiate the synthesis of prostacyclin, upregulate the expression of phospholipase A₂ and cyclo-oxygenase, and inhibit the expression of ICAM-1 and VCAM-1 induced by exposure to oxLDL.’ (pg 223)

Reference 2.0:

‘Vitamin E modulates many of the important events in atherogenesis, including the oxidative modification of LDL. In the absence of vitamin E, LDL is easily oxidized and is taken up by macrophages in the arterial intima, leading to the accumulation of lipid-laden foam cells and later, fatty streaks. Its presence in the intima may also result in the release of cytotoxic compounds which damage the endothelial layer, leading to platelet aggregation, release of growth factors, and migration and proliferation of smooth muscle cells.’ (pg 1882,1883)

Reference 4.3:

‘The clinical manifestations of vitamin E deficiency vary considerably between species. In general, however, the targets are the neuromuscular, vascular and reproductive systems.’ (pg 6)

ANNEX 4.4

Vitamin K

Source documents for reviewing vitamin K

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.5:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin K. April 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out196_en.pdf).

Reference 4.4:

Revised Review of Vitamin K. Expert Group on Vitamins and Minerals. April 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm-01-09p.pdf>).

Reference 6.1:

Nutrition and Bone Health: with particular reference to calcium and vitamin D. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of Food and Nutrition Policy. Report on Health and Social Subjects No. 49. DH: The Stationery Office, 1998.

1) Coagulation

Code	Proposed statement
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VK1:	<i>Vitamin K is necessary for normal coagulation (blood clotting).</i>
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Reference 1.1:

‘Vitamin K functions as a coenzyme during the synthesis of the biologically active form of a number of proteins involved in blood coagulation and ...’ (pg 162)

‘Vitamin K plays an essential role in the posttranslational conversion of specific glutamyl residues in a limited number of proteins to γ -carboxyglutamyl (Gla) residues (Suttie, 1993). These proteins include plasma prothrombin (coagulation factor II) and the plasma procoagulants, factors VII, IX, and X. Because under- γ -carboxylated forms of these proteins lack biological activity, the classical sign of a vitamin K deficiency has been a vitamin K-responsive increase in prothrombin time and, in severe cases, a hemorrhagic event.’ (pg 163)

'A clinically significant vitamin K deficiency has usually been defined as a vitamin K-responsive hypoprothrombinemia and is associated with an increase in prothrombin time (PT) and, in severe cases, bleeding....There are numerous case reports of bleeding episodes in antibiotic-treated patients, and these have often been ascribed to an acquired vitamin K deficiency resulting from a suppression of menaquinone-synthesizing organisms. However, these reports are complicated by the possibility of general malnutrition in this patient population and by the antiplatelet of many of the same drugs (Suttie, 1995).' (pg 164)

'Although there is some interference in the hepatic synthesis of the vitamin K-dependent clotting factors that can be measure by sensitive assays, standard clinical measure of procoagulant potential are not changed.' (pg 165)

'In humans, an insufficiency of vitamin K leads to the secretion into plasma of biologically inactive, under- γ -carboxylated forms of the vitamin K-dependent clotting factors.' (pg 167)

'Concentrations of vitamin K in cord blood are usually less than 0.1 nmol/L or undetectable (Mandelbrot et al., 1988; Widdershoven et al., 1988), and elevated concentrations of undercarboxylated prothrombin (PIVKA-II) have been reported (Greer, 1995). Poor vitamin K status added to the fact that the concentrations of most plasma clotting factors are low at the time of birth increases the risk of bleeding during the first weeks of life, a condition known as hemorrhagic disease of the newborn (HDNB). Because HDNB can be effectively prevented by administration of vitamin K, infants born in the United States and Canada routinely receive 0.5 to 1 mg of phyloquinone intramuscularly or 2.0 mg orally within 6 hours of birth.' (pg 176)

Reference 2.0:

'Vitamin K serves as a cofactor of an enzyme that posttranslationally γ -carboxylates specific glutamate residues in a few proteins. These γ -carboxylglutamate (Gla) residues confer to proteins the ability of binding calcium with high affinity and specificity. The carboxylase itself is a Gla protein. Several of the blood coagulation factors (factors II, VII, IX and X) and coagulation inhibitors (proteins C and S) contain Gla without which they cannot be activated.' (pg 1928)

'Traditionally, blood-clotting assays have been used to monitor vitamin K activity...Blood-clotting assays are based on the fact that the coagulation factors II, VII, IX and X are vitamin-K dependent proteins which are incompletely carboxylated during vitamin K deficiency and thus have reduced activity.' (pg 1930)

'The earliest and most devastating effect of vitamin K deficiency concerns infants during their first weeks of life...This naturally low vitamin K status during intrauterine life is associated with low blood coagulability...For this reason most health services now either recommend or require prophylactic vitamin K administration for all newborns...' (pg 1931)

'Normal or near normal blood coagulation is usually maintained in older children and adults even in the absence of dietary vitamin K, presumably because the small amounts of bacterial menaquinones from the lower intestine are sufficient for this function....However, if vitamin K production by the normal intestinal flora is reduced

at the same time, as during antibiotic treatment or as a consequence of diarrhoea, significant and even dangerous bleeding may occur within days. In the absence of liver disease normal blood coagulation is restored within one or two days by the administration of vitamin K.' (pg 1931,1932)

Reference 3.5:

'The prime physiological relevance of phylloquinone is the synthesis of coagulation proteins (Ferland, 1998; Olson, 1999 and 2000).'

Reference 4.4:

'Vitamin K was first identified in 1935 by Dam, who identified it as the fat-soluble factor necessary for the coagulation of blood. The primary function of vitamin K is to catalyse the synthesis of prothrombin by the liver. In the absence of vitamin K, hypoprothrombinaemia occurs in which blood clotting time may be greatly prolonged. Blood coagulation is a highly complex process, the mechanism of which is not fully understood. It involves cells such as thrombocytes, platelets and erythrocytes, numerous protein factors and Ca^{2+} . Essentially, a cascade of protein factors catalyses the reaction prothrombin to thrombin, the latter protein then converting soluble fibrinogen into insoluble fibrin which forms the basis of the blood clot. Vitamin K is known to be involved in the hepatic synthesis of at least four of the protein factors, which include prothrombin (factor II), proconvertin (factor VII), thromboplastin (factor IX) and the Stuart-Prower factor (factor X) (Committee on Nutrition 1961, Basu and Dickerson 1996).'

'Vitamin K is thought to be necessary for formation of Ca^{2+} binding sites on prothrombin (Gallop *et al* 1980, Olson 1984). These are essential for prothrombin to be bound to phospholipids, for activation to thrombin. In the presence of dicoumarol, a very potent antagonist of vitamin K the prothrombin produced *in vivo* has a very low Ca^{2+} binding capacity. The Ca^{2+} binding sites of prothrombin are formed by the introduction of a second carboxyl group into the glutamyl side-chains, located in the amino-terminal region of the protein. Once carboxylated, the glutamates are referred to as γ -carboxyglutamic acid (GLA). When the action of vitamin K is blocked by dicoumarol, calcium ions cannot bind to prothrombin because the protein lacks added carboxyl groups. The formation of vitamin K epoxide is an obligatory step in the action of vitamin K in the biosynthesis of prothrombin.'

'Like prothrombin, factors VII, IX, and X have been found to have a series of glutamic acid residues and vitamin K is also needed for the carboxylation of these residues (Gallop *et al* 1980, Olson 1984). The vitamin K-dependent carboxylation is carried out by a liver microsomal enzyme, through a molecular mechanism that is not fully understood. It is believed to require reduced vitamin K (or its epoxide) and CO_2 . The process appears to be coupled with the simultaneous epoxidation of vitamin K hydroquinone, the active form of the vitamin.'

2) Bone

Code	Proposed statement
VK2:	<i>Vitamin K contributes to the normal structure of bone</i>

Reference 1.1:

‘Vitamin K functions as a coenzyme during the synthesis of the biologically active form of a number of proteins involved in ... and bone metabolism.’ (pg 162)

‘Two structurally related vitamin K-dependent proteins (Price, 1988), osteocalcin found in bone and matrix Gla protein originally found in bone but now known to be more widely distributed, have received recent attention as proteins with possible roles in the prevention of chronic disease (Ferland, 1998).’ (pg 163)

‘Small amounts of the bone protein, osteocalcin, circulate in plasma, and like PIVKA-II, under- γ -carboxylated osteocalcin (ucOC) has been considered an indicator of suboptimal vitamin K status....Only recently has direct assessment of ucOC been possible with the development of a monoclonal antibody specific for the undercarboxylated form of osteocalcin (Vergnaud et al., 1997).’ (pg 168,169)

‘...a number of reports have correlated decreased bone mineral density (BMD) or increased fracture rate with a five to eight-fold increase in ucOC. Concurrently, it has been observed that vitamin K intakes similar to those reported for the general population did not ensure complete carboxylation of osteocalcin (Bach et al., 1996; Sokoll and Sadowski, 1996) and that ucOC could be decreased by increasing vitamin K intake (Binkley et al., 1999); Booth et al., 1999b; Douglas et al., 1995; Knapen et al., 1989, 1993). These reports have led to the suggestion that vitamin K requirements for bone function are probably much higher than those needed to maintain normal hemostasis and that the recommendation for vitamin K should be much higher than current recommendations (Weber, 1997).’ (pg 169)

‘Although there is little doubt that vitamin K intake affects the degree of osteocalcin λ -carboxylation the physiological significance of diet-induced changes prevent the use of ucOC for estimating an average requirement for vitamin K.’ (pg 170)

‘More recently, lower circulating phylloquinone and menaquinone concentrations have been observed in subjects with reduced BMD (Kanai et al., 1997; Tamatani et al., 1998) though other studies have not confirmed this finding (Rosen et al., 1993)....The role of vitamin K in bone metabolism has also been investigated by studying the vitamin K bone protein osteocalcin and its undercarboxylated form ucOC. The extent to which osteocalcin is undercarboxylated has been assessed with respect to age, bone status, and risk of hip fracture (Binkley and Suttie, 1995; Vermeer et al., 1996). Although ucOC was reported to increase with age in some studies (Knapen et al., 1998; Liu and Peacock, 1998; Plantalech et al., 1991), other reports have not confirmed this finding (Sokoll and Sadowski, 1996). Negative correlations have also been reported between ucOC and BMD, but the strength of the associations has varied depending on the population studied (Knapen et al., 1998; Liu and Peacock, 1998; Vergnaud et al., 1997). Although the observed relationship between ucOC and BMD is of interest, it requires further investigation as significant inverse relationships have also been observed between BMD and total osteocalcin (Liu and Peacock, 1998; Ravn et al., 1996) and between BMD and the active (carboxylated) form of osteocalcin (Knapen et al., 1998).’ (pg 170, 171)

‘Whether vitamin K intake is a significant etiological component of osteoporosis is difficult to establish on the basis of the studies performed thus far.’ (pg 172)

Reference 2.0:

‘Three additional Gla proteins have been completely characterized, but have functions not related to haemostasis; bone Gla protein (BGP, osteocalcin), matrix Gla protein (MGP) and ...The function of BGP, which is produced almost exclusively in osteoblasts and odontoblasts, is still obscure; it is most likely related to the control of mineralizing activities by these cells.’ (pg 1928)

‘At least three vitamin K-dependent proteins (BGP, MGP and protein S) are produced in bone, suggesting that vitamin K may be important for bone health...the improvement of vitamin K status has been shown to minimize loss of bone minerals. While it remains to be seen whether supplemental vitamin K in later age actually reduces bone fracture risk, long-term vitamin K status appears to be important for bone health.’ (pg 1932)

‘Little is known about the mechanism(s) through which vitamin K status influences bone. Bone is constantly remodelled by osteoclastic breakdown and subsequent osteoblastic rebuilding. BGP has been suggested as a mediator that links osteoclastic and osteoblastic activities; undercarboxylated BGP has been found to be ineffective for this function. As a consequence, the osteoclastic breakdown cycle may continue longer during suboptimal than during optimal vitamin K status. Another mechanism may be an inhibiting effect of vitamin K on interleukin 6 (IL-6) production. IL-6 relays the action on osteoblasts of various mediators such as parathyroid hormone (PTH) to osteoclasts (which do not have PTH receptors themselves). Vitamin K might thus dampen the catabolic effect of such hormones and limit bone mineral loss. Finally, there may be a more or less direct effect of vitamin K on PTH levels. It has recently been observed that secondary hyperparathyroidism due to renal failure is much less prevalent among patients with optimal vitamin K status compare to those with poorer vitamin K status.’ (pg 1932)

Reference 3.5:

‘...vitamin K is also essential for the synthesis of a number of proteins...the bone Gla-protein, osteocalcin, which is exclusively synthesised by osteoblasts and odontoblasts, and which is a negative regulator of bone formation.’ (pg 4)

‘Vitamin K is required for the α -carboxylation of glutamate in 2 proteins induced by the vitamin D hormone in bone. Osteocalcin is a 49-residue protein with 3 carboxyglutamic acid residues, is water soluble, adheres to the bone mineral hydroxyapatite and is secreted by osteoblasts.’ (pg 4)

‘The level of osteocalcin carboxylation has been proposed as an indicator of the nutritional state of the bone with respect to vitamin K. Circulating levels of undercarboxylated osteocalcin may be a sensitive marker of vitamin K inadequacy. These levels of undercarboxylate osteocalcin have been reported to be increased both in postmenopausal women and in individuals who sustain hip fracture (Binkley and Suttie, 1995; Vermeer et al., 1995; Szulc et al., 1993 and 1994; Knäpen et al., 1998; Luukinen et al., 2000).’ (pg 4)

Reference 4.4:

‘Proteins containing GLA have been identified in bone (Price 1988). There appear to be at least two GLA-containing proteins in bone, called bone GLA protein (BGP) or osteocalcin, and matrix GLA protein (MGP). The functions of these proteins have not been clearly defined, but there is an accumulation of evidence suggesting that they may participate in the modulation of bone mineralisation.’ (pg 10)

‘Osteocalcin is one of the most abundant non-collagenous proteins in the extra-cellular matrix of the bone. Its precise function is uncertain but it appears to be a marker of osteoblast activity (Shearer, 1995). Osteocalcin contains three GLA residues spaced at the same interval as calcium ions in the hydroxyapatite lattice. The appearance of osteocalcin in bones has been shown, using embryonic chick bones, to coincide with the beginning of mineralisation. Injection of vitamin K antagonists into eggs containing developing embryos, has been shown to result in a reduction of the GLA content of osteocalcin by 20-50% (Hauschka *et al* 1978).’ (pg 10)

‘Undercarboxylated (partially functional) osteocalcin may be associated with low bone mineral density and risk of hip fracture (Shearer, 1995, DH 1998) in older women....Schaafsma *et al* (2000) studied the effect of daily vitamin D₃ and phylloquinone supplements in postmenopausal women with normal and low bone mineral density....At baseline, women with normal BMD had significantly higher percentage carboxylated osteocalcin (%carbOC) and across the whole group, %carbOC was positively correlated with BMDs of the lumbar spine and femoral neck. After 6 and 12 months women with normal BMD who had received phylloquinone (alone or with vitamin D) had significantly higher %carbOC compared to the placebo group and to baseline. In women with low BMD %carbOC rose significantly from baseline values in both groups but the phylloquinone-vitamin D group were not significantly different to the vitamin D group.’ (pg 11)

‘The function of MGP is unclear, but it has been related to the action of the active metabolite of vitamin D (1,25-(OH)₂D₃) and therefore the mobilisation and deposition of bone calcium (Price and Baukol 1980).’ (pg 11)

‘It has been suggested that kidney GLA protein (KGB) is involved in the reabsorption of Ca²⁺ by the kidney tubules, a function related to vitamin D action. It is thought that KGB may solubilise calcium salts in urine. Sakamoto *et al* (1999) reported that urinary calcium excretion was lower in subjects considered to have high dietary vitamin K intakes.’ (pg 11)

Reference 6.1:

‘Vitamin K status might influence bone health as several vitamin K-dependent proteins, including osteocalcin and matrix gla-protein, are involved in bone mineralisation. Low dietary intake of vitamin K is associated with an elevated proportion of under-carboxylated (partially functional) osteocalcin and this has been associated with low BMD and increased risk of hip fracture in older women.’ (pg 53)

3) Arteries

Code	Proposed statement
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VK3:	<i>Vitamin K contributes to the normal function of arteries</i>
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Reference 1.1:

‘A role for vitamin K in atherosclerosis was hypothesized when proteins containing Gla residues were isolated from hardened atherosclerotic plaque (Gijsbers et al., 1990; Levy et al, 1979). These were later identified as osteocalcin and matrix Gla proteins (Ferland, 1998).’ (pg 173)

Reference 2.0:

‘Three additional Gla proteins have been completely characterized, but have functions not related to haemostasis; bone Gla protein (BGP, osteocalcin), matrix Gla protein (MGP) and ...MGP has been shown to modulate the nucleation of calcium crystals in a variety of tissues and is essential for the prevention of arterial wall calcification.’ (pg 1928)

Reference 3.5:

‘...vitamin K is also essential for the synthesis of a number of proteins...matrix Gla-protein (MGP) which is synthesised in most soft tissues, but predominantly in cartilage (by chondrocytes) and in vessel wall (by vascular smooth muscle cells) and which is a potent inhibitor of soft tissue calcification’. (pg 4)

‘Matrix carboxyglutamic acid (Gla) protein contains 79 amino acid residues of which 5 are Gla residues. It is hydrophobic, insoluble in plasma, and is associated with the matrix of cartilage and bone as well as with the tunica media of the arterial vessel wall (Olson, 2000).’ (pg 4)

4) Embryonic development

Code	Proposed statement
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VK4:	<i>Vitamin K contributes to normal embryonic development</i>
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Reference 2.0:

‘Three additional Gla proteins have been completely characterized, but have functions not related to haemostasis; ...and growth-arrest specific protein 6 (gas6)...Gas6 is the specific ligand of the tyrosine-kinase receptor axl which participates in growth and differentiation modulating cell signalling.’ (pg 1928)

Reference 3.5:

‘...vitamin K is also essential for the synthesis of a number of proteins...growth arrest-specific gene 6 protein (Gas6), which is a ligand for tyrosine kinases and has strong apoptotic activity in cultured cells.’ (pg 4)

Reference 4.4:

‘Growth arrest-specific protein (Gas 6) is vitamin K dependent and may be a ligand for tyrosine kinases. In addition, sequence analysis suggests a possible role for vitamin K in cell signalling. It has been suggested (Israels, *et al*, 1997) that the level

of vitamin K in the newborn is tightly regulated because of the involvement of vitamin K dependent proteins in tyrosine kinases signalling and thus in growth regulation in the developing foetus. Tight control of vitamin K levels would be necessary to ensure normal embryonic development.’ (pg 12)

Summary Table

Reference 2.0, (pg 1929):

Human vitamin K-dependent proteins:

Name	No. of Gla residues	Function
Factor II	10	Blood coagulation cascade
Factor VII	10	Blood coagulation cascade
Factor IX	12	Blood coagulation cascade
Factor X	11	Blood coagulation cascade
Protein C	9	Inhibitor of coagulation
Protein S Receptor	11	Activator of protein C, ligand of dte (growth modulation)
Protein Z	13	Enhancement of blood coagulation
gas6	5	Ligand of axl receptor (growth modulation)
Osteocalcin (bone Gla protein, BGP)	2-3	Regulation of bone mineralization (mechanism unknown)
Matrix Gla protein (MGP)	5	Modulator of calcium crystal nucleation
Vitamin K dependent carboxylase	?	Carboxylation of Gla proteins
Galactocerebroside sulfotransferase	?	Sulfatide biosynthesis in brain
n-Sulfatidase	?	Sphingolipid catabolism

ANNEX 4.5

Thiamin (B1)

Source documents for reviewing thiamin (B1)

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.6:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B₁. July 2001.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out93_en.pdf).

Reference 4.5:

Revised Review of Thiamin. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm0014p.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

1) Carbohydrate metabolism

Code	Proposed statement
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Th1:	<i>Thiamin is necessary for the normal metabolism of carbohydrates</i>
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Reference 1.3:

‘Thiamin functions as a coenzyme in the metabolism of carbohydrates and branched-chain amino acids.’ (pg 58)

‘Chemically, thiamin consists of substituted pyrimidine and thiazole rings linked by a methylene bridge. It exists mainly in various inter-convertible phosphorylated forms, chiefly thiamin pyrophosphate (TPP). TPP, the coenzymatic form of thiamin, is involved in two main types of metabolic reactions: decarboxylation of α -ketoacids (e.g., pyruvate, α ketoglutarate, and branched-chain keto acids) and transketolation (e.g., among hexose and pentose phosphates).’ (pg 58, 59)

Reference 2.0:

‘The active form of thiamin in the body is TDP which acts as a coenzyme/cofactor in the oxidative phosphorylation of α -ketoacids and in the transketolase reactions. These

two reaction pathways are involved in glucose metabolism so that thiamin is mainly required for energy metabolism.’ (pg 1859)

‘The decarboxylation and oxidation of pyruvate gives acetyl-S-Coenzyme A, which then enters the tricarboxylic acid (Krebs) cycle where further oxidation yields carbon dioxide and water. This oxidative decarboxylation is accomplished by a multienzyme pyruvate dehydrogenase complex (PDHC), comprising three enzymes, a TDP-dependent pyruvate decarboxylase, a lipoic acid-bound dihydrolipoyl transacetylase and a dihydrolipoyl dehydrogenase (an FAD-dependent enzyme) which reoxidizes the reduced lipoic acid. An analogous series of reactions involving the α -ketoglutarate dehydrogenase complex (α KGDHC) catalyses the conversion of α -ketoglutarate to succinyl-S-CoA in the tricarboxylic acid (TCA) cycle. The decarboxylation of the three branched-chain α -ketoacids derived from the deamination of leucine, isoleucine and valine, namely α -ketoisocaproic acid, α -keto- β -methylvaleric acid and α -ketoisovaleric acid, is achieved by a multienzyme complex similar to those described above.’ (pg 1859)

‘Here the transketolase TDP reacts with the appropriate ketosugars to break the carbon-to-carbon bond between C2 and C3 to form a TDP-glycoaldehyde intermediate which is transferred to a suitable acceptor aldehyde in the pentose or hexose monophosphate shunt (HMPS) pathway for the oxidation of glucose. Glycolysis is the main pathway for the oxidation of glucose and this second metabolic pathway for glucose is important, not so much for energy production (as is the TCA cycle), as for the production of pentoses for RNA and DNA synthesis and NADPH for the biosynthesis of fatty acids and other products, while also supplying intermediate sugars for glycolysis.’ (pg 1859-1861)

‘The UK dietary reference value (DRV) for thiamin is expressed per energy intake because of its essential role in energy metabolism....The thiamin requirement is related to metabolic rate and is greatest when carbohydrate is the energy source.’ (pg 1862)

‘In maple syrup urine disease (MSUD), also called branched-chain ketoaciduria, where the branched-chain α -ketoacids derived from the three amino acids are not decarboxylated but are excreted in the urine, patients are not thiamin-deficient but many illustrate a dependency and a response to large doses of thiamin or TDP.’ (pg 1863)

Reference 3.6:

‘Vitamin B₁ mainly acts in α -ketoacid decarboxylation (e.g. pyruvate, α -ketoglutarate and branched-chain α -ketoacid acids), in transketolation (e.g. among hexose and pentose phosphates) ...’ (pg 2)

‘Animal experiments have shown that the rate of vitamin B₁ utilisation depends on the amount of carbohydrate metabolised. Because the principal metabolic role is in energy-yielding metabolism the requirement is related to energy intake.’ (pg 3)

Reference 4.5:

'The major coenzymatic form of thiamin is thiamin pyrophosphate (TPP), which requires ATP, Mg²⁺ and thiaminpyrophosphokinase for its synthesis. TPP functions as coenzyme in the following enzymic reactions:

- (i) Non-oxidative decarboxylation of α -ketoacids catalysed by pyruvate decarboxylase (mainly plants and yeast, first step in alcoholic fermentation)
 $\text{RCOCOOH} \rightarrow \text{RCHO} + \text{CO}_2$.
- (ii) Oxidative decarboxylation – catalysed by the pyruvate, α -ketoglutarate (and other α -keto acids) and branched-chain amino acid (leucine, isoleucine and valine) dehydrogenase multienzyme complex systems. All these enzymes are intramitochondrial and produce acetyl-coenzyme A (CoA), succinyl CoA and the appropriate derivatives of branched chain amino acids, respectively, which are important in carbohydrate and lipid metabolism.
- (iii) Transketolation – catalysed by cytosolic transketolase. This is an important reaction in the pentose phosphate pathway, and allows the reversible conversion of three-, four-, five-, six- and seven- carbon sugars by the transfer of two- or three-carbon moieties. This pathway provides the major source of pentose sugars for the synthesis of nucleic acids and NADPH for fatty acid synthesis. (Rindi, 1996 and references therein).' (pg 6)

2) Neurological and cardiac systems

Code	Proposed statement
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Th2:	<i>Thiamin is necessary for normal neurological and cardiac function.</i>
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Reference 1.3:

'The clinical signs of deficiency include ... mental changes such as apathy, decrease in short-term memory, confusion, and irritability; muscle weakness; and cardiovascular effects such as an enlarged heart (Horwitt et al., 1948; Inouye and Katsura, 1965; Platt, 1967; Williams et al., 1942; Wilson, 1983).' (pg 59)

'A controlled-diet, dose-response experiment was conducted with nine girls aged 16 to 18 years to examine the thiamin requirement (Hart and Reynolds, 1957)...The authors noted that the subjects became irritable and uncooperative and lost the ability to concentrate when fed the low-thiamin diet – symptoms also noted by others in the early stage of thiamin deficiency.' (pg 67, 68)

Reference 2.0:

'Thiamin, as TTP, may have a part to play in nerve cell transmissions.' (pg 1859)

'Polyneuritis, which is so often a feature of thiamin deficiency, is evidence of a specific function in neural tissues, and the existence of TPP in brain and other neural tissues suggests a direct role for thiamin in neural excitation. It has been repeatedly demonstrated that the stimulation of nerves or treatment with certain neuroactive drugs results in a decrease in the level of TDP and particularly TTP in the nerve, concomitant with an increase in free TMP in the surrounding fluid. It has been postulated that TTP plays an essential role in nerve transmission involving a gating

mechanism for Na⁺ and K⁺ transport via (Na⁺-K⁺) - ATPase. This is supported by the fact that nerves contain a constant and significant level of TTP (10%) and also that patients with Leigh disease (Subacute necrotizing encephalomyelopathy) have a deficiency of TTP (but normal TDP), and this is accompanied by severe neurological involvement. The presence of an inhibitor of TTP synthesis from TDP is thought to be the contributing factor here.' (pg 1861)

'Thiamin has also been shown to bear a relationship to the levels and functions of various neurotransmitters namely the serotonergic, adrenergic and cholinergic systems. Whether, or to what extent, changes in these systems are responsible for the neurologic symptoms of thiamin deficiency remains to be established.' (pg 1861)

'Beriberi is the traditional thiamin deficiency disease and ... is characterized by involvement of the nervous and cardiac systems, with one or other system being predominantly affected: the cardiac system in wet beriberi and the nervous system in dry beriberi. In addition to beriberi, the decreased activity of cerebral thiamin-dependent congenital lactic acidosis, intermittent ataxia of childhood, Leigh disease and the Wernicke-Korsakoff syndrome. Recent evidence also suggests that thiamin neurochemistry is disrupted in Alzheimer's disease. The role of thiamin deficiency in Wernicke-Korsakoff syndrome is well established. This syndrome and Alzheimer's disease are both associated with marked loss of cholinergic neurons in the nucleus basalis and with memory loss, suggesting that alterations of thiamin-dependent enzymes and/or disrupted neurotransmissions could also be implicated in the pathophysiology of Alzheimer's disease.' (pg 1861)

Reference 3.6:

'Vitamin B₁ mainly acts in ... and possibly in nerve conduction.' (pg 2)

Reference 4.5:

'Thiamin is a pharmacologic antagonist of acetylcholine, which may explain the nervous lesions caused by thiamin deficiency (Baugartner, 1991 and references therein).' (pg 6)

'A non-coenzymatic function for TPP has been proposed in nervous tissue. TPP is concentrated in neuronal cells and other excitable tissues such as skeletal muscle ...' (pg 6)

'Clinical deficiency in humans, and various animals, results in the disease known as beriberi, the major manifestations of which mainly affect the cardiovascular (wet beriberi) and nervous systems (dry beriberi). Cardiovascular manifestations of beriberi include cardiac hypertrophy and dilatation, particularly of the right ventricle, tachycardia, respiratory stress and oedema of the legs. Neurological manifestations typically affect the lower extremities and include exaggerated tendon reflexes, polyneuritis and sometimes paralysis. In later stages of the disease, the upper extremities are also affected, resulting in muscle weakness and pain and convulsions. "Burning feet" syndrome may also be a manifestation of thiamin deficiency, appearing early on in the course of polyneuropathy. In more severe cases, both cardiovascular and neurological symptoms may be present and the disease can be fatal.' (pg 7)

Reference 5.0:

‘Deficiency symptoms: ...abnormalities of the electrocardiogram. Severe thiamin deficiency of long duration culminates in beriberi, the symptoms of which are...disturbances of heart function...’ (pg 84)

‘More recent evidence suggests that thiamin has a role beyond that of a coenzyme in regulating transmission of impulses in peripheral nerves. ...The symptoms, as they progress, are often followed by... and tachycardia. When thiamin deficiency is advanced, the patient usually exhibits prominent cardiovascular and neurological features. Cardiac findings include an enlarged heart, tachycardia, edema, and ST-segment and T-wave changes. There is high output failure due, at least in part, to the peripheral vasodilation. The clinical syndrome has a number of similarities to apathetic hyperthyroidism, with which it often confused.’ (pg 1321)

ANNEX 4.6

Riboflavin (B₂)

Source documents for reviewing riboflavin (B₂)

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.7:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B₂. November 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80i_en.pdf).

Reference 4.6:

Revised Review of Riboflavin. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/reviewriboflavin>).

Reference 7.0:

Introduction to Human Nutrition. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.

1) Release of energy from food

Code Proposed statement

Ri1: Riboflavin contributes to the normal release of energy from food.

Reference 1.3:

‘The primary form of the vitamin is an integral component of the coenzymes, flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD) (McCormick, 1994; McCormick and Greene, 1994; Merrill et al., 1981). It is in these bound coenzyme forms that riboflavin functions as a catalyst for redox reactions in numerous metabolic pathways and in energy production (McCormick and Greene, 1994).’ (pg 87, 88)

‘The redox reactions in which flavocoenzymes participate include flavoprotein-catalyzed dehydrogenations that are both pyridine nucleotide (niacin) dependent and independent, reactions with sulfur-containing compounds, hydroxylations, oxidative decarboxylations (involving thiamin as its pyrophosphate), dioxygenations, and reduction of oxygen to hydrogen peroxide (McCormick and Greene, 1994). There are obligatory roles of flavocoenzymes in the formation of some vitamins and their coenzymes. For example, the biosynthesis of two niacin-containing coenzymes from tryptophan occurs via FAD-dependent kynurenine hydroxylase, an FMN-dependent oxidase catalyzes the conversion of the 5’-phosphates of vitamin B₆ to coenzymic

pyridoxal 5'-phosphate, and an FAD-dependent dehydrogenase reduces 5,10-methylene-tetrahydrofolate to the 5'-methyl product that interfaces with the B₁₂-dependent formation of methionine from homocysteine and thus with sulfur amino acid metabolism.' (pg 88)

'Riboflavin interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B₆, which requires FN for conversion to the coenzyme pyridoxal 5'-phosphate (McCormick, 1989).' (pg 96)

Reference 2.0:

'The first example of serious metabolic disturbance, seen in moderate riboflavin deficiency, is the disturbance of fatty acid oxidation. The normal first stage in the spiral process of β -oxidation of fatty acids within the mitochondria is the removal of two hydrogen atoms from the two carbons located α and β to the activated carboxyl end of the chain. The fatty acyl coenzyme A substrate is acted upon by one of several fatty acyl-CoA dehydrogenase flavoprotein enzymes (e.g. long-chain acyl-CoA: (acceptor) 2,3- oxidoreductase EC 1.3.99.13), each of which is specific for a small range of acyl chains. The second stage in this process involves transfer of the electrons via another flavoenzyme, known as 'electron transferring flavoprotein dehydrogenase' (electron-transferring-flavoprotein; ubiquinone oxidoreductase EC 1.5.5.1), and thence to the cytochrome chain and to oxygen. These flavoenzymes, unlike the flavoenzymes that are linked to carbohydrate oxidation, are highly sensitive to dietary riboflavin depletion.' (pg 1726)

'Several studies have documented an apparent increase in riboflavin requirements accompanying an increase physical exercise in human subjects. This may reflect the fact that anabolic influences and the accretion of new lean body mass creates a demand for the vitamin, for mitochondrial accretion.' (pg 1727)

'Although even a severe riboflavin deficiency is less obviously life-threatening than some other types of malnutrition that are commonly encountered in the Third World, it can nevertheless be a major source of debility, through skin lesions and metabolic dysfunctions, and riboflavin nutrition thus deserves an important place in future public health programmes.' (pg 1729)

Reference 3.7:

'Riboflavin is a precursor of certain essential coenzymes such as flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD). In these coenzyme forms riboflavin functions as a catalyst for redox reactions including flavoprotein-catalyzed dehydrogenations that are either pyridine nucleotide dependent or independent reactions with sulphur-containing compounds, hydroxylations, oxidative carboxylations, dioxygenations and the reduction of oxygen to hydrogen peroxide. Flavo-coenzymes are also involved in the biosynthesis of niacin-containing coenzymes from tryptophan via FAD-dependent kynurenine hydroxylase, the FMN dependent conversion of the 5'-phosphates of vitamin B₆ to pyridoxal 5'-phosphate and the FAD-dependent dehydrogenation of 5,10-methylene-tetrahydrofolate to the 5'-methyl product, with the vitamin B₁₂ - dependent formation of methionine and sulphur amino metabolism.' (pg 2)

Reference 4.6:

‘Clinically, riboflavin promotes normal growth, is required for the breakdown of fat, and assists in the synthesis of steroids and glycogen and formation of red blood cells. FAD plays roles in oxidation-reduction reactions as well, interacting with a group of enzymes known as flavoproteins.’ (pg 6)

2) Transport and metabolism of iron

Code	Proposed statement
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<i>Ri2:</i>	<i>Riboflavin contributes to the normal transport and metabolism of iron in the body.</i>
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Reference 1.3:

‘The signs of riboflavin deficiency (ariboflavinosis) in humans are ... normochromic, normocytic anemia associated with pure erythrocyte cytoplasia of the bone marrow (Wilson, 1983). Riboflavin deficiency is most often accompanied by other nutrient deficiencies....’ (pg 90)

Reference 2.0:

‘An important interaction of riboflavin with iron economy has been suspected for many years, partly because iron-deficient animals failed to respond readily to iron supplements if they were also riboflavin-deficient, and also because the redox system involving riboflavin and its coenzymes has been shown to interact very readily with the redox system between ferric and ferrous iron.’ (pg 1726)

‘Some studies in experimental animals have shown that not only is there evidence for some impairment of absorption of iron in riboflavin-deficient animals, and of its distribution between discrete compartments within the body, but also – more surprisingly and strikingly – a major increase in rates of iron loss from the intestinal mucosa, resulting in impaired retention of the body iron stores. This enhanced rate of iron loss is accompanied by hyperproliferation of crypt cells and increased cellular transit along the villi, leading to an excessive proportion of immature villi, and probably also to a reduction in absorptive area. These studies begin to explain how a combination of iron deficiency and riboflavin deficiency, which is frequently encountered in human populations in many developing countries, may lead to a gradual deterioration of iron status, which is often accompanied by other intestinal lesions and by impaired gut function.’ (pg 1726,1727)

Reference 3.7:

‘Riboflavin ...is thought also to be necessary for the absorption of iron, since it is common for iron deficiency to accompany a deficiency in riboflavin (Butler and Topham 1993).’ (pg 6)

Reference 7.0:

‘Riboflavin deficiency is sometimes associated with hypochromic anaemia as a result of impaired iron absorption...the mobilisation of iron bound to ferritin in mucosal cells for transfer to transferrin requires oxidation by a flavin-dependent enzyme.’ (pg 149)

3) Mucous membranes

Code	Proposed statement
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<i>Ri3:</i>	<i>Riboflavin contributes to the normal structure of mucous membranes (such as the surface of the tongue, the mouth, eyes and intestines).</i>
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Reference 1.3:

‘The signs of riboflavin deficiency (ariboflavinosis) in humans are sore throat; hyperemia and edema of the pharyngeal and oral mucous membranes; cheilosis; angular stomatitis; glossitis (magenta tongue); seborrheic dermatitis; and...’ (pg 90)

‘...lens opacities in humans have been associated with high glutathione reductase activity (with FAD) (Leske et al., 1995)...’ (pg 94)

Reference 2.0:

‘Some studies in experimental animals have shown that not only is there evidence for some impairment of absorption of iron in riboflavin-deficient animals, and of its distribution between discrete compartments within the body, but also – more surprisingly and strikingly – a major increase in rates of iron loss from the intestinal mucosa, resulting in impaired retention of the body iron stores. This enhanced rate of iron loss is accompanied by hyperproliferation of crypt cells and increased cellular transit along the villi, leading to an excessive proportion of immature villi, and probably also to a reduction in absorptive area. These studies begin to explain how a combination of iron deficiency and riboflavin deficiency, which is frequently encountered in human populations in many developing countries, may lead to a gradual deterioration of iron status, which is often accompanied by other intestinal lesions and by impaired gut function.’ (pg 1726,1727)

Reference 4.6:

‘Riboflavin helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system...’ (pg 6)

Reference 7.0:

Riboflavin deficiency is characterized by lesions of the margin of the lips (cheilosis) and corners of the mouth (angular stomatitis), a painful desquamation of the tongue, so that it is red, dry and atrophic (magenta tongue), and seborrheic dermatitis, with filiform excrescences, affecting especially the nasolabial folds, eyelids and ears, with abnormalities of the skin around the vulva and anus and at the free border of prepuce. There may also be conjunctivitis with vascularization of the cornea and opacity of the lens. This last is the only lesion of ariboflavinosis for which the biochemical basis is known: glutathione is important in maintaining the normal clarity of crystalline in the lens, and glutathione reductase is a flavoprotein that is particularly sensitive to riboflavin depletion.’ (pg 148)

4) Fetal Growth

Code	Proposed statement
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<i>Ri4:</i>	<i>Riboflavin contributes to normal fetal growth</i>
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Reference 1.3:

‘Maternal riboflavin intake (estimated from a crosscheck dietary history) was positively associated with foetal growth in a study of 372 pregnant women (Badart-Smook et al., 1997), but the data are insufficient to warrant use of foetal growth as an indicator for setting the riboflavin requirement for pregnant women. For pregnancy an additional riboflavin requirement of 0.3mg/day is estimated based on increased growth in maternal and fetal compartments and a small increase in energy utilization.’ (pg 110, 111)

Reference 2.0:

‘Riboflavin is secreted into milk, the concentration being species-specific and to a moderate extent dependent on maternal status and intake. Riboflavin is also required by the developing fetus, which implies a need for active transport from the maternal to the fetal circulation during pregnancy, the flavin concentration being greater on the fetal side. Studies from India have identified a specific riboflavin concentration carrier protein (RCP) present in bird (e.g. chicken) eggs, which is considered to be specific for riboflavin, and is essential for normal embryonic development....A homologous protein...has been shown to occur in...two species of monkeys and also in humans. There remains some controversy over the interpretation of these data and other, less specific, riboflavin binders in blood may also play an important role. These studies have provided an intriguing example of the role of specific vitamin-transporting mechanisms, designed to ensure that the vitamin needs of developing embryos has been provided by the demonstration that riboflavin analogues can cause teratogenic changes, even in the absence of any detectable damage to maternal tissues.’ (pg 1724,1725)

Reference 4.6:

‘Clinically, riboflavin promotes normal growth...’ (pg 6)

5) Eyes

Code Proposed statement

Ri5: Riboflavin contributes to the normal structure of eyes

Reference 1.3:

‘...lens opacities in humans have been associated with high glutathione reductase activity (with FAD) (Leske et al., 1995)...’ (pg 94)

Reference 4.6:

‘Riboflavin helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system...’ (pg 6)

Reference 7.0:

‘Riboflavin deficiency is characterized by ...There may also be conjunctivitis with vascularization of the cornea and opacity of the lens. This last is the only lesion of ariboflavinosis for which the biochemical basis is known: glutathione is important in maintaining the normal clarity of crystalline in the lens, and glutathione reductase is a flavoprotein that is particularly sensitive to riboflavin depletion.’ (pg 148)

6) Red blood cells

Code	Proposed statement
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<i>Ri6:</i>	<i>Riboflavin contributes to the normal structure of red blood cells</i>
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Reference 1.3:

‘...assessing riboflavin status involves the determination of erythrocyte glutathione reductase (EGR) activity...The EGR value is an enzymatic and hence functional indicator that is conventionally determined with and without the addition on flavin-adenine dinucleotide (FAD) – the coenzyme required for the activity of EGR...’ (pg 90 - 91)

‘Erythrocyte flavin has been used as an indicator of the cellular concentration of the vitamin in its coenzyme forms because these coenzymes comprise over 90 percent of flavin (Burch et al., 1948).’ (pg 91)

Reference 2.0:

‘... Riboflavin is also required by the developing fetus, which implies a need for active transport from the maternal to the fetal circulation during pregnancy, the flavin concentration being greater on the fetal side. Studies from India have identified a specific riboflavin concentration carrier protein (RCP) present in bird (e.g. chicken) eggs, which is considered to be specific for riboflavin, and is essential for normal embryonic development....A homologous protein...has been shown to occur in...two species of monkeys and also in humans. There remains some controversy over the interpretation of these data and other, less specific, riboflavin binders in blood may also play an important role...’ (pg 1724 - 1725)

Reference 4.6:

‘Clinically, riboflavin ... assists in the ... formation of red blood cells.’ (pg 6)

ANNEX 4.7

Niacin

Source documents for reviewing niacin

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.8:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Levels of Nicotinic Acid and Nicotinamide (Niacin) April 2002.

(http://www.europa.eu.int/comm/food/fs/sc/scf/out80j_en.pdf).

Reference 4.7:

Review of Niacin – Revised Version. Expert Group on Vitamins and Minerals. August 2002.

(<http://www.food.gov.uk/multimedia/pdfs/evm-01-11r.pdf>).

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

Reference 7.0:

Introduction to Human Nutrition. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.

1) Release of energy from food

Code	Proposed statement
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Nil:	<i>Niacin is necessary for the normal release of energy from food.</i>
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Reference 1.3:

‘In the form of the coenzymes NAD and NADP, niacin functions in many biological redox reactions. NAD functions in intracellular respiration and as a codehydrogenase with enzymes involved in the oxidation of fuel molecules such as glyceraldehyde 3-phosphate, lactate, alcohol, 3-hydroxybutyrate, pyruvate, and α -ketoglutarate... Three classes of enzymes cleave the β -N-glycosylic bond of NAD to free nicotinamide and catalyze the transfer of ADP-ribose in non-redox reactions (Lautier et al., 1993). Two of the three classes catalyze ADP-ribose transfer to proteins; mono-ADP-ribosyltransferases and poly-ADP-ribose polymerase (PARP). The third class

promotes the formation of cyclic ADP-ribose, which mobilizes calcium from intracellular stores in many types of cells (Kim et al., 1994).’ (pg 124)

Reference 2.0:

‘Some of the most important and characteristic functions of NAD manifest in the principal cellular catabolic pathways, responsible for liberation of energy during the oxidation of energy-producing fuels.’ (pg 1293)

Reference 3.8:

‘Niacin is the term used to describe two related compounds, nicotinic acid and nicotinamide, both of which have biological activity. Niacin is not strictly speaking a vitamin because it is formed from the metabolism of tryptophan, and is not *per se* essential to the body, providing that there is an adequate supply of the essential amino acid tryptophan (Horwitt *et al.*, 1981). Niacin is the precursor for two cofactors, NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate), which are essential for the functioning of a wide range of enzymes involved in redox reactions.’ (pg 2)

‘The co-enzymes NAD and NADPH are involved in a large number of redox reactions essential for the normal functioning of mammalian cells.’ (pg 2)

Reference 4.7:

‘Niacin is the functional component of two important coenzymes, NAD and NADP (nicotinamide adenine dinucleotide and its phosphorylated relative), which activate over 20 dehydrogenase enzymes essential to electron transport and other cellular respiratory reactions. Most dehydrogenases are specific to either NAD or NADP, however, a small number of dehydrogenases use both nicotinamide coenzymes (Levy *et al* 1983).’ (pg 6)

‘In spite of their great structural similarity, NAD and NADP have quite different metabolic roles. NAD functions as an electron carrier for intracellular respiration as well as a co-dehydrogenase with enzymes involved in the oxidation of fuel molecules, such as glyceraldehyde 3-phosphate, lactate, pyruvate and α -ketoglutarate dehydrogenases.’ (pg 6)

Reference 7.0:

‘The best defined role of niacin is in the metabolism of metabolic fuels, and the functional nicotinamide part of the coenzymes NAD and NADP, which play a major role in oxidation and reduction reactions.’ (pg 151)

‘In general, NAD^+ is involved as an electron acceptor in energy-yielding metabolism, being oxidized by the mitochondrial electron transport chain, while the major coenzyme for reductive synthetic reactions is NADPH. An exception to this general rule is the pentose phosphate pathway of glucose metabolism, which results in the reduction of NADP^+ to NADPH, and is the principal metabolic source of reductant for fatty acid synthesis.’ (pg 151)

2) DNA replication and growth

Code	Proposed statement
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<i>Ni2a:</i>	<i>Niacin is necessary for the normal repair and replication of DNA</i>
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<i>Ni2b:</i>	<i>Niacin contributes to normal growth in the developing fetus</i>
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Reference 1.3:

‘The enzyme PARP is found in the nuclei of eukaryotic cells and catalyzes the transfer of many ADP-ribose units from NAD to an acceptor protein and also to the enzyme itself. These nuclear poly ADP-ribose proteins seem to function in DNA replication and repair and in cell differentiation. DNA damage greatly enhances the activity of PARP (Stierum et al., 1994); PARP activity is strongly correlated with cellular apoptosis (Stierum et al., 1994).’ (pg 124)

‘A possible functional measure for niacin status could be polyadenosine diphosphate (ADP) ribosylation, because ADP ribosylation may contribute to gene stability (poly-ADP-ribose polymerase in the nucleus) and may function in deoxyribonucleic acid (DNA) replication and repair (Stierum et al., 1994).’ (pg 127)

‘To derive the EAR for pregnant women, it is estimated that the need for niacin increases by 3mg/day of NEs to cover increased energy utilization and growth in maternal and fetal compartments, especially during the second and third trimesters.’ (pg 136)

Reference 2.0:

‘NAD is essential for the synthesis and repair of DNA. NAD has, in addition, a role in supplying ADP ribose moieties to lysine, arginine and asparagine residues in proteins such as histones, DNA lyase II and DNA-dependent RNA polymerase, and to polypeptides such as the bacterial diphtheria and cholera toxins. In the nucleus, poly (ADP ribose) synthetase is activated by binding to DNA breakage points and is involved in DNA repair. It is also concerned with condensation and expansion chromatin during the cell cycle and in DNA replication. Niacin status affects the level of ADP ribolysation of proteins. A high level of poly (ADP ribose) synthetase activity, which is found in some tumours, can result in low levels of NAD.’ (pg 1293)

‘The two pyridine nucleotide coenzymes, ... and known nowadays as ‘NAD’ and ‘NADP’ (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate), are involved in hundreds of enzyme-catalysed redox reactions *in vivo*. Although a minority of these diverse reactions can use either of the two niacin-derived cofactors, most are highly specific for one or the other.’ (pg 1292)

Reference 3.8:

‘In addition, NAD is the source for ADP-ribose, which is used in repairing DNA breakage caused by mutagens and other toxins.’ (pg 2)

Reference 4.7:

‘The niacin co-factor NAD is also required for important non-redox reactions. It is the substrate for three classes of enzymes that cleave the β -N-glycosylic bond of NAD to free nicotinamide and catalyse the transfer of ADP-ribose to proteins (Jacob and Swendseid, 1996).’ (pg 6)

Reference 7.0:

‘In addition to its coenzyme role, NAD has a function as the source of ADP-ribose for the ADP-ribosylation of a variety of proteins and poly(ADP-ribosylation) and hence activation of nucleoproteins involved in the DNA repair mechanism.’ (pg 151)

‘In the nucleus, poly(ADP-ribose)polymerase is activated by binding to breakage points in DNA. The enzyme is involved in activation of the DNA repair mechanism in response to strand breakage caused by radical attack or UV radiation. In cells that have suffered considerable DNA damage, the activation of poly(ADP-ribose)polymerase may deplete intracellular NAD to such an extent that ATP formation is impaired, leading to cell death.’ (pg 151, 152)

3) Fatty acid and steroid synthesis

Code	Proposed statement
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<i>Ni3:</i>	<i>Niacin contributes to the normal structure of some steroids, which are required to make hormones</i>
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Reference 1.3:

‘NADP functions in reductive biosyntheses such as in fatty acid and steroid syntheses and, like NAD, as a coenzyme – as in the oxidation of glucose 6-phosphate to ribose 5-phosphate in the pentose phosphate pathway.’ (pg 124)

Reference 2.0:

‘NADP, however, functions mainly in the reductive reactions of lipid biosynthesis, and the reduced form of this coenzyme is generated via the pentose phosphate cycle.’ (pg 1293)

Reference 4.7:

‘NADP functions as a hydrogen donor in reductive biosyntheses, such as in fatty acid and steroid syntheses, and like NAD as a coenzyme, such as in the oxidation of glucose-6-phosphate to ribose 5-phosphate in the pentose phosphate pathway (Jacob and Swendseid 1996).’ (pg 6)

4) Skin and mucous membranes

Code	Proposed statement
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<i>Ni4:</i>	<i>Niacin is necessary for the normal structure and function of skin and mucous membranes (such as in the intestines).</i>
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Reference 1.3:

‘Pellagra is the classic manifestation of a severe niacin deficiency. It is characterised by a pigmented rash that develops symmetrically in areas exposed to sunlight; changes in the digestive tract that are associated with vomiting, constipation or diarrhea, and a bright red tongue; ...’ (pg 125-126)

Reference 2.0:

'The most characteristic clinical signs of severe niacin deficiency in humans are dermatosis (hyperpigmentation, hyperkeratosis, desquamation – especially where exposed to the sun), anorexia, achlorhydria, diarrhoea, angular stomatitis, cheilosis, magenta tongue, anaemia, ...The picture in other species is not radically different; however deficient dogs and cats typically exhibit 'black tongue' (pustules in the mouth, excessive salivation) and bloody diarrhoea; ... fowl exhibit inflammation of the upper gastrointestinal tract, dermatitis, diarrhoea and damage to the feathers.' (pg 1295)

Reference 3.8:

'The condition that is characteristic of a deficiency of both tryptophan and preformed niacin is pellagra...and is characterised by spinal pains, "magenta tongue", digestive disturbances and subsequently erythema with drying and expurgation of the skin....' (pg 2)

Reference 4.7:

'The most common symptoms of niacin deficiency are divided into three categories: changes in the skin; mucosa of the mouth, stomach and intestinal tract; and... The changes in the skin are amongst the most characteristic in human beings. They are called 'pellagra', which means 'raw skin'. These symptoms are most pronounced in the parts of the skin which are exposed to sunlight. In severe deficiency, the human tongue and gastric mucosa become inflamed; the tongue becomes bright red and swells. ...' (pg 7)

Reference 6.3:

'Niacin deficiency results in pellagra, which is characterised by a severe sunburn-like skin lesion in areas of the body exposed to sunlight, and in areas such as the knees, ankles, wrists and elbows which are subjected to pressure. Diarrhoea is a characteristic, but not inevitable, symptom of pellagra...' (pg 99)

Reference 7.0:

'Pellagra is characterized by a photosensitive dermatitis, like severe sunburn, typically with a butterfly-like pattern of distribution over the face, affecting all parts of the skin that are exposed to sunlight. Similar skin lesions may also occur in areas not exposed to sunlight, but subject to pressure, such as the knees elbows, wrists and ankles. ... and there may be diarrhoea. Untreated pellagra is fatal...' (pg 152)

5) Neurological system

Code Proposed statement

Ni5: Niacin is necessary for normal neurological function.

Reference 1.3:

'Pellagra is the classic manifestation of a severe niacin deficiency. It is characterised by ... neurological symptoms including depression, apathy, headache, fatigue and loss of memory.' (pg 125-126)

Reference 2.0:

‘The most characteristic clinical signs of severe niacin deficiency in humans are ... neuropathy (headache, dizziness, tremor, neurosis, apathy). ... The picture in other species is not radically different; ... pigs show neurological lesions affecting the ganglion cells; rats exhibit damage to the peripheral nerves (cells and axons); ...’ (pg 1295)

Reference 3.8:

‘The condition that is characteristic of a deficiency of both tryptophan and preformed niacin is pellagra... Various nervous manifestations, such as spasms, ataxic paraplegia and mental disturbances occur in severe cases.’ (pg 2)

Reference 4.7:

‘The most common symptoms of niacin deficiency are divided into three categories: changes in the skin; mucosa of the mouth, stomach and intestinal tract; and changes in the nervous system.... The neurological symptoms experienced can include fatigue, sleeplessness, depression, loss of memory and visual impairment (Gopalan and Rao).’ (pg 7)

Reference 6.3:

‘Niacin deficiency results in pellagra,... In advanced cases there may be dementia with intermittent periods of lucidity.’ (pg 99)

Reference 7.0:

‘Advanced pellagra is also accompanied by dementia (more correctly a depressive psychosis), ... The depressive psychosis is similar to schizophrenia and the organic psychoses, but clinically distinguishable by the sudden lucid phases which alternate with the most florid psychiatric signs. It is probable that these mental symptoms can be explained by a relative deficit of the essential amino acid tryptophan, and hence reduced synthesis of the neurotransmitter 5-hydroxytryptamine (serotonin), and not to a deficiency of niacin per se.’ (pg 152)

ANNEX 4.8

Pantothenic Acid

Source documents for reviewing pantothenic acid

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.9:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Pantothenic Acid. April 2002. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80k_en.pdf).

Reference 4.8:

Review of Pantothenic Acid. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/panto.pdf>).

1) Fat metabolism

Code	Proposed statement
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<i>Pa1:</i>	<i>Pantothenic acid is necessary for the normal metabolism of fat.</i>
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Reference 1.3:

‘Pantothenic acid is vital to the synthesis and maintenance of co-enzyme A (CoA), a cofactor and acyl group carrier for many enzymatic processes, and acyl carrier protein, a component of the fatty acid synthase complex (Tahiliani and Beinlich, 1991). As such, pantothenic acid is essential to almost all forms of life. Most tissues transport pantothenic acid into cells for the synthesis of CoA.’ (pg 357)

‘The synthesis of CoA from pantothenate is regulated primarily by pantothenate kinase, an enzyme that is inhibited by the pathway end products, CoA and acyl CoA. Thus CoA production does not reflect the amount of available pantothenate (Tahiliani and Beinlich, 1991).’ (pg 358)

Reference 2.0:

‘The primary role of pantothenic acid is in acyl group activation for lipid metabolism, involving thiol acylation of CoA or of ACP both of which contain 4-phosphopantotheine, the active group of which is β -mercaptoethylamine. Coenzyme A is essential for oxidation of fatty acids, of pyruvate and of α -oxoglutarate, for metabolism of sterols, and for acetylation of other molecules, so as to modulate their transport characteristics or functions.’ (pg 1512)

'Beta-oxidation within the peroxisomes is also CoA-dependent, and is downregulated by pantothenate deficiency. The rate of CoA synthesis is under close metabolic control by energy-yielding substrates, such as glucose and free fatty acids, at the initial activation step, catalysed by pantothenate kinase (ATP: pantothenate 4-phosphotransferase, EC 2.7.1.33). This feedback control is thought to be a mechanism for conservation of cofactor requirements.' (pg 1512)

'In addition to the now well-established roles of CoA in the degradation and synthesis of fatty acids, of sterols and of other compounds synthesized from isoprenoid precursors, there are also a number of acetylation and long-chain fatty acylation processes which seem to require CoA as part of their essential biological catalytic sites... The acetylation of amino sugars and some other basic reactions of acetyl-CoA and succinyl-CoA in intermediary metabolism have been known since the 1980's. (pg 1512,1513)

Reference 3.9:

'Pantothenic acid plays a central role in intermediary metabolism as part of the coenzyme A (CoA) molecule and as part of the pantotheine functional group in the acyl-carrier protein (Acyl-CP). This vitamin serves therefore as a cofactor in acyl-group activation and transfer in fatty acid and carbohydrate metabolism, as well as in a wide range of (other) acylation reactions (see Fox, 1984 and Plesofsky-Vig, 1996 for reviews).' (pg 2)

Reference 4.8:

'Pantothenate, usually in the form of CoA-containing species (e.g. acetyl CoA, succinyl CoA) performs multiple roles within cellular metabolism and in the synthesis of many essential molecules... (reviewed by Plesofsky-Vig, 1999) ...Within the tricarboxylic acid cycle, β -oxidation of fatty acids and oxidative degradation of amino acids.' (pg 8)

2) Molecule structure

Code Proposed statement

Pa2: Pantothenic acid contributes to the normal structure of numerous essential molecules in the body

Reference 1.3:

'... and in the synthesis of fatty acids and membrane phospholipids, amino acids, steroid hormones, vitamins A and D, porphyrin and corrin rings, and neurotransmitters. It is also required for the acetylation and acylation of proteins and the synthesis of α -tubulin (Plesofsky-Vig, 1996) (pg 358)

Reference 2.0:

'Acyl carrier protein, which is synthesized from apo-ACP and coenzyme A, is involved specifically in fatty acid synthesis. Its role is to activate acetyl, malonyl and intermediate-chain fatty acyl groups during their anabolism by the biotin-dependent fatty acid synthase complex (i.e. acyl-CoA; malonyl-CoA-acyl transferase (decarboxylating, oxoacyl and enoyl-reducing and thioester-hydrolysing), EC 2.3.1.85). (pg 1512)

‘The organ with the highest concentration of pantothenate is liver, followed by adrenal cortex, because of the requirement of steroid hormone metabolism there.’ (pg 1512)

‘... the biochemical functions, and hence the basis for the dietary requirement of pantothenic acid, arise entirely from its occurrence as an essential component of CoA and of ACP, which cannot be synthesized *de novo* in mammals from simpler precursors.’ (pg 1512)

‘... the addition of acetyl or fatty acyl groups to certain proteins in order to modify and control their specific and essential properties is a more recent discovery. The first category of these modifications comprises the acetylation of the *N* terminal amino acid in certain proteins, which actually occurs in at least half of all the known proteins that are found in higher organisms. The specific amino acids that are recipients of these acetyl groups are most commonly methionine, alanine or serine. The purposes of this terminal acetylation process are not entirely clear and may be multiple, including modifications of function (e.g. of hormone function), of binding and site recognition, of tertiary peptide structure, and of eventual susceptibility to degradation. Another possible site of protein acetylation is the side chain of certain internal lysine residues, whose side chain ϵ -amino group may become acetylated in some proteins, notably the basic histone proteins of the cell nucleus, and the α -tubulin proteins of the cytoplasmic microtubules, which help to determine cell shape and motility.’ (pg 1513)

‘Proteins can also be modified by acylation with certain long-chain fatty acids, notably the 16-carbon saturated fatty acid, palmitic acid, and the 14-carbon saturated fatty acid, myristic acid. Although structurally very similar to each other, these two fatty acids seek entirely different protein locations for acylation and also have quite different functions. They have recently been explored with particular emphasis on viral and yeast proteins, although proteins in higher animals, in organs such as lung and brain, can also become acylated with palmitoyl moieties. Palmitoyl-CoA is also required for the transport of residues through the Golgi apparatus during protein secretion. It is believed that these protein acylations may enable and control specific protein interactions, especially in relation to cell membranes, and proteins which are palmitoylated are generally also found to be associated with the plasma membrane. Signal transduction (e.g. of the human β_2 -adrenergic receptor) is one process which appears to be controlled by palmitoylation, and other palmitoylated proteins possess some structural importance, for example in the case of the protein-lipid complex of brain myelin. Clearly these subtle protein-modifications, all of which depend on CoA and hence on pantothenic acid, have a wide-ranging significance for many biological processes which is still being actively explored.’ (pg 1513)

Reference 4.8:

‘Pantothenate, usually in the form of CoA-containing species (e.g. acetyl CoA, succinyl CoA) performs multiple roles within cellular metabolism and in the synthesis of many essential molecules....(reviewed by Plesofsky-Vig, 1999):...Fatty acid and membrane phospholipid synthesis; Amino acid synthesis (leucine, arginine, methionine); Synthesis of isoprenoid derivatives, such as cholesterol, steroid hormones, dolichol, vitamin A, vitamin D, haem A; Synthesis of δ -amino-laevulinic acid, the precursor of porphyrin and corrin rings (vitamin B₁₂, haemoglobin,

cytochromes); Synthesis of neurotransmitters (eg acetylcholine); Acetylation, acylation, myristolation, palmitoylation and isoprenylation of proteins.' (pg 8)

ANNEX 4.9

Vitamin B₆

Source documents for reviewing vitamin B₆

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.10:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B₆. October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80c_en.pdf).

Reference 4.9:

Revised review of Vitamin B₆. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/EVM/00/19/P.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

1) Protein metabolism

Code	Proposed statement
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VB ₆ 1:	<i>Vitamin B₆ is necessary for the normal metabolism of protein</i>
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Reference 1.3:

‘Vitamin B₆ (B₆) comprises a group of six related compounds: pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM), and their respective 5’-phosphates (PLP, PNP, and PMP).’ (pg 150)

‘PLP is a coenzyme for more than 100 enzymes involved in amino acid metabolism, including aminotransferases, decarboxylases, race-mases, and dehydratases. It is a coenzyme for δ-aminolevulinatase synthase ... and for cystathionine β-synthase and cystathioninase, enzymes involved in the transsulfuration pathway from homocysteine to cysteine. The carbonyl group of PLP binds to proteins as a Schiff’s base with the ε-amine of lysine. For practically all PLP enzymes the initial step in catalysis involves formation of a Schiff’s base between an incoming amino acid, via its α-amino group, and the carbonyl group of PLP. Much of the total PLP in the body is found in muscle bound to phosphorylase. PLP is a coenzyme in the phosphorylase reaction and is also directly involved in catalysis.’ (pg 151)

‘The major pathway of tryptophan catabolism proceeds via the PLP-dependent kynureninase reaction (Shane and Contractor, 1980). The xanthurenic acid pathway also involves PLP-dependent enzymes. However, under conditions of B₆ deficiency, this minor pathway is used to a greater extent, leading to the increased excretion of abnormal tryptophan metabolites.’ (pg 157)

‘Homocysteine catabolism proceeds via transsulfuration to cysteine and involves two PLP-dependent enzymes.’ (pg 158)

‘Because of PLP’s role as a coenzyme for many enzymes involved in amino acid metabolism, it has been proposed that B₆ requirements are influenced by protein intake. Many studies have demonstrated that increased protein intake causes a relative decrease in B₆ status indicators (Baker et al., 1964; Hansen et al., 1996b; Linkswiler, 1978; Miller et al., 1985; Sauberlich, 1964).’ (pg 161)

Reference 2.0:

‘Vitamin B₆ has a central role in amino acid metabolism as it is the coenzyme for a variety of reactions, including transamination and decarboxylation.’ (pg 1916)

‘The metabolically active vitamer is pyridoxal phosphate. This is involved in many reactions of amino acid metabolism, where the carbonyl group is the reactive moiety; in glycogen phosphorylase, where it is the phosphate group which is important in catalysis; and in the release of hormone receptors from tight nuclear binding, where again it is the carbonyl group that is important. Glycogen phosphorylase catalyses the sequential phosphorolysis of glycogen to release glucose-1-phosphate; it is thus the key enzyme in the utilization of muscle and liver glycogen reserves.’ (pg 1918)

‘Pyridoxal phosphate-dependent enzymes catalyse a number of important reactions in amino acid metabolism, including transamination to yield oxo (keto) acids, decarboxylation to yield amines, and a variety of side chain elimination and rearrangement reactions.’ (pg 1918)

‘In the absence of the substrate, pyridoxal phosphate is bound to the enzyme by the formation of a Schiff base to the ε-amino group of a lysine residue. The first reaction between the substrate and the coenzyme is transfer of the aldimine linkage from this ε-amino group to the α-amino group of the substrate. The ring nitrogen of pyridoxal phosphate exerts a strong electron-withdrawing effect on the aldimine, and this leads to weakening of all three bonds about the α-carbon of the substrate; which bond is cleaved will depend on the orientation of the Schiff base relative to reactive groups of the catalytic site.’ (pg 1918)

‘Cleavage of the α-carbon-carboxyl bond of the Schiff base leads to decarboxylation of the amino acid, followed by release of the corresponding amine and reformation of the internal Schiff base to lysine. A number of the products of the decarboxylation of amino acids are important as neurotransmitters and hormones, and as the diamines and polyamines involved in the regulation of DNA metabolism; the decarboxylation of phosphatidylserine to phosphatidylethanolamine is important in phospholipid metabolism.’ (pg 1918)

‘Hydrolysis of the α -carbon-amino bond of the Schiff base results in the release of the 2-oxo-acid corresponding to the amino acid substrate, and leaves pyridoxamine phosphate at the catalytic site of the enzyme. In this case there is no reformation of the internal Schiff base to the reactive lysine residue. This is the half-reaction of transamination. The process is completed by reaction of pyridoxamine phosphate with a second oxo-acid substrate, followed by the reverse of the reaction sequence.’ (pg 1920)

‘Transamination is of central importance in amino acid metabolism, providing pathways for the catabolism of all amino acids other than lysine (which does not undergo transamination). Many of these reactions are linked to the amination of 2-oxoglutarate to glutamate or glyoxylate to glycine, which are substrates for oxidative deamination, reforming the oxo-acids. Equally, transamination reactions provide a pathway for the synthesis of those amino acids for which there is an alternative source of the oxo-acid (the nonessential amino acids).’ (pg 1920)

Reference 3.10:

‘Pyridoxal phosphate plays an essential role in the metabolism of many amino acids, and deficiency of this coenzyme can lead to many manifestations. Clinical signs include retarded growth, acrodynia, alopecia, skeletal changes and anaemia, while changes in neurotransmitters, such as dopamine, serotonin, norepinephrine (noradrenaline), tryptamine, tyramine, histamine, GABA and taurine, affect brain function and can lead to seizures and convulsions.’ (pg 2)

‘The active form of the vitamin is pyridoxal phosphate, which is a coenzyme that is recognised as being required for the function of more than 60 enzymes involved with transamination, deamination, decarboxylation or desulfuration reactions.’ (pg 2)

‘Tryptophan metabolism is dependent on vitamin B₆ status, because the enzyme kynureninase, requires pyridoxal phosphate. This enzyme is especially sensitive to vitamin B₆ depletion.’ (pg 3)

‘Vitamin B₆ is involved in the metabolism of sulphur-containing amino acids (methionine, taurine and cysteine (Sturman, 1986).’ (pg 3)

Reference 4.9:

‘As with the other B complex vitamins, pyridoxine is involved in the functioning of enzymes involved in the release of energy from food. The active, coenzyme forms of pyridoxine are pyridoxal 5’ phosphate and pyridoxamine 5’ phosphate. Pyridoxal phosphate is involved as a coenzyme in over 60 enzyme reactions (Basu and Dickerson, 1996) particularly in the metabolic transformation of amino acids, including decarboxylation, transamination, dehydration, desulphydration, cleavage, racemisation and synthesis.’ (pg 11)

‘Transaminases (aminotransferases) catalyse the transamination reaction whereby α -amino acids are converted to α -keto acids resulting in the formation of different α -amino acids. Most amino acids can undergo these reversible reactions, and the reaction is responsible for the formation of non-essential amino acids from keto acids. Examples of this are aspartate, which is formed from oxaloacetate, and alanine which is formed from pyruvate (Basu and Dickerson, 1996).’ (pg 12)

‘Pyridoxine is involved in several enzyme reactions in the metabolism of tryptophan. In individuals who are vitamin B₆ deficient, a number of metabolites of tryptophan, in particular xanthurenic acid, are excreted in urine in abnormally large quantities. This phenomenon is employed in the diagnostic tryptophan load test. The enzyme kynureinase is involved in the catabolism of tryptophan, cleaving the 3-hydroxyanthranilate ring, a pathway via which nicotinamide is formed in the body.’ (pg 12)

‘Vitamin B₆ is also a cofactor in the conversion of tryptophan to 5-hydroxytryptamine and of methionine to cysteine (Sturman, 1978) ...’ (pg 12)

2) Transport and metabolism of iron

Code	Proposed statement
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<i>VB62:</i>	<i>Vitamin B6 is necessary for the normal transport and metabolism of iron in the body.</i>
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Reference 1.3:

‘...It is a coenzyme for δ -aminolevulinic acid synthase which catalyzes the first step in heme biosynthesis...’ (pg 151)

‘The classical clinical symptoms of B₆ deficiency are ... microcytic anemia (Snyderman et al., 1953)...Microcytic anemia reflects decreased hemoglobin synthesis. The first enzyme and committed step in heme biosynthesis, aminolevulinic acid synthase, uses PLP as a coenzyme...’ (pg 153)

Reference 2.0:

‘A number of studies have shown that between 10 and 20% of the apparently healthy population have low plasma concentrations of pyridoxal phosphate or abnormal erythrocyte transaminase activation coefficient...’ (pg 1922)

Reference 3.10:

‘Pyridoxal phosphate plays an essential role in the metabolism of many amino acids, and deficiency of this coenzyme can lead to many manifestations. Clinical signs include ... anaemia...’ (pg 2)

Reference 4.9:

‘... Pyridoxal and pyridoxal phosphate also bind to haemoglobin increasing the oxygen-binding capacity and preventing sickling in sickle-cell haemoglobin. Vitamin B₆ is also involved in the biosynthesis of haem and ... (Bender, 1999).’ (pg 12)

3) Hormones

Code	Proposed statement
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<i>VB63:</i>	<i>Vitamin B6 is necessary for the normal function of some hormones</i>
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Reference 1.3:

'The classical clinical symptoms of B₆ deficiency are ...epileptiform convulsions (Bessey et al., 1957); Coursin, 1954) and depression and confusion (Hawkins and Barsky, 1948)... Because PLP is also a coenzyme of decarboxylases that are involved in neurotransmitter synthesis, defects in some of these enzymes could explain the onset of convulsions in B₆ deficiency...However, it has not been definitely shown whether the convulsions are due to the reduced level of one of these neurotransmitters in particular. Guilarte (1993) proposed that the convulsions are caused by abnormal tryptophan metabolites that accumulate in the brain in B₆ deficiency.' (pg 153)

Reference 2.0:

'...It is also the coenzyme of glycogen phosphorylase, and has a role in the actions of steroid and other hormones which act by modulation of gene expression.' (pg 1916)

'The metabolically active vitamer is pyridoxal phosphate. This is involved in many reactions of amino acid metabolism, where the carbonyl group is the reactive moiety; in glycogen phosphorylase, where it is the phosphate group which is important in catalysis; and in the release of hormone receptors from tight nuclear binding, where again it is the carbonyl group that is important...' (pg 1918)

'Vitamin B₆ has a role in the action of those hormones which act by binding to a nuclear receptor protein and modulating gene expression. Such hormones include androgens, oestrogens, progesterone, glucocorticoids, calcitriol (the active metabolite of vitamin D), retinol and retinoic acid, and the thyroid hormones. Target tissue specificity of hormone action is ensured by the presence of receptor proteins which are responsible for both nuclear uptake and the interaction with control regions of DNA. Pyridoxal phosphate reacts with a lysine residue in the receptor protein and releases the hormone-receptor complex from tight nuclear binding. It thus acts to terminate hormone action and release receptor proteins for reutilization. In experimental animals, vitamin B₆ deficiency results in increased and prolonged nuclear uptake and retention of steroid hormones in target tissues, and there is enhanced sensitivity to low doses of hormones. In cells in culture, pyridoxal phosphate depletion results in enhanced induction of marker enzymes, while high intracellular concentrations of pyridoxal phosphate impair enzyme induction in response to the hormone.' (pg 1920)

Reference 3.10:

'Pyridoxal phosphate plays an essential role in the metabolism of many amino acids, and deficiency of this coenzyme can lead to many manifestations... changes in neurotransmitters, such as dopamine, serotonin, norepinephrine (noradrenaline), tryptamine, tyramine, histamine, GABA and taurine, affect brain function and can lead to seizures and convulsions.' (pg 2)

Reference 4.9:

'Decarboxylases catalyse the decarboxylation of amino acids to amines, these reactions include the formation of a number of neuroactive amines such as histamine from histidine, serotonin from tryptophan, γ -aminobutyric acid (GABA) from glutamic acid and adrenaline/noradrenaline and tyramine/dopamine from tyrosine (Bender, 1999, Basu and Dickerson, 1996).' (pg 12)

‘Vitamin B₆ is also a cofactor in the conversion of tryptophan to 5-hydroxytryptamine and of methionine to cysteine (Sturman, 1978) and has been claimed to modify the action of steroid hormones *in vivo* by interacting with steroid-receptor complexes (Disorbo *et al.*, 1980). This interaction occurs via B₆ inhibiting the induction of hepatic tyrosine aminotransferase by glucocorticoids, probably by formation of a Schiff base link to the DNA-binding site of the complex (Basu and Dickerson, 1996) ...Vitamin B₆ is also involved ...in the decarboxylation of phosphatidylserine to phosphatidylethanolamine in phospholipid synthesis (Bender, 1999).’ (pg 12)

4) Homocysteine metabolism

Code	Proposed statement
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<i>VB₆4:</i>	<i>Vitamin B₆ contributes to the maintenance of normal blood homocysteine levels</i>
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Reference 1.3:

‘It is a coenzyme for ... cystathionine β-synthase and cystathioninase, enzymes involved in the transsulfuration pathway from homocysteine to cysteine.’ (pg 151)

‘Inadequate intakes of B₆ have also been reported to impair platelet function and clotting mechanisms (Branstrom *et al.*, 1990; Subarao and Kakkar, 1979), but these effects may also be due to the hyperhomocysteinemia noted in such patients (Brattstrom *et al.*, 1990).’ (pg 154)

‘...fundamental tests for B₆ status, including the increase in homocysteine after a methionine load...’ (pg 155 – 156)

‘Homocysteine catabolism proceeds via transsulfuration to cysteine and involves two PLP-dependent enzymes. Homocysteine can also be remethylated to methionine via folate and vitamin B₁₂-dependent enzymes. Thus plasma concentrations of homocysteine are influenced by B₆ and folate, and to a lesser extent, B₁₂ intakes (Selhub *et al.*, 1993).’ (pg 158)

‘The increase in plasma homocysteine concentration after a methionine load or a meal is responsive to and primarily affected by B₆ status...Results from population-based studies using data adjusted for folate and B₁₂ status and for age indicate that B₆ status as measured by PLP is inversely correlated with nonfasting plasma homocysteine concentration (Selhub *et al.*, 1993).’ (pg 159)

‘For B₆ the data are compatible with the Framingham study (Selhub *et al.*, 1993), in which the lowest deciles of B₆ intake were associated with higher circulating homocysteine. ... At these high B₆ intakes, there is little effect of B₆ intake on homocysteine levels, which are mainly affected by changes in intake at much lower intakes.’ (pg 159)

Reference 3.10:

'Vitamin B₆ is involved in the metabolism of sulphur-containing amino acids (methionine, taurine and cysteine (Sturman, 1986). The disease states of homocystinuria and cystathioninuria are due to inborn errors of metabolism involving the enzymes cystathionine β -synthase (EC 4.2.1.22) and gamma-cystathionase (EC 4.4.1.1). ' (pg 3)

Reference 4.9:

'Vitamin B₆ is also a cofactor in the conversion of tryptophan to 5-hydroxytryptamine and of methionine to cysteine (Sturman, 1978) ...' (pg 12)

Reference 5.0:

'Vitamin B₆ has a significant role to play, along with folate and vitamin B₁₂, the reduction of elevated homocysteine levels associated with increased risk of cardiovascular disease – specifically, coronary artery disease and stroke.' (pg 1338)

ANNEX 4.10

Folate

Source documents for reviewing folate

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.11:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Folate. October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80e_en.pdf).

Reference 4.10:

Review of Folic Acid. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0018p.pdf>).

Reference 6.2:

Folic Acid and the Prevention of Disease Report of the Committee on Medical Aspects of Food and Nutrition Policy. Report on Health and Social Subjects No. 50. DH: The Stationery Office, 2000

1) Cell division

Code	Proposed statement
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<i>Fo1:</i>	<i>Folate is necessary for normal cell division (such as in the gastrointestinal tract).</i>
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Reference 1.3:

‘The folate coenzymes are involved in numerous reactions that involve (1) deoxyribonucleic acid (DNA) synthesis, which depends on a folate coenzyme for pyrimidine nucleotide biosynthesis (methylation of deoxyuridylic acid to thymidylic acid) and thus is required for normal cell division; (2) purine synthesis (formation of glycinamide ribonucleotide and 5-amino-4-imidazole carboxamide ribonucleotide); (3) generation of formate into the formate pool (and utilization of formate); ...’ (pg 197)

‘Both folate and vitamin B₁₂ are required for the formation of 5,10-methylenetetrahydrofolate and involved in thymidylate synthesis by way of a vitamin B₁₂-containing enzyme. The formation of 5,10-methylene tetrahydrofolate depends on the regeneration of the parent compound (tetrahydrofolate) in the homocysteine-to-methionine conversion. This reaction involves the removal of a methyl group from methyl folate and the delivery of this group to homocystine for the synthesis of

methionine. Folate is involved as a substrate (5-methyl-tetrahydrofolate) and vitamin B₁₂ as a coenzyme. The 5,10-methylenetetrahydrofolate delivers its methyl group to deoxyuridylate to convert it to thymidylate for incorporation into DNA. In either a folate or vitamin B₁₂ deficiency, the megaloblastic changes occurring in the bone marrow and other replicating cells result from lack of adequate 5,10-methylene-tetrahydrofolate.' (pg 199)

'Folate requirements increase substantially during pregnancy because of the marked acceleration in single-carbon transfer reactions, including those required for nucleotide synthesis and thus cell division. During pregnancy, cells multiply in association with uterine enlargement, placental development, expansion of maternal erythrocyte number, and fetal growth (Cunningham et al., 1989). (pg 233)

Reference 2.0:

'Folate functions metabolically as an enzyme cofactor in the synthesis of nucleic acids and amino acids. Deficiency of the vitamin leads to impaired cell replication and other metabolic alterations particularly related to methionine synthesis.' (pg 803)

'Single carbon units are removed from folate by a number of reactions. The ... single-carbon units from 10-formyl-THF are used for the biosynthesis of purines... one-carbon transfer from 5,10-methylene-THF to deoxyuridylate to form thymidylic acid, a precursor of DNA, is of crucial importance to the cell.' (pg 804)

'In summary, the biochemical function of folate coenzymes is the transfer and utilization of these one-carbon units in a variety of essential reactions including (1) *de novo* purine biosynthesis (formylation of glycinamide ribonucleotide (GAR) and 5-amino-4-imidazole carboxamide ribonucleotide (AICAR)); (2) pyrimidine nucleotide biosynthesis (methylation of deoxyuridylic acid to thymidylic acid); ...; and (4) generation and utilization of formate.' (pg 806)

'An important determinant of folate uptake into cells is their mitotic activity, as would be expected given the dependence of DNA biosynthesis on folate coenzyme function. Folate accumulation is more rapid in actively dividing cells than in quiescent cells, a factor probably related to the induction and activity of folylpoly- γ -glutamate synthase. This enzyme catalyses the addition of glutamate by γ -peptide linkage to the initial glutamate moiety of the folate molecule. Although polyglutamate derivatization may be considered a storage strategem, this elongation is the most efficient coenzyme form for normal one-carbon metabolism.' (pg 807)

Reference 3.11:

'Folates play an important role in the transfer of C₁-groups (i.e. methyl-, methylene- and formyl-groups), maintaining the methylation balance, such as in the biosynthesis of DNA bases and ...' (pg 2)

'In tissues folates are retained as polyglutamates and the folate coenzymes can be interconverted in numerous (de-)methylation reactions, such as in DNA synthesis (formation of thymidilate from deoxyuridine)...' (pg 3)

Reference 4.10:

‘Deficiency of folate results in a reduction in *de novo* DNA biosynthesis and, thus, impairment of cell replication, with the most obvious effects apparent in rapidly dividing cell-types, such as red blood cells and other cells generated by the bone marrow, enterocytes, and skin cells. This condition is recognised, haematologically, as a macrocytic anaemia, with characteristic red cell precursors (megaloblasts) present in bone marrow aspirates, and the clinical manifestation of megaloblastic anaemia.’ (pg 15)

‘Various THF-polyglutamates function metabolically as coenzymes and substrates in one-carbon metabolism. Transfer of a one-carbon unit from serine to THF *via* pyridoxal phosphate (PLP)-dependent serine hydroxymethyltransferase (SHMT), in the coupled serine to glycine conversion pathway, produces 5,10 methylene-THF. This reduced folate cofactor serves as the substrate to generate 5,10 methenyl-THF (5, 10 formyl-THF, anhydroleucovorin) (dehydrogenase reaction) and 10-formyl-THF (cyclohydrolase reaction), which are required for synthesis of the purine ring. 5,10 Methylene-THF also provides the methyl group for methylation of deoxyuridine monophosphate (dUMP, deoxyuridylic acid) for the *de novo* synthesis of deoxythymide monophosphate (dTMP, thymidylic acid) catalysed by thymidylate synthase. This reaction generates DHF, which is reduced to THF by the enzyme dihydrofolate reductase (DHFR). (pg 14)

‘Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine (SAM).’ (pg 15)

‘Deficiency of folate results in a reduction in *de novo* DNA biosynthesis and, thus, impairment of cell replication, with the most obvious effects apparent in rapidly dividing cell-types, such as red blood cells and other cells generated by the bone marrow, enterocytes, and skin cells...’ (pg 15)

‘Various THF-polyglutamates function metabolically as coenzymes and substrates in one-carbon metabolism. Transfer of a one-carbon unit from serine to THF *via* pyridoxal phosphate (PLP)-dependent serine hydroxymethyltransferase (SHMT), in the coupled serine to glycine conversion pathway, produces 5,10 methylene-THF. This reduced folate cofactor serves as the substrate to generate 5,10 methenyl-THF (5, 10 formyl-THF, anhydroleucovorin) (dehydrogenase reaction) and 10-formyl-THF (cyclohydrolase reaction), which are required for synthesis of the purine ring. 5,10 Methylene-THF also provides the methyl group for methylation of deoxyuridine monophosphate (dUMP, deoxyuridylic acid) for the *de novo* synthesis of deoxythymide monophosphate (dTMP, thymidylic acid) catalysed by thymidylate synthase. This reaction generates DHF, which is reduced to THF by the enzyme dihydrofolate reductase (DHFR). (pg 14)

Reference 6.2:

‘The one-carbon units that are attached to intracellular folate include formyl, methylene and methenyl methyl groups; they are derived from serine and also probably from formate. They are used in the biosynthesis of pyrimidines and purines, and thus for the synthesis of DNA in cell division...’ (pg 18)

2) Developing neural tube

Code	Proposed statement
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<i>Fo2:</i>	<i>Folate is necessary for the normal structure of the neural tube in developing embryos</i>
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Reference 1.3:

‘Folate requirements increase substantially during pregnancy because of the marked acceleration in single-carbon transfer reactions, including those required for nucleotide synthesis and thus cell division. During pregnancy, cells multiply in association with uterine enlargement, placental development, expansion of maternal erythrocyte number, and fetal growth (Cunningham et al., 1989).’ (pg 233)

‘...A defect in enzymes involved in homocysteine metabolism is suggested by altered folate, vitamin B₁₂, homocysteine, and methylmalonate values in mothers of infants with NTDs (Mills et al., 1995; Steegers-Theunissen et al., 1994); the prevention of some human NTDs by folate administration; and the prevention of NTDs in some rodent models by methionine (Essien, 1992; Vanaerts et al., 1994). These enzymes are 5,10-methylenetetrahydrofolate reductase (MTHFR), cystathionine β-synthase, and methionine synthase. Interestingly, families with homocystinuria caused by severe mutations in genes for each of these enzymes do not exhibit NTDs (Haworth et al., 1993; Kang et al., 1991b).’ (pg 244, 245)

‘The mechanism by which folate could reduce NTD risk is not known. Increasing folate intake and thus the concentrations of folate derivatives in tissues might overcome a metabolic deficiency in the production of proteins or in DNA synthesis at the time of neural tube closure (Mills et al., 1995). Another hypothesis is that folate does not prevent the occurrence of NTD but selectively increases the abortion rate of affected fetuses (Hook and Czeizel, 1997). Certainly, more research is needed to understand the effect of folate on embryonic and fetal development.’ (pg 258)

‘To summarize the data, a reduced risk of NTD has been observed for women who took a folate supplement of 360 to 800 μg/day in addition to a dietary folate intake of 200 to 300 μg/day. Folate intake is positively associated with erythrocyte folate concentration (Bower and Stanley, 1989; Brown et al., 1997; Cuskelly et al., 1996; Daly et al., 1997), and NTD risk is inversely associated with both folate intake (Bower and Stanley, 1989; Shaw et al., 1995c; Werler et al., 1993) and erythrocyte folate concentration (Daly et al., 1995).’ (pg 258, 259)

‘Although it is recognized that there are still uncertainties about the relationship among folate intake, erythrocyte folate, and NTD risk and the extent to which there are differences in the absorption of folate from food compared with supplements, the evidence is still judged sufficient to support a recommendation to reduce the risk of NTD.’ (pg 259)

Reference 2.0:

‘The debate on folate requirements of normal pregnancy has been overtaken by the finding that periconceptual consumption of folic acid has a significant protective effect against the occurrence and recurrence of neural tube defects (NTD). ...’ (pg 809)

‘... It appears that folic acid exerts its protective effect by overcoming a partial block in folate metabolism rather than by correcting a nutritional deficiency. A functional variant of the gene for 5,10-methylene-THF reductase, the ‘thermolabile variant’ associated with NTD, may express its aberrancy through an inability to bind its flavin cofactor properly, an inability shown to be corrected experimentally (in the bacterial enzyme at least) by increasing the folate concentration. It is likely that other variants of this gene and/or variants of other genes associated with folate metabolism are involved not only in NTD but also in vascular diseases related to hyper-homocysteinaemia.’ (pg 809)

Reference 3.11:

‘... an intake >400µg/day is considered protective against neural tube defect (NTD).’ (pg 3)

Reference 4.10:

‘Health experts recommend peri-conceptual folic acid supplementation in women for the prevention of neural tube defects in developing foetuses. The Department of Health recommends that all women planning a pregnancy take 400µg of folic acid from when they cease contraception to the twelfth week of pregnancy to reduce the risk of them having a NTD-affected pregnancy.’ (pg 5)

Reference 6.2:

‘Low folate status, even when serum and red blood cell folate levels are in the conventional normal range, is associated with an increased risk of neural tube defects.’ (pg 19)

3) Neurotransmitters

Code Proposed statement

Fo3: Folate is necessary for the normal structure of some neurotransmitters

Reference 1.3:

‘The folate coenzymes are involved in numerous reactions that involve ... amino acid interconversions, including the catabolism of histidine to glutamic acid, interconversion of serine and glycine, and conversion of homocysteine to methionine. Folate-mediated transfer of single-carbon units from serine provides a major source of substrate in single-carbon metabolism. The conversion of homocysteine to methionine serves as a major source of methionine for the synthesis of *S*-adenosylmethionine, an important in vivo methylating agent (Wagner, 1996).’ (pg 197)

‘The mechanism by which folate modifies brain functions has been sought for more than two decades and is generally hypothesized to be related to its role in single-carbon metabolism (Alpert and Fava, 1997). In particular, methylene tetrahydrofolate is the methyl donor in methionine synthesis from homocysteine and is postulated to be important in maintaining adequate methionine pools for *S*-adenosylmethionine (SAM) biosynthesis (Bottiglieri et al., 1994). SAM is the cofactor in key methylation reactions in catecholamine synthesis and metabolism in brain (Turner, 1977); catecholamines are transmitters known to be important in maintaining affective state,

and exogenous SAM has been shown by some to elevate mood (Bell et al., 1998). Folate has also been linked to the maintenance of adequate brain levels of tetrahydropterin (Hamon et al., 1986), a key cofactor in the hydroxylation reactions leading the synthesis of transmitters such as serotonin and the catecholamines (Turner, 1977). Methylation reactions involving folate may be important in maintaining neuronal and glial membrane lipids (Hirata and Axelrod, 1980), which could have effects on more general brain functions as reflected in changes in mood, irritability, and sleep.’ (pg 268, 269)

‘Although available information may suggest that a link exists between folate deficiency and abnormal mental function, more than three decades of research have not produced a definitive connection.’ (pg 269)

Reference 2.0:

‘Folate functions metabolically as an enzyme cofactor in the synthesis of nucleic acids and amino acids. Deficiency of the vitamin leads to impaired cell replication and other metabolic alterations particularly related to methionine synthesis.’ (pg 803)

‘In summary, the biochemical function of folate coenzymes is the transfer and utilization of these one-carbon units in a variety of essential reactions including ... (3) amino acid interconversions – the interconversion of serine to glycine, catabolism of histidine to glutamic acid, and conversion of homocysteine to methionine (which also requires vitamin B₁₂); ...’ (pg 806)

Reference 3.11:

‘Folates play an important role in the transfer of C₁-groups (i.e. methyl-, methylene- and formyl-groups), maintaining the methylation balance, such as in ... amino acid metabolism.’ (pg 2)

‘In tissues folates are retained as polyglutamates and the folate coenzymes can be interconverted in numerous (de-)methylation reactions, such as in ... amino acid interconversions, such as the remethylation of homocysteine to methionine. In this latter methionine synthase (MS) reaction vitamin B₁₂ is also involved as a cofactor.’ (pg 3)

Reference 4.10:

‘Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism ... and the formation of the primary methylating agent, S-adenosylmethionine (SAM).’ (pg 15)

‘Chronic, severe folate deficiency has also, rarely, been associated with neurological changes and depression (*cited in* Weir & Scott 1999).’ (pg 15)

‘The other pathway that requires 5,10 methylene-THF is the biosynthesis of 5-methyl-THF, catalysed by the enzyme methylene THF reductase (MTHFR), using NADPH as a cofactor. This pathway is:- 1] an absolute requirement for the *de novo* synthesis of 5-methyl-THF, the predominant form of intracellular folate, and 2] irreversible under normal physiological conditions. The N-5 methyl group of 5-methyl-THF can be used metabolically only for transfer to homocysteine, resulting in the regeneration of methionine. In addition to its role as an amino acid, methionine serves as a methyl

group donor *via* conversion to S-adenosyl methionine (SAM), an important biological methylating agent involved in many methyltransferase reactions. The conversion of homocysteine to methionine, *via* methyl transfer from 5-methyl-THF, is catalysed by the enzyme methionine synthase (homocysteine-methyl-transferase) and requires methyl-Cobalamin (vitamin B₁₂) as a cofactor.' (pg 14)

Reference 6.2:

'...As well as being essential for the action of the enzyme thymidylate synthase, and thus having a crucial role in DNA synthesis, 5,10-methylene-THF also indirectly supplies methyl groups for the "methylation cycle". 5-Methyl-THF is formed by reduction of 5,10-methylene-THF under the action of the enzyme 5,10-methylene-THF reductase. The methyl group is transferred from 5-methyl-THF to methionine, catalysed by the vitamin B₁₂ –dependent enzyme methionine synthase. S-adenosylmethionine (SAM) is synthesised from methionine, and acts as a methyl donor in the methylation of a range of diverse compounds with different functions. The methyltransferase reactions give rise to S-adenosylhomocysteine (SAH), which is immediately metabolised to homocysteine.' (pg 18)

'The folate level in cerebrospinal fluid is three times higher than that in plasma, and nerve tissue concentrates folate at the expense of other organs. This may account for the apparent protection of neural tissue from folate deficiency...' (pg 19)

'The concentration of folate in the form of methylfolate in the cerebrospinal fluid is approximately three times higher than that in the serum. Through the homocysteine/methionine pathway and ultimately through S-adenosylmethionine, methylfolate provides the methyl group in innumerable methylation reactions in the nervous system, involving, for example, nucleoproteins, proteins, phospholipids, monoamines and neurotransmitters. Methylfolate may also influence monoamine metabolism and mood through the biopterin pathway.' (pg 51)

4) Blood formation

Code Proposed statement

Fo4: Folate is necessary for normal blood formation.

Reference 1.3:

'Both folate and vitamin B₁₂ are required for the formation of 5,10-methylenetetrahydrofolate and involved in thymidylate synthesis by way of a vitamin B₁₂-containing enzyme. The formation of 5,10-methylene tetrahydrofolate depends on the regeneration of the parent compound (tetrahydrofolate) in the homocysteine-to-methionine conversion. This reaction involves the removal of a methyl group from methyl folate and the delivery of this group to homocystine for the synthesis of methionine. Folate is involved as a substrate (5-methyl-tetrahydrofolate) and vitamin B₁₂ as a coenzyme. The 5,10-methylenetetrahydrofolate delivers its methyl group to deoxyuridylate to convert it to thymidylate for incorporation into DNA. In either a folate or vitamin B₁₂ deficiency, the megaloblastic changes occurring in the bone marrow and other replicating cells result from lack of adequate 5,10-methylene-tetrahydrofolate.' (pg 199)

‘Within weeks of the development of early morphological abnormalities in the marrow, subtle changes appear in the peripheral blood (Eichner et al., 1971) when hypersegmentation of the neutrophils becomes apparent.’ (pg 200)

‘... When folate supply to the bone marrow becomes rate limiting for erythropoiesis, macrocytic cells are produced. ...’ (pg 200)

‘As folate depletion progresses further, the mean cell volume increases above normal. Neutrophil hypersegmentation (defined as more than 5 percent five-lobed or any six-lobed cells per 100 granulocytes) is typically present in the peripheral blood at this stage of macrocytosis and the neutrophil lobe average is elevated.’ (pg 200)

‘Macrocytic anemia then develops, as first evidenced by a depression of the erythrocyte count. Eventually, all three measures of anemia (hematocrit, hemoglobin concentration, and erythrocyte concentration) are depressed. At this point, macroovalocytes and macrocytes are usually detectable in the peripheral blood, and hypersegmentation is more impressive (Lindenbaum et al., 1988).’(pg 200)

‘Because folate is taken up only by the developing erythrocyte in the bone marrow and not by the circulating mature erythrocyte during its 120-day lifespan, erythrocyte folate concentration is an indicator of long-term status.’ (pg 201)

Reference 2.0:

‘Folate deficiency alone, manifested clinically as megaloblastic anaemia, is the most common vitamin deficiency in developed countries.’ (pg 803)

Reference 4.10:

‘Deficiency of folate results in a reduction in *de novo* DNA biosynthesis and, thus, impairment of cell replication, with the most obvious effects apparent in rapidly dividing cell-types, such as red blood cells and other cells generated by the bone marrow, enterocytes, and skin cells. This condition is recognised, haematologically, as a macrocytic anaemia, with characteristic red cell precursors (megaloblasts) present in bone marrow aspirates, and the clinical manifestation of megaloblastic anaemia.’ (pg 15)

Reference 6.2:

‘... Deficiency of this vitamin particularly affects rapidly dividing tissues such as bone marrow and mucous membranes, leading to symptoms of anaemia and sore tongue. The diagnosis is based on macrocytic red blood cells and hypersegmented neutrophils in the peripheral blood, on typical morphological changes of megaloblastosis in bone marrow, on low concentrations of folate in serum and red blood cells, and on the exclusion of vitamin B₁₂ deficiency.’ (pg 53)

5) Homocysteine metabolism

Code **Proposed statement**

Fo5: *Folate contributes to the maintenance of normal blood homocysteine levels*

Reference 1.3:

‘The conversion of homocysteine to methionine serves as a major source of methionine for the synthesis of S-adenosyl-methionine, an important in vivo methylating agent (Wagner, 1996).’ (pg 197)

‘The formation of 5,10-methylene tetrahydrofolate depends on the regeneration of the parent compound (tetrahydrofolate) in the homocysteine-to-methionine conversion. This reaction involves the removal of a methyl group from methyl folate and the delivery of this group to homocysteine for the synthesis of methionine.’ (pg 199)

‘Inadequate folate intake first leads to a decrease in serum folate concentration, then to a ... rise in homocysteine concentration, ...’ (pg 199)

‘Plasma homocysteine concentration increase when inadequate quantities of folate are available to donate the methyl group that is required to convert homocysteine to methionine. Controlled metabolic and epidemiological studies provide evidence that plasma homocysteine rises with reductions in blood folate indices. ... Many investigators have reported that plasma homocysteine is significantly elevated in individuals who have been diagnosed as folate deficient on the basis of established serum folate, plasma folate, or erythrocyte folate norms (Allen et al., 1993; Chadeaux et al., 1994; Curtis et al., 1994; Kang et al., 1987; Savage et al., 1994; Stabler et al., 1988; Ubbink et al., 1993).’ (pg 201/202)

‘Thus, in studies of different types, a similar inverse relationship between folate intake and plasma homocysteine values is seen for pre- and postmenopausal women, adult men, and the elderly.’ (pg 202)

‘Folate is required in the form of methyltetrahydrofolate as a substrate for methionine synthase. Therefore, the remethylation of homocysteine depends on adequate quantities of folate.’ (pg 261)

‘The inverse relationship between folate intake and homocysteine concentration is well established. However, there are conflicting data on the association among indicators of folate status or metabolism, homocysteine concentration, and risk of vascular disease. Whether increasing intake of folate could reduce the risk of vascular disease remains to be demonstrated. Folate may reduce the risk of cardiovascular disease through other mechanisms.’ (pg 263)

Reference 2.0:

‘A solitary transfer of one-carbon units takes place at the methanol level of oxidation. It involves the transfer of the methyl group from 5-methyl-THF to homocysteine to form methionine and THF. This reaction is catalysed by the enzyme methionine synthase and requires vitamin B₁₂ as cofactor.’ (pg 804)

‘An important consequence of folate deficiency is the inability to remethylate homocysteine. Indeed, there is an inverse correlation between the levels of folate and homocysteine in the blood of humans. Many clinical studies, beginning with the observations of the children with homocysteinuria presenting with vascular abnormalities and thromboembolism, have demonstrated an association between

hyperhomocysteinaemia and increased risk of premature atherosclerosis in the coronary, carotid and peripheral vasculature. The prevalence of hyperhomocysteinaemia compared with normal controls. Even mild hyperhomocysteinaemia is recognized to be an independent risk factor for cardiovascular disease. Metabolically, homocysteine may be disposed of by the methionine synthase reaction (dependent on folate as well as vitamin B₁₂), the transsulfuration pathway (dependent on vitamin B₆) and by the choline degradation pathway. Marginal deficiencies of these three vitamins are associated with hyperhomocysteinaemia. Of the three, however, folic acid administration has been shown to be the most effective in lowering homocysteine, blood levels. Convincing evidence of the potential role of folate intake in the prevention of vascular disease, probably by lowering blood levels of homocysteine, has been demonstrated by a significant inverse relationship between serum folate levels and fatal coronary heart disease.’ (pg 806)

Reference 3.11:

‘...amino acid interconversions, such as the remethylation of homocysteine to methionine.’ (pg 3)

Reference 4.10:

‘The N-5 methyl group of 5-methyl-THF can be used metabolically only for transfer to homocysteine, resulting in the regeneration of methionine. In addition to its role as an amino acid, methionine serves as a methyl group donor *via* conversion to S-adenosyl methionine (SAM), an important biological methylating agent involved in many methyltransferase reactions. The conversion of homocysteine to methionine, *via* methyl transfer from 5-methyl-THF, is catalysed by the enzyme methionine synthase (homocysteine-methyl-transferase) and requires methyl-Cobalamin (vitamin B₁₂) as a cofactor.’ (pg 14)

Reference 6.2:

‘The 5-methyl-THF needed for methionine synthase is provided by 5,10-methylene-THF reductase. The activities of this enzyme and those of methionine synthase and cystathionine synthase keep the levels of homocysteine in cells and in plasma normally within a narrow range. These three enzymes depend, respectively, on folate and vitamin B₁₂ and vitamin B₆. Thus reduced status of any of these nutrients can cause an elevation in plasma homocysteine. In practice, high plasma homocysteine levels are most likely to be related to low folate status, rather than low status of vitamins B₆ or B₁₂.’ (pg 18)

ANNEX 4.11

Vitamin B₁₂

Source documents for reviewing vitamin B₁₂

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.12:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B₁₂. October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80d_en.pdf).

Reference 4.11:

Revised review of Vitamin B₁₂. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/EVM/00/20/P>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

1) Cell division and blood formation

Code	Proposed statement
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VB ₁₂ 1a:	<i>Vitamin B12 is necessary for normal cell division (such as in the gastrointestinal tract).</i>
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VB ₁₂ 1b:	<i>Vitamin B12 contributes to normal blood formation.</i>
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Reference 1.3:

‘B₁₂ is a cofactor for two enzymes: methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase requires methylcobalamin as a cofactor for the methyl transfer from methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate...An adequate supply of B₁₂ is essential for normal blood formation and...’ (pg 307)

‘As in folate deficiency, the underlying mechanism of anemia is an interference with normal deoxyribonucleic acid (DNA) synthesis. This results in megaloblastic change, which causes production of larger-than-normal erythrocytes (macrocytosis). This leads first to an increase in the erythrocyte distribution width index and ultimately to an elevated mean cell volume. Oval macrocytes and other abnormally shaped erythrocytes are present in the blood... By the time anemia has become established, there is usually also some degree of neutropenia and thrombocytopenia because the

megaloblastic process affects all rapidly dividing marrow elements. The hematological complications are completely reversed by treatment with B₁₂.’ (pg 311)

Reference 2.0:

‘Methionine synthase or N^5 – methyl tetrahydrofolate: homocysteine methyltransferase, which uses methyl-Cbl as a cofactor, forms an integral link between two essential metabolic processes of internal metabolism: the synthesis of the nucleic acids (RNA and DNA) via purines and pyrimidines, and the methylation reactions via *S*-adenosylmethionine.’ (pg 397)

‘Serine, which is synthesized from glucose passes its beta carbon moiety to tetrahydrofolate (THF) to produce N^5, N^{10} –methylene-THF and glycine in the cytoplasm of the cell. The product N^5, N^{10} –methylene-THF then stands at a metabolic crossroads. On the one hand, it can either (1) in conjunction with deoxyuridine monophosphate synthesize thymidylate which in turn produces a pyrimidine base of DNA, or (2) produce N^{10} –formyl-THF which inserts carbons 2 and 3 into the purine ring. On the other hand, it can be reduced to N^5 – methyl-THF which is required for the remethylation of homocysteine to methionine via methionine synthase, which is subsequently converted to *S*-adenosylmethionine (Ado-Met) via *S*-adenosylmethionine synthetase. Ado-Met, the universal methylator is essential for 35 methylation reactions in internal metabolism which have important synthetic and regulatory functions...Another essential function of methionine synthase is to act as a gatekeeper for the entry of folate into the cell.’ (pg 397)

‘Ado-Met (*S*-adenosylmethionine) function is also controlled by the level of its product, adenosylhomocysteine (Ado-Hcy), which is its main inhibitor. Thus, the ratio of the level of Ado-Met to that of Ado-Hcy has often been described as the ‘methylation ratio’. When homocysteine levels rise, the back reaction of *S*-adenosylhomocysteine hydrolase is favoured and Ado-Hcy levels increase. This leads to inhibition of the methylation reactions and their regulation of internal metabolism.’ (pg 397, 398)

‘Inhibition of methionine synthase as a result of methyl-Cbl deficiency leads to reduced synthesis of THF and methionine, and to the accumulation of homocysteine and N^5 – methyl-THF. This leads to reduced availability of N^5, N^{10} –methylene-THF for thymidylate synthesis. Also ... deficiency of methionine and Ado-Met leads to enhance conversion of N^5, N^{10} –methylene-THF to N^5 – methyl-THF which under physiological conditions is irreversible, thus further depleting the supply of N^5, N^{10} –methylene-THF and thymidylate for nucleic acid synthesis. This forms the basis of what has been termed the ‘methyl-folate trap’ hypothesis. The resulting nucleic acid deficiency induces a megaloblastic anaemia in the bone marrow which is identical to that induced by folate deficiency.’ (pg 398, 399)

Reference 3.12:

‘The key symptom in vitamin B₁₂ deficiency is macrocytic megaloblastic anemia. These haematological abnormalities are indistinguishable from those seen in folate deficiency, because of the interrelated function of both vitamins (Herbert, 1986).’ (pg 2)

Reference 4.11:

‘In the form of methyl Cbl, vitamin B₁₂ participates as a cofactor to the enzyme methionine synthase in the methylation of homocysteine (Hcy) which involves transfer of the methyl group from N⁵-methyltetrahydrofolate (N⁵-methyl-THF-glu₁₋₅). As such, vitamin B₁₂ plays a pivotal role in one-carbon (methyl donor) metabolism, vital to many aspects of cellular metabolism, including the synthesis of the building block precursors to DNA and RNA.’ (pg 12)

‘The methionine formed may be converted to S-adenosylmethionine (SAM). SAM acts as the universal methyl donor in more than 100 methylation reactions within the cell, all of which are essential for internal metabolism. In particular, SAM is the major direct donor of methyl groups in the synthesis of polyamines (e.g. spermidine and putrescine [*important in cell and tissue growth*]).’ (pg 13)

‘The N⁵-THF-glu₁ formed in the methionine synthase reaction is converted to the polyglutamated form N⁵-THF-glu₅ by folyl- γ -glutamate synthetase which is the central folate acceptor molecule in the folate one-carbon cycle. In turn, N⁵-THF-glu₅ receives the β -carbon moiety from serine, via serine hydroxymethyltransferase, to give glycine and N⁵,N¹⁰ – methylene-THF-glu₅. N⁵,N¹⁰ – methylene-THF-glu₅ either acts as a methyl donor in the conversion of deoxyuridylate monophosphate to thymidylate monophosphate (the precursor to the pyrimidine base thymidine) in a reaction catalysed by thymidine synthetase, is converted to N¹⁰ formyl-THF-glu₅, which provides carbons 2 and 8 in the synthesis of the purine bases, or is reduced to methyl-THF-glu₅, which can serve to re-methylate homocysteine to methionine.’ (pg 13)

2) Neurological system

Code	Proposed statement
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VB ₁₂ 2:	<i>Vitamin B12 is necessary for the normal structure and function of the neurological system.</i>
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Reference 1.3:

‘An adequate supply of B₁₂ is essential for normal ...and neurological function.’ (pg 307)

‘Neurological complications are present in 75 to 90 per cent of individuals with clinically observable B₁₂ deficiency and may, in about 35 per cent of cases, be the only clinical manifestation of B₁₂ deficiency.’ (pg 311)

‘Neurological manifestations include sensory disturbances in the extremities (tingling and numbness), which are worse in the lower limbs. Vibratory and position sense are particularly affected. Motor disturbances, including abnormalities of gait, also occur. Cognitive changes may occur, ranging from loss of concentration to memory loss, disorientation, and frank dementia, with or without mood changes. In addition, visual disturbances, insomnia, impotency, and impaired bowel and bladder control may develop.’ (pg 312)

'In some cases, neurological manifestations may be the earliest clinical sign of low B₁₂ values (Beck, 1991; Karnaze and Carmel, 1990; Lindenbaum et al., 1998; Martin et al., 1992).' (pg 328)

Reference 2.0:

'The other effect of methionine synthase inhibition by Cbl deficiency is both to reduce the endogenous supply of methionine and Ado-Met and to increase the levels of homocysteine and Ado-Hcy. This causes a reduction of the Ado-Met/Ado-Hcy ratio (the methylation ratio), which as explained above will inhibit the methylation reactions which in turn produces the neuropathy.' (pg 399)

Reference 3.12:

'Another key symptom of vitamin B₁₂ deficiency are neurological complications, such as paraesthesia, leg weakness, memory loss, etc, due to progressive lesions in the lateral and posterior columns of the spinal cord (subacute combined degeneration of the spinal cord). Neurological symptoms occur in about 75-90% of all individuals with (untreated) vitamin B₁₂ deficiency, and appear generally at a later stage.' (pg 2)

Reference 4.11:

'The methionine formed may be converted to S-adenosylmethionine (SAM). SAM acts as the universal methyl donor in more than 100 methylation reactions within the cell, all of which are essential for internal metabolism. In particular, SAM is the major direct donor of methyl groups in the synthesis of polyamines (e.g. spermidine and putrescine [*important in cell and tissue growth*]).' (pg 13)

3) Energy production

Code Proposed statement

VB₁₂3: *Vitamin B₁₂ contributes to normal energy production*

Reference 1.3:

'B₁₂ is a cofactor for two enzymes: methionine synthase and L-methylmalonyl-CoA mutase. ... L-Methylmalonyl-CoA mutase requires adenosylcobalamin to convert L-methylmalonyl-CoA to succinyl-CoA in an isomerization reaction...' (pg 307)

Reference 2.0:

'Methylmalonyl-CoA mutase requires adenosyl-Cbl. Methylmalonyl semialdehyde is produced by a series of compounds which include amino acids (valine, isoleucine, methionine and threonine), along with cholesterol, thymine and odd-chain fatty acids. Methylmalonyl semialdehyde is then metabolized via propionyl-CoA to methylmalonyl-CoA. Methylmalonyl-CoA is normally converted to succinyl-CoA and propionic acid via *SR*-methylmalonyl-CoA racemase, and subsequently the adenosylcobalamin-dependent *R*-methylmalonyl-CoA mutase. However, when Cbl deficiency is present, (1) the mutase function is impaired and *S*-methylmalonyl-CoA is converted to methylmalonic acid (MMA) via *S*-methylmalonyl-CoA hydrolase, a vitamin-independent enzyme; and (2) propionyl-CoA levels will be elevated, and in association with oxaloacetic acid and citrate synthase will produce 2-methylcitric acids 1 and 2. Consequently, in Cbl deficiency states concentrations of

methylmalonyl-CoA, its hydrolytic product MMA, and 2-methylcitric acids 1 and 2 are raised.’ (pg 397)

Reference 3.12:

Vitamin B₁₂ plays a specific role in amino acid metabolism, i.e. in methylation reactions, together with folate, in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA into succinyl CoA (for review see Herbert, 1984; Ellenbogen & Cooper, 1991). (pg 3)

Reference 4.11:

‘Methionine synthase also acts as gatekeeper for the entry of folate into the cell. Folate enters in the form of N⁵-methyl-THF-glu₁ and can only remain inside the cell following demethylation via methionine synthase. Consequently, the uptake of folate into the cell is also dependent on the methyl Cbl form of vitamin B₁₂.’ (pg 13)

‘As deoxyadenosylCbl (adoCbl), vitamin B₁₂ has the role of obligate cofactor for the enzymatic conversion of L-methylmalonyl CoA to succinyl CoA by methylmalonyl CoA mutase.’ (pg 13)

4) Homocysteine metabolism

Code Proposed statement

VB₁₂4: Vitamin B₁₂ contributes to the maintenance of normal blood homocysteine levels

Reference 1.3:

‘Vitamin B₁₂ (cobalamin) functions as a coenzyme for a critical methyl transfer reaction that converts homocysteine to methionine.’ (pg 306)

‘B₁₂ is a cofactor for two enzymes: methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase requires methylcobalamin as a cofactor for the methyl transfer from methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate...’ (pg 307)

‘Serum total homocysteine concentration is commonly elevated in elderly persons whose folate status is normal but who have a clinical response to treatment with B₁₂ (Stabler et al., 1996). Because a lack of folate, vitamin B₆, or both also results in an elevated serum and plasma homocysteine concentration, this indicator has poor specificity...’ (pg 314)

‘Lindenbaum and colleagues (1990) reported that metabolites that arise from B₁₂ insufficiency are more sensitive indicators of B₁₂ deficiency than is the serum B₁₂ value. This was found in patients with pernicious anemia or previous gastrectomy who experienced early haematological relapse: serum methylmalonic acid (MMA), total homocysteine, or both were elevated in 95 percent of the instances of relapse whereas the serum B₁₂ value was low (less than 150 pmol/L [200pg/mL] in 69 percent....At present, the techniques developed to measure serum MMA and homocysteine (capillary gas chromatography and mass spectrometry) are costly and may be beyond the scope of routine laboratories.’ (pg 316)

Reference 2.0:

‘Serine, which is synthesized from glucose passes its beta carbon moiety to tetrahydrofolate (THF) to produce N^5,N^{10} -methylene-THF and glycine in the cytoplasm of the cell. The product N^5,N^{10} -methylene-THF then stands at a metabolic crossroads. ... it can be reduced to N^5 -methyl-THF which is required for the remethylation of homocysteine to methionine via methionine synthase, which is subsequently converted to *S*-adenosylmethionine (Ado-Met) via *S*-adenosylmethionine synthetase.’ (pg 397)

‘The availability of methyl-Cbl and the substrates of the reaction controlled by methionine synthase – namely homocysteine and N^5 -methyl-THF – control its function. The availability of these substrates is in turn tightly controlled by the availability of dietary methionine and its products Ado-Met.’ (pg 397)

‘Plasma homocysteine is derived from intracellular homocysteine which occurs as a product of dietary methionine metabolism. The level is maintained by four enzymes, *S*-adenosylhomocysteine hydrolase (which increases it), and cystathionine synthase, methylene reductase and methionine synthase (which act to reduce it). The latter three enzymes have as cofactors the vitamins pyridoxine, folate and cobalamin respectively.’ (pg 399)

Reference 3.12:

Vitamin B₁₂ plays a specific role in amino acid metabolism, i.e. in methylation reactions, together with folate, in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA into succinyl CoA (for review see Herbert, 1984; Ellenbogen & Cooper, 1991). (pg 3)

Reference 4.11:

‘In the form of methyl Cbl, vitamin B₁₂ participates as a cofactor to the enzyme methionine synthase in the methylation of homocysteine (Hcy) which involves transfer of the methyl group from N^5 -methyltetrahydrofolate (N^5 -methyl-THF-glu₁₋₅).’ (pg 12)

‘ N^5,N^{10} -methylene-THF-glu₅ either ...or is reduced to methyl-THF-glu₅, which can serve to re-methylate homocysteine to methionine.’ (pg 13)

Reference 5.0:

‘Higher blood levels of vitamins B₆, B₁₂, and folic acid are associated with low levels of homocysteine, and supplementing with these vitamins helps to lower homocysteine levels.’ (pg 1339)

ANNEX 4.12

Biotin

Source documents for reviewing biotin

Reference 1.3:

Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. Washington D.C. National Academy Press 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.13:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Biotin. September 2001. (http://www.europa.eu.int/comm/food/fs/sc/scf/out106_en.pdf).

Reference 4.12:

Revised Review of Biotin. Expert Group on Vitamins and Minerals. April 2002. (<http://www.food.gov.uk/multimedia/pdfs/biotin.pdf>).

1) Energy metabolism

Code	Proposed statement
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<i>Bi1:</i>	<i>Biotin contributes to normal fat metabolism and energy production</i>
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Reference 1.3:

‘The second biotin-dependent carboxylase, pyruvate carboxylase, catalyzes the carboxylation of pyruvate to form oxaloacetate, which serves as an intermediate in the tricarboxylic acid cycle. Oxaloacetate thus formed is converted to glucose in the liver, kidney, and other gluconeogenic tissues. A third biotin-dependent carboxylase, β -methylcrotonyl-CoA carboxylase, is required for the degradation of leucine, a branch-chained amino acid...A fourth biotin-dependent carboxylase, propionyl-CoA carboxylase, carboxylates propionyl-CoA to form D-methylmalonyl-CoA, which is racemized to the L-isomer, then undergoes isomerization to succinyl-CoA, and subsequently enters the tricarboxylic acid cycle.’ (pg 375)

Reference 2.0:

‘Pyruvate carboxylase (EC 6.4.1.1) catalyses the incorporation of bicarbonate into pyruvate to form oxaloacetate, an intermediate in the Krebs tricarboxylic acid cycle. Thus, pyruvate carboxylase (PC) catalyses an anaplerotic reaction. In gluconeogenic tissues (i.e. liver and kidney), the oxaloacetate can be converted to glucose. Deficiency of PC is probably the cause of the lactic acidaemia, central nervous system lactic acidosis and abnormalities in glucose regulation observed in biotin deficiency and biotinidase deficiency.’ (pg 173)

'Methylcrotonyl-CoA carboxylase (EC 6.4.1.4) catalyses an essential step in the degradation of the branched chain amino acid leucine... (pg 173)

Propionyl-CoA carboxylase (EC 6.4.1.3) catalyses the incorporation of bicarbonate into propionyl-CoA to form methylmalonyl-CoA, which undergoes isomerization to succinyl-CoA and enters the tricarboxylic acid cycle.' (pg 173)

Reference 3.13:

'In main biotin is an essential co-factor for four carboxylases which catalyse the incorporation of bicarbonate into a substrate and are involved in gluconeogenesis and provision of intermediates into the citric acid cycle (pyruvate carboxylase, PC, EC 6.4.1.1)... leucine catabolism (3-methylcrotonyl-CoA carboxylase, MCC EC 6.4.1.4) and propionate catabolism (propionyl-CoA carboxylase, PCC, EC 6.4.1.3). The propionate to be carboxylated has various sources: catabolism of valine, isoleucine, threonine, methionine, the side chain of cholesterol, odd-numbered saturated fatty acids, and metabolism of intestinal bacteria.' (pg 5)

Reference 4.12:

'Biotin acts as an essential cofactor for the... propionyl-CoA, β -methylcrotonyl-CoA, and pyruvate carboxylase (... PCC, MCC and PC) enzymes. These ... enzymes catalyse critical steps in pathways of intermediary metabolism... PC catalyses the incorporation of bicarbonate into pyruvate to form oxaloacetic acid (OAA), a Krebs' tricarboxylic acid cycle intermediate. In gluconeogenic tissues, such as liver and kidney OAA can be converted into glucose. MCC catalyses a critical step in the degradation of the branch-chain amino acid, leucine. PCC catalyses the carboxylation of propionyl-CoA to form D-methylmalonyl-CoA. D-methylmalonyl-CoA is racemised to the L-isomer and subsequently undergoes isomerisation to form the tricarboxylic acid intermediate succinyl-CoA.' (pg 13, 14)

2) Fatty acids

Code Proposed statement

Bi2: Biotin is necessary for the synthesis of fatty acids, which are important for the normal structure of cell membranes

Reference 1.3:

'Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl CoA to form malonyl CoA. Malonyl CoA then serves as a substrate for fatty acid elongation.' (pg 375)

Reference 2.0:

'Acetyl-CoA carboxylase (EC 6.4.1.2) catalyses the incorporation of bicarbonate into acetyl-Co-A to form malonyl-CoA. This three-carbon compound then serves as a substrate for the fatty acid synthetase complex; the net result is the elongation of the fatty acid substrate by two carbons and the loss of the third carbon as CO₂.' (pg 173)

'Odd-chain fatty acid accumulation is also a marker of biotin deficiency. The accumulation of odd-chain fatty acid is thought to result from PCC deficiency.' (pg 174)

Reference 3.13:

'In main biotin is an essential co-factor for four carboxylases which catalyse the incorporation of bicarbonate into a substrate and are involved in ...fatty acid synthesis (acetyl-CoA carboxylase, ACC, EC 6.4.1.2)...' (pg 5)

Reference 4.12:

'Biotin acts as an essential cofactor for ... acetyl-CoA...ACC catalyses the incorporation of bicarbonate into acetyl-CoA to form malonyl-CoA. Malonyl-CoA, in turn, serves as the substrate for the fatty acid synthetase complex, donating two of its carbons to the fatty acid elongation process with the loss of the third as carbon dioxide.' (pg 14)

3) Cell proliferation and growth

Code Proposed statement

Bi3a: *Biotin is necessary for normal cell proliferation*

Bi3b: *Biotin is contributes to normal growth in the developing embryo and infant*

Reference 1.3:

'In infants on biotin-free TPN, symptoms of biotin deficiency begin to appear within 3 to 6 months after initiation of the TPN regimen, which is earlier than that seen in adults, probably because of the increased biotin requirement related to growth (Mock, 1996).' (pg 377)

Reference 2.0:

'In the normal turnover of cellular proteins, holocarboxylases are degraded to biocytin or biotin linked to an oligopeptide containing at most a few amino acid residues. Because the amide bond between biotin and lysine is not hydrolysed by cellular proteases, the specific hydrolase biotinidase (biotin-amide hydrolase, EC 3.5.1.12) is required to release biotin for recycling. The biotinidase gene is a single copy gene of 1629 bases encoding a 543 amino acid protein. Biotinidase mRNA is present in many tissues including heart, brain, liver, lung, skeletal muscle, kidney, pancreas and placenta. The greatest biotinidase activities are found in serum, liver, kidney and the adrenal gland. The observation that serum concentrations of biotinidase are decreased in patients with impaired liver function suggest that if the liver is the source of serum biotinidase.' (pg 173)

Reference 3.13:

'Biotinidase is able to recycle biotin bound to carboxylases and to cleave biotin bound to dietary proteins. Apart from this important function of biotinidase in providing biotin for intermediary metabolism, a function of this enzyme, is the transfer of biotin to nucleophilic acceptor proteins such as histones, thereby affecting gene expression (Hymes and Wolf, 1996) and e.g. embryological development (Bender, 1999; Zempleni and Mock, 2000b). Biotin is essential for cell proliferation. Its proliferative effect in immune cells can become of clinical relevance in biotin deficiency (Zempleni and Mock, 2001).' (pg 5, 6)

Reference 4.12:

'In addition to its role in the hydrolysis of biotin...biotinidase has been shown in vitro to catalyse the biotinylation of histone proteins...The specific transfer of biotin to histones may explain the presence of the vitamin inside the nucleus and suggests a role in the regulation of protein transcription.' (pg 14)

ANNEX 4.13

Vitamin C

Source documents for reviewing vitamin C

Reference 1.2:

Institute of Medicine Dietary Reference Intakes for Vitamin C, Vitamin E, selenium and carotenoids. Washington D.C. National Academy Press, 2000.

Reference 2.0:

'Encyclopedia of Human Nutrition 2E'.

Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego: Academic Press, c1999.

Reference 3.23:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Reference 4.13:

Revised review of Vitamin C. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/vitaminc.pdf>).

1) Connective tissue

Code	Proposed statement
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<i>VC1:</i>	<i>Vitamin C is necessary for the normal structure and function of connective tissue (such as that required for normal gums, skin, healing processes, bone and cartilage).</i>
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Reference 1.2:

'It is a co-factor for enzymes involved in the biosynthesis of collagen...' (pg 95)

'Vitamin C is known to be an electron donor for eight human enzymes. Three participate in collagen hydroxylation ...' (pg 96)

'Evidence also suggests that ascorbate plays a role in or influences collagen gene expression, cellular procollagen secretion, and the biosynthesis of other connective tissue components besides collagen, including elastin, fibronectin, proteoglycans, bone matrix, and elastin-associated fibrillin.' (pg 98).

'Lack of ascorbate-related hydroxyproline and hydroxylysine formation needed for collagen cross-linking may explain many of the connective tissue and hemorrhagic manifestations of scurvy, however, the specific histologic defects have not been identified.' (pg 101)

‘Ascorbic acid is required along with iron as a cofactor for the post-translation hydroxylation of proline and lysine to effect cross-linking of mature collagen... despite the important role of the vitamin in collagen formation, no collagen-related measures are available to use as a functional indicator for the dietary vitamin C requirement.’ (pg 118-119)

Reference 2.0:

‘Proline and lysine hydroxylases are required for the postsynthetic modification of collagen ...’ (pg 146)

‘The best studied of this class of enzymes is procollagen proline hydroxylase; it is assumed that the others follow essentially the same mechanism. The first step in the reaction is an attack on the substrate by oxygen, followed by condensation with 2-oxoglutarate, the release of the hydroxylated substrate and decarboxylation to release succinate. There is oxidation of ascorbate during the reaction, but not stoichiometrically, with the decarboxylation of 2-oxoglutarate and hydroxylation of the substrate. The purified enzyme is active in the absence of ascorbate, but after some 5-10 seconds (about 15-30 cycles of enzyme action) the rate of reaction begins to fall. At this stage the iron in the catalytic site has been oxidized to Fe³⁺, which is catalytically inactive. Activity is only restored by ascorbate, which reduces the iron back to Fe²⁺. This oxidation of the catalytic iron in the enzyme is the consequence of a side reaction rather than the main reaction of the enzyme. Nevertheless, ascorbate is essential for the activity of these enzymes *in vivo*. (pg 146-147)

Reference 4.13:

‘The functions of vitamin C include synthesis of collagen...’ (pg 6)

‘Clinical signs of scurvy are due to inhibition of collagen synthesis, this leads to failure to maintain the cellular structure of supporting tissues of mesenchymal origin, such as bone, dentine, cartilage and connective tissues.’ (pg 7)

2) & 3) Wound healing & scar tissue formation

Code	Proposed statement
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VC2:	<i>Vitamin C is necessary for the normal structure of wounds</i>
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VC3:	<i>Vitamin C is necessary for the normal structure of scar tissue</i>
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Reference 1.2:

‘Clinical features of scurvy include ... impaired wound healing’ (pg 101)

Reference 4.13:

‘Wound healing is impaired, since in deficiency, although fibroblasts proliferate they remain immature and fail to synthesise collagen.’ (pg 7)

4) Gums

Code	Proposed statement
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VC4:	<i>Vitamin C is necessary for the normal structure of gums</i>
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Reference 1.2:

‘In experimental subjects made vitamin C deficient,... gingival inflammation and fatigue were amongst the most sensitive markers of deficiency’ (pg 101)

‘Epidemiological studies have failed to demonstrate an association between Vitamin C intake and periodontal disease. Controlled experimental studies of patients with gingivitis and apparently healthy adults with vitamin C intakes of 5 to 1500 mg/day have shown mixed results with regards to the influence of vitamin C status on periodontal integrity. Other studies, with animals and humans, have shown that vitamin C intake can affect the structural integrity of gingival tissue...’ (pg 120)

‘Overall, while evidence suggests that Vitamin C deficiency is linked to some aspects of periodontal disease, the relationship of vitamin C intake to periodontal health in the population is unclear.’ (pg 120)

Reference 4.13:

‘Specific signs [of scurvy] such as swollen, bleeding and sensitive gums,...’ (pg 7)

5) Blood vessels

Code Proposed statement

VC5: *Vitamin C is necessary for the normal structure and function of blood vessels*

Reference 1.2:

‘Clinical features of scurvy include ... Ecchymoses ...(*the skin discoloration caused by the escape of blood into the tissues from ruptured blood vessels*). Vitamin C deficiency in infants may result in ... and hemorrhagic symptoms and resultant anaemia.... Oxidative degradation of some blood coagulation factors due to low plasma ascorbate concentrations may contribute to hemorrhagic symptoms.’ (pg 101)

‘Impaired vascular function is crucial to the clinical manifestation of atherosclerosis... numerous investigators have reported a beneficial effect of high dose (*up to 3g per day*) vitamin C administration...on vasodilation. Vitamin C improves endothelial function and vasodilation, possibly by scavenging superoxide radicals, conserving intracellular glutathione, or potentiating intracellular NO synthesis.’ (pg 103)

Reference 4.13:

‘Specific signs [of scurvy] such as,...petechial haemorrhages under the skin...A loss of blood may also be observed due to petechiae, perifollicular haemorrhages and bleeding gums.’ (pg 7)

6) Skin

Code	Proposed statement
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VC6:	<i>Vitamin C is necessary for the normal structure of skin</i>
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Reference 1.2:

‘Clinical features of scurvy include follicular hyperkeratosis ... and coiled hairs’. (pg 101)

Reference 4.13:

‘Specific signs [of scurvy] include hardening and roughness around hair follicles (hyperkeratosis),...’ (pg 7)

7) & 8) Bone and joints

Code	Proposed statement
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VC7:	<i>Vitamin C is necessary for the normal structure of connective tissue in bone</i>
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VC8:	<i>Vitamin C contributes to the normal structure of joints</i>
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Reference 1.2:

‘Evidence also suggests that ascorbate plays a role in or influences...and the biosynthesis of other connective tissue components beside collagen, including, elastin, fibroconectin, proteoglycans, bone matrix and elastin-associated fibrillin.’ (pg 98).

‘Clinical features of scurvy include... joint effusions (*pouring out of fluid*), arthralgia (*joint pain*),...’ (pg 101)

‘Vitamin C deficiency in infants may result in bone abnormalities such as impaired bone growth and disturbed ossification,...’ (pg 101)

Reference 2.0:

‘... proline hydroxylase is also required for the postsynthetic modification of osteocalcin in bone and the C1q component of complement.’ (pg 146)

Reference 3.23:

In childhood scurvy, the bone tissue is most obviously involved, especially in the breast cage and the stressed epiphyseal cartilage of the extremities. (pg 124)

Vitamin C status can be evaluated from signs of clinical deficiency such as joint pain. (pg 124)

Reference 4.13:

‘Early signs of deficiency, that is scurvy, are relatively non-specific. They often include..., aching bones, joints,...As the dentine becomes porous, alveolar bone becomes osteoporotic, and the teeth loosen and fall out. The cartilage matrix of the epiphyseal plate builds up between long bones and can become calcified; this results in compressed and brittle bone.’ (pg 7)

9) Iron absorption

Code	Proposed statement
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<i>VC9:</i>	<i>Vitamin C contributes to the absorption of iron from food</i>
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Reference 1.2:

‘Ascorbic acid modulates iron absorption, transport and storage.’ (pg 99)

Reference 2.0:

‘Inorganic dietary iron is absorbed as Fe²⁺, not as Fe³⁺; ascorbic acid in the intestinal lumen will not only maintain iron in the reduced state but also chelate it, thus increasing absorption considerably. A dose of 25mg of vitamin C taken with a meal increases the absorption of iron by some 65%, while a 1g dose gives a nine-fold increase. This is an effect of ascorbic acid present together with the test meal; neither intravenous administration of vitamin C nor supplements several hours before the test meal have any effect on iron absorption. The endogenous vitamin C in foods has the same effect on iron absorption. This is not a specific effect of ascorbate; a variety of other reducing agents also enhance the absorption of inorganic iron.’ (pg 147)

Reference 4.13:

‘Ascorbic acid is a potent enhancer of non-haem iron absorption from food. Ascorbic acid in the intestine is thought to keep iron in its reduced form, preventing the formation of insoluble ferric hydroxide and hence aids absorption. Ascorbic acid may also be involved in the transfer of iron into the blood, as well as mobilising it from its stores.’ (pg 7)

10) Antioxidant activity

Code	Proposed statement
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<i>VC10:</i>	<i>Vitamin C contributes to cell protection from the damage caused by free radicals (such as during the immune response)</i>
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Reference 1.2:

‘Vitamin C functions physiologically as a water-soluble antioxidant by virtue of its very high reducing power... and facile regeneration via ubiquitous reductants such as glutathione...’ (pg 43)

‘Evidence for in vivo antioxidant functions of ascorbate include the scavenging of reactive oxidants in activated leukocytes, lung, and gastric mucosa, and diminished lipid peroxidation as measured by urinary isoprostane excretion.’ (pg 95)

‘Because of its ability to donate electrons, ascorbic acid is an effective antioxidant. The vitamin readily scavenges reactive oxygen species (ROS) and reactive nitrogen species (RNS)... The relatively high tissue levels of ascorbate provide substantial antioxidant protection in the eye, ... in neutrophils, ... and in semen...’ (pg 98).

‘Ascorbic acid protects against plasma and low-density lipoprotein oxidation by scavenging ROS ... and possibly by sparing or regenerating vitamin E. Evidence

suggests that ascorbate also provides antioxidant protection indirectly by regenerating other biological antioxidants such as glutathione and α -tocopherol back to their active state.’(pg 98)

‘The most convincing evidence that vitamin C functions as an antioxidant *in vivo* is the study by Reilly et al. (1996), showing that supplementation of smokers with 2g/day for 5 days was associated with a significant reduction in urinary isoprostanes, an indicator of oxidative stress... Vitamin C supplementation (2g/day for 4-12 months) in 41 patients with non-atrophic gastritis decreased gastric mucosal nitrotyrosine, a measure of RNS activity Mannick et al, 1996. Thus, from this study and the study by Reilly et al. (1996) it can be concluded that supplementation with vitamin C results in an antioxidant effect *in vivo* because it significantly reduces nitrotyrosine and urinary isoprostanes.’ (pg 102).

‘The content of vitamin C in leukocytes is especially important because the ROS generated during phagocytosis and neutrophil activation are associated with infectious and inflammatory stresses... Along with pituitary and adrenal glands and eye lens, leucocytes contain the highest vitamin C concentrations of all body tissues...’ (pg 103).

‘The high intra-cellular concentration of ascorbate in leukocytes provides cellular protection against oxidant damage associated with the respiratory burst.’ (pg 108)

‘The ratio of oxidised to reduced ascorbate was found to be increased in the knee synovial fluid of active rheumatoid arthritis patients, which suggests that ascorbate is acting to scavenge phagocyte-derived oxidants in this locally inflamed area’.(pg 108)

‘Increased ascorbate oxidation in the plasma of patients with adult respiratory distress syndrome and in smokers indicates protection against oxidant damage from activated neutrophils and other sources in the lung.These results imply that ascorbate protects against inflammatory oxidative stress induced by ozone.’ (pg 108)

‘Ascorbate scavenging of myeloperoxidase-derived oxidants from phagocytic white cells may also be protective against *in vivo* LDL oxidation because HOCl oxidised proteins have also been identified in human atherosclerotic lesions.’ (pg 109)

Reference 2.0:

‘Ascorbic acid functions as a relatively nonspecific, radical-trapping antioxidant ...’ (pg 144)

‘Ascorbate reacts with nitrite and other nitrosating reagents *in vitro*, forming nitric oxide, nitrous oxide and nitrogen. This may be important in preventing the formation of carcinogenic nitrosamines by reaction between nitrites and amines present in foods in the acid conditions of the stomach. Again, this is an effect of ascorbate present in the stomach together with the dietary nitrites and amines, rather than an effect of vitamin C nutritional status. However, while ascorbate can deplete nitrosating compounds under anaerobic conditions, the situation may be reversed in the presence of oxygen. Nitric oxide reacts with oxygen to form N_2O_3 and N_2O_4 , both of which are nitrosating reagents, and can also react with ascorbate to form NO and monodehydroascorbate. It is thus possible for ascorbate to be depleted, with no

significant effect on the total concentration of nitrosating species. It remains to be determined whether or not ascorbate has any significant effect in reducing the risk of nitrosamine formation and carcinogenesis.’ (pg 147)

‘Ascorbate also acts nonenzymically to reduce oxidized vitamin E...Vitamin C thus has a vitamin E-sparing antioxidant action, coupling lipophilic and hydrophilic antioxidant reactions. The antioxidant efficiency of ascorbate is variable...it is only at very low concentrations of ascorbate that it tends towards the theoretical 2 : 1 ratio. This is probably because, as well as its antioxidant role, ascorbate can be a source of hydroxyl and superoxide radicals.’ (pg 147)

Reference 4.13:

‘Vitamin C plays a major role as an antioxidant and free-radical scavenger...Vitamin C is a strong reducing agent and hence has a general importance as an antioxidant, affecting the body’s ‘redox potential’...In fact it forms part of the body’s antioxidant defences against reactive oxygen species and free radicals, thereby preventing tissue damage.’ (pg 6)

‘Regeneration of ascorbic acid from its oxidation products, by reducing agents such as glutathione and nicotinamide-adenine dinucleotide (NAD) potentiate its antioxidant potential.’ (pg 6)

11) Carnitine

Code Proposed statement

VC11: *Vitamin C is necessary for the normal structure of carnitine*

Reference 1.2:

‘It is a co-factor for enzymes involved in the biosynthesis of,... carnitine,...’ (pg 95)

‘Vitamin C is known to be an electron donor for eight human enzymes... two [participate] in carnitine biosynthesis.’ (pg 96)

‘...ascorbate is required along with iron at two steps in the pathway of carnitine biosynthesis’ (pg 99)

Reference 2.0:

‘Trimethyllysine and γ -butyrobetaine hydroxylases are required for the synthesis of carnitine.’ (pg 146)

Reference 4.13:

The functions of vitamin C include the synthesis of...and carnitine.’ (pg 6)

12) Neurological system

Code **Proposed statement**

VC12: *Vitamin C is necessary for the normal neurological function*

Reference 1.2:

‘It is a co-factor for enzymes involved in the biosynthesis of ...neurotransmitters in vitro,...’ (pg 95)

‘Vitamin C is known to be an electron donor for eight human enzymes. The three enzymes that participate in hormone [and amino acid] biosynthesis are dopamine β -hydroxylase, necessary for the biosynthesis of the catecholamines norepinephrine and epinephrine and; peptidyl-glycine monooxygenase, necessary for amidation of peptide hormones; and’ (pg 96)

‘Ascorbic acid is involved in the synthesis and modulation of some hormonal components of the nervous system. The vitamin is ...involved in the biosynthesis of neuropeptides. Other nervous system components modulated by ascorbate concentrations include neurotransmitter receptors, the function of glutamatergic and dopaminergic neurons, and synthesis of glial cells and myelin.’ (pg 98)

‘The vitamin is involved in the biosynthesis of corticosteroids and aldosterone and...’ (pg 99)

‘Although vitamin C’s role as an antioxidant and cofactor for catecholamines biosynthesis might suggest that it protects cognitive function, there is little valid, consistent evidence that it does.’ (pg 127)

Reference 2.0:

‘It also has a specific metabolic function as the redox coenzyme for dopamine β -hydroxylase and peptidyl glycine hydroxylase ...’ (pg 144)

‘Dopamine β -hydroxylase (EC 1.14.17.1) is a copper-containing enzyme involved in the synthesis of the catecholamines, noradrenaline and adrenaline, from tyrosine in the adrenal medulla and central nervous system. The active enzyme contains Cu^+ , which is oxidized to Cu^{2+} during the hydroxylation of the substrate; reduction back to Cu^+ specifically requires ascorbate, which is oxidized to monodehydroascorbate.’ (pg 146)

Reference 4.13:

‘The functions of vitamin C include the synthesis of..., neurotransmitters, ...’ (pg 6)

‘Additional clinical manifestations observed in vitamin C deficiency include behavioural changes, often apathy, depression and emotional disturbances. Such observations are thought to be the consequence of a depression in catecholamine synthesis.’ (pg 7)

13) Metabolism of foreign compounds

Code	Proposed statement
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<i>VC13:</i>	<i>Vitamin C contributes to the breakdown of undesirable chemicals</i>
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Reference 1.2:

‘Ascorbic acid functions as a reducing agent for mixed-function oxidases in the microsomal drug-metabolising system that inactivates a wide variety of substrates, such as endogenous hormones or xenobiotics (i.e., other chemical compounds such as drugs, pesticides, or carcinogens that are foreign to humans). The activity of both microsomal drug-metabolising enzymes and cytochrome P-450 electron transport is lowered by ascorbate deficiency.’ (pg 99)

Reference 4.13:

‘Vitamin C is... involved in the detoxification of many foreign compounds.’ (pg 6)

14) Muscle function

Code	Proposed statement
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<i>VC14:</i>	<i>Vitamin C is necessary for the normal function of muscles</i>
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Reference 1.2:

‘Muscle carnitine is significantly depleted in scorbutic guinea pigs, suggesting that loss of energy derived from carnitine-related β -oxidation of fatty acids may explain the fatigue and muscle weakness observed in human scurvy. However, neither guinea pig nor human studies show a consistent relationship between vitamin C status and carnitine levels. Although vitamin C deficiency appears to alter carnitine metabolism, the specific interactions and their relevance to functional carnitine status in humans are unclear.’ (pg 120)

Reference 3.23:

‘Prescorbutic symptoms include weakness, lassitude, fatigue’. (pg 124)

Reference 4.13:

‘Early signs of deficiency, that is scurvy, are relatively non-specific. They often include..., aching bones, joints and muscles...’ (pg 7)

ANNEX 4.14

Calcium

Source documents for reviewing calcium

Reference 1.4:

Institute of Medicine Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington D.C. National Academy Press, 1997.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.14:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Calcium. April 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out194_en.pdf).

Reference 4.14:

Review of Calcium. Expert Group on Vitamins and Minerals. March 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm0112p.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference 6.1:

Nutrition and Bone Health: with particular reference to calcium and vitamin D. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of Food and Nutrition Policy. Report on Health and Social Subjects No. 49. DH: The Stationery Office, 1998.

Reference 8.1:

Calcium. The Report of the British Nutrition Foundation Task Force. The British Nutrition Foundation, 1989.

Reference 9.0:

Calcium in Nutrition. Michael Gurr. ILSI Europe Concise Monograph Series. 1999 International Life Sciences Institute.

1) Bones and teeth

Code	Proposed statement
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<i>Ca1:</i>	<i>Calcium is necessary for the normal structure of bones and teeth.</i>
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Reference 1.4:

‘The skeleton has an obvious structural role and it also serves as a reservoir for calcium’ (pg 71)

‘Chronic calcium deficiency resulting from inadequate intake or poor intestinal absorption is one of several important causes of reduced bone mass and osteoporosis (DHSS, 1990; NIH, 1994; NRC, 1989b; Osteoporosis Society of Canada, 1993)’ (pg 82)

‘Thus, although PTH (*parathyroid hormone*) maintains a normal circulating calcium concentration during calcium deprivation, it does so at the expense of skeletal mass.’ (pg 83)

‘Ideally the optimal calcium intake for skeletal health would be defined as that which leads to the fewest osteoporotic fractures later in life.’ (pg 84)

‘*Bone mineral content* (BMC) is the amount of mineral at a particular skeletal site such as the femoral neck, lumbar spine, or total body. *Bone mineral density* (BMD) is BMC divided by the area of the scanned region. Recent studies have indicated that both measures are strong predictors of fracture risk (Black et al., 1992; Cummings et al., 1993; Melton et al., 1993a).’ (pg 85)

‘In contrast, randomised, placebo-controlled calcium intervention studies that measure change in BMC or BMD provide valuable evidence for the calcium requirement. A major strength of such longitudinal studies is that the increment in calcium intake (the intervention) is known. In addition, their generally large sample sizes and subject randomisation greatly reduce confounding of the results by other factors that influence bone mass.’ (pg 86)

‘...99 percent of body calcium is located in the skeleton which has an essential structural function. To maximize skeletal size and strength, one must have adequate calcium retention to provide the substrate (along with other minerals) for bone mineral expansion during growth and maintenance after peak bone mass has been achieved. To a great extent, the retention of calcium in bone is under strong homeostatic control), which is regulated by genetics, calciotropic hormones and weight bearing exercise. The target intake of dietary calcium to achieve the desirable and optimal calcium accretion in bone is difficult to estimate because of all of the other factors which play a role in bone mineral homeostasis.’ (pg 97)

‘Adults continue to lose bone despite high intakes of calcium for other reasons such as lack of estrogen smoking, or sedentary lifestyle. Thus not all bone loss can be prevented by additional dietary calcium.’ (pg 87/88)

‘No published studies indicate that increasing bone accretion using high calcium-containing formulas or cow milk during infancy leads to greater bone mineralization in later childhood or adolescence.’ (pg 95)

‘Several randomised trials have been conducted in children through adolescence which provide evidence that increasing dietary intakes of calcium of girls above their habitual intake of about 900 mg (22.5 mmol)/day is associated with positive effects on bone mineral accretion, especially during the pre-pubertal stage.’ (pg 101/102)

‘Mounting evidence from randomised clinical trials suggest that the bone mass gained during childhood and adolescence through calcium or milk supplementation is not retained postintervention (Fehily et al., 1992; Lee et al., 1996; Slemenda et al., 1997).’ (pg 102)

Reference 2.0:

‘The most obvious role of calcium is to provide structure and strength in bones and teeth. About 99% of the total body calcium content is used for this purpose.’ (pg 216)

Reference 3.14

‘Over 99% of the total calcium of the body is located in the bones, where it accounts for 39% of the total body bone mineral content (Weaver, 2001), and in teeth, mostly as hydroxyapatite. Bone mineral provides structure and strength to the body, and, very important, a reservoir of calcium that helps to maintain a constant concentration of blood calcium.’ (pg 2)

Reference 4.14:

‘...In the vertebrate skeleton, calcium provides rigidity in the form of calcium phosphate ($\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$, also known as hydroxyapatite), this mineral is embedded in collagen fibrils. Calcium is also a key component in the maintenance of cell structure. Membrane rigidity, viscosity and permeability are partly dependent on local calcium concentrations.’ (pg 10)

Reference 5.0:

‘The primary function of calcium is to build the bones and teeth and to maintain the bones. Other functions are...’ (pg 87)

Reference 6.1:

‘More than 99 per cent of the body’s calcium resides in the skeleton, mainly as crystalline hydroxyapatite...’ (pg 21)

Reference 8.1:

‘Since about 99% of calcium is contained in bone, it is assumed that total body calcium very accurately represents the total quantity of skeletal mineral.’ (pg 20)

‘Calcium is the most abundant mineral element in the body. It is an essential nutrient, not only for the mineralisation of bones and teeth but for the regulation of intracellular events in most, if not all, body tissues. It is therefore fulfils many functions crucial to survival, in addition to its role in the skeleton.’ (pg 99)

Reference 9.0:

‘The element is present in two body “compartments” with quite distinct functions (Figure 1). The greater part (99%) is present as a type of calcium phosphate in the skeleton, where it contributes to the mechanical properties of bone (“structural” role); about 7g is also present in the teeth.’ (pg 2)

‘Bone consists of protein fibres encased in a crystalline mineral. The latter is a complex salt, which is mainly a calcium phosphate but also contains other minerals. The mineral part contributes to the strength of bone because it is particularly good at resisting compression.’ (pg 2)

‘Builds and maintains skeleton and teeth.’ (Fig 1 pg 3)

‘Preterm babies are particularly at risk of not being able to absorb enough calcium for optimal bone growth. However, the reduced bone mineralization of prematurity is now regarded as predominately due to a deficiency of phosphorus, not of calcium.’ (pg 18)

‘Whereas recent studies have revealed that bone mass is positively associated with calcium intake in mainland Chinese people, it has to be stressed that bone mass is not the only determinant of osteoporosis risk.’ (pg 18)

‘The concept that osteoporosis is a result of calcium deficiency does not necessarily imply that there is insufficient calcium in the diet; poor absorption, defective bone metabolism or increased urinary excretion despite high calcium intakes may all be involved.’ (pg 24)

‘Calcium intake affects bone mineral content, but how does it affect the structure and physical characteristics of bone? Research is needed on the interactive effects of nutrients other than calcium. How is bone structure related to bone strength? What is the relative influence of calcium compared with other factors such as hormones and physical activity?’ (pg 33)

2) Nevers and muscle

Code	Proposed statement
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<i>Ca2:</i>	<i>Calcium is necessary for normal nerve and muscle function.</i>
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Reference 1.4:

‘...it plays a role in mediating ...muscle contraction...’ (pg 71)

Reference 2.0:

‘Almost all of the intracellular calcium is bound within organelles such as the nucleus, endoplasmic reticulum and vesicles. This means that cytosolic calcium concentrations are very low and influenced greatly by release of some calcium from cellular organelles. This release occurs through a variety of signals and triggers events such as muscle contraction... ’ (pg 216)

Reference 3.14

Intracellular calcium rises in response to stimuli interacting with the cell surface receptor. The increase of intracellular calcium comes from influx of extracellular calcium or from release of intracellular calcium stores. This activates specific responses like hormone or neurotransmitter release, muscle contraction, cellular differentiation and many others.’ (pg 2)

Reference 4.14:

‘Changes in calcium concentration, in response to a physiological stimulus such as a hormone or neurotransmitter, can give rise to an intracellular signal. This controls events such as cell aggregation, muscle contraction and cell movement, ... (Macrae *et al.*, 1993).’ (pg 10, 11)

Reference 5.0:

‘Other functions are... muscle contraction and relaxation, especially the heartbeat.’ (pg 87)

‘Regulates the heartbeat’. (pg 1351)

Reference 6.1:

‘... it is essential for maintaining biomembrane integrity and permeability (which is important for normal neuromuscular function), ...’ (pg 21)

Reference 8.1:

‘Electrically excitable cells contain channels which are selective for calcium, and open when the membrane is depolarised. Thus calcium currents play an important role in the action potential of the heart, provoking contraction, and in provoking transmitter release at nerve terminals. In both cases, depolarisation of the plasma membrane causes a rise in cytosolic calcium, which is the internal signal causing a muscle cell to contract or a nerve terminal to secrete.’ (pg 14)

‘The main mobilisable intracellular calcium store in all cells is the endoplasmic reticulum. In skeletal muscle, this is released directly by an electrical signal. In heart muscle, it is released by the small amount of calcium which moves into the cell through the action potential. In all other cells, calcium is released by inositol trisphosphate (IP₃) (Berridge and Irvine, 1984).’ (pg 16)

3) Coagulation

Code Proposed statement

Ca3: *Calcium is necessary for normal coagulation (blood clotting).*

Reference 3.14

‘Extracellular calcium... participates in blood clotting and intercellular adhesion’. (pg 2)

Reference 4.14:

‘Calcium also plays two important regulatory roles in the body (Macrae *et al.*, 1993). Firstly, a passive role as ... an important component of the blood clotting mechanism.

Secondly, an active role as an intracellular signal (Lipkin *et al.*, 1999a). Changes in calcium concentration, in response to a physiological stimulus such as a hormone or neurotransmitter, can give rise to an intracellular signal. This controls events such as cell aggregation...(Macrae *et al.*,1993).’ (pg 10, 11)

Reference 5.0:

‘Assists in blood clotting.’ (pg 1351)

Reference 8.1:

‘Removal of calcium will prevent blood clotting or the activation of complement in the test tube. This is because calcium is required for four enzymes in the blood clotting pathway, and for the first enzyme complex of complement, C1, binding to an antibody-antigen complex.’ (pg 13)

Reference 9.0:

‘Many enzymes require a chemical association between calcium and an enzyme’s protein(s) for full catalytic activity to occur. Several digestive enzymes in the blood-clotting cascade and the “complement” system of enzymes involved in immune defence. Such functions are not affected by changes in plasma calcium concentration (there is always sufficient calcium present for full activity), hence the term passive”.’ (pg 2)

4) Nerve transmission

Code Proposed statement

Ca4: *Calcium is necessary for normal nerve signals and messages.*

Reference 1.4:

‘...it plays a role in mediating ...nerve transmission...’ (pg 71)

Reference 2.0:

‘Almost all of the intracellular calcium is bound within organelles such as the nucleus, endoplasmic reticulum and vesicles. This means that cytosolic calcium concentrations are very low and influenced greatly by release of some calcium from cellular organelles. This release occurs through a variety of signals and triggers events such as ... nerve conduction.... In these roles calcium acts as an intracellular messenger.’ (pg 216)

Reference 4.14:

‘Calcium also plays...an active role as an intracellular signal (Lipkin *et al.*, 1999a). Changes in calcium concentration, in response to a physiological stimulus such as a hormone or neurotransmitter, can give rise to an intracellular signal. This controls events such as cell aggregation, muscle contraction and cell movement, muscle protein degradation, secretion, transformation and cell division (Macrae *et al.*,1993).’ (pg 10, 11)

Reference 5.0:

‘Other functions are...nerve transmission.’ (pg 87)

Reference 6.1:

‘... it is essential for maintaining biomembrane integrity and permeability (which is important for normal neuromuscular function), intercellular and intra-cellular signalling and enzyme regulation.’ (pg 21)

Reference 8.1:

‘Electrically excitable cells contain channels which are selective for calcium, and open when the membrane is depolarised. Thus calcium currents play an important role in the action potential of the heart, provoking contraction, and in provoking transmitter release at nerve terminals. In both cases, depolarisation of the plasma membrane causes a rise in cytosolic calcium, which is the internal signal causing a muscle cell to contract or a nerve terminal to secrete.’ (pg 14)

5) Cell wall permeability

Code Proposed statement

Ca5: *Calcium is necessary for the normal permeability of cell membranes.*

Reference 5.0:

‘Other functions are...cell wall permeability’ (pg 87)

Reference 6.1:

‘... it is essential for maintaining biomembrane integrity and permeability (which is important for normal neuromuscular function), intercellular and intra-cellular signalling and enzyme regulation.’ (pg 21)

Reference 8.1:

‘...Calcium bound to catechol- and other amines also plays a structural role in the secretory granules of endocrine cells and nerve terminals. Bound to phospholipids and proteins, calcium is necessary for maintaining the integrity and permeability properties of biological membranes, and when bound to DNA, for determining some of the structural features of chromosomes.’ (pg 12)

Reference 9.0:

‘Calcium is one of the most important and widely occurring of these intracellular signals. Calcium that crosses the cell membrane by leakage through “calcium channels” or is released from intracellular stores can cause a very large proportionate rise in calcium concentration in the cell fluid. This can either activate the cell or, if too high, can injure it.’ (pg 4, 5)

6) Hormone secretion, especially insulin

Code Proposed statement

Ca6: *Calcium contributes to the normal release of hormones, such as insulin.*

Reference 1.4:

‘...it plays a role in...glandular secretion.’ (pg 71)

Reference 2.0:

‘Almost all of the intracellular calcium is bound within organelles such as the nucleus, endoplasmic reticulum and vesicles. This means that cytosolic calcium concentrations are very low and influenced greatly by release of some calcium from cellular organelles. This release occurs through a variety of signals and triggers events such as ... secretion of hormones such as insulin. In these roles calcium acts as an intracellular messenger.’ (pg 216)

Reference 4.14:

‘Calcium also plays ... an active role as an intracellular signal (Lipkin *et al.*, 1999a). Changes in calcium concentration, in response to a physiological stimulus such as a hormone or neurotransmitter, can give rise to an intracellular signal. This controls events such as ... secretion, ...(Macrae *et al.*,1993).’ (pg 10, 11)

‘Cellular calcium fluxes are important mediators of hormonal effects on target organs and are closely linked with cyclic adenosine-mono-phosphate system.’ (pg 11)

Reference 5.0:

‘Other functions are...secretion of a number of hormones and hormone releasing factors’ (pg 87)

Reference 8.1:

‘...Calcium bound to catechol- and other amines also plays a structural role in the secretory granules of endocrine cells and nerve terminals. Bound to phospholipids and proteins, calcium is necessary for maintaining the integrity and permeability properties of biological membranes, and when bound to DNA, for determining some of the structural features of chromosomes.’ (pg 12)

Reference 9.0:

‘Calcium acts as an intracellular “messenger” in many different types of cells carrying out many different functions or “end responses”. ...The calcium cloud then reacts at another point on the cell membrane to produce an end response, depending on the function of the cell. For example, in the cells of the adrenal gland whose function is to make steroid hormones, ... the end response is the production of aldosterone from cholesterol.’ (Fig 5, pg 5)

7) Blood pressure

Code Proposed statement

Ca7: *Calcium contributes to maintaining normal blood pressure.*

Reference 1.4:

‘...it plays a role in mediating vascular contraction and vasodilation...’ (pg 71)

‘In a review of 22 randomised intervention trials, calcium supplementation was found to reduce systolic blood pressure modestly-by 1.68 mm Hg in hypertensive adults-and had no significant effect in normotensive adults (Allender et al., 1996). Diastolic blood pressure was not altered in either group. More recently, a diet with increased low-fat dairy products, fruits and vegetables, and with reduced saturated and total fat, lowered blood pressure when fed to normotensive and hypertensive adults (Appel et al., 1997). In this study, the increase in dairy product consumption provided a mean dietary calcium increase from 443 to 1,265 mg (11.1 to 31.6 mmol)/day.’ (pg 89)

‘The influence of dietary calcium on pregnancy-induced hypertension has been investigated extensively. A meta-analysis of 14 randomised controlled trials of calcium supplementation during pregnancy found that supplements of 1,500 to 2,000 mg (37.5 to 50 mmol)/day of calcium may result in a significant lowering of both diastolic and systolic blood pressure (Bucher et al., 1996). However, the randomised controlled trial of Calcium for Preeclampsia Prevention (CPEP) in 4,589 pregnant women found no effect of calcium supplementation on hypertension, blood pressure, or preeclampsia (Levine et al., 1997), perhaps because the intake of the control group was above a threshold value.’ (pg 89/90)

‘Because the effect of dietary calcium on blood pressure may be modest and variable in the general population, and because the calcium intake needed to reduce blood pressure is very likely below the threshold necessary for desirable skeletal retention (McCarron et al., 1991), blood pressure will not be used as a primary indicator for estimating calcium requirements.’ (pg 90)

Reference 5.0:

‘May lower high blood pressure in individuals with calcium-poor diet.’ (pg 1351)

Reference 8.1:

‘Based on epidemiological studies, the case for implicating dietary calcium in hypertension is not proven. It is possible that calcium intake may influence other dietary factors which in turn influence blood pressure, but, at present, there is no information on which to judge this hypothesis.’ (pg 74)

‘It has been suggested that an abnormality of calcium metabolism contributes to the genesis of essential hypertension,’ (pg 75)

Reference 9.0:

‘Calcium in the body is involved in the maintenance of normal blood pressure; it works in conjunction with several other ions. Calcium and sodium are the predominant divalent and monovalent cations outside cells, whereas magnesium and potassium predominate inside cells. Together these ions influence blood pressure by affecting vascular tone (the normal state of balanced tension in muscles) through the regulation of contractile proteins and the transport of substances through membranes. They also regulate metabolic activities in the smooth muscles surrounding blood vessels, which in turn control the transmission of signals between cells and the generation of energy for muscular contraction.’ (pg 26)

‘People with raised blood pressure tend to have lower than normal concentrations of calcium ions in the blood, although this observation has not been confirmed in all

studies. More consistently, high blood pressure is accompanied by higher intracellular calcium concentrations, especially in red blood cells, platelets and lymphocytes, higher circulating levels of parathyroid hormone and elevated excretion of calcium in urine. These observations suggest that at least some people with hypertension have a defect in their ability to use calcium in normal cellular functions.' (pg 26)

8) Digestion

Code	Proposed statement
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<i>Ca8:</i>	<i>Calcium is necessary for the normal function of enzymes, such as those required for digestion.</i>
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Reference 4.14:

'Calcium also plays...a passive role as a cofactor for many enzymes (eg, lipase)...'
(pg 10)

Reference 5.0:

'Other functions are...enzyme activation.' (pg 87)

Reference 8.1:

'Calcium is also required for the maximum activity of several extracellular digestive enzymes, including proteases, phospholipases and nucleases.' (pg 14)

Reference 9.0:

'Extraskeletal: Metabolic control of ...enzyme production and activation.' (Fig 1, pg 3)

ANNEX 4.15

Magnesium

Source documents for reviewing magnesium

Reference 1.4:

Institute of Medicine Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington D.C. National Academy Press, 1997.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.15:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Magnesium. September 2001. (http://www.europa.eu.int/comm/food/fs/sc/scf/out105_en.pdf).

Reference 4.15:

Review of Magnesium. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-01-13.pdf>).

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Energy metabolism

Code	Proposed statement
Mg1:	<i>Magnesium is necessary for normal energy metabolism</i>

Reference 1.4:

‘Magnesium is a required cofactor for over 300 enzyme systems (Wacker and Parisi, 1968). It is required for both anaerobic and aerobic energy generation and for glycolysis, either indirectly as a part of the Mg-ATP complex or directly as an enzyme activator (Garfinkel and Garfinkel, 1985). Magnesium has also been shown to be required for mitochondria to carry out oxidative phosphorylation (Wacker and Parisi, 1968). The mitochondrial enzymes utilize the magnesium chelate of ATP and ADP as the actual substrates for phosphate transfer reactions.’ (pg 190)

‘...determination of intracellular magnesium concentration should be a more physiologically relevant measurement of magnesium status, as it is thought to play a critical role in enzyme activation within the cell.’ (pg 203, 204)

Reference 2.0:

‘...magnesium is essential for oxidative phosphorylation and for all reactions in which adenosine triphosphate (ATP) is required. Processes with enzymatic reactions that require ATP include the synthesis of fat, protein, nucleic acid and coenzymes.’ (pg 1227)

Reference 3.15:

‘Depending on the degree of the deficiency, symptoms are latent, moderate or even life threatening because Mg is a cofactor in hundreds of enzymatic reactions, many of which involve energy metabolism.’ (pg 2)

Reference 4.15:

‘Magnesium is a required cofactor for many enzyme systems. It is required for protein synthesis and for both anaerobic and aerobic energy generation and for glycolysis, either indirectly as a part of magnesium-ATP complex or directly as an enzyme activator (Bronzetti *et al.*, 1995; Food and Nutrition Board, 1997).’ (pg 6)

Reference 6.3:

‘Biochemically Mg acts as a co-factor for enzymes requiring ATP, in the replication of DNA and the synthesis of RNA¹.’ (pg 146)

2) Cell replication

Code Proposed statement

Mg2: *Magnesium contributes to normal cell replication.*

Reference 1.4:

‘Magnesium presence is important for maintaining an adequate supply of purine and pyrimidine nucleotides required for the increased DNA and RNA synthesis that occurs during cell proliferation (Rubin, 1975; Switzer, 1971). Replicating cells must be able to synthesize new protein, and this synthesis has been reported to be highly sensitive to magnesium depletion. Many hormones, neurotransmitters, and other cellular effectors regulate cellular activity via the adenylate cyclase system, and the activation of adenylate cyclase requires the presence of magnesium. There is also evidence for magnesium binding through which magnesium directly increases adenylate cyclase activity (Maguire, 1984).’ (pg 191)

Reference 2.0:

‘Many intracellular changes are mediated by second messengers. The formation of the second messenger cyclic adenosine monophosphate by adenylate cyclase is dependent on the presence of magnesium. Magnesium itself may also serve as a second messenger in the insulin stimulation of protein synthesis. Numerous genetic processes are dependent on magnesium, including the synthesis of the purine and pyrimidine precursors of nucleic acids. Magnesium is also required for enzymes involved in DNA replication, transcription and RNA translation.’ (pg 1227)

‘During pregnancy, an additional 40 mg per day of magnesium are recommended to supply the magnesium needs of the fetus.’ (pg 1227)

‘Unfortunately, only one short-lived radiotracer of magnesium exists ($^{28}\text{Mg}, t_{1/2}=21.3$ h) and its availability is limited. Use of this radiotracer, however, has provided much of the available data on the *in vivo* magnesium metabolism in adults. The limitation to the use of this radiotracer is that it cannot be safely administered to all population groups, especially those who may have increased needs because of growth and increased physiological demands such as infants, children, and pregnant and lactating women.’ (pg 1229)

Reference 3.15:

‘It also plays an important role in protein and nucleic acid synthesis and has a stabilizing and protecting effect on membranes.’ (pg 2)

Reference 4.15:

‘Magnesium plays a multifunctional role in cell metabolism, particularly at the level of key phosphorylations. The role of magnesium in cell division is also well recognised and it has been suggested that cell division of various cell types is highly dependent on the availability of extracellular magnesium (Rubin, 1975).’ (pg 6)

‘It has also been suggested that the presence of magnesium is important for maintaining an adequate supply of purine and pyrimidine nucleotides for RNA and DNA synthesis (Rubin, 1975).’ (pg 6)

Reference 6.3:

‘Biochemically Mg acts as a co-factor for enzymes requiring ATP, in the replication of DNA and the synthesis of RNA¹.’ (pg 146)

3) Electrolyte balance

Code	Proposed statement
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<i>Mg3:</i>	<i>Magnesium is necessary for normal electrolyte balance</i>
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Reference 1.4:

‘Magnesium is necessary for sodium, potassium-ATPase activity, which is responsible for active transport of potassium (Dorup and Clausen, 1993). Magnesium regulates the outward movement of potassium in myocardial cells (Matsuda, 1991). The arrhythmogenic effect of magnesium deficiency may be related to magnesium’s role in maintaining intracellular potassium.’ (pg 191)

‘Although cardiac arrhythmia may be associated with the primary cardiac disorders, magnesium depletion may further predispose to cardiac arrhythmias, by decreasing intracellular potassium.’ (pg 198)

Reference 3.15:

‘Finally, Mg is also considered essential in maintaining Ca, K and Na homeostasis (Aikawa, 1981; Durlach, 1988; Seelig, 1989; Wacker, 1980).’ (pg 2)

‘Intracellularly, K is decreased and the concentration of Na and Ca is increased (owing to decreased activity of Mg-ATP-dependent ionic pumps and “leaky” membranes).’ (pg 4)

Reference 4.15:

‘It is also necessary for sodium potassium-ATPase activity, which is responsible for active transport of potassium (Dorup, and Clausen, 1993). Magnesium regulates the movement of potassium in myocardial cells (Matsuda, 1991) and is also known to act as a calcium channel blocker (Iseri and French, 1984). Thus magnesium depletion is linked to muscle cramps, hypertension and coronary and cerebral vasospasms.’ (pg 6)

4) Nerves and muscle

Code Proposed statement

Mg4: Magnesium is necessary for normal nerve and muscle function.

Reference 1.4:

‘Magnesium has been called “nature’s physiological calcium channel blocker: (Iseri and French, 1984). During magnesium depletion, intracellular calcium rises. Since calcium plays an important role in skeletal and smooth muscle contraction, a state of magnesium depletion may result in muscle cramps, hypertension, and coronary and cerebral vasospasms. Magnesium depletion is found in a number of diseases of cardiovascular and neuromuscular function, in malabsorption syndromes, in diabetes mellitus, in renal wasting syndromes, and in alcoholism (Ma et al., 1995).’ (pg 191)

‘Neuromuscular hyperexcitability is the initial problem cited in individuals who have or are developing magnesium deficiency (Rude and Singer, 1980). Latent tetany, as elicited by a positive Chvostek’s and Trousseau’s sign, or spontaneous carpal-pedal spasm may be present. Frank, generalized seizures may also occur. Although hypocalcemia may contribute to the neurological signs, hypomagnesemia without hypocalcemia may result in neuromuscular hyperexcitability.’ (pg 197)

‘In normal subjects, experimental magnesium depletion results in increased urinary thromboxane concentration, angiotensin II-induced plasma aldosterone levels, and blood pressure – indicating a potential effect of magnesium deficiency on vascular function (Nadler et al., 1993; Rude et al., 1989). Magnesium depletion is associated with cardiac complications, including electrocardiographic changes, arrhythmias, and increased sensitivity to cardiac glycosides (Rude, 1993).’ (pg 197)

Reference 2.0:

‘Magnesium is also essential for muscle contraction and methyl group transfers.’ (pg 1227)

‘The remaining body reserves of magnesium (approximately 40%) are located in muscle and nonmuscular soft tissues. Only 1% of total body magnesium is present in the extracellular fluid; of this; less than 0.3% circulates in serum.’ (pg 1227)

Reference 3.15:

‘Finally, Mg is also considered essential in maintaining Ca, K and Na homeostasis (Aikawa, 1981; Durlach, 1988; Seelig, 1989; Wacker, 1980).’ (pg 2)

‘Intracellularly, K is decreased and the concentration of Na and Ca is increased (owing to decreased activity of Mg-ATP-dependent ionic pumps and “leaky” membranes).’ (pg 4)

‘In the CNS, the activity of excitatory amino acids (especially glutamate) is enhanced because Mg is a specific blocker of the glutamate-NMDA receptor. Consequently central-nervous and spastic symptoms predominate in Mg deficiency.’ (pg 4)

Reference 4.15:

‘...and is also known to act as a calcium channel blocker (Iseri and French, 1984).’ (pg 6)

‘Magnesium deficiency has been linked to several disease states involving the cardiovascular, skeletal, gastrointestinal and central nervous systems.’ (pg 6)

Reference 6.3:

‘The physiological importance of magnesium (Mg) lies in its role in skeletal development and in the maintenance of electrical potential in nerve and muscle membranes.’ (pg 146)

‘Magnesium is intimately involved with calcium in metabolism. Calcium homeostasis is controlled in part by a Mg-requiring mechanism which releases parathyroid hormone. Several Mg activated enzymes are inhibited by calcium while in others Mg can be replaced by manganese².’ (pg 146)

5) Vitamin D metabolism

Code	Proposed statement
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Mg5:	<i>Magnesium is necessary for the normal activation of vitamin D in the body.</i>
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Reference 1.4:

‘Magnesium is also important in vitamin D metabolism and/or action. Patients with hypocalcemia and magnesium deficiency are resistant to pharmacological doses of vitamin D, 1 α hydroxyvitamin D, and 1,25 (OH)₂D (for a review, see Fatemi et al. [1991]).’ (pg 197)

Reference 2.0:

‘Furthermore, magnesium is required for the hepatic 3 β -hydroxylase enzyme, which converts 25-hydroxyvitamin D into the biologically active form of vitamin D (1,25-dihydroxyvitamin D).’ (pg 1229)

Reference 3.15:

‘Extracellularly, hypomagnesaemia is frequently associated with hypocalcemia (as a consequence of disturbed vitamin D metabolism and disturbed parathyroid hormone activity) and sometimes with hypokalaemia (renin-aldosterone-interactions).’ (pg 4)

‘... as Mg is required for the renal hydroxylation of vitamin D and for the activity of parathyroid hormone, Ca-resistant hypocalcaemia can be compensated by Mg supplements (Schimatschek *et al.*, 1997).’ (pg 5)

6) Bone and teeth

Code	Proposed statement
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<i>Mg6:</i>	<i>Magnesium is necessary for the normal structure of bone and teeth.</i>
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Reference 1.4:

‘Total body magnesium (Mg) content is approximately 25g (1,000 mmol), of which 50 to 60 percent resides in bone in the normal adult. One-third of skeletal magnesium is exchangeable, and it is this fraction that may serve as a reservoir for maintaining a normal extracellular magnesium concentration (Elin, 1987).’ (pg 190)

‘Many hormones, neurotransmitters, and other cellular effectors regulate cellular activity via the adenylate cyclase system, and the activation of adenylate cyclase requires the presence of magnesium. There is also evidence for magnesium binding through which magnesium directly increases adenylate cyclase activity (Maguire, 1984).’ (pg 191)

‘Magnesium plays a major role in bone and mineral homeostasis and can also directly affect bone cell function as well as influence hydroxyapatite crystal formation and growth (Cohen, 1988).’ (pg 200)

‘Significant reductions in the serum magnesium and bone mineral content (BMC), but not red blood cell magnesium concentration or bone magnesium content, have been described in women with postmenopausal osteoporosis compared to age-matched controls (Reginster *et al.*, 1989).’ (pg 200)

‘..... observations suggest that dietary magnesium may be related to osteoporosis and indicate a need for further investigation of the role of magnesium in bone metabolism (Sojka and Weaver, 1995).’ (pg 201)

Reference 2.0:

‘... The majority of magnesium in the body (approximately 60%) is located in bone. Of this, approximately 30% is freely exchangeable and the remainder is found as an integral part of the bone crystal.’ (pg 1227)

‘During pregnancy, an additional 40 mg per day of magnesium are recommended to supply the magnesium needs of the fetus.’ (pg 1227)

‘Although high calcium intakes do not adversely alter magnesium retention, magnesium plays an important role in the metabolism of several calcitropic hormones. Magnesium is required for parathyroid hormone secretion in humans and is also needed for parathyroid hormone to illicit its effects on kidney, bone and gut.’ (pg 1229)

‘Unfortunately, only one short-lived radiotracer of magnesium exists ($^{28}\text{Mg}, t_{1/2}=21.3$ h) and its availability is limited. Use of this radiotracer, however, has provided much of the available data on the *in vivo* magnesium metabolism in adults. The limitation to the use of this radiotracer is that it cannot be safely administered to all population groups, especially those who may have increased needs because of growth and increased physiological demands such as infants, children, and pregnant and lactating women.’ (pg 1229)

Reference 4.15:

‘Magnesium deficiency has been linked to several disease states involving the cardiovascular, skeletal, gastrointestinal and central nervous systems.’ (pg 6)

Reference 6.3:

‘The physiological importance of magnesium (Mg) lies in its role in skeletal development and in the maintenance of electrical potential in nerve and muscle membranes.’ (pg 146)

ANNEX 4.16

Iron

Source documents for reviewing iron

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 4.16:

Review of Iron – Revised Version. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm-01-12r.pdf>)

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

Reference 8.2:

Iron. Nutritional and physiological significance. The Report of the British Nutrition Foundation Task Force. T.J. Press (Padstow) Ltd, 1995.

1) & 7) Oxygen transport and blood formation

Code	Proposed statement
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<i>Fe1:</i>	<i>Iron is necessary for the normal transport of oxygen in the body</i>
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<i>Fe7:</i>	<i>Iron contributes to normal blood formation</i>
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Reference 1.1:

‘Iron functions as a component of a number of proteins, including enzymes and hemoglobin, the latter being important for the transport of oxygen to tissues throughout the body for metabolism.’ (pg 290)

‘Almost two-thirds of iron in the body is found in hemoglobin present in circulating erythrocytes. A readily mobilizable iron store contains another 25 percent. Most of the remaining 15 percent is in the myoglobin of muscle tissue and a variety of enzymes necessary for oxidative metabolism and many other functions in all cells.’ (pg 290)

‘The interconversion of iron oxidation states is a mechanism whereby iron participates in electron transfer, as well as a mechanism whereby iron can reversibly

bind ligands. The common biological ligands for iron are oxygen, nitrogen, and sulfur atoms.’ (pg 291)

‘Important subclinical and clinical consequences of iron deficiency are impaired physical work performance, developmental delay, cognitive impairment, and adverse pregnancy outcomes.’ (pg 295)

‘... it has been shown that anemia and tissue iron deficiency exert independent effects on skeletal muscle (Davies et al., 1984; Finch et al., 1976). Anemia primarily affects maximal oxygen consumption. Endurance exercise is markedly impaired by intracellular iron deficiency in the muscle cells (Willis et al., 1988). From a practical point of view, the distinction may be relatively unimportant since anemia and tissue iron deficiency develop simultaneously in humans who suffer from nutritional iron deficiency.’ (pg 295)

‘Various factors may contribute to impaired work performance with iron deficiency. It has been shown that anemia and tissue iron deficiency exert independent effects on the function of organs such as skeletal muscle (Davies et al., 1984; Finch et al., 1976). Anemia primarily affects maximal oxygen consumption. Mild anemia reduces performance during brief but intense exercise (Viteri and Torun, 1974) because of the impaired capacity of skeletal muscle for oxidative metabolism. Endurance exercise is more markedly impaired by intracellular iron deficiency in skeletal muscle cells (Willis et al., 1988).’ (pg 295, 296)

‘Heme is formed in developing erythrocytes by the incorporation of iron into protoporphyrin IX by ferrochetalase. If there is insufficient iron for optimal hemoglobin synthesis, erythrocytes accumulate an excess of protoporphyrin, which remains in the cells for the duration of their lifespans (Cook, 1999).’ (pg 304)

‘Iron deficiency leads to the formation of small erythrocytes. Mean corpuscular hemoglobin (MCH) is the amount of hemoglobin in erythrocytes. The mean corpuscular volume (MCV) is the volume of the average erythrocyte. Both MCH and MCV are reduced in iron deficiency, but their values are not specific for it. They occur in all conditions that cause impaired hemoglobin synthesis, particularly the thalassemias (Chalevelakis et al., 1984).’ (pg 305, 306)

Reference 2.0:

‘Iron plays a central role in metabolic processes involving oxygen transport and storage as well as oxidative metabolism and cellular growth. The fact that it readily serves as an electron donor or acceptor accounts both for its critical metabolic role and its potential toxicity. Iron-containing compounds function as carriers for oxygen and electrons and as catalysts for oxidation and hydroxylation reactions.’ (pg 1153)

‘Most of the functional iron in the body is present in the form of haem proteins, i.e. proteins with an iron protoporphyrin IX prosthetic group.’ (pg 1154)

‘*Haemoglobin*, which is made up of four globin chains, each with an attached haem group, transports oxygen to the tissues. It is quantitatively the most important haem protein and contains 80% of all functional iron.’ (pg 1154)

Myoglobin is found in the sarcoplasm of muscles. It has a structure similar to haemoglobin but contains only one globin chain attached to a single haem group and accounts for a further 10% of functional iron. Myoglobin functions as an oxygen store, ensuring an adequate oxygen supply during muscle contraction.’ (pg 1154)

‘Since 80% of the body’s functional iron is in the haemoglobin of the circulating red blood cells, measurements of internal iron exchange are dominated by the requirements of this compartment. Complete exchange of the iron in the circulating red blood cell compartment occurs every 4 months. This involves rapid transfer of iron by plasma transferrin. Only 3-4 mg iron is found in the plasma at any one time, but 35 mg is transported through this compartment each day. Most of it comes from haemoglobin catabolism in macrophages. Two-thirds (24 mg per day) is delivered to erythroid precursors in the bone marrow for the synthesis of new haemoglobin. While there is some iron loss owing to ineffective red cell production or the removal of iron not used for haemoglobin synthesis from red cell precursors, most of the iron (70%) is returned to the circulation as haemoglobin in erythrocytes. Erythrocytes have a lifespan of about 120 days. The senescent cells are catabolized in the macrophages of the liver, spleen and bone marrow, completing the cycle.’ (pg 1155)

‘A significant limitation of the ability to perform endurance physical activity has emerged as an important consequence of chronic iron deficiency. Animal studies conducted by Finch and coworkers demonstrated that iron-deficient rats show a marked impairment of running ability which is unrelated to haemoglobin level. It results from impaired oxidative metabolism in iron-depleted muscles. Field studies from many developing countries suggest that a similar disability reduces an iron individual’s ability to carry out prolonged physical work.’ (pg 1158)

Reference 4.16:

‘The majority of functional iron within the body exists in the form of haem proteins, in which iron is associated with a porphyrin prosthetic group. Approximately 85% of functional iron is in the form of haemoglobin, a 68,000 kDa compound, comprising four haem subunits, each with a polypeptide chain of globin attached, which plays a critical role in transferring oxygen from the lungs to the tissues. Myoglobin, the haem oxygen-carrying compound of muscle, has a similar structure to haemoglobin, except that it consists of only one haem and one globin chain.’ (pg 6)

Reference 8.2:

‘Functionally important forms of iron in the body are haemoglobin, myoglobin, cytochromes, iron-sulphur proteins, iron enzymes and lactoferrin.’ (pg 1)

‘Iron is carried to the bone marrow as ferric iron bound to transferrin: it is released within the red cell precursors, reduced to the ferrous form and transferred to protoporphyrin.’ (pg 1)

‘Defects in haemoglobin production encompass impaired haem synthesis due to inadequate iron supply or defects in porphyrin synthesis, and defective globin chain synthesis. By far the commonest defect is impaired haem synthesis resulting from iron deficiency where the reduced iron supply to the mitochondria leads to an accumulation of free protoporphyrin in the red cells. (pg 45)

‘Iron deficiency anaemia is the final and most obvious stage of a progressive ‘negative iron balance’, in which iron uptake from the gastrointestinal tract is insufficient to meet the need for an expanding volume of red blood cells (e.g. in growing infants or in pregnancy), or to keep pace with obligatory iron losses (predominantly through loss of gut enterocytes or desquamation of skin cells) or with pathological iron losses (e.g. haemorrhage into the gastrointestinal tract).’ (pg 46)

‘Such iron deficient erythropoiesis is associated with an increased concentration of serum transferrin receptors, derived from the increased expression on the cell membranes of iron deficient developing erythroblasts (Skikne *et al.*, 1990), as well as an accumulation of free erythrocyte protoporphyrin, and an increase in red cell heterogeneity as assessed by the red cell size distribution width, RDW (Bessman *et al.*, 1983). (pg 46)

2) Energy production

Code	Proposed statement
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Fe2:	<i>Iron contributes to normal energy production</i>
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Reference 1.1:

‘Various factors may contribute to impaired work performance with iron deficiency. It has been shown that anemia and tissue iron deficiency exert independent effects on the function of organs such as skeletal muscle (Davies *et al.*, 1984; Finch *et al.*, 1976). Anemia primarily affects maximal oxygen consumption. Mild anemia reduces performance during brief but intense exercise (Viteri and Torun, 1974) because of the impaired capacity of skeletal muscle for oxidative metabolism. Endurance exercise is more markedly impaired by intracellular iron deficiency in skeletal muscle cells (Willis *et al.*, 1988).’ (pg 295, 296)

Reference 2.0:

‘Despite its vital metabolic role, all other tissue iron represents only a very small fraction of total body iron. The *cytochromes* are a group of *haem-containing electron transport enzymes* that are essential for the oxidative metabolism necessary to generate adenosine triphosphate (ATP) as well as for the oxidative degradation of drugs and endogenous substrates. *Catalase and peroxidase* are involved in the reduction of endogenously generated hydrogen peroxide.’ (pg 1154)

‘A significant limitation of the ability to perform endurance physical activity has emerged as an important consequence of chronic iron deficiency. Animal studies conducted by Finch and coworkers demonstrated that iron-deficient rats show a marked impairment of running ability which is unrelated to haemoglobin level. It results from impaired oxidative metabolism in iron-depleted muscles. Field studies from many developing countries suggest that a similar disability reduces an iron individual’s ability to carry out prolonged physical work.’ (pg 1158)

Reference 4.16:

‘Many of the key biological functions of iron in living systems, for example its role in oxygen and energy metabolism, rely on the high redox potential, enabling it to switch rapidly between the Fe²⁺ and Fe³⁺ forms.’ (pg 6)

‘Iron-containing proteins also serve major functions in respiration and energy metabolism. The cytochromes are haem-containing compounds critical to cellular energy production through their role in mitochondrial electron transport, and the non-haem iron-containing enzymes such as the iron-sulphur complexes of NADH dehydrogenase and succinate dehydrogenase are also involved in energy metabolism.’ (pg 6)

‘Hydrogen peroxidases are a group of iron-containing enzymes, which act on reactive by-products of oxygen metabolism. Other enzymes that require iron for their function include aconitase, an enzyme of the tricarboxylic acid cycle; phosphoenolpyruvate carboxykinase, a rate-limiting enzyme in the gluconeogenic pathway; ...’ (pg 6)

Reference 8.2:

‘The nutritional need for iron in living organisms is derived from the central role that it plays in the energy metabolism of living cells.’ (pg 1)

‘Myoglobin, the red pigment of muscle, is a single peptide homologue of haemoglobin Its function is to store oxygen delivered to the tissues by haemoglobin for utilization during muscle contraction.’ (pg 2)

‘Cytochromes, the electron-transport enzymes, are located in the mitochondria as well as in other cellular membranes. They are able to undergo reversible oxidation by way of changes in the oxidation state of iron. Cytochromes a, b and c are present in all aerobic cells, within the cristae of mitochondria, and are essential for the oxidative production of cellular energy in the form of ATP.’ (pg 2)

‘... studies suggest the possibility that the iron therapy may be having effects on cellular function/oxidative metabolism which are not simply a result of correction of the anaemia.’ (pg 55)

3) Antioxidation / detoxification

Code Proposed statement

Fe3: *Iron contributes to the body’s ability to breakdown undesirable chemicals.*

Reference 2.0:

‘The *cytochromes* are a group of *haem-containing electron transport enzymes* that are essential for the oxidative metabolism necessary ... for the oxidative degradation of drugs and endogenous substrates.’ (pg 1154)

Reference 4.16:

‘The haem-containing P450 enzymes catalyse the breakdown of various endogenous compounds and chemicals or toxins from external sources by oxidative degradation.’ (pg 6)

Reference 8.2:

'Extra-mitochondrial cytochromes include cytochrome P-450, located within microsomal membranes of the liver, which is involved in oxidative degradation of drugs and endogenous substrates, e.g. steroids.' (pg 2)

4) DNA synthesis and growth

Code	Proposed statement
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<i>Fe4:</i>	<i>Iron contributes to normal DNA synthesis, required for growth.</i>
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Reference 1.1:

'The cytochromes contain heme as the active site with the iron-containing porphyrin ring functioning to reduce ferric iron to ferrous iron. Cytochromes act as electron carriers. The 40 different proteins that constitute the respiratory chain contain six different heme proteins, six with iron sulfur centers, two with copper centers, and ubiquinone to connect nicotinamide adenine dinucleotide hydride to oxygen.' (pg 291, 292)

Reference 2.0:

'Iron plays a central role in ... cellular growth.' (pg 1153)

'In mitochondria, nonhaem compounds account for more iron than do those containing haem. This group of enzymes includes the iron sulfur flavoproteins such as xanthine oxidase, NADH (the reduced form of nicotinamide adenine dinucleotide) dehydrogenase and succinate dehydrogenase, as well as other nonhaem enzymes, e.g. ribonucleotide reductase and phenylalanine hydroxylase.' (pg 1154)

'Iron is also required for the synthesis of DNA, which, in turn, is obligatory for the proliferation of lymphocytes involved in immune responses. The rate-limiting enzyme for DNA synthesis is ribonucleotide reductase, an iron metalloenzyme which must be continuously synthesized and is therefore dependent on a continuous supply of iron. This may be physiological reason for the expression of transferrin receptors on lymphocytes activated by interleukin 2. Iron deficiency, then, may lead to impaired lymphocytes proliferative responses because transferrin iron, essential for DNA synthesis, is in limiting concentrations. Diminished expansion of lymphocyte clones involved in the immune response in iron deficiency states could underlie the defects in immune function that have been reported.' (pg 1082)

Reference 4.16:

'... and ribonucleotide reductase, an enzyme required for DNA synthesis (Yip & Dallman, 1996).' (pg 6)

5) Immune system

Code	Proposed statement
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<i>Fe5:</i>	<i>Iron is necessary for the normal function of the immune system</i>
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Reference 1.1:

‘With use of in vitro tests and animal models, iron deficiency is associated with impaired host defense mechanism against infection such as cell-mediated immunity and phagocytosis (Cook and Lynch, 1986). The clinical relevance of these findings is uncertain although iron deficiency may be a predisposing factor for chronic mucocutaneous candidiasis (Higgs, 1973)’ (pg 300)

Reference 2.0:

‘Lymphocyte proliferation in response to the mitogens, phytohaemagglutinin and concanavalin A, are impaired, demonstrating defective T cell immunity. Impaired intracellular bacterial killing by polymorphonuclear leucocytes, and decreased reduction of the dye, nitroblue tetrazolium, have also been documented. These defects appear to result from diminished myeloperoxidase activity.’ (pg 1158)

‘Iron is also required for the synthesis of DNA, which, in turn, is obligatory for the proliferation of lymphocytes involved in immune responses. The rate-limiting enzyme for DNA synthesis is ribonucleotide reductase, an iron metalloenzyme which must be continuously synthesized and is therefore dependent on a continuous supply of iron. This may be physiological reason for the expression of transferrin receptors on lymphocytes activated by interleukin 2. Iron deficiency, then, may lead to impaired lymphocytes proliferative responses because transferrin iron, essential for DNA synthesis, is in limiting concentrations. Diminished expansion of lymphocyte clones involved in the immune response in iron deficiency states could underlie the defects in immune function that have been reported.’ (pg 1082)

‘Other iron-dependent host defence mechanisms may be affected by iron deficiency. For example, the neutrophil iron metalloenzyme myeloperoxidase is clearly inhibited in iron deficiency states. This enzyme catalyses generation of bactericidal reactive halide radicals during the oxidative burst initiated by phagocytosis by neutrophils. While myeloperoxidase may contribute to host defences by this mechanism it turns out not to be necessary, since patients with congenital deficiency of the enzyme do not exhibit an increased susceptibility to infection. Iron also catalyses the production of bactericidal oxygen radicals by other mechanisms, and a defect in this pathway may explain the reduced ability of neutrophils from iron-deficient subjects to reduce the dye nitroblue tetrazolium under conditions of cell activation. Consistent with this, some but not all studies have shown a modest decrease in bacterial killing capacity by iron –deficient neutrophils, although other iron-independent functions such as chemotaxis, phagocytosis and degranulation are normal.’ (pg 1082, 1083)

Reference 4.16:

‘Iron deficiency has also been associated with reduced resistance to infection, although a cause and effect relationship has not been established (Dallman, 1987)...’ (pg 24)

Reference 8.2:

‘The glycoprotein lactoferrin (molecular weight 80,000) is a cationic iron carrier, very similar to transferrin, that is present in high concentrations in human breast milk (1 mg/ml). It binds two atoms of ferric iron per molecule and is found in neutrophilic granulocytes and on mucosal surfaces as part of their protective coat. Lactoferrin is believed to participate in the defence of the breast-fed infant against infection by depriving bacteria of the iron needed for growth, and by donating iron to generate

reactive oxygen radicals to enhance the microbicidal mechanisms of phagocytes.’ (pg 2)

‘Iron is required for the growth of many pathogenic microorganisms, but under normal conditions iron binding proteins in the host prevent the metal becoming available to the organisms. This probably prevents the growth of many pathogens *in vivo*.... Metabolic activity associated with activation of the immune system is a process requiring iron. Iron deficiency, and perhaps also iron overload, may impair immune function. Severe iron deficiency is associated with reduced T lymphocyte and neutrophil function.’ (pg 138)

6) Taste

Code	Proposed statement
<i>Fe6:</i>	<i>Iron contributes to normal taste function.</i>

Reference 1.1:

‘Iron deficiency is also associated with abnormalities of the mucosa of the mouth and gastrointestinal tract leading to angular stomatitis, glossitis, esophageal webs, and chronic gastritis (Jacobs, 1971)...The eating of nonfood material (pica) or a craving for ice (pagophagia) are also associated with iron deficiency (Ansell and Wheby, 1972).’ (pg 300)

Reference 2.0:

‘An intriguing sensory disturbance encountered both in children and adults who are iron-deficient is the perversion of taste leading to the consumption of nonfood items (pica) or compulsive ice eating (pagophagia). The specificity of the association has been confirmed by the study of patients of whom iron deficiency was induced by phlebotomy alone, making the contribution of confounding nutritional and social factors unlikely. It is corrected by iron repletion.’ (pg 1158)

8) Neurological development in embryos

Code	Proposed statement
<i>Fe8:</i>	<i>Iron is necessary for normal neurological development in embryos.</i>

Reference 1.1:

‘Studies of iron deficiency anemia and behaviour in the developing human and in animal models suggest persistent functional changes. Investigators have demonstrated lower mental and motor test scores and behavioural alterations in infants with iron deficiency anemia (Idjradinata and Pollitt, 1993; Lozoff et al., 1982a, 1982b, 1985, 1987, 1996; Noakes et al., 1998; Walter et al., 1989).’ (pg 296)

‘Specific central nervous system processes (e.g., slower nerve conduction and impaired memory) appear to remain despite correction of the iron deficiency anemia. There is a general lack of specificity of effect and of information about which brain regions are adversely affected. Recent data from Chile showed a decreased nerve conduction velocity in response to an auditory signal in formerly iron-deficient

children despite haematological repletion with oral iron therapy (Roncagliolo et al., 1998). This is strongly suggestive evidence for decreased myelination of nerve fibers, though other explanations could also exist. Current thinking about the impact of early iron deficiency anemia attributes some role for “functional isolation”, a paradigm in which the normal interaction between stimulation and learning from the physical and social environment is altered (Pollitt et al., 1993; Strupp and Levitsky, 1995).’ (pg 297)

Reference 2.0:

‘Recently and increasing body of evidence connecting iron deficiency in early childhood with impaired psychomotor development and cognitive function has accumulated. While the nature and the extent of the problem remains controversial, there is considerable cause for concern. Animal studies indicate that a brief period of iron deficiency in young animals reduces brain iron content. The later administration of iron readily corrects body iron stores but has much less effect on brain iron. Some recent observations suggest that long-term effects on behaviour and cognitive function resulting from iron deficiency in early childhood may not be corrected completely by later iron administration.’ (pg 1158)

Reference 4.16:

‘Consequences of functional iron deficiency which may occur without anaemia, include adverse effects on work capacity, intellectual performance, behaviour...’ (pg 24)

Reference 8.2:

‘Although there are methodological and conceptual difficulties surrounding all the studies discussed here, and the results are not always in the same direction, there is considerable support for the conclusion that iron deficiency sufficient to cause anaemia is associated with impaired performance in children. There is sufficient evidence also to indicate the possibility that iron deficiency without anaemia may impair functioning.’ (pg 71-72)

Iron deficient children have often been reported to be irritable, listless, perceptually restricted, distractible and apathetic. Walter et al. (1989a) found that iron deficient anaemic infants were more unhappy than controls. Infants with low ferritin values were more fearful, less attentive and more vocal (Deinhard et al., 1986). Lozoff et al found that anaemic infants were more withdrawn, hesitant, fearful and tense and less reactive. These behaviours undoubtedly influence test performance and the children’s general ability to make use of their environments.’ (pg 77-78)

‘The functioning brain is dependent on several iron-related process, of which efficient cell mitochondrial oxidative activity and neuronal synaptic transmission are among the most crucial. Behavioural abnormalities in human infants with iron deficiency which may be, but are not always reversed by subsequent iron supplementation, are indicative of iron-related disturbances in neurological function. However the consequences of low iron intakes on cognitive functioning in adults and senile neurodegenerative processes remain to be determined.’ (pg 90)

‘Iron is involved in the synthesis and degradation of catecholamines and serotonin, which act as neurotransmitters. Monoamine oxidases (MAO) are involved in the

catabolism of catecholamines and their activity is reduced in iron deficient rats (Youdim et al., 1980). Symes et al. (1971) found tissue levels of MAO activity were reduced to 60% of control levels in iron deficient rats and were restored to normal with iron supplementation. Reduced levels have also been demonstrated in platelets from iron deficient humans. (Youdim et al., 1975). Noradrenaline acts as a neurotransmitter at sympathetic nerve terminals and is metabolized by MAO's. If MAO activity is reduced, an increased excretion of noradrenaline occurs in the urine. Voorhess et al. demonstrated such increased excretion in iron deficient children which is responsive to iron therapy (Voorhess et al., 1975). Levels of noradrenaline in the urine did not vary directly with haemoglobin, iron or saturation. Children with anaemia due to other causes, e.g. thalassaemia, did not have elevated noradrenaline excretion, which suggests that iron deficiency per se was responsible, rather than anaemia.' (pg 90)

'The balance of evidence from intervention studies supports a causal relationship between iron deficiency anaemia and impaired mental and psychomotor performance.' (pg 139)

'Iron performs a crucial role in the functioning of the brain involving key metabolic enzymic reactions associated with mitochondrial oxidation, neurotransmitter synthesis and myelin formation.' (pg 140)

ANNEX 4.17

Copper

Source documents for reviewing copper

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.16:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Copper. March 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out176_en.pdf).

Reference 4.17:

Draft Review of Copper. Expert Group on Vitamins and Minerals. May 2002.
(<http://www.food.gov.uk/multimedia/pdfs/reviewofcopper.pdf>).

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Connective tissues

Code Proposed statement

Cu1: Copper contributes to the normal structure of connective tissues (such as in bone, lungs and the vascular system).

Reference 1.1:

‘Lysyl oxidase uses lysine and hydroxylysine found in collagen and elastin as substrates for posttranslational processing to produce cross-linkages needed for the development of connective tissues, including those of bone, lung and the circulatory system.’ (pg 225)

‘Physiologic consequences resulting from copper deficiency include defects in connective tissue that lead to vascular and skeletal problems, ...’ (Harris, 1997; Turnlund, 1999).’ (pg 226)

‘Osteoporosis was observed in copper-deficient infants and growing children.’ (pg 227)

‘A report of changes in some, but not other, markers of bone metabolism with a dietary copper intake of 700 µg/day deserves further investigation (Baker et al., 1999).’ (pg 229)

‘Lysyl oxidase activity in the skin, which declined with low dietary copper and increased with repletion, is potentially a useful indicator of copper status (Werman et al., 1997).’ (pg 232)

Reference 2.0:

‘It is well established that copper is required for ...skeletal health, for ... Several amine and diamine oxidases contain copper. Lysyl oxidase is particularly important for proper crosslinking of collagen and elastin; thus, copper deficiency, particularly in animals is associated with bone and vascular abnormalities.’ (pg 443)

Reference 3.16:

‘Studies have also shown that copper is required for infant growth, ...bone strength, ...’(Uauy *et al.*, 1998) (pg 2)

‘...copper deficiency are similar to those seen in experimental animals and include ... bone abnormalities (Danks, 1988), ...’ (pg 5)

Reference 4.17:

‘Copper is an essential trace element. Animal and human studies have shown that copper is involved in the function of several enzymes (Olivares and Uuay, 1996), including cytochrome *c* oxidase, amino acid oxidase, superoxide dismutase and monoamine oxidase. Studies have also shown that copper is required for infant growth, ... bone strength...’ (pg 6)

‘The most usual clinical manifestations of acquired copper deficiency are ...bone abnormalities (Percival, 1995).’ (pg 6)

Reference 6.3:

‘Features of Cu deficiency in infants and the young include leucopenia and skeletal fragility and ...’ (pg 171)

2) Transport and metabolism of iron

Code	Proposed statement
Cu2:	<i>Copper contributes to the normal transport and metabolism of iron in the body.</i>

Reference 1.1:

‘Ferroxidases are copper enzymes found in plasma, with a function in ferrous iron oxidation ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$) that is needed to achieve iron’s binding to transferrin (Linder and Hazegh-Azam, 1996).’ (pg 225)

‘Ferroxidase I, also called ceruloplasmin, is the predominant copper protein in plasma and may also have antioxidant functions. Defects in ceruloplasmin function produce cellular iron accumulation, a result that supports its ferroxidase role (Harris and

Gitlin, 1996).’ Ferroxidase II is found in human plasma, but it may have a role in iron metabolism in specific cellular sites. A transmembrane copper containing protein (hephaestatin) with ferroxidase activity has been described (Pena et al., 1999; Vulpe et al., 1999).’ (pg 225)

‘Physiologic consequences resulting from copper deficiency include ...anemia associated with defective iron utilization, and ...(Harris, 1997; Turnlund, 1999).’ (pg 226)

Reference 2.0:

‘It is well established that copper is required for... iron metabolism, and...’ (pg 443)

‘Copper deficiency has been mistaken for iron deficiency because of the low haemoglobin values. However, the anaemia of copper deficiency does not respond to iron supplements and hence is called ‘iron refractory’ anaemia.’ (pg 445)

Reference 3.16:

‘Studies have also shown that copper is required for ... iron transport...(Uauy *et al.*, 1998).’ (pg 2)

‘...copper deficiency are similar to those seen in experimental animals and include anaemia, ...’ (pg 5)

Reference 4.17:

‘Copper is an essential trace element. Animal and human studies have shown that copper is involved in the function of several enzymes (Olivares and Uuay, 1996), including cytochrome *c* oxidase, amino acid oxidase, superoxide dismutase and monoamine oxidase. Studies have also shown that copper is required for ...iron transport, ...’ (pg 6)

‘The most usual clinical manifestations of acquired copper deficiency are anemia, ... (Percival, 1995).’ (pg 6)

Reference 6.3:

‘Anaemia may develop if deficiency is prolonged and severe.’ (pg 171)

3) Red blood cells

Code Proposed statement

Cu3: Copper contributes to the normal structure of red blood cells.

Reference 1.1:

‘Symptoms accompanying the copper deficiency included normocytic, hypochromic anemia, leukopenia, and neutropenia (Fujita et al., 1989).’ (pg 227)

Reference 2.0:

‘It is well established that copper is required for ...formation of red blood cells.’ (pg 443)

'The low copper diet was associated with low red cell superoxide dismutase activity, low platelet cytochrome *c* oxidase activity, low red cell glutathione peroxidase activity and elevated plasma factor VIII.' (pg 444)

Reference 3.16:

'Studies have also shown that copper is required for ... red and white cell maturation, ... (Uauy *et al.*, 1998) (pg 2)

Reference 4.17:

'Copper is an essential trace element. Animal and human studies have shown that copper is involved in the function of several enzymes (Olivares and Uauy, 1996), including cytochrome *c* oxidase, amino acid oxidase, superoxide dismutase and monoamine oxidase. Studies have also shown that copper is required for ...red and white cell maturation, ...' (pg 6)

4) Energy production

Code Proposed statement

Cu4: Copper is necessary for normal energy production

Reference 1.1:

'Cytochrome *c* oxidase is a multisubunit enzyme in mitochondria that catalyzes reduction of O₂ to H₂O. This establishes a high-energy proton gradient required for adenosine triphosphate (ATP) synthesis. This copper enzyme is particularly abundant in tissues of greatest metabolic activity including heart, brain and liver.'(pg 225)

Reference 2.0:

'Copper functions as a component of enzymes and proteins in the human body summarizes those copper enzymes known in mammals. ... Cytochrome *c* oxidase is essential for energy production in mitochondria...' (pg 443)

5) Antioxidant activity

Code Proposed statement

Cu5: Copper contributes to cell protection from the damage caused by free radicals.

Reference 1.1:

'Ferroxidase I, also called ceruloplasmin, is the predominant copper protein in plasma and may also have antioxidant functions.' (pg 225)

'Copper/zinc superoxide dismutase (Cu/Zn SOD) uses two copper atoms for conversion of the superoxide anion (O₂⁻) to H₂O₂ and O₂. Zinc atoms have a structural role in the enzyme. The enzyme is localized in the cytosol and, along with the mitochondrial manganese-containing form, provides a defense against oxidative damage from superoxide radicals that, if uncontrolled, can lead to other damaging reactive oxygen species.' (pg 225)

‘The role of copper as an antioxidant has led to interest in the possibility that copper deficiency impairs antioxidant status (Johnson et al., 1992).’ (pg 229)

‘Ceruloplasmin carries between 60 and 95 percent of serum copper. ... The dietary copper intake at which ceruloplasmin concentration no longer increases in response to increased dietary copper might be considered the copper requirement for ceruloplasmin synthesis. Ceruloplasmin is an acute phase protein and increases markedly with a number of diseases, including liver disease, malignancy, inflammatory diseases, myocardial infarction, and a variety of infectious diseases (Mason, 1979). It also increases with pregnancy and oral contraceptive use.’ (pg 230)

Reference 2.0:

‘Copper functions as a component of enzymes and proteins in the human body summarizes those copper enzymes known in mammals. ... Superoxide dismutases function as antioxidants. ’ (pg 443)

‘The low copper diet was associated with low red cell superoxide dismutase activity, ..., low red cell glutathione peroxidase activity and ...’ (pg 444)

Reference 3.16:

‘It is well established that the trace element copper is essential for life. Copper in living organisms, including humans, forms an essential component of many enzymes (cuproenzymes) and proteins. The biochemical role for copper is primarily catalytic, with many copper metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen, for example cytochrome-C-oxidase and superoxide dismutase.’ (pg 2)

Reference 4.17:

‘Copper is an essential trace element. Animal and human studies have shown that copper is involved in the function of several enzymes (Olivares and Uuay, 1996), including ... superoxide dismutase and monoamine oxidase.’ (pg 6)

Reference 6.3:

‘Copper (Cu) is a component of many enzymes, including cytochrome oxidase, and superoxide dismutase (Cu/Zn SOD)’ (pg 171)

6) Neurological system

Code Proposed statement

Cu6: Copper is necessary for normal neurological function.

Reference 1.1:

‘Monoamine oxidase (MAO) is important in serotonin degradation to excretable metabolites and in the metabolism of catecholamines (epinephrine, norepinephrine, and dopamine). MAO inhibitors are used as antidepressant drugs.’ (pg 225)

‘Dopamine β monooxygenase uses ascorbate, copper, and O₂ to convert dopamine to norepinephrine, a neurotransmitter, produced in neuronal and adrenal gland cells.

Dopa, a precursor of dopamine, and metabolites used in melanin formation are oxidatively produced from tyrosine by the copper enzyme tyrosinase.’ (pg 225)

‘ α -Amidating monooxygenase (α -AE, uses copper and ascorbate to remove two carbons from a C-terminal glycine of peptides, thus generating an amide. A number of peptide hormones are postrationally modified by α -AE (Harris, 1997).’ (pg 225)

‘Physiologic consequences resulting from copper deficiency include ...and possibly specific aspects of central nervous system dysfunction (Harris, 1997; Turnlund, 1999).’ (pg 226)

‘Changes in catecholamine metabolism have been investigated, but results are inconsistent (Bhathena et al., 1998).’ (pg 229)

Reference 2.0:

‘Copper functions as a component of enzymes and proteins in the human body summarizes those copper enzymes known in mammals. ... Several amine and diamine oxidases contain copper...Copper can also bind to low molecular weight ligands such as amino acids and small peptides.’ (pg 443)

Reference 3.16:

‘Copper plays additional roles that are less well understood and may be in part non-enzymatic, such as in angiogenesis, nerve myelination and endorphin action (Linder and Hazegh-Azam, 1996).’ (pg 2)

Reference 6.3:

‘Synthesis of a range of neuroactive amines and peptides (eg catecholamines and enkephalins) also involves Cu enzymes.’ (pg 171)

7) Immune system

Code Proposed statement

Cu7: Copper is necessary for the normal function of the immune system.

Reference 1.1:

‘Some evidence suggests that immune and cardiac dysfunction occurs in experimental copper deficiency and the development of such signs of deficiency has been demonstrated in infants (Graham and Cordano, 1969; Olivares and Uauy, 1996; Turnlund, 1999).’ (pg 226)

‘Symptoms accompanying the copper deficiency included ... leukopenia, and neutropenia (Fujita et al., 1989).’ (pg 227)

‘An index of immune function declined in a depletion study with copper intakes of 380 μ g/day that resulted in decreases in indexes of copper status, but other indexes of immune function did not decline and repletion did not result in reversal of the change (Kelley et al., 1995)’ (pg 229)

‘Ceruloplasmin carries between 60 and 95 percent of serum copper ... The dietary copper intake at which ceruloplasmin concentration no longer increases in response to increased dietary copper might be considered the copper requirement for ceruloplasmin synthesis. Ceruloplasmin is an acute phase protein and increases markedly with a number of diseases, including liver disease, malignancy, inflammatory diseases, myocardial infarction, and a variety of infectious diseases (Mason, 1979).’ (pg 230)

‘Leukocyte copper concentration was found to decline along with other indexes of copper status in one study (Turnlund et al., 1997), but it has not been reported in others.’ (pg 232)

Reference 2.0:

‘It is well established that copper is required for proper functioning of the immune system,’ (pg 443)

Reference 3.16:

‘Studies have also shown that copper is required for red and white cell maturation, ... (Uauy et al., 1998).’ (pg 2)

‘...copper deficiency are similar to those seen in experimental animals and include ..., neutropaenia (Williams, 1983) ... while less frequent signs are ... increased incidence of infections (Castillo-Duran et al., 1983), alterations of phagocytic capacity of neutrophils (Heresi et al., 1985) ...’ (pg 5)

Reference 4.17:

‘Copper is an essential trace element. Animal and human studies have shown that copper is involved in the function of several enzymes (Olivares and Uauy, 1996), including cytochrome *c* oxidase, amino acid oxidase, superoxide dismutase and monoamine oxidase. Studies have also shown that copper is required for ...red and white cell maturation, ...’ (pg 6)

‘The most usual clinical manifestations of acquired copper deficiency are ..., neutropenia and ... (Percival, 1995).’ (pg 6)

Reference 6.3:

‘Features of Cu deficiency in infants and the young include leucopenia and skeletal fragility and increased susceptibility to respiratory tract and other infections.’ (pg 171)

‘Apart from neutropenia, such signs are seen rarely. Studies with adults suggest that early features of Cu deficiency can include defects in cardiovascular function.’ (pg 171)

8) Fetal development

Code	Proposed statement
Cu8:	<i>Copper contributes to the normal development of the fetus, including the brain.</i>

Reference 1.1:

‘Ceruloplasmin is an acute phase protein and increases markedly with a number of diseases,... It also increases with pregnancy and oral contraceptive use.’ (pg 230)

Reference 2.0:

‘In experimental animals, some disorders of the vascular and nervous systems induced by copper deficiency during prenatal development cannot be reversed by copper supplementation after birth. Thus, it is absolutely essential that sufficient copper be provided during pregnancy.’ (pg 443)

‘Menkes’ syndrome is an X-linked disorder that results in mental deterioration, hypothermia, failure to thrive, connective tissue disorders and death in early childhood. The degree of neuronal degeneration at birth suggests that the disease begins *in utero* and that copper is required for development of the nervous system in the foetus.’ (pg 446)

Reference 3.16:

‘Studies have also shown that copper is required for infant growth... (Uauy *et al.*, 1998).’ (pg 2)

‘...copper deficiency are similar to those seen in experimental animals ... less frequent signs are ... impaired growth (Castillo-Duran and Uauy, 1988)...’ (pg 5)

Reference 4.17:

‘Studies have also shown that copper is required for infant growth...and brain development.’ (pg 6)

9) Allergic reaction

Code Proposed statement

Cu9: Copper contributes to the normal control of an allergic reaction.

Reference 1.1:

‘Diamine oxidase inactivates histamine released during allergic reactions.’ (pg 224, 225)

Reference 2.0:

‘Diamine oxidase: Inactivates histamine and polyamines; highest activity in small intestine; also high activity in kidney and placenta.’ (Table 2, pg 444)

10) Cholesterol and glucose metabolism

Code Proposed statement

Cu10: Copper contributes to the normal metabolism of glucose and cholesterol.

Reference 1.1:

‘A report of increased blood cholesterol concentrations in one young man consuming 830 µg/day of copper (Klevay et al., 1984) suggested that elevated blood cholesterol concentration may be associated with marginal amounts of dietary copper. This effect was not observed in other subjects or in a number of other studies with this or lower levels of dietary copper. In one study, blood cholesterol concentration decreased with lower dietary copper (Milne and Nielsen, 1996), and in a copper supplementation study investigators found increased blood cholesterol concentrations with supplementation (Medeiros et al., 1991).’ (pg 228)

‘Glucose tolerance was lower in two of a group of eight men consuming 80 µg/day of copper than in men consuming higher levels of copper (Klevay et al., 1986), but similar observations have not been reported at lower intakes of copper in other studies.’ (pg 229)

Reference 2.0:

‘... the possible role of copper in other functions such as the regulation of cholesterol, glucose ... are not well understood.’ (pg 443)

Reference 3.16:

‘Studies have also shown that copper is required for ... cholesterol and glucose metabolism (Uauy et al., 1998).’ (pg 2)

‘...copper deficiency are similar to those seen in experimental animals and include anaemia, neutropaenia (Williams, 1983) and bone abnormalities (Danks, 1988), while less frequent signs are ... abnormalities of glucose (Klevay et al., 1986) and cholesterol metabolism (Reiser et al., 1987).’ (pg 5)

Reference 4.17:

‘Studies have also shown that copper is required for ... cholesterol and glucose metabolism, ...’ (pg 6)

11) Coagulation

Code Proposed statement

Cu11: Copper contributes to the normal structure of blood clots.

Reference 1.1:

‘Changes in blood clotting factors V and VIII were observed in one study with copper intakes of 570 µg/day (Milne and Nielsen, 1996).’ (pg 229)

‘... research suggests that platelet copper concentration and platelet cytochrome *c* oxidase activity, when measured in controlled studies, may be more sensitive to changes in copper dietary intake.’ (pg 231)

Reference 2.0:

‘... the possible role of copper in other functions such as ...blood clotting are not well understood.’ (pg 443)

‘Copper-binding proteins include metallothionein, albumin, transcuprein, and blood clotting factors V and VIII.’ (pg 443)

‘The low copper diet was associated with ..., low platelet cytochrome *c* oxidase activity, ... and elevated plasma factor VIII.’ (pg 444)

12) Skin, hair pigment

Code	Proposed statement
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<i>Cu12:</i>	<i>Copper is necessary for the normal colouration of skin and hair.</i>
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Reference 1.1:

‘Dopamine β monoxygenase uses ascorbate, copper, and O₂ to convert dopamine to norepinephrine, a neurotransmitter, produced in neuronal and adrenal gland cells. Dopa, a precursor of dopamine, and metabolites used in melanin formation are oxidatively produced from tyrosine by the copper enzyme tyrosinase.’ (pg 225)

Reference 2.0:

‘During copper deficiency in animals with dark hair, the deficiency of tyrosinase can lead to changes in hair colour.’ (pg 443)

‘Involved in melanin synthesis; deficiency of this enzyme in skin leads to albinism; catalyses conversion of tyrosine to dopamine and oxidation of dopamine to dopaquinone. Present in eye and skin and forms colour in hair, skin and eyes.’ (Table 2, pg 444)

Reference 3.16:

‘...copper deficiency are similar to those seen in experimental animals and include anaemia, neutropaenia (Williams, 1983) and bone abnormalities (Danks, 1988), while less frequent signs are hypopigmentation (Danks, 1988),...’ (pg 5)

Table 2: Human enzymes and proteins that contain copper

Enzyme or protein	Function
Cytochrome c oxidase	Mitochondrial enzyme involved in the electron transport chain; reduces oxygen to water and allows formation of ATP; activity is highest in the heart and also high in the brain, liver and kidney
Caeruloplasmin (ferroxidase 1)	Glycoprotein with six to seven copper atoms; four copper atoms involved in oxidation/reduction reactions; role of other copper atoms not fully know; scavenges free radicals; quencher of superoxide radicals generated in the circulation; oxidizes some aromatic amines and phenols; catalyses oxidation of ferrous iron to ferric iron; assists with iron transport from storage to sites of haemoglobin synthesis; about 60% of plasma copper bound to caeruloplasmin; primarily extracellular; activity will be low during severe copper restriction
Ferroxidase 11	Catalyses oxidation of iron; no other functions known; in human plasma is only about 5% of ferroxidase activity
Monoamine oxidase	Inactivates catecholamines; reacts with serotonin, noradrenaline, tyramine, and dopamine; activity inhibited by some antidepressant medications.
Diamine oxidase	Inactivates histamine and polyamines; highest activity in small intestine; also high activity in kidney and placenta
Lysyl oxidase	Acts on lysine and hydroxylysine found in immature collagen and elastin: important for integrity of skeletal and vascular tissue; use of oestrogen increases activity
Dopamine β -hydroxylase	Catalyses conversion of dopamine to noradrenaline, a neurotransmitter; contains two to eight copper atoms; important in brain and adrenal glands
Copper, zinc superoxide dismutase	Contains two copper atoms; primarily in cytosol, protects against oxidative damage by converting superoxide ion to hydrogen peroxide; erythrocyte levels are somewhat responsive to changes in copper intake
Extracellular superoxide dismutase	Protects against oxidative damage by scavenging superoxide ion radicals and converting them to hydrogen peroxide; small amounts in plasma; larger amounts in lungs, thyroid and uterus.
Tyrosinase	Involved in melanin synthesis; deficiency of this enzyme in skin leads to albinism; catalyses conversion of tyrosine to dopamine and oxidation of dopamine to dopaquinone. Present in eye and skin and forms colour in hair, skin and eyes
Metallothionein	Cysteine rich protein that binds zinc, cadmium, and copper; important for sequestering metal ions and preventing toxicity
Albumin	Binds and transports copper in plasma and interstitial fluids; about 10-15% of copper in plasma is bound to albumin
Transcuprein	Binds copper in human plasma; may transport copper
Blood clotting factors V and VIII	Role in clotting and thrombogenesis not well understood; part of structure homologous with caeruloplasmin

Adapted from Linder (1996) and Turnlund (1995).

(Reference 2.0, pg 444)

ANNEX 4.18

Iodine

Source documents for reviewing iodine

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.17:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Iodine. September 2002. (http://www.europa.eu.int/comm/food/fs/sc/scf/out146_en.pdf).

Reference 4.18:

Revised Review of Iodine. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0006p.pdf>)

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Production of thyroid hormones

Code	Proposed statement
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II:	<i>Iodine is necessary for the normal production of thyroid hormones.</i>
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Reference 1.1:

‘Iodine is an essential component of the thyroid hormones, throxine (T4) and triiodothyronine (T3), comprising 65 and 59 percent of their respective weights. Thyroid hormones, and therefore iodine, are essential for mammalian life. They regulate many key biochemical reactions, especially protein synthesis and enzymatic activity. Major target organs are the developing brain, muscle, heart, pituitary, and kidney.’ (pg 258)

‘Once in the circulation, iodine is removed principally by the thyroid gland and the kidney. The thyroid selectively concentrates iodide in amounts required for adequate thyroid hormone synthesis, and most of the remaining iodine is excreted in urine. Several other tissues can also concentrate iodine, including salivary glands, breast,

choroid plexus, and gastric mucosa. Other than the lactating breast, these are minor pathways of uncertain significance.’ (pg 259)

‘Iodide in the thyroid gland participates in a complex series of reactions to produce thyroid hormones. Thyroglobulin, a large glycoprotein of molecular weight 660,000, is synthesized within the thyroid cell and serves as a vehicle for iodination. Iodide and thyroglobulin meet at the apical surface of the thyroid cell. There thyroperoxidase and hydrogen peroxide promote the oxidation of the iodide and its simultaneous attachment to tyrosyl residues within the thyroglobulin molecule to produce the hormone precursors diiodotyrosine and monoiodotyrosine. Thyroperoxidase further catalyzes the intramolecular coupling of two molecules of diiodotyrosine to produce tetraiodothyronine (T_4). A similar coupling of one monoiodotyrosine and one diiodotyrosine molecule produces triiodothyronine (T_3).’ (pg 259, 260)

‘About two-thirds of thyroglobulin’s iodine is in the form of the inactive precursors, monoiodotyrosine and diiodotyrosine. This iodine is not released into the circulation, but instead is removed from the tyrosine moiety by a specific deiodinase and then recycled within the thyroid gland. This process is an important mechanism for iodine conservation, and individuals with impaired or genetically absent deiodinase activity risk iodine deficiency.’ (pg 260)

‘Thyroid enlargement (goiter) is usually the earliest clinical feature of iodine deficiency. It reflects an attempt to adapt the thyroid to the increased need, brought on by iodine deficiency, to produce thyroid hormones.’ (pg 262)

Reference 2.0:

‘...it is the critical importance of iodine in the formation of the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) that makes any discussion of this element and human physiology of necessity bound up with a review of thyroid function.’ (pg 1138)

‘Iodine entering the circulation is actively trapped by the thyroid gland. This remarkable capacity to concentrate iodine is a reflection of the fact that the most critical physiological role for iodine is the normal functioning of the thyroid gland.’ (pg 1139)

‘In addition to trapping iodine, follicular cells also synthesize the glycoprotein, thyroglobulin (Tg), from carbohydrates and amino acids (including tyrosine) obtained from the circulation. Thyroglobulin moves into the lumen of the follicle where it becomes available for hormone production. ... Thyroglobulin is very concentrated in the follicles through a process of compaction, making the concentration of iodine in the thyroid gland very high. ...This remarkable ability of the thyroid to concentrate and store iodine allows the gland to be very rapidly responsive to metabolic needs for thyroid hormones.’ (pg 1139)

‘Separating the role of iodine from the complex and pervasive function of the thyroid gland is difficult since iodine is a critical component of the hormones that mediate these functions, and whatever other roles iodine may have are poorly understood. Thyroid hormones affect a wide range of physiological functions, from liver and kidney to heart and brain. Earlier work supported a role for thyroid hormones in affecting the energy generating capacity of cells through biochemical changes in mitochondria. More recent work has shown, however, that these hormones act on specific genetic receptors in cell nuclei, and perhaps through other extranuclear mechanisms. The nuclear receptors belong to a large family of receptors that bind other extranuclear molecules including vitamins A and D and steroids. Through this interaction, along with a number of other proteins, thyroid hormones modify genetic expression. A great deal of research currently focuses on these thyroid hormone receptors, and the effect primarily of T₃ on the physiological function of the target organ through genetic transcription. These receptors are present in pituitary, liver, heart, kidney and brain cells.’ (pg 1140, 1141)

‘In the most simplistic physiological model, inadequate intake of iodine results in a reduction in thyroid hormone production, which stimulates increased TSH production. TSH acts directly on thyroid cells, and without the ability to increase hormone production, the gland becomes hyperplastic.’ (pg 1142)

Reference 4.18:

‘Iodine deficiency is therefore partly compensated for by the larger epithelial cells and the enlarged thyroid gland. This enlargement is known as a goitre.’ (pg 10)

‘Goitre occurs when in response to low circulating concentrations of T₄, thyrotrophin (TSH) secretion by the pituitary is increased stimulating both iodide uptake into the thyroid and enlargement of the gland itself.’(pg 10)

2) Growth

Code	Proposed statement
I2:	<i>Iodine is necessary for normal growth.</i>

Reference 1.1:

‘The so-called iodine deficiency disorders (IDD) include mental retardation, hypothyroidism, goiter, cretinism, and varying degrees of other growth and developmental abnormalities. These result from inadequate thyroid hormone production from lack of sufficient iodine.’ (pg 261)

Reference 2.0:

‘In the pituitary gland, thyroid hormones, along with many cofactors, regulate the synthesis and secretion of growth hormone by increasing gene transcription. Similarly, as part of the feedback loop for hormone regulation and release, thyroid hormones affect transcription of TSH in the pituitary.’ (pg 1141)

‘Myxoedematous cretinism presents as disturbances of growth and development including short stature, coarse facial features, retarded sexual development, mental retardation and other signs of hypothyroidism.’ (pg 1143)

‘Iodine as a trace element in low concentrations in most environments plays a critical role in the normal growth and development of many species.’ (pg 1145)

Reference 3.17:

‘... clinical effects are seen at all stages of development and are particularly noticeable in the foetus, the neonate and the infant as goitre, this being the commonest cause of human thyroid disease.’ (pg 9)

‘Foetal iodine deficiency. ... The major hazard is endemic cretinism associated with iodine intakes of <25 µg/day. The more common neurological type is characterised by mental deficiency, deaf mutism, spastic diplegia, the less common myxoedematous type by apathy, hypothyroidism, puffy features, growth retardation, delayed bone maturation, retarded sexual maturation and dwarfism.’(pg 9)

‘Iodine deficiency in children. ... School performance and IQ’s are impaired even if allowance is made for confounding factors. Growth is reduced and psychomotor development lags behind normal children noticeable already from age 2.5 years onward.’(pg 10)

3) Neurological development

Code Proposed statement

I3: Iodine is necessary for normal neurological development.

Reference 1.1:

‘ The so-called iodine deficiency disorders (IDD) include mental retardation, hypothyroidism, goiter, cretinism, and varying degrees of other growth and developmental abnormalities. These result from inadequate thyroid hormone production from lack of sufficient iodine.’ (pg 261)

‘The most damaging effect of iodine deficiency is on the developing brain. Thyroid hormone is particularly important for myelination of the central nervous system, which is most active in the perinatal period and during fetal and early postnatal development. Numerous population studies have correlated an iodine-deficient diet with increased incidence of mental retardation.... The effects of iodine deficiency on brain development are similar to those of hypothyroidism from any other cause. ... Iodine treatment can reverse cretinism especially when the treatment is begun early (Klein et al., 1972). Cretinism is an extreme form of neurological damage from fetal hypothyroidism. It occurs in severe iodine deficiency and is characterized by gross mental retardation along with varying degrees of short stature, deaf mutism and spasticity.’ (pg 261, 262)

Reference 2.0:

‘In the adult brain, receptors have been identified, but the specific genes affected by thyroid hormones have not yet been located. However, in the developing brain of the fetus and neonate, the effects of thyroid hormones are significant even though the exact mechanisms are still not fully understood. The effects of thyroid hormones on brain development are suggested by failure in development of the nerve elements, failure of differentiation of cerebellar cells, and reduced development of other brain cells, in hypothyroid states. It is this early effect that has recently elevated the status of iodine from an element whose deficiency caused goitre, to one whose deficiency is the leading cause of mental impairment worldwide.’ (pg 1141)

‘There may also be a direct effect of thyroid hormones on brain enzymatic activity....In addition, thyroid hormones stimulate growth and development and, as noted earlier are critical for the normal proliferation, growth and development of brain cells....Iodine deficiency is the most common cause of preventable mental retardation in the world.’ (pg 1141, 1142)

‘The most important clinical effect of deficiency relates to the fact that thyroid hormone is required for the normal development of the brain in both humans and other animals. Numerous studies have demonstrated reduced psychomotor skills and intellectual development in the presence of iodine deficiency, and most experts now believe that there is a continuum of deficits, from mild impairment in IQ to severe mental retardation. ... In Europe, where mild deficiency still exists, studies have demonstrated decreased psychomotor, perceptual integrative motor ability as well as lower verbal IQ scores in schoolchildren. Studies in Iran showed similar findings. A recent meta-analysis of 18 studies demonstrated a strong relationship, with an overall 13.5 IQ point difference between deficient and nondeficient populations. These findings, coupled with the high prevalence of deficiency in many countries, have major implications for development.’ (pg 1142, 1143)

‘The most severe effect of iodine deficiency is cretinism, which is rare in areas of mildly endemic deficiency, but may have reached 5-10% or more in areas with severe deficiency. There are general classifications of cretinism, the symptoms of which frequently overlap. Neurological cretinism presents as extreme mental retardation, deaf-mutism, and impaired motor function including spastic gait. Myxoedematous cretinism presents as disturbances of growth and development including short stature, coarse facial features, retarded sexual development, mental retardation and other signs of hypothyroidism. It appears likely that severe deficiency resulting in decreased maternal T₄ may be responsible for the impaired neurological development of the fetus occurring early in pregnancy. The effect of deficiency on the fetus after 20 weeks’ gestation may result in hyperstimulation of the developing fetal thyroid, with the extreme manifestation being thyroid failure causing myxoedematous cretinism. Other factors may affect thyroid hormone metabolism.’ (pg 1143)

'In humans, iodine is critical for brain development and correction of global deficiencies is an unparalleled opportunity to improve the wellbeing of our global community.' (pg 1145)

Reference 3.17:

'... in the integrity of the connective tissue, and are necessary for optimum cellular metabolism particularly during early growth, development and maturation of most organs especially the brain.' (pg 6)

'... clinical effects are seen at all stages of development and are particularly noticeable in the foetus, the neonate and the infant as goitre, this being the commonest cause of human thyroid disease.' (pg 9)

'Foetal iodine deficiency: ... The major hazard is endemic cretinism associated with iodine intakes of <25 µg/day. The more common neurological type is characterised by mental deficiency, deaf mutism, spastic diplegia, the less common myxoedematous type by apathy, hypothyroidism, puffy features, growth retardation, delayed bone maturation, retarded sexual maturation and dwarfism.'"(pg 9)

'Neonatal iodine deficiency: This is associated with increased perinatal and neonatal mortality and more frequent congenital abnormalities. It constitutes a threat to early brain development with consequent physical and mental retardation and possible later depressed cognitive and motor performance.' (pg 10)

'Iodine deficiency in children: ... School performance and IQ's are impaired even if allowance is made for confounding factors. Growth is reduced and psychomotor development lags behind normal children noticeable already from age 2.5 years onward.'"(pg 10)

Reference 4.18:

'Receptors binding T3 and T4 have been found in the nucleus where they are involved in protein synthesis and in the mitochondria, suggesting that there are many sites of action (Cavalieri, 1980) for thyroid hormones. In the developing foetus and infant, iodine is necessary for the development of the nervous system.' (pg 9)

Reference 6.3:

'In the fetus I is necessary for the development of the nervous system during the first three months of gestation; infants born to severely deficient mothers are likely to suffer from cretinism.' (pg 183)

4) Energy metabolism

Code	Proposed statement
I4:	<i>Iodine is necessary for normal metabolism.</i>

Reference 2.0:

‘Carbohydrate metabolism and formation of certain fats (lipogenesis) are affected through hormone-induced changes in gene transcription in liver cells.’ (pg 1141)

‘Thyroid hormones stimulate glucose transport, and again though originally attributed to a direct action on the plasma membrane, recent evidence suggests a genetic mechanism. There may also be a direct effect of thyroid hormones on brain enzymatic activity.’ (pg 1141)

‘The overall effects of these cellular and systemic actions is to stimulate respiratory and other enzyme synthesis, which results in increased oxygen consumption and resultant increased basal metabolic rate. This affects heart rate, respiratory rate, mobilization of carbohydrates, cholesterol metabolism, and a wide variety of other physiological activities.’ (pg 1141)

Reference 3.17:

‘The biological function of the thyroid hormones T₄, T₃ and of iodotyrosines encompasses the regulation of energy metabolism and endocrine function by cellular oxidation, calorogenesis, thermoregulation, intermediate metabolism, protein and enzymes synthesis, nitrogen retention, gluconeogenesis and pituitary gonadotropins. Thyroid hormones also play a role in the intestinal absorption of glucose and galactose, in lipolysis and in the uptake of glucose by adipocytes,’ (pg 6)

‘... T₃ and T₄ may interact at receptor and gene expression level with sex hormones. They may upregulate hepatic oestrogen receptor levels in the rat. They also regulate the same subset of genes involved in lipid homeostasis (NNT, 2002).’ (pg 6)

‘Iodine deficiency disorders (IDD) in adults ... Hypothyroidism (myxoedema), another form of IDD, also results from hormone deficiency and is associated with reduced metabolic rate, cold intolerance, weight gain, puffy face, oedema, hoarse voice and mental sluggishness.’ (pg 9)

Reference 4.18:

‘... iodine forms part of the hormones thyroxine (T₄) and triiodothyronine (T₃) which are necessary for the maintenance of metabolic rate, cellular metabolism and integrity of connective tissue (COMA, 1991)’ (pg 9)

Reference 6.3:

‘Iodine (I) forms part of the hormones thyroxine (T₄) and triiodothyronine (T₃), which are necessary for the maintenance of metabolic rate, cellular metabolism and ...’ (pg 183)

ANNEX 4.19

Zinc

Source documents for reviewing zinc

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.18:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Zinc. March 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out177_en.pdf).

Reference 4.19:

Revised Review of Zinc. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/reviewofzinc.pdf>).

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Immune system

Code	Proposed statement
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Zn1:	<i>Zinc is necessary for the normal function of the immune system.</i>
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Reference 1.1:

‘Zinc also provides a structural function for some enzymes; copper-zinc superoxide dismutase is the most notable example. In this instance, copper provides catalytic activity, whereas zinc’s role is structural. Also of potential relevance as a structural role is the essentiality of zinc for intracellular binding of tyrosine kinase to T-cell receptors, CD4 and CD8 α , which are required for T-lymphocyte development and activation (Hurse et al., 1998; Lin et al., 1998).’ (pg 443)

‘Up-regulation of metallothionein by specific cytokines and some hormones suggests a function that is critical to a stress response.’ (pg 444)

‘Other functions that respond to zinc supplementation include ...immune function (Bogden et al., 1987). Evidence of the efficacy of zinc lozenges in reducing the duration of common colds is still unclear (Jackson et al., 2000).’ (pg 447)

‘Zinc is essential of the integrity of the immune system, and inadequate zinc intake has many adverse effects (Shankar and Prasad, 1998).’ (pg 453)

Reference 2.0:

‘The cytokine mediators of the acute-phase response to injury, infection or inflammation produce a profound decrement of up to 60% in plasma zinc, sequestering the entire loosely bound fraction in liver. A teleological explanation that zinc is assisting in the rapid hepatic production of acute-phase proteins has been proffered for this redistribution.’ (pg 1968)

‘There is evidence for regulation of the expression of other genes by heavy metals, notably zinc, in systems related to the transcription of certain acute-phase proteins. ...Sperm counts and sex hormone levels will be low in men, resulting in problems of libido and fertility. Immunosuppression of various parts of the host defence system can also be seen on laboratory evaluation.’ (pg 1969)

‘The hypozincaemia of infection is, like iron, part of the acute-phase response mediated by cytokines such as interleukin 1, which in this case induces the synthesis of the intracellular zinc-binding protein metallothioneine. As a result, zinc is rapidly removed from the circulation and taken up by cells in the liver, thymus and bone marrow. The physiological role of this acute transfer of zinc from the extracellular to the intracellular compartment is not clear but it may be related to the dependency of DNA transcription and RNA translation on zinc metalloenzymes. Because of this, shifts of zinc to the intracellular compartment of lymphocytes could help increase the proliferative efficiency of lymphocytes involved in the immune response to infections and thus enhance host defence. Zinc deficiency, on the other hand, might be expected to diminish the lymphocyte proliferative response and impair host defence.’ (pg 1083)

Severe zinc deficiency has significant clinical and biological effects. ... In addition, defects in cell-mediated immunity and increased susceptibility to infections are well described in the human disease acrodermatitis enteropathica, and a similar syndrome exists in Friesian cattle. ... Experimental zinc deprivation in animals results in thymic involution, splenic atrophy and lymphopenia. As a consequence the proliferative response to both T dependent and T independent mitogens is reduced. In addition to the inhibitory effect of zinc deficiency on DNA synthesis, the depressed lymphocyte proliferative response may be related to altered cytokine metabolism. Whatever the mechanism, zinc deficiency leads to depression of delayed hypersensitivity responses.’ (pg 1083-1084)

‘Zinc also plays a role in regulating the activation of acute-phase genes via its ability to bind to RNA finger loop domains known as ‘zinc fingers’ involved in the

conformational stabilization of transcription factor proteins that allow sequence-specific DNA recognition and gene expression. If zinc deficiency blocks these regulatory signals as well, host responses may be altered as the translation of genes normally activated during the acute-phase reaction is impaired. Another essential role of zinc in the immune response is its binding to certain thymus-derived peptides that appear to function as hormones in the differentiation of T cells. Reversible inhibition of thymulin function has been described in human zinc deficiency. Several studies have reported that the synthesis of thymic peptide hormones is decreased in protein-energy malnutrition (which is almost always accompanied by zinc deficiency), suggesting that this defect may underlie the decreased number of mature T cells and increased number of immature T cells found in the circulation of these patients. Similar mechanisms may lead to the depletion of mature T cells observed in other zinc deficiency states, and heighten susceptibility to infection.’ (pg 1084)

‘A few studies have suggested that zinc status may affect the production and/or membrane binding of certain cytokine regulators of immune responses, including interleukins 1 and 2, and interferon. Systematic studies of zinc status, cytokines and immune function will help to unravel these interactions.’ (pg ’ (pg 1084)

‘Although zinc plays an essential role in biology and immunology and there is a relatively large amount of zinc in the body, there is no physiologically regulated store of the metal. Continuous ingestion of zinc is necessary to sustain zinc-dependent functions at normal levels.’ (pg 1084)

‘Because of the consistent finding of the dependency of normal immune function on adequate zinc availability, the association of zinc deficiency with increased infection morbidity is explicable. ... Community-based prospective zinc supplementation of poorly nourished infants and children, and therapeutic trials in acutely ill children, suggest a decrease in susceptibility to diarrhoea, respiratory infection and possibly malaria in the former studies, and a more rapid improvement in patients in some of the latter studies.’ (pg 1084)

Reference 3.18:

‘Zinc is essential for ... and immunocompetence.’ (pg 2)

‘The main clinical manifestations of zinc deficiency are ..., increased susceptibility to infections, ...’ (pg 4)

‘Symptoms of mild/marginal zinc deficiency include delayed wound healing, impaired resistance to infection and ... (Walsh *et al.*, 1994; WHO, 1996).’ (pg 4)

Reference 4.19:

‘Zinc has many diverse functions in the human body, being essential for ...and immunocompetence.’ (pg 12)

‘In addition, zinc is recognised for its anti-infective and anti-cancer properties (Walsh *et al.*, 1994; Vallee and Fulchuk, 1993 and references therein).’ (pg 12)

Reference 6.3:

‘Early features of deficiency include ... defects of rapidly dividing tissues such as ... and the immune system.’ (pg 167)

2) Cell division

Code Proposed statement

Zn2: *Zinc is necessary for normal cell division.*

Reference 1.1:

‘The structural role of zinc involves proteins that form domains capable of zinc coordination, which facilitates protein folding to produce biologically active molecules. The vast majority of such proteins form a “zinc finger-like” structure created by chelation centers, including cysteine and histidine residues (Klug and Schwabe, 1995). Some of these proteins have roles in gene regulation as dioxyribonucleic acid binding transcription factors. Examples include nonspecific factors such as Sp1 and specific factors such as retinoic acid receptors and vitamin D receptors. These structural motifs are found throughout biology and include the zinc-containing nucleocapsid proteins of viruses such as the human immunodeficiency virus (Berg and Shi, 1996).’ (pg 443)

‘The role of zinc as a regulator of gene expression has received less attention than its other functions. Metallothionein expression is regulated by a mechanism that involves zinc’s binding to the transcription factor, metal response element transcription factor (MTF1), which activates gene transcription (Cousins, 1994; Dalton et al., 1997).’ (pg 443)

‘Zinc transporter proteins associated with cellular zinc accumulation and release may be along the metal response element–regulated family of genes (McMahon and Cousins, 1998). Zinc has been shown to influence both apoptosis and protein kinase C activity (McCabe et al., 1993; Telford and Fraker, 1995; Zalewski et al., 1994), which is within the regulatory function. The relationship of zinc to normal synaptic signaling processes also falls within the regulatory role (Cole et al., 1999). The most widely studied MTF-regulated gene is the metallothionein gene. An unequivocal function has not been established, but this metalloprotein appears to act as a zinc trafficking molecule for maintaining cellular zinc concentrations (Cousins, 1996) and perhaps as part of a cellular redox system for zinc donation to zinc finger proteins (Jacob et al., 1998; Roesijadi et al., 1998). Upregulation of metallothionein by specific cytokines and some hormones suggests a function that is critical to a stress response.’ (pg 443, 444)

‘Monocyte metallothionein messenger RNA responds rapidly to in vivo zinc supplementation (Sullivan et al., 1998) and merits additional research.’ (pg 453)

Reference 2.0:

‘Zinc exercises part of its physiological role as a component of zinc metalloenzymes. The first to be characterized was carbonic anhydrase. Alcohol dehydrogenase was the second. The number of enzymes in which zinc is claimed to be a tightly coordinated, essential constituent for the structural conformation, catalytic function or both (i.e. a metalloenzyme) ranges from 200 to 700. ... Pancreatic proteases such as carboxypeptidases contain zinc. Many of the enzymes involved in transcription and translation of the genetic message and in the synthesis of nucleotide bases are zinc metalloenzymes.’ (pg 1969)

‘A pervasive role for zinc’s regulation of both genetic and protein-protein interactions has emerged in the advent of our understanding of the ‘zinc finger’ motif of regulatory proteins. This is a clustered sequence along a peptide chain with four cysteine residues alternating with other amino acids (or with cysteine and histidine residues); it allows zinc to form a tetrahedral complex, providing structural rigidity to the protein. The knuckling of the protein strand around this complex provides the origin of the term ‘zinc finger’. Zinc finger domains are found in regulatory proteins. Some of the proteins are transcription factors in the nucleus that are sensitive to hormonal messengers such as retinoic acid or vitamin D₃. Zinc fingers are found in cytosolic proteins as well, suggesting a role for zinc in protein-protein interaction or cellular translocation of proteins and organelles.’ (pg 1969)

‘There is evidence that ionic zinc plays a role in membrane stabilization and in the assembly of polyribosomes. Soluble zinc may also be important in the regulation of gene expression, specifically of metallothioneine (MT), in which zinc inhibits the expression of MT. A subcellular mechanism involves (1) a metal regulatory element as part of the promoter of the regulatory gene for MT expression on the DNA strand of the chromosome; and (2) a metal-binding transcription factor which is sensitive to – and binds – zinc in the cell cytosol and nucleus. This function may be more generalized than just the regulation of zinc. There is evidence for regulation of the expression of other genes by heavy metals, notably zinc, in systems related to the transcription of certain acute-phase proteins.’ (pg 1969)

‘Zinc also plays a role in regulating the activation of acute-phase genes via its ability to bind to RNA finger loop domains known as ‘zinc fingers’ involved in the conformational stabilization of transcription factor proteins that allow sequence-specific DNA recognition and gene expression. If zinc deficiency blocks these regulatory signals as well, host responses may be altered as the translation of genes normally activated during the acute-phase reaction is impaired.’ (pg 1084)

Reference 3.18:

‘Additionally, it maintains the configuration of a number of non-enzyme proteins such as ..., some mammalian gene transcription proteins (Struhl, 1989) and’ (pg 2)

Reference 4.19:

‘Zinc is also an important component of DNA, acting to stabilise phosphate groups and co-ordinate with bases. About 1% of the human genome codes for zinc finger proteins that play an important regulatory function in gene expression.’ (pg 12)

Reference 6.3:

‘It is an essential component of a number of enzymes in which it has structural, regulatory, or catalytic roles. Additionally, it has a structural role in a number of non-enzymic proteins for example in maintaining ...the configuration of mammalian gene transcription proteins, and ...’ (pg 167)

3) Enzyme function

Code Proposed statement

Zn3: *Zinc is necessary for the normal function of numerous enzymes.*

Reference 1.1:

‘Nearly 100 specific enzymes (e.g., EC 1.1.1.1 alcohol dehydrogenase) depend on zinc for catalytic activity. Zinc removal results in loss of activity, and reconstitution of the holoenzyme with zinc usually restores activity. Examples of zinc metalloenzymes can be found in all six enzyme classes (Vallee and Galles, 1984). Well-studied zinc metalloenzymes include the ribonucleic acid (RNA) polymerases, alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase. Zinc is defined as a Lewis acid, and its action as an electron acceptor contributes to its catalytic activity in many of these enzymes.’ (pg 443)

‘In mild human zinc deficiency states, the detectable features and laboratory/functional abnormalities of mild zinc deficiency are diverse. This diversity is not altogether surprising in view of the biochemistry of zinc and the ubiquity of this metal in biology with its participation in an extra-ordinarily wide range of vital metabolic processes. Impaired growth velocity is a primary clinical feature of mild zinc deficiency and can be corrected with zinc supplementation (Hambidge et al., 1979b; Walrevens et al., 1989).’ (pg 446,447)

‘...the activities of zinc-dependent enzymes, including alkaline phosphatase, copper-zinc superoxide dismutase, and lymphocyte 5'-nucleotidase, can at most serve as supportive indicators of dietary zinc requirements ...’ (pg 453)

Reference 2.0:

‘Zinc exercises part of its physiological role as a component of zinc metalloenzymes. The first to be characterized was carbonic anhydrase. Alcohol dehydrogenase was the second. The number of enzymes in which zinc is claimed to be a tightly coordinated, essential constituent for the structural conformation, catalytic function or both (i.e. a metalloenzyme) ranges from 200 to 700. ... Pancreatic proteases such as carboxypeptidases contain zinc. Many of the enzymes involved in transcription and

translation of the genetic message and in the synthesis of nucleotide bases are zinc metalloenzymes.’ (pg 1069)

Reference 3.18:

‘Over 300 zinc enzymes have been discovered covering all six classes of enzymes and in different species of all phyla (Christianson, 1991; Coleman, 1992; Vallee and Auld, 1990). Zinc has structural, regulatory or catalytic roles in many enzymes (Vallee and Galdes, 1984; Hambridge et al., 1986).’ (pg 2)

4) Growth

Code	Proposed statement
Zn4:	<i>Zinc contributes to normal growth.</i>

Reference 1.1:

‘In mild human zinc deficiency states, the detectable features and laboratory/functional abnormalities of mild zinc deficiency are diverse. This diversity is not altogether surprising in view of the biochemistry of zinc and the ubiquity of this metal in biology with its participation in an extra-ordinarily wide range of vital metabolic processes. Impaired growth velocity is a primary clinical feature of mild zinc deficiency and can be corrected with zinc supplementation (Hambidge et al., 1979b; Walrevens et al., 1989).’ (pg 446,447)

‘Because of the ubiquity of zinc and the involvement of this micronutrient in so many core areas of metabolism, it is not surprising that the feature of zinc deficiency are frequently quite basic and nonspecific, including growth retardation, alopecia, diarrhea, delayed sexual maturation and impotence, eye and skin lesions, and impaired appetite. Clinical features and laboratory criteria are not always consistent.’ (pg 447)

‘... studies of the effects of zinc supplementation on physical growth velocity in children are useful in evaluating dietary zinc requirements for several reasons...First, confirmation of the effect of zinc supplements on growth velocity (linear growth and weight) in children with varying degrees of growth retardation has been shown in a number of studies from many countries (Brown et al., 1998; Umeta et al., 2000). Second, because a sufficient number of these studies have been undertaken in North America, growth is applicable as a functional/clinical indicator of zinc requirement in North American children (Gibson et al., 1989; Walravens and Hambidge, 1976; Walrevens et al., 1983, 1989). Third, baseline dietary data typically included in these studies are adequate to use for group analyses.’ (pg 449, 450)

‘Zinc supplementation has been associated with an increase in both circulating IGF-1 concentration and growth velocity (Ninh et al., 1996).’ (pg 454)

Reference 2.0:

‘What could be termed ‘mild’ deficiency would not be detected on clinical examination. It represents functions that are being limited by a less than adequate availability of zinc, probably at the level of the rapidly exchangeable intra and extracellular pools. Growth retardation and delayed development of children is one manifestation. ... Teratogenesis is worth mentioning in the context of mild human zinc deficiency. The adverse effects of experimental maternal deficiency of zinc on embryonic and fetal development and on the birthing process have been well defined in rodent and small ruminant models. It has been suggested that up to half of the idiopathic anatomical birth defects in human newborns worldwide, including those of the neural tube, could be the result of marginal zinc status of the mothers.’ (pg 1969)

‘Severe deficiency leads to anatomical and physiological ‘lesions’ that can be documented in a review of symptoms and an examination of the patient for physical signs. The growth effects are more profound, and frank failure to thrive may be manifest in infants and toddlers.’ (pg 1969)

‘The hypozincaemia of infection is, like iron, part of the acute-phase response mediated by cytokines such as interleukin 1, which in this case induces the synthesis of the intracellular zinc-binding protein metallothioneine. As a result, zinc is rapidly removed from the circulation and taken up by cells in the liver, thymus and bone marrow. The physiological role of this acute transfer of zinc from the extracellular to the intracellular compartment is not clear but it may be related to the dependency of DNA transcription and RNA translation on zinc metalloenzymes. Because of this, shifts of zinc to the intracellular compartment of lymphocytes could help increase the proliferative efficiency of lymphocytes involved in the immune response to infections and thus enhance host defence. Zinc deficiency, on the other hand, might be expected to diminish the lymphocyte proliferative response and impair host defence.’ (pg 1083)

Reference 3.18:

‘Zinc is essential for growth and development, ...’ (pg 2)

‘The main clinical manifestations of zinc deficiency are growth retardation, delay in sexual maturation, diarrhoea, increased susceptibility to infections, dermatitis, the appearance of behavioural change and alopecia.’ (pg 4)

‘Symptoms of mild/marginal zinc deficiency include delayed wound healing, impaired resistance to infection and reduced growth rate (Walsh *et al.*, 1994; WHO, 1996).’ (pg 4)

Reference 4.19:

‘Zinc has many diverse functions in the human body, being essential for growth and development,’ (pg 12)

‘Zinc is also essential for the activities of the ..., growth and sex hormones and’ (pg 12)

Reference 6.3:

'Early features of deficiency include growth retardation, ...' (pg 167)

5) Neurological function

Code **Proposed statement**
Zn5: *Zinc contributes to normal brain function.*

Reference 1.1:

'... nonspecific laboratory functional tests of zinc status (e.g., tests of neuro-cognitive function [Sandstead et al., 1988]) ...' (pg 449)

Reference 2.0:

'... Neurological and mental status examinations may reveal seizures in zinc-deficient infants...' (pg 1969)

Reference 3.18:

'Zinc is essential for ... neurological function, ...' (pg 2)

Reference 4.19:

'Zinc has many diverse functions in the human body, being essential for ... neurological function, ...' (pg 12)

'Zinc is an absolute requirement for normal brain development and' (pg 12)

6) Insulin action

Code **Proposed statement**
Zn6: *Zinc is necessary for the normal synthesis and action of insulin.*

Reference 3.18:

'Additionally, it maintains the configuration of a number of non-enzyme proteins such as pre-secretory granules of insulin,' (pg 2)

Reference 4.19:

'Zinc plays a key role in the synthesis and action of insulin. Hyperzincuria (increased zinc excretion due to hypozincaemia) may arise from the effect diabetes has on zinc homeostasis (Chausmer, 1998).' (pg 12)

'Zinc is also essential for the activities of the thymic, growth and sex hormones and for glucagon and insulin.' (pg 12)

Reference 6.3:

'It is an essential component of a number of enzymes in which it has structural, regulatory, or catalytic roles. Additionally, it has a structural role in a number of non-

enzymic proteins for example in maintaining the aggregation of the presecretory insulin granules and' (pg 167)

7) Reproductive development

Code	Proposed statement
Zn7:	<i>Zinc contributes to normal reproductive development.</i>

Reference 1.1:

'Because of the ubiquity of zinc and the involvement of this micronutrient in so many core areas of metabolism, it is not surprising that the feature of zinc deficiency are frequently quite basic and nonspecific, including ...delayed sexual maturation and ... Clinical features and laboratory criteria are not always consistent.' (pg 447)

Reference 2.0:

'Hormonal influences also affect zinc concentration in the circulation: oestrogens lower zinc levels.' (pg 1068)

'Sperm counts and sex hormone levels will be low in men, resulting in problems of libido and fertility.' (pg 1969)

Reference 3.18:

'Zinc is essential for growth and development, testicular maturation... ' (pg 2)

'The main clinical manifestations of zinc deficiency are ...delay in sexual maturation,... ' (pg 4)

Reference 4.19:

'Zinc has many diverse functions in the human body, being essential for ...testicular maturation,... ' (pg 12)

'Zinc is also essential for the activities of the thymic, growth and sex hormones and for glucagon and insulin.' (pg 12)

'Zinc is an absolute requirement for normal brain development and for the reproductive process.' (pg 12)

8) & 9) Skin and wound healing

Code	Proposed statement
Zn8:	<i>Zinc contributes to the normal structure of skin and normal wound healing.</i>
Zn9:	<i>Zinc contributes to normal wound healing.</i>

Reference 2.0:

‘There is evidence that ionic zinc plays a role in membrane stabilization and in the assembly of polyribosomes.’ (pg 1969)

‘Zinc deficiency has dermatological manifestations ranging from mild, generalized drying to a specific hyperkeratosis in the areas of pressure and stress points such as over elbow and knee joints and in the perioral area.’ (pg 1969)

Reference 3.18:

‘Zinc is essential for ...wound healing ... Over 300 zinc enzymes have been discovered covering all six classes of enzymes and in different species of all phyla (Christianson, 1991; Coleman, 1992; Vallee and Auld, 1990). Zinc has structural, regulatory or catalytic roles in many enzymes (Vallee and Galdes, 1984; Hambridge *et al.*, 1986).’ (pg 2)

‘Symptoms of mild/marginal zinc deficiency include delayed wound healing, impaired resistance to infection and reduced growth rate (Walsh *et al.*, 1994; WHO, 1996)...The main clinical manifestations of zinc deficiency are ... dermatitis,’ (pg 4)

Reference 4.19:

‘Zinc has many diverse functions in the human body, being essential for ...skin integrity, wound healing ...’ (pg 12)

Reference 6.3:

‘It is an essential component of a number of enzymes in which it has structural, regulatory, or catalytic roles. Additionally, it has a structural role in a number of non-enzymic proteins for example in ...the integrity of biomembranes.’ (pg 167)

‘Early features of deficiency include ... defects of rapidly dividing tissues such as skin, intestinal mucosa, and ...’ (pg 167)

10) Antioxidant activity

Code	Proposed statement
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Zn10:	<i>Zinc contributes to cell protection from the damage caused by free radicals.</i>
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Reference 1.1:

‘...the activities of zinc-dependent enzymes, including alkaline phosphatase, copper-zinc superoxide dismutase, and lymphocyte 5'-nucleotidase, can at most serve as supportive indicators of dietary zinc requirements ...’ (pg 453)

Reference 3.18:

‘Additionally, it maintains the configuration of a number of non-enzyme proteins such as pre-secretory granules of insulin, some mammalian gene transcription proteins (Struhl, 1989) and thymulin. Well known zinc containing enzymes include superoxide dismutase, alkaline phosphatase and alcohol dehydrogenase.’ (pg 2)

Reference 4.19:

‘Zinc is a cofactor of the superoxide dismutase enzymes, which play an important antioxidant role in the detoxication of reactive oxygen species (ROS).’ (pg 12)

‘In addition, zinc is recognised for its anti-infective and anti-cancer properties (Walsh *et al.*, 1994; Vallee and Fulchuk, 1993 and references therein).’ (pg 12)

ANNEX 4.20

Manganese

Source documents for reviewing manganese

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.19:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Manganese. October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80f_en.pdf).

Reference 4.20:

Review of Manganese – Revised Version. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm9922p.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

1) Bone

Code	Proposed statement
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<i>Mn1:</i>	<i>Manganese contributes to normal bone formation.</i>
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Reference 1.1:

‘Manganese is an essential nutrient involved in the formation of bone and in amino acid cholesterol, and carbohydrate metabolism. Manganese metalloenzymes include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase. Glycosyltransferases and xylosyltransferases, which are important in proteoglycan synthesis and thus bone formation, are sensitive to manganese status in animals.’ (pg 394)

‘... manganese deficiency interferes with normal skeletal development in various animal species (Freeland-Graves, 1994; Hurley and Keen, 1987; Keen et al., 1994).’ (pg 396)

‘Decreased plasma manganese concentrations have been reported in osteoporotic women. Furthermore, bone mineral density was improved when trace minerals, including manganese, were included with calcium in their diets or supplements (Freeland-Graves and Turnlund, 1996; Strause and Saltman, 1987; Strause et al., 1986, 1987).’ (pg 396)

Reference 2.0:

‘Manganese deficiency has been demonstrated in several species, including rats, mice, pigs and cattle. Signs of manganese deficiency include impaired growth, skeletal abnormalities, ...’ (pg 1263)

‘The effects of manganese deficiency on bone development have been studied extensively. In most species, manganese deficiency can result in shortened and thickened limbs, curvature of the spine, and swollen and enlarged joints. The basic biochemical defect underlying the development of these bone defects is a reduction in the activities of glycosyltransferase; these enzymes are necessary for the synthesis of the chondroitin sulfate side chains of proteoglycan molecules. In addition, manganese deficiency in adult rats can result in an inhibition of both osteoblast and osteoclast activity. This observation is particularly noteworthy, given the reports that women with osteoporosis tend to have low blood manganese concentrations and that the provision of manganese supplements might be associated with an improvement in bone health in postmenopausal women.’ (pg 1263)

‘There is considerable debate as to the extent to which manganese deficiency affects humans under free living conditions. Manganese deficiency can be induced in humans under highly controlled experimental conditions. In one study, manganese deficiency was induced in adult male subjects by feeding a manganese-deficient diet (<0.1 mg Mn per day) for 39 days. The subjects developed temporary dermatitis, as well as increased serum calcium and phosphorus concentrations and increased alkaline phosphatase activity, suggestive of bone resorption.’ (pg 1264)

Reference 3:

‘Manganese has been shown to be essential for various species. It is a component of arginase, pyruvate carboxylase and superoxide dismutase and plays a role as co-factor of certain enzyme systems. Accordingly, manganese-deficient animals exhibit adverse effects, e.g. impaired growth, skeletal abnormalities,’ (pg 2)

Reference 5.0:

‘Formation of bone and the growth of other connective tissues.’ (pg 93)

‘... only one description of an unequivocal case of human manganese deficiency has been reported. A child with a postoperative short bowel receiving over 90% of her nutrition parenterally, which was low in manganese, developed short stature and brittle bones. Because manganese deficiency has been so difficult to identify in humans, manganese is considered not of nutritional concern.’ (pg 1467)

2) Energy metabolism

Code	Proposed statement
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<i>Mn2:</i>	<i>Manganese contributes to normal energy metabolism.</i>
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Reference 1.1:

‘Manganese is an essential nutrient involved in ... amino acid, cholesterol, and carbohydrate metabolism. Manganese metalloenzymes include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase.’ (pg 394)

‘Manganese deficiency has been observed in various species of animals with the signs of deficiency, including ...alterations in carbohydrate and lipid metabolism.’ (pg 396)

‘In a manganese depletion study, ...(Friedman et al., 1987). ... Plasma cholesterol concentrations declined during the depletion period, perhaps because manganese is required at several sites in the biosynthetic pathway of cholesterol (Krishna et al., 1966).’ (pg 396)

Reference 2.0:

‘Pyruvate carboxylase, the enzyme that catalyses the first step of carbohydrate synthesis from pyruvate, contains 4 mol Mn²⁺ per mol enzyme.’ (pg 1262)

‘An exception to the nonspecific manganese activation of enzymes is the manganese-specific activation of enzymes is the manganese-specific activation of glycosyltransferases. Several manganese deficiency-induced pathologies have been attributed to a low activity of this enzyme class. A second example of an enzyme that may be specifically activated by manganese is phosphoenolpyruvate carboxykinase (PEPCK), the enzyme which catalyses the conversion of oxaloacetate to phosphoenolpyruvate, GDP and CO₂.’ (pg 1263)

‘A third example of a manganese-activated enzyme is glutamine synthetase. This enzyme, found in high concentrations in the brain, catalyses the reaction NH₃ + glutamate + ATP → glutamine + ADP + P_i.’ (pg 1263)

‘Manganese deficiency has been demonstrated in several species, including rats, mice, pigs and cattle. Signs of manganese deficiency include ... defects in lipid and carbohydrate metabolism.’ (pg 1263)

Reference 3.19:

‘Manganese has been shown to be essential for various species. It is a component of arginase, pyruvate carboxylase and superoxide dismutase and plays a role as co-factor of certain enzyme systems. Accordingly, manganese-deficient animals exhibit adverse effects, e.g. ... defects in lipid and carbohydrate metabolism.’ (pg 2)

Reference 4.20:

'Manganese is a component of enzymes such as pyruvate carboxylase, mitochondrial superoxide dismutase and arginase. It also activates many other enzymes including hydrolases, glycosyl transferases, kinases, prolinase and phosphotransferases (COMA, 1991). A number of the enzymes activated by manganese are non-specific and can be activated by other metal ions, particularly Mg^{2+} and are not significantly affected during manganese deficiency (ILSI 1994). Glycosyl transferases are specifically activated by manganese. Manganese is involved in both carbohydrate and lipid metabolism (Leach and Lilburn , 1978).' (pg 4)

Reference 5.0:

'Activator of various enzymes in the metabolism of carbohydrates, fats, proteins and nucleic acids.' (pg 93)

'Cholesterol synthesis.' (pg 93)

'Signs of Deficiency: Biochemical – Possible signs include hypocholesterolemia, and increased serum calcium, phosphorus and alkaline phosphatase activity.' (pg 1471)

'Manganese is a cofactor for enzymes involved in protein and energy metabolism, antioxidant action, and mucopolysaccharide synthesis. These enzymes include the metalloenzymes manganese-dependent superoxide dismutase, pyruvate carboxylase and arginase, and the manganese-activated enzymes phosphoenolpyruvate carboxykinase, glucosyl transferases, glutamine synthetase, and farnesyl pyrophosphate synthetase.' (pg 1471)

3) Antioxidant activity

Code	Proposed statement
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<i>Mn3:</i>	<i>Manganese contributes to cell protection from the damage caused by free radicals.</i>
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Reference 1.1:

'Manganese-deficient animals have low manganese-superoxide dismutase (MnSOD) activity (Davis et al., 1992; Malecki et al., 1994; Zidenberg-Cherr et al., 1983). Davis and Greger (1992) demonstrated that lymphocyte MnSOD activity was elevated in 47 women supplemented with 15 mg/day of manganese for more than 90 days.' (pg 401)

Reference 2.0:

'MnSOD catalyses the disproportionation of O_2 to H_2O_2 and O_2 . The essential role of MnSOD in the normal biological function of tissues has been clearly demonstrated by the homozygous inactivation of the SOD2 gene for MnSOD in mice.' ...It has been shown that this decrease in MnSOD activity after manganese deficiency is owing to the downregulation of MnSOD at the (pre)-transcriptional level, supporting a role for manganese in the control of this enzyme by a mechanism of gene activation. Tissue MnSOD activity can be increased by several diverse stressors including

alcohol, ozone, irradiation, interleukin 1 and tumour necrosis factor α , presumably as a consequence of stressor-associated increases in cellular free radical (or oxidized target(s)) concentrations.’ (pg 1262, 1263)

Reference 3.19:

‘Manganese has been shown to be essential for various species. It is a component of ...and superoxide dismutase ...’ (pg 2)

Reference 5.0:

‘Manganese is a cofactor for enzymes involved in ..., antioxidant action, and These enzymes include the metalloenzymes manganese-dependent superoxide dismutase,’ (pg 1471)

4) pH regulation

Code	Proposed statement
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<i>Mn4:</i>	<i>Manganese contributes to the normal regulation of pH levels in the body.</i>
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Reference 2.0:

‘Arginase, the cystolic enzyme responsible for urea formation, contains 4 mol Mn^{2+} per mol of enzyme. Although the activity of this enzyme can be lower in manganese deficient animals than in controls, the functional significance of the reduction has not been defined; however, reductions in arginase activity owing to manganese deficiency have been shown to result in elevated plasma concentrations of ammonia and lowered plasma concentrations of urea. In addition, it has been found that manganese binding by arginase is critical for the pH-sensing function of this enzyme in the ornithine cycle, suggesting that manganese plays a role in the regulation of body pH.’ (pg 1262)

Reference 5.0:

‘Signs of Deficiency: Biochemical – Possible signs include ...increased serum calcium, phosphorus and alkaline phosphatase activity.’ (pg 1471)

5) Insulin action

Code	Proposed statement
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<i>Mn5:</i>	<i>Manganese contributes to the normal action of insulin, required for energy metabolism.</i>
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Reference 1.1:

‘Manganese deficiency has been observed in various species of animals with the signs of deficiency, including ... impaired glucose tolerance, and ...’ (pg 396)

Reference 2.0:

‘Defects in carbohydrate metabolism, in addition to those described above, have been shown in manganese-deficient rats and guinea pigs... In addition to its effect on pancreatic tissue integrity, manganese deficiency can directly impair pancreatic insulin synthesis and secretion as well as enhance intracellular insulin degradation. The mechanism (s) underlying the effects of manganese on pancreatic insulin metabolism have not been fully delineated, but they are thought to be multifactorial. For example, the flux of islet cell manganese from the cell surface to an intracellular pool may be a critical signal for insulin release. It is known that insulin mRNA levels are reduced in the deficient animal, which is consistent with the depressed insulin synthesis observed in these animals.’ (pg 1263, 1264)

‘Although the majority of studies concerning the influence of manganese deficiency on carbohydrate metabolism have been conducted with experimental animals, there is one report in the literature of an insulin-resistant diabetic patient who responded to oral doses of manganese (doses ranged from 5 to 10 mg) with decreasing blood glucose concentrations. While this is an intriguing case report, others have reported a lack of an effect of oral manganese supplements (up to 30 mg) in diabetic subjects, and low blood manganese concentrations have not been found to be a characteristic of diabetics.’ (pg 1264)

Reference 5.0:

‘Insulin action.’ (pg 93)

ANNEX 4.21

Sodium

Source documents for reviewing sodium

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michel J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.23:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Reference 4.21:

Review of Sodium Chloride. Expert Group on Vitamins and Minerals. April 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm0108p.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

Reference 6.4:

Nutritional Aspects of Cardiovascular Disease. Report of the Cardiovascular Review Group. Report on Health and Social Subjects No. 46. DH: The Stationery Office, 1994.

1) Water and electrolyte balance

Code	Proposed statement
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<i>Na1:</i>	<i>Sodium is necessary for normal water and electrolyte balance throughout the body.</i>
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Reference 2.0:

'Despite the fact that the body contains more calcium and potassium, sodium is arguably the most important cation because it dictates the volume of extracellular fluid (ECF) and its concentration affects osmotic concentration of both ECF and intracellular fluid (ICF). Abnormalities of ECF sodium concentration cause movement of water into or out of cells, thus altering the osmotic concentration of ICF in parallel and causing swelling or shrinkage of cells. The main impact of this is on the brain because its cells are rigidly enclosed by the cranium.' (pg 1774)

‘Sodium behaves physiologically as a cation, i.e. a positively charged ion; its distribution and effects are fairly independent of the negative ions (anions) which originally accompanied its ingestion though they affect its absorption and excretion. Most sodium is in ECF kept there by the sodium pump, an enzyme system, Na⁺/K⁺-exchanging ATPase, which uses substantial amounts of energy (adenosine triphosphate; ATP) in maintaining a low intracellular sodium concentration and a high intracellular potassium (K⁺) concentration. Sodium transport is a central issue in the physiology of sodium for a number of reasons:

1. It helps to maintain the ionic environment of ICF and the volume of ECF.
2. It prevents cell swelling (the Na⁺ efflux exceeds the K⁺ influx).
3. It establishes gradients which, in various tissues, allow transport of other cations in exchange, other anions in parallel or organic solutes – these are often cotransported with sodium down concentration gradients which are secondary to the low sodium environment created by the pump.’ (pg 1775)

‘The main effects of excess ECF volume are seen as expanded ISF, visible clinically as oedema (or ascites when fluid accumulates in the abdomen rather than the tissue spaces). Mild oedema is merely a cosmetic problem in itself but pulmonary or cerebral oedema, or severe ascites, are potentially serious forms. ...The main effect of inadequate ECF volume is to reduce plasma volume and thus to compromise cardiovascular function, in extreme cases by causing circulatory shock.’ (pg 1775)

Reference 3.23:

‘Sodium is the principal cation in extracellular fluid. Its physiological roles include the maintenance of (i) extracellular fluid volume (ECF), which is related closely to total body sodium content, (ii) extracellular fluid oncotic pressure, (iii) acid base balance, ... (pg 165)

Reference 4.21:

‘Sodium, along with potassium, is an essential mineral for regulating the body’s fluid balance. Sodium is the most abundant cation in the extracellular fluid and sodium salts account for more than 90% of the osmotically active solute in the plasma and interstitial fluid. Consequently, the sodium load is the major determinant of extracellular volume.’ (pg 5)

Reference 5.0:

‘Helps to maintain the balance of water, acids, and bases in the fluid outside the cells.’ (pg 88)

Reference 6.3:

‘Its physiological roles include the maintenance of (i) extracellular fluid (ECF) volume which is closely related to total body Na content, (ii) extracellular fluid oncotic pressure, (iii) acid base balance, ...’ (pg 152)

2) Blood pressure

Code **Proposed statement**
Na2: *Sodium contributes to normal blood pressure.*

Reference 2.0:

‘Excretion of excess sodium involves not only suppression of salt-retention mechanisms but also activation of sodium-shedding (natriuretic) mechanisms. Two types of hormones are involved: atrial natriuretic peptide (ANP), produced by the cardiac atria when they are overstretched (reduction of ECF volume being an appropriate response to cardiac overload), and active sodium transport inhibitors (ASTIs), probably produced within the brain. ... Atrial natriuretic peptide has various effects which essentially oppose those of the salt retention induced by aldosterone: it increases sodium excretion, lowers arterial pressure and promotes movement of ECF towards the interstitial compartment.’ (pg 1777)

‘...there are numerous studies which, when rigorously analysed, indicate that human arterial pressure and salt intake are positively correlated; sufficiently to anticipate reductions in the prevalence of hypertension in response to manageable reductions in dietary sodium.’ (pg 1777)

Reference 3.23:

‘A recent meta-analysis of studies of the relationship between sodium intake and blood pressure strongly implies that the causal association has been underestimated. When published epidemiological studies and clinical trials in economically advanced and non-advanced populations were analysed separately to minimise the socio-economic variables mentioned, there was apparent for both types of community, and amongst individuals within such communities, an association between sodium intake, and increasing systolic and diastolic blood pressures.’ (pg 166)

Reference 4.21:

‘Sodium deficiency is highly unusual, but can lead to low blood pressure, dehydration and muscle cramps.’ (pg 5)

Reference 5.0:

‘A large body of data relates salt intake to the level of blood pressure ... studies have convinced the medical community that salt may be responsible for the higher prevalence of hypertension in modern society compared to more primitive communities. However, sodium may not be the sole culprit. Studies suggest that the chloride anion with sodium is necessary for an increase in blood pressure, since giving sodium with other anions does not increase blood pressure.’ (pg 962)

Reference 6.4:

‘There is evidence for causal relationships between the consumption of sodium and both the level of blood pressure and the rise in blood pressure with age.’ (pg 12)

‘A meta-analysis by Law *et al* re-examined data from 24 previous studies around the world, although they did not include the Intersalt data on the grounds that the blood pressures measure in this study were lower than in other studies. ... The relationship observed in this analysis was considerably stronger than that seen in the Intersalt study. Furthermore, based on the relationship seen in the observational studies, Law *et al* were able to predict the reduction seen in clinical studies of sodium restriction.’ (pg 137)

3) Nerve and muscle

Code	Proposed statement
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<i>Na3:</i>	<i>Sodium is necessary for the normal function of nerves and muscle.</i>
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Reference 2.0:

‘Sodium transport is a central issue in the physiology of sodium for a number of reasons: ...It establishes the membrane voltages on which excitability and secretory activities frequently depend.’ (pg 1775)

Reference 3.23:

‘Sodium is the principal cation in extracellular fluid. Its physiological roles include the maintenance of ...(iv) electrophysiological phenomena in muscle, neuromuscular and nerve impulse transmission ...’ (pg 165)

Reference 4.21:

‘Sodium deficiency is highly unusual, but can lead to low blood pressure, dehydration and muscle cramps.’ (pg 5)

‘Sodium chloride is an essential mineral for the normal functioning of the body. It is very important for nerve conduction, muscle contractions, correct osmotic balance of ECF and absorption of glucose and other nutrients.’ (pg 5)

‘Passage of sodium through the sodium channels in excitable tissue is greatly increased by a decrease in membrane potential, and is therefore voltage gated in these tissues.’ (pg 7)

Reference 5.0:

‘Associated with muscle contraction and nerve functions.’ (pg 88)

‘Deficiency symptoms ... muscle cramps ...’ (pg 88)

Reference 6.3:

‘Its physiological roles include the maintenance of ...(iv) electrophysiological phenomena in muscle and nerves ...’ (pg 152)

4) Nutrient absorption

Code	Proposed statement
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<i>Na4:</i>	<i>Sodium is necessary for the normal absorption of nutrients during digestion.</i>
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Reference 3.23:

‘Sodium is the principal cation in extracellular fluid. Its physiological roles include the maintenance of ... (v) generation of transmembrane gradients essential for the energy-dependent carrier-mediated uptake of nutrients and substrates by cells, including hepatocytes and those in the intestinal mucosa and renal tubules.’ (pg 165)

Reference 4.21:

‘Sodium chloride is an essential mineral for the normal functioning of the body. It is very important for ... absorption of glucose and other nutrients.’ (pg 5)

Reference 5.0:

‘Plays a specific role in the absorption of carbohydrates.’ (pg 88)

‘As a constituent of pancreatic juice, bile, sweat and tears.’ (pg 88)

Reference 6.3:

‘Its physiological roles include the maintenance of ... (v) the generation of transmembrane gradients which enable the energy dependent uptake of nutrients (eg amino acids and hexoses) by cells including those of the intestinal mucosa and renal tubules.’ (pg 152)

5) Metabolic rate

Code	Proposed statement
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<i>Na5:</i>	<i>Sodium contributes to the body's normal metabolic rate.</i>
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Reference 2.0:

‘The energy expenditure of the pump is a substantial portion of total metabolic activity and contributes to thermogenesis.’ (pg 1775)

Reference 4.21:

Na^+ - K^+ ATPase utilises APT for energy and active transport of Na^+ and K^+ is one of the major energy-using processes in the body and accounts for a large part of the basal metabolism. The sodium-potassium pump is found in all parts of the body.’ (pg 7)

ANNEX 4.22

Potassium

Source documents for reviewing potassium

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michel J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.23:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Reference 4.22:

Revised review of Potassium. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/potassium.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Water and electrolyte balance

Code	Proposed statement
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K1:	<i>Potassium is necessary for normal water and electrolyte balance throughout the body.</i>
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Reference 2.0:

'Many of the functions of potassium are due to its ionic character, whereby it generates gradients of concentration, potential and pressure, and K⁺ participates in regulation of the acid-base balance. By virtue of being the predominant osmotically active species within cells, K⁺ plays a major role in the distribution of fluids inside and outside the cell and hence in the maintenance of cellular volume.' (pg 1578)

Reference 3.23:

'Potassium is predominantly an intracellular cation. This compartmentalisation is maintained by the energy-dependent cellular uptake of the element and simultaneous excretion of sodium by the cell membrane bound enzyme Na-K ATPase. This

process is fundamental to the cellular uptake of molecules against electrochemical and concentration gradients, ...' (pg 170)

Reference 4.22:

'Potassium, together with its close relative sodium, is important in maintaining normal osmotic pressure within cells.' (pg 8)

Reference 5.0:

'Intracellular potassium is the major cation responsible for establishing the membrane potential. The blood pressure of normotensives increases with potassium depletion. Observational studies suggest an inverse relationship between potassium intake and blood pressure. Often there is an inverse relationship with dietary potassium and sodium or a positive relationship between urinary Na⁺/K⁺ ration and blood pressure. ... Explanations for the hypotensive effects of potassium include direct vasodilatation, a direct natriuretic effect, altered baroreceptor function, increased urinary kallikrein, or suppression of the renin-angiotensin-aldosterone axis or sympathetic nervous system.' (pg 970)

2) Blood pressure

Code	Proposed statement
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K2:	<i>Potassium contributes to normal blood pressure.</i>
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Reference 2.0:

'A strong inverse association between potassium intake and hypertension and stroke has been described, suggesting the possibility that elevation of K⁺ inhibits free radical formation, smooth muscle proliferation and thrombus formation. In addition, potassium plays an important role in decreasing blood pressure under hypertensive conditions through some of the following actions; its natriuretic properties; the suppression of the sympathetic nervous and renin-angiotensin systems; a direct vasodilator effect; and an increase in glomerular filtration rate. Thus a high-potassium diet may prevent lesions of the artery walls and subsequent cerebral haemorrhage and infarctions.' (pg 1578)

Reference 3.23:

'An inverse correlation exists between increased blood pressure and urinary potassium excretion or urinary Na:K excretion ratios. An adequate potassium intake is needed to achieve effective homoeostasis of sodium. ... Increasing potassium intakes to levels achievable with customary diets {i.e. 65 and 100 mmol/d (2.5 and 3.9 g/d)} reduced blood pressure in normotensive and hypertensive individuals and increased urinary sodium loss. This effect of potassium on blood pressure is supported by a recent meta-analysis of published reports.' (pg 171)

Reference 4.22:

'Hypokalaemia can also predispose to hypertension. For example, Krishna et al. (1989) found that young normotensive men on a potassium intake of 390 mg/day (10 mmol/day) were less able to excrete an imposed sodium excess than when they had potassium intake of 90 mmol/day. At the same time, their blood pressures increased.' (pg 8)

Reference 5.0:

'Intracellular potassium is the major cation responsible for establishing the membrane potential. The blood pressure of normotensives increases with potassium depletion. Observational studies suggest an inverse relationship between potassium intake and blood pressure. Often there is an inverse relationship with dietary potassium and sodium or a positive relationship between urinary Na⁺/K⁺ ratio and blood pressure. ... Explanations for the hypotensive effects of potassium include direct vasodilatation, a direct natriuretic effect, altered baroreceptor function, increased urinary kallikrein, or suppression of the renin-angiotensin-aldosterone axis or sympathetic nervous system.' (pg 970)

3) Nerves and muscle

Code Proposed statement

K3: Potassium contributes to normal nerve and muscle function, including those involved in digestion.

Reference 2.0:

'Another system for active K⁺ transport is controlled by H⁺/K⁺-exchanging ATPase which ejects H⁺ in exchange for K⁺. This pump has an important role in some gastrointestinal cells and in the renal collecting duct.' (pg 1574)

'Hypokalaemia can be the result of either or both intracellular shift and potassium depletion. Among its clinical sequelae are hyperpolarization of membrane, which affects the activity of nerves and muscles, as well as cardiac, renal and metabolic alterations.' (pg 1577)

'A difference of potassium concentration across cell membrane is essential for the normal polarization of the cell, and the latter is crucial for maintaining cell excitability and muscle contraction. The transmembrane electrical potential of the cell is determined by the ratio of the intracellular to extracellular potassium and sodium concentrations, mainly potassium. These concentration differences of K⁺ and Na⁺ across cell membranes are maintained by the specific permeability to each one of these ions and by the operation of Na⁺/K⁺-exchanging APTase pump. Thus K⁺ is critical for the excitability of nerve and muscle cells.' (pg 1578)

'Potassium released from contracting skeletal muscle cells facilitates ongoing muscle contraction but also leads to muscular fatigue. Training reduces the exercise-induced rise in plasma [K⁺] and also increases the total activity of Na⁺/K⁺ pumps in human

muscle. The potassium internal balance helps to delay the onset of fatigue during exercise and to restore homeostasis during recovery.’ (pg 1578)

‘Finally, potassium can modify both the mechanical and electrical properties of the heart, because it is critical for the contractility of cardiac cells. In chronic heart failure, potassium can reduce the potentially lethal ventricular tachyarrhythmias. However, as mentioned above, hyperkalaemia can produce a cardiac arrest.’ (pg 1578)

Reference 3.23:

‘Potassium is predominantly an intracellular cation. This compartmentalisation is maintained by the energy-dependent cellular uptake of the element and simultaneous excretion of sodium by the cell membrane bound enzyme Na-K ATPase. This process is fundamental to the cellular uptake of molecules against electrochemical and concentration gradients, to the electro-physiology of nerves and muscle, and to acid-base regulation.’ (pg 170)

‘Potassium deficiency alters the electrophysiological phenomena of cell membranes. This causes weakness of skeletal muscles and the effect on cardiac muscle is reflected by electrocardiographic changes characteristic of impaired polarisation, which may lead to arrhythmia and cardiac arrest. Similar functional changes in intestinal muscle cause intestinal ileus. Mental depression and confusion can also develop.’ (pg 170, 171)

Reference 4.22:

‘The concentration in the extracellular fluid is a critical determinant of neuromuscular excitability (Sterns et al, 1981).’ (pg 8)

‘Hypokalaemia may cause rapid and irregular heartbeats, muscle weakness, irritability and occasionally paralysis, nausea and vomiting, diarrhoea and swollen abdomen (Ensminger et al., 1995).’ (pg 8)

Reference 5.0:

‘Relaxes the heart muscle – action opposite to that of calcium which is stimulatory. ...Potassium deficiency may cause rapid and irregular heartbeats and abnormal electrocardiograms; muscle weakness irritability, and occasionally paralysis;’ (pg 90)

Reference 6.3:

‘Potassium (K) is predominantly an intracellular cation. This compartmentalisation of K is maintained by the energy dependent cellular uptake of the element and simultaneous extrusion of sodium by the cell membrane bound enzyme Na: K adenosine triphosphatase. This process is fundamental to the cellular intake of molecules against electrochemical and concentration gradients to the electrophysiology of nerves and muscle,....’ (pg 156)

‘Potassium deficiency alters the electrophysiological characteristics of cell membranes causing weakness of skeletal muscles. The effect on cardiac muscle is

reflected by electrocardiographic changes characteristic of impaired polarisation which may lead to arrhythmias and cardiac arrest. Similar changes in intestinal muscle cause intestinal ileus (loss of motility)' (pg 156)

4) Energy metabolism

Code	Proposed statement
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<i>K4:</i>	<i>Potassium contributes to normal energy metabolism, required for cell activity.</i>
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Reference 2.0:

'Hypokalaemia can be the result of either or both intracellular shift and potassium depletion. Among its clinical sequelae are ... metabolic alterations.' (pg 1577)

'A high intracellular potassium concentration is essential for optimal cellular metabolism: metabolic control; growth and division; DNA, protein and carbohydrate synthesis; and since this element is a cofactor for enzymes of energy transduction, glycogenesis, cellular growth and other pathways.' (pg 1578)

Reference 4.22:

'Potassium is a cofactor for a number of enzymes including glycerol dehydrogenase, mitochondrial pyruvate carboxylase, pyruvate kinase, vitamin B₁₂-dependent diol dehydratase, L-threonine dehydratase, adenosine triphosphatase and aminoacyl transferase.' (pg 8)

'Potassium is also involved in phosphorylation of creatine, in carbohydrate metabolism and protein synthesis (Ensminger *et al*, 1995).' (pg 8)

5) Secretion of insulin

Code	Proposed statement
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<i>K5:</i>	<i>Potassium is necessary for the normal secretion of insulin by the pancreas.</i>
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Reference 2.0:

'A rise in plasma [K⁺] together with the hormones insulin, adrenaline (by activating β₂- receptors) and aldosterone promote the uptake of K⁺ into skeletal muscle, liver, bone and red blood cells. ... Moreover, hyperkalaemia stimulates insulin, aldosterone and adrenaline secretions, while hypokalaemia has the opposite effect.' (pg 1576)

Reference 4.22:

'Potassium is required for the secretion of insulin by the pancreas (Ensminger *et al*, 1995).' (pg 8)

Reference 5.0:

'Required for the secretion of insulin by the pancreas in enzyme reactions involving the phosphorylation.' (pg 90)

6) Growth

Code Proposed statement

K6: Potassium is necessary for normal growth.

Reference 2.0:

'A high intracellular potassium concentration is essential for optimal cellular metabolism: metabolic control; growth and division; DNA, protein and carbohydrate synthesis; and since this element is a cofactor for enzymes of energy transduction, glycogenesis, cellular growth and other pathways. Potassium deficiency causes growth retardation through a decrease in plasma levels of growth hormone and somatomedin C and a concomitant deterioration of protein synthesis; it may also contribute to growth retardation through a relatively organ-specific resistance to some growth factors.' (pg 1578)

Reference 4.22:

'Potassium is also involved in phosphorylation of creatine, in carbohydrate metabolism and protein synthesis (Ensminger *et al*, 1995).' (pg 8)

'It can also induce growth retardation, with pronounced decrease in circulating somatomedin C and concomitant inhibition of protein synthesis (Macrae *et al*, 1993).' (pg 8)

Reference 6.3:

'Potassium is needed for lean tissue synthesis and an adequate K intake is needed to achieve effective homeostasis of sodium and renal function.' (pg 156)

7) pH regulation

Code Proposed statement

K7: Potassium contributes to normal pH regulation (acid-base balance).

Reference 2.0:

'Proton secretion by H⁺/K⁺ -exchanging ATPase, together with that by H⁺ -ATPase, contribute to acidification of the urine. These enzymes are probably located at the luminal and basolateral sites and participate in the acid-base balance and potassium status.' (pg 1576)

‘Other factors also influence K^+ movements across the cell, but these are not normal homeostatic mechanisms. Metabolic acidosis promotes movement of K^+ out of cells, whereas metabolic alkalosis has the opposite effect.’ (pg 1576)

‘Acid-base balance is another factor that regulates K^+ secretion: alkalosis increases secretion, whereas acidosis decreases it.’ (pg 1577)

‘Many of the functions of potassium are due to its ionic character, whereby it generates gradients of concentration, potential and pressure, and K^+ participates in regulation of the acid-base balance..’ (pg 1578)

Reference 3.23:

‘Potassium is predominantly an intracellular cation. This compartmentalisation is maintained by the energy-dependent cellular uptake of the element and simultaneous excretion of sodium by the cell membrane bound enzyme Na-K ATPase. This process is fundamental to ... acid-base regulation.’ (pg 170)

Reference 5.0:

‘Involved in the maintenance of proper acid-base balance ...’ (pg 90)

Reference 6.3:

‘Potassium (K) is predominantly an intracellular cation. This compartmentalisation of K is maintained by the energy dependent cellular uptake of the element and simultaneous extrusion of sodium by the cell membrane bound enzyme Na: K adenosine triphosphatase. This process is fundamental to ...acid-base regulation.’ (pg 156)

8) Nutrient transfer

Code Proposed statement

K8: Potassium is necessary for the normal transfer of nutrients in and out of cells.

Reference 2.0:

‘... the basolateral K^+ channels have higher conductance than the apical ones, which is essential for maintaining a negative intracellular membrane potential and hence promoting reabsorption of sodium and organic substrates, such as glucose and amino acids.’ (pg 1575)

‘In all parts of the nephron preceding the distal tubule, potassium transport is strongly related to the reabsorption of other nutrients.’ (pg 1576)

‘Potassium is also implicated in the metabolism or utilization of several nutrients, particularly in sodium metabolism, so an adequate potassium intake is needed to achieve effective homeostasis of sodium. The enzyme Na^+/K^+ -exchanging ATPase provides the driving force for the transport of other solutes, such as amino acids, sugar

and phosphate. An increase in dietary potassium reduces urinary calcium and results in a more positive calcium balance.’(pg 1578)

Reference 5.0:

‘Involved in ... the transfer of nutrients in and out of individual cells.’ (pg 90)

Reference 6.3:

‘Potassium (K) is predominantly an intracellular cation. This compartmentalisation of K is maintained by the energy dependent cellular uptake of the element and simultaneous extrusion of sodium by the cell membrane bound enzyme Na: K adenosine triphosphatase. This process is fundamental to the cellular intake of molecules against electrochemical and concentration gradients...’ (pg 156)

ANNEX 4.23

Selenium

Source documents for reviewing selenium

Reference 1.2:

Institute of Medicine Dietary Reference Intakes for Vitamin C, Vitamin E, selenium and carotenoids. Washington D.C. National Academy Press, 2000.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michel J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.20:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Selenium. October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80g_en.pdf).

Reference 4.23:

Revised review of Selenium. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/selenium.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Antioxidant activity

Code	Proposed statement
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<i>Se1:</i>	<i>Selenium is necessary for cell protection from some types of damage caused by free radicals.</i>
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Reference 1.2:

‘The four known selenium-dependent glutathione peroxidases designated as GSHPx 1 through 4 defend against oxidative stress (Flohe, 1988). Selenoproteins P and W are postulated to do so as well (Arteel et al., 1998; Burk et al., 1995; Saito et al., 1999; Sun et al., 1999).’ (pg 285)

‘Thus, the known biological functions of selenium include defense against oxidative stress,...’ (pg 285)

‘... induction of vitamin E deficiency in selenium-deficient animals causes lipid peroxidation and liver necrosis in rats and pigs and cardiac injury in pigs, sheep and cattle (Van Vleet, 1980).’ (pg 287)

‘Keshan disease, a cardiomyopathy that occurs almost exclusively in children is the only human disease that is firmly linked to selenium deficiency (Keshan Disease Research Group, 1979).’ (pg 288)

Reference 2.0:

‘Glutathione peroxidase (cGSHPx) was the first selenoenzyme to be identified; in 1973 it was shown to contain Se as a functional component. It occurred in erythrocytes, where its role was apparently to protect haemoglobin against oxidative damage by hydrogen peroxide (H₂O₂). It was later shown that GSHPx is not a single enzyme, but has several forms with different, though related functions.’(pg 1754)

‘Cytosolic intracellular glutathione peroxidase (cGSHPx) was the first of the different forms of GSHPx to be clearly classified. It consists of four identical 22 kDa subunits, each of which contains one selenocysteine residue. It can metabolize a wide range of hydroxides, though not if they are esterified to phospholipids. It is the major selenoprotein in mammalian cells accounting for about 60% of total Se in Se-adequate animals. In Se-deficient rats, however hepatic cGSHPx can fall to less than 1% of levels in controls, without any ill effects. Consequently, it is believed that the major role of cGSHPx may be as a buffer to provide Se for other selenoproteins which are more important for maintaining health, rather than as a cellular antioxidant.’ (pg 1754)

‘Gastrointestinal glutathione peroxidase is similar in structure, but not identical with cGSHPx. It is believed to have an antioxidant function specific to intestinal tissue in which it mainly occurs.’ (pg 1755)

‘Phospholipid hydroperoxide glutathione peroxidase (PGSHPx) is a monomer of 20-23 kDa, similar to the subunits of other GSHPx enzymes, but unlike them it can metabolize fatty acid hydroperoxides esterified to phospholipids. It is associated with cell membranes and is involved, along with vitamin E, in the protection of membranes against oxidative damage. It may also have a more general role in cellular metabolism, possibly through involvement in eicosanoid metabolism.’ (pg 1755)

‘Extracellular glutathione peroxidase (eGSHPx), also tetramer of four identical 22 kDa subunits, each containing a selenocysteine residue, is a glycoprotein. It is synthesized mainly in the kidneys and is thought to protect extracellular spaces from oxidative damage.’ (pg 1755)

‘Selenoprotein P (Se-P) is a single polypeptide chain containing 10 selenocysteine residues. Between 70 and 80% of plasma Se may be in this form. Its function has not

yet been elucidated but it may have an antioxidant role in plasma and extracellular spaces.’ (pg 1755)

‘Selenoprotein W (Se-W) is a low molecular weight protein with a single selenocysteine residue per molecule. It is found in animal muscles and its loss is associated with myopathy in Se deficiency. It may also have antioxidant functions.’ (pg 1755)

‘It was not until 1973 that a satisfactory explanation for the role of Se was proposed following the discovery by Rotruck and his colleagues that the element is an integral part of the antioxidant enzyme GSHPx.’ (pg 1755)

‘It is postulated that damage to the arterial endothelium caused by free radicals produced by peroxidation of lipids can initiate formation of atheromatous plaques leading to coronary vascular disease (CVD). There is some evidence that antioxidant nutrients, including Se, can reduce peroxidation, and thus have a protective effect against CVD.’ (pg 1756)

Reference 3.20:

At least eleven selenoproteins containing the amino acid selenocysteine have been identified in mammals: cellular glutathione peroxidase (cGSHPx), extracellular glutathione peroxidase (eGSHPx), phospholipid hydroperoxide glutathione peroxidase (phGSHPx), gastrointestinal glutathione peroxidase (giGSHPx), iodothyronine deiodinase types I, II and III, prostatic epithelial selenoprotein (PES), selenoprotein P (SeP), selenoprotein W, thioredoxin reductase (Alexander and Meltzer, 1995; Johansson *et al.*, 1997).’ (pg 3)

Reference 4.23:

‘Selenium is an essential trace element. It is necessary for the functioning of the enzyme glutathione peroxidase (GPX), which protects against oxidative damage to intracellular structures. The biologically active form of selenium is selenocysteine.’ (pg 5)

‘Selenoprotein P is a plasma protein containing 7.5 atoms of selenium per mole protein. It has been suggested that it may have a transport as well as an anti-oxidant function but its precise role is unclear.’ (pg 5)

Reference 5.0:

‘Component of the enzyme glutathione peroxidase, the metabolic role of which is to protect against oxidation of polyunsaturated fatty acids and resultant tissue damage.’ (pg 94)

‘There are no clear-cut deficiencies of selenium, because this mineral is so closely related to vitamin E that it is difficult to distinguish deficiency due to selenium alone.’ (pg 94)

‘An epidemiological association between increased selenium intakes and reduced cancer risk, as well as the antioxidant role of selenium in glutathione peroxidase, have provided a basis for research on the potential anticarcinogenic effects of selenium.’ (pg 1353)

‘Antioxidant via glutathione peroxidase.’ (pg 1354)

Reference 6.3:

‘Selenium (Se) is an integral part of the enzyme glutathione peroxidase (GSHPx), one of the mechanisms whereby intracellular structures are protected against oxidative damage. Selenium deficiency results both in a decrease in GSHPx activity and in GSHPx protein.’ (pg 174)

2) Utilization of iodine in the production of thyroid hormones

Code	Proposed statement
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<i>Se2:</i>	<i>Selenium is necessary for the normal utilization of iodine in the production of thyroid hormones.</i>
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Reference 1.2:

‘Three selenium-dependent iodothyronine deiodinases regulate thyroid hormone metabolism (Berry and Larsen, 1992). (pg 285)

‘Thus, the known biological functions of selenium include...regulation of thyroid hormone action, ...’ (pg 285)

‘Keshan disease, a cardiomyopathy that occurs only in selenium-deficient children, appears to be triggered by an additional stress, possibly an infection or a chemical exposure (Ge et al., 1983). Clinical thyroid disorders have not been reported in selenium-deficient individuals with adequate iodine intake, but based on observations in Africa, it has been postulated that infants born to mothers deficient in both selenium and iodine are at increased risk of cretinism (Vanderpas et al., 1992).’ (pg 287)

Reference 2.0:

‘The iodothyronine deiodinases are a class of ‘non-antioxidant’ selenoproteins which are involved in thyroid metabolism. Type 1, type 2 and type 3 iodothyronine deiodinases (ID-I, ID-II and ID-III) are responsible for the regulation of the interconversion of active and inactive forms of iodothyronines. All three enzymes have selenocysteine at their active sites, but show considerable differences in their sensitivities to the effects of various inhibitors. They also are affected to different extents by Se deficiency.’ (pg 1755)

‘Normal function of the thyroid gland is dependent on an adequate supply of dietary selenium. It has been shown that the myxoedematous cretinism which occurs in regions of endemic goitre in tropical Africa is related to a combined deficiency of

both Se and iodine (I). It is believed that Se deficiency increases some indicators of hypothyroid stress associated with inadequate dietary I. The connection between the two elements is complex and is not yet fully understood.’ (pg 1756)

Reference 3.20:

At least eleven selenoproteins containing the amino acid selenocysteine have been identified in mammals: ... iodothyronine deiodinase types I, II and III, ... (Alexander and Meltzer, 1995; Johansson et al., 1997).’ (pg 3)

Reference 4.23:

‘Other selenoproteins have been isolated, these include peripheral terathiodothyronone 5’I-deiodinase I which converts thyroxine to T3 in the thyroid and other peripheral organs. Selenium status also appears to affect terathiodothyronone 5’II-deiodinase activity but the enzyme itself (at least in the rat) does not contain selenium.’ (pg 5)

Reference 6.3:

‘Other selenoproteins have been isolated from mammalian tissues and of particular interest is the role of Se in the hepatic microsomal deiodination of thyroxine.’ (pg 174)

3) Regeneration of molecules

Code Proposed statement

Se3: *Selenium contributes to the body’s normal ability to re-use some molecules such as vitamin C.*

Reference 1.2:

‘Three thioredoxin reductases have been identified (Sun et al., 1999). Their functions include reduction of intramolecular disulfide bonds and regeneration of ascorbic acid from its oxidized metabolites (May et al., 1998). Thus, the known biological functions of selenium include defense against oxidative stress, regulation of thyroid hormone action, and regulation of the redox status of vitamin C and other molecules.’ (pg 285)

Reference 2.0:

‘Thioredoxin reductase has recently been identified as a selenoprotein. It is involved in redox regulation of a range of enzymes and transcription factors and is required for the expression of several different proteins.’ (pg 1755)

Reference 3.20:

At least eleven selenoproteins containing the amino acid selenocysteine have been identified in mammals: ...thioredoxin reductase (Alexander and Meltzer, 1995; Johansson et al., 1997).’ (pg 3)

4) Muscle

Code	Proposed statement
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<i>Se4:</i>	<i>Selenium contributes to normal muscle function</i>
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Reference 2.0:

‘Selenoprotein W (Se-W) is a low molecular weight protein with a single selenocysteine residue per molecule. It is found in animal muscles and its loss is associated with myopathy in Se deficiency. It may also have antioxidant functions.’ (pg 1755)

‘The use of Se in rations and fertilizers has had a major impact in reducing levels of incidence of Se-dependent conditions such as white muscle disease (WMD).’ (pg 1755)

‘... several nonendemic Se-responsive conditions occur in humans. These include cardiomyopathies and muscular problems in patients receiving total parenteral nutrition (TPN) where there is inadequate Se in the infusion fluid.’ (pg 1755)

Reference 3.20:

‘A suspected selenium deficiency syndrome has also been demonstrated in a few patients treated with parenteral nutrition without added selenium (see Rannem *et al.*, 1996). Muscular pain and muscular and cardiac dysfunction has been demonstrated in some patients, but no uniform symptomatology has been described.’ (pg 4)

Reference 5.0:

‘Helps prevent Keshan disease (a cardiomyopathy).’ (pg 1354)

5) Embryonic development

Code	Proposed statement
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<i>Se5:</i>	<i>Selenium contributes to normal embryonic development.</i>
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Reference 1.2:

‘Selenium functions largely through an association with proteins, known as selenoproteins (Stadtman, 1991), and disruption of their synthesis is lethal for embryos (Bosl *et al.*, 1997). A selenoprotein is a protein that contains selenium in stoichiometric amounts.’ (pg 285)

Reference 2.0:

‘Normal function of the thyroid gland is dependent on an adequate supply of dietary selenium. It has been shown that the myxoedematous cretinism which occurs in regions of endemic goitre in tropical Africa is related to a combined deficiency of both Se and iodine (I). It is believed that Se deficiency increases some indicators of

hypothyroid stress associated with inadequate dietary I. The connection between the two elements is complex and is not yet fully understood.’ (pg 1756)

6) Sperm development

Code	Proposed statement
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<i>Se6a:</i>	<i>Selenium contributes to the normal development of sperm.</i>
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<i>Se6b:</i>	<i>Selenium contributes to normal reproduction.</i>
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Reference 2.0:

‘Sperm capsule selenoprotein contains three selenoprotein residues per molecule. It appears to be required for sperm development and may play a structural role in the sperm tail. Se deficiency can cause abnormal sperm formation and infertility in male rats.’ (pg 1755)

Reference 3.20:

‘...selenium has been related to ... reproduction and ...(WHO, 1987; Flohe, 1989; Knekt *et al.*, 1990; Virtamo and Huttunen, 1991; Willett *et al.*, 1991; Kok *et al.*, 1991; Clarke *et al.*, 1996, Rayman, 2000).’ (pg 4)

7) Immune system

Code	Proposed statement
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<i>Se7:</i>	<i>Selenium is necessary for the normal function of the immune system.</i>
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Reference 2.0:

‘Se plays a part in the body’s response to infection and contributes to the integrity of the immune system. In children with Down syndrome, for instance, who are particularly prone to bacterial infection, Se supplementation has been shown to increase levels of serum IgG₂. Se supplementation may also help to prevent age-related immunosuppression. Decreased immunocompetence in patients suffering from kwashiorkor may be related to low serum Se.’ (pg 1756)

Reference 5.0:

‘While the exact mechanism by which selenium exerts its preventative effect against certain types of cancer in humans is unknown, selenium supplementation in animal experiments has been shown to result in enhanced primary immune response in mice, as measured by the plaque-forming cell test and hemagglutination.’ (pg 1353)

‘Needed for proper immune system response.’ (pg 1354)

ANNEX 4.24

Chromium

Source documents for reviewing chromium

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.21:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Trivalent Chromium. April 2003.

(http://www.europa.eu.int/comm/food/fs/sc/scf/out197_en.pdf).

Reference 4.24:

Review of Chromium. Expert Group on Vitamins and Minerals. August 2002.

(<http://www.food.gov.uk/multimedia/pdfs/reviewofchrome.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Insulin regulation

Code Proposed statement

Cr1: Chromium is necessary for the normal regulation of insulin.

Reference 1.1:

‘Chromium potentiates the action of insulin in vivo and in vitro (Mertz, 1969, 1993; Mertz et al., 1961). Schwarz and Mertz (1959) identified chromium as the element that restored glucose tolerance in rats. Impaired glucose tolerance of malnourished infants responded to an oral dose of chromium chloride (Hopkins and Majaj, 1967); Hopkins et al., 1968); subsequently, benefits of chromium chloride were reported in a patient receiving total parenteral nutrition (TPN) (Jeejeebhoy et al., 1977). A number of studies have demonstrated beneficial effects of chromium on circulating glucose,

insulin and lipids in a variety of human subjects and animal species; however, not all reports of supplementation are positive (Anderson, 1997; Anderson et al., 1991)...’ (pg 198)

‘Recent work by Davis and Vincent (1997a, 1997b) and Vincent (1999) suggests that a low molecular weight chromium-binding substance (LMWCr) may amplify insulin receptor tyrosine kinase activity in response to insulin. It is proposed that the inactive form of the insulin receptor (IR) is converted to the active form by binding insulin, which stimulates the movement of chromium from the blood into the insulin-dependent cells and results in the binding of apoLMWCr to chromium. The holoLMWCr then binds to the insulin receptor tyrosine kinase. The ability of LMWCr to activate insulin receptor tyrosine kinase depends on its chromium content. When insulin concentration drops, the holoLMWCr is possibly released from the cell to terminate its effects.’ (pg 198)

‘Because chromium potentiates the action of insulin and chromium deficiency in TPN patients, impairs glucose utilisation, and raises insulin requirements, it has been hypothesized that poor chromium status is a factor contributing to the incidence of impaired glucose tolerance and Type II diabetes.’ (pg 201)

‘Consumption of diets high in simple sugars (35 percent of total kcal) increased urinary chromium excretion in adults (Kozlovsky et al., 1986). Urinary chromium excretion was found to be related to the insulinogenic properties of carbohydrates (Anderson et al., 1990).’ (pg 204)

Reference 2.0:

‘Chromium in the trivalent form is essential nutrient that functions in carbohydrate, lipid and nucleic acid metabolism...Chromium functions in glucose and insulin metabolism primarily through its role in regulation of insulin. Adequate dietary Cr leads to a normalization of insulin, with reductions in blood glucose concentrations in subjects with elevated blood glucose levels, increases in subjects with low blood glucose levels, and no effect on subjects with near-optimal glucose tolerance.’ (pg 388)

‘The essentiality of Cr in human nutrition was documented in 1977 when diabetic signs and symptoms of a patient on total parenteral nutrition (TPN) were reversed by supplemental Cr. Diabetic symptoms, in addition to elevated blood glucose levels, included weight loss, impaired nerve conduction, brain disorders and abnormal respiratory quotient, refractory to exogenous insulin. Upon daily addition of supplemental Cr to the patient’s TPN solution for 2 weeks, diabetic symptoms were alleviated and exogenous insulin requirement dropped from 45 units per day to zero. These findings have been repeated and documented on several occasions. (pg 389)

‘The hallmark of marginal Cr deficiency is impaired glucose tolerance... Chromium leads to a decrease in blood glucose concentration in people with elevated glucose levels and an increase in those with low blood glucose levels owing to its role in normalizing insulin. In the presence of Cr in a physiologically active form, insulin is

more efficient, and much lower levels of insulin are required. During periods of elevated blood glucose, more efficient insulin leads to a decrease in blood glucose levels. In people with low blood glucose (reactive hypoglycaemia), more efficient insulin leads to a rapid rise in response to a glucose challenge and a more rapid return to baseline values. This leads to less of a drop or a raising of the hypoglycaemic glucose values. Supplemental Cr also leads to increased insulin binding and increased insulin receptor number, and recent evidence suggests that Cr may be involved in the phosphorylation and dephosphorylation of the insulin receptor proteins.’ (pg 389)

Reference 3.21

‘Chromium deficiency has not been seen in humans except in patients during long-term parenteral nutrition without substitution of chromium. The deficiency symptoms (impaired glucose tolerance and glucose utilisation...) disappeared rapidly after oral supplementation (200µg/day) (Jeejeebhoy et al., 1977; Freund et al., 1979).’ (pg 5)

‘Trivalent chromium is considered to be an essential element in both animal feeding and human nutrition. It influences carbohydrate, lipid and protein metabolism via an effect on insulin action. However the mechanism is not quite clear neither is the exact structure of the biologically active form of chromium, the “Glucose Tolerance Factor” (GTF) (WHO, 1996).’ (pg 5)

Reference 4.24:

‘Chromium is an essential nutrient that potentiates insulin action and thus, influences carbohydrate, lipid and protein metabolism. However the nature of the relationship between chromium and insulin function has not been clearly defined. Mertz et al., (1974) suggested that the biologically active form of chromium (glucose tolerance factor) is a complex of chromium, nicotinic acid and possibly the amino acids glycine, cysteine and glutamic acid. Many attempts have been made to isolate or synthesise the glucose tolerance factor; none has been successful. Thus, the precise structure of the glucose tolerance factor and whether it is the biologically active form of chromium, remains uncertain.’ (pg 7)

‘Low molecular weight chromium-binding substance (LMWCr) is a naturally occurring oligopeptide which has recently been proposed as the biologically active form of chromium. Its primary function is proposed to be the activation of insulin receptor tyrosine kinase in response to insulin. Chromium is essential for LMWCr to perform this function. (Vincent, 2000).’ (pg 7)

Reference 5.0:

‘Chromium is an essential micronutrient for utilization of glucose, since it activates phosphoglucosmutase and increases insulin activity.’ (pg 1020)

‘(Emerging benefit): Hyperglycaemic control.’ (pg 1355)

Reference 6.3:

‘Chromium (Cr) appears to function biologically in an organic complex which potentiates the action of insulin^{1,2}. It may also participate in lipoprotein metabolism and in maintaining the structure of nucleic acids, and in gene expression³.’ (pg 181)

2) Lipid metabolism

Code **Proposed statement**

Cr1: Chromium contributes to the normal metabolism of lipids.

Reference 2.0:

‘Chromium in the trivalent form is essential nutrient that functions in carbohydrate, lipid and nucleic acid metabolism...’ (pg 388)

Reference 3.21

‘Chromium deficiency has not been seen in humans except in patients during long-term parenteral nutrition without substitution of chromium. The deficiency symptoms (...elevated plasma fatty acids...) disappeared rapidly after oral supplementation (200µg/day) (Jeejeebhoy et al., 1977; Freund et al., 1979).’ (pg 5)

Reference 5.0:

‘(Emerging benefit): Improved lipid profiles’. (pg 1355)

Reference 6.3:

‘Chromium...may also participate in lipoprotein metabolism and....’ (pg 181)

3) DNA synthesis

Code **Proposed statement**

Cr3: Chromium contributes to normal DNA synthesis and the expression of some genes.

Reference 2.0:

‘Chromium in the trivalent form is essential nutrient that functions in... nucleic acid metabolism...’ (pg 388)

Reference 4.24:

‘... it has been found that in vitro, RNA synthesis directed by free DNA is enhanced by the binding of chromium to template (Okada et al., 1981); this suggests that chromium may act similarly to zinc in regulating gene expression, so it may be regulating the synthesis of a molecule that potentiates insulin action. This suggestion is supported by the finding that there is a four-hour lag period between the administration of biologically active chromium and its optimal effect on insulin action in vivo (Tunman and Doisy, 1977).’ (pg 8)

Reference 5.0:

‘(Established benefits): Important for cell formation.’ (pg 1355)

Reference 6.3:

‘Chromium ... may also participate ... in maintaining the structure of nucleic acids, and in gene expression³.’ (pg 181)

ANNEX 4.25

Molybdenum

Source documents for reviewing molybdenum

Reference 1.1:

Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington D.C. National Academy Press, 2001.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michel J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.22:

Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Molybdenum. October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80h_en.pdf).

Reference 4.25:

Review of Molybdenum. Expert Group on Vitamins and Minerals. August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0009p.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Enzyme activity

Code Proposed statement

Mo1: Molybdenum is necessary for the normal activity of some enzymes in the body.

Reference 1.1:

‘Molybdenum has been shown to act as a cofactor for a limited number of enzymes in humans: sulfite oxidase, which is believed to be most important for health, xanthine oxidase, and aldehyde oxidase. In all mammalian molybdoenzymes, functional molybdenum is present as an organic component called molybdopterin (Rajagopalan, 1988). These enzymes are involved in catabolism of sulfur amino acids and heterocyclic compounds, including purines and pyridines. A clear molybdenum deficiency syndrome producing physiological signs of molybdenum restriction has

not been achieved in animals despite major reduction in the activity of these molybdoenzymes. Rather, molybdenum essentiality is based on a genetic defect that prevents sulfite oxidase synthesis. Because sulfite is not oxidized to sulfate, severe neurological damage leading to early death occurs with this inborn error of metabolism (Johnson, 1997). Further support for an essential metabolic role for molybdenum relates to amino acid intolerance in a patient who received long-term total parenteral nutrition without molybdenum (Abumrad et al., 1981). The intolerance, which was probably due to abnormal sulfur amino acid metabolism, was reversed with intravenous repletion of ammonium molybdate.’ (pg 421)

‘Molybdenum deficiency has not been observed in healthy people. A severe metabolic defect, molybdenum cofactor deficiency, had been identified in 47 patients by 1993. The disease results in deficiency in the three molybdoenzymes known to occur in humans: sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Few infants with these defects survive the first days of life (Johnson et al., 1993), and those who survive have severe neurological abnormalities and a variety of other abnormalities.’ (pg 421, 422)

Reference 2.0:

‘The evidence for the essentiality of molybdenum is substantial and conclusive. Molybdenum functions as a cofactor or in enzymes that catalyse the hydroxylation of various substrates. Aldehyde oxidase oxidizes and detoxifies various pyrimidines, purines, pteridines and related compounds. Xanthine oxidase/dehydrogenase catalyses the transformation of hypoxanthine to xanthine, and xanthine to uric acid. Sulfite oxidase catalyses the transformation of sulfite to sulfate.’ (pg 1894)

Reference 3.22:

‘Xanthine dehydrogenase (XD) converts tissue purines, pyrimidines, pteridins and pyridins by oxidative hydroxylation to uric acid as an irreversible process. Its normal action is that of a dehydrogenase, but when reacting with O₂ during proteolysis, freezing/thawing or in the presence of reactive –SH reagents it changes into Xanthine oxidase (XO), which produces free radicals of oxygen known to be involved in tissue damage following physical injury, reperfusion, injury by toxins or Mo excess. Avian XD is stable, hence birds excrete uric acid. Allopurinol oxidises metabolically to alloxanthine, which inhibits XD.’ (pg 4)

‘Reduced XD activity is associated with xanthinuria, low urinary uric acid, high blood xanthine levels, high urinary and blood hypoxanthine levels, renal calculi and depositions in muscles with myopathy. Low Mo intake reduces tissue XD activity, however the intake variations from normal diet are insufficient to exert on XD activity, which can cause overt clinical changes. ... It is not known whether high Mo intake stimulates tissue XD activity (Rajagopalan, 1987; WHO, 1996a).’ (pg 5)

‘Aldehyde oxidase is structurally and chemically similar to XO, has a similar tissue distribution and shares some substrates, e.g.: aldehydes, substituted pyridines, pyrimidines, quinolines and purine derivatives. Its principal metabolic role is unknown (Rajagopalan, 1987).’ (pg 5)

‘Sulphite oxidase (SO) is a haem-containing molybdoprotein located in the intermembrane space of mitochondria. SO converts sulphite to sulphate. Sulphite derives metabolically from S-amino acids, e.g. cysteine, methionine. SO occurs in the liver of man and other species (WHO, 1996a).’ (pg 5)

Reference 4.25:

‘The basis of the biological importance of molybdenum is in its role in at least six metalloenzymes; nitrate reductase and nitrogenase in plants and bacteria and xanthine oxidase, sulphate oxidase, sulphite oxidase and aldehyde oxidase. All of the molybdoenzymes are oxidoreductases, exploiting the variable valency states of molybdenum. The molybdenum in molybdoenzymes is inserted as part of a prosthetic group, known as the ‘molybdenum cofactor’ (Solomons, 1984).’ (pg 6)

‘In man, xanthine oxidase acts in the pathway of degradation of purine nucleic acids to uric acid, acting to oxidise the hypoxanthine and xanthine. Sulphite oxidase is responsible for the terminal oxidation of the degraded sulphur from organic sulphur compounds (cysteine, methionine, taurine etc) from sulphite to sulphate. Sulphate is usually the major urinary form of sulphur.’ (pg 6, 7)

‘Naturally occurring molybdenum deficiency has never been identified in free-living human or animal species.’ (pg 7)

‘Studies in rodents have indicated, however, that diets with a low molybdenum content (approximately 20 µg/kg) adversely affect growth (WHO, 1993).’ (pg 7)

‘Molybdenum found in sulphite oxidase detoxifies sulphite to the inert and harmless sulphate which can benefit those people with sulphite sensitivity (Papaioannou and Pfeiffer, 1984).’ (pg 7)

‘Molybdenum IV injections (increasing from 250 µg to 750 µg) for 3 months were beneficial to a female asthmatic by reducing her wheezing and her use of inhalers from 4 times daily to twice daily (Wright and Littleton, 1989). In an Australian study of 1,750 asthma patients (Birkmayer and Beyer, 1990), 41.5% of the sample were found to be molybdenum deficient. The addition of molybdenum to the diet was found to improve the symptoms of those asthmatics who experienced an elevated ratio of sulphites to sulphates in their urine.’ (pg 7)

Reference 5.0:

‘As a component of three different enzyme systems which are involved in the metabolism of carbohydrates, fats, proteins, sulfur-containing amino acids, nucleic acids (DNA and RNA,) and iron.’ (pg 93)

Reference 6.3:

‘Molybdenum (Mo) is essential for the enzymes xanthine oxidase/dehydrogenase, aldehyde oxidase and sulphite oxidase which are involved in the metabolism of DNA and sulphites.’ (pg 178)

ANNEX 4.26

Fluoride

Source documents for reviewing fluoride

Reference 1.4:

Institute of Medicine Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington D.C. National Academy Press, 1997.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.23:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Reference 5.0:

Introduction to Human Nutrition. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Teeth

Code	Proposed statement
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<i>F11a:</i>	<i>Fluoride contributes to the maintenance of healthy teeth</i>
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<i>F11b:</i>	<i>Fluoride is necessary for the normal function of enamel in teeth.</i>
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Reference 1.4:

'Owing to its high affinity for calcium, fluoride is mainly associated with calcified tissues. Its ability to inhibit, and even reverse, the initiation and progression of dental caries is well known. It also has the unique ability to stimulate new bone formation, and as such, it has been used as an experimental drug for the treatment of osteoporosis (Kleerekoper and Mendlovic, 1993).' (pg 288)

'The ingestion of fluoride during the pre-eruptive development of teeth as a cariostatic effect (it reduces the risk of dental caries) due to the uptake of fluoride by enamel crystallites and formation of fluorhydroxyapatite, which is less acid soluble than

hydroxyapatite (Brown et al., 1977; Chow, 1990). Fluoride in the oral fluids, including saliva and dental plaque, also contributes to the cariostatic effect. This posteruptive effect is due mainly to reduced acid production by plaque bacteria and to an increased rate of enamel remineralization during an acidogenic challenge (Bowden, 1990; Hamilton, 1990; Marquis, 1995).’ (pg 288, 289)

‘About 99 percent of the body’s fluoride is found in calcified tissues-to which it is strongly but not irreversibly bound. Fluoride in bone appears to exist in both a rapidly exchangeable pool and a slowly exchangeable pool. ... For young children, as much as 80 percent can be retained owing to increased uptake by the developing skeleton and teeth (Ekstrand et al., 1994a, b).’ (pg 289)

‘The cariostatic action of fluoride on erupted teeth of children and adults is due to its effects on the metabolism of bacteria in dental plaque and on the dynamics of enamel de- and remineralization during an acidogenic challenge (Marquis, 1995; Tatevossian, 1990). Plaque fluoride concentrations are directly related to the fluoride concentrations in and frequencies of exposure to water, beverages, foods and dental products. Fluoride can be deposited in plaque by direct uptake from these sources as well as from the saliva and gingival crevicular fluid after ingestion and absorption from the gastrointestinal tract. Its effects on plaque bacteria involved inhibition of several enzymes, which limits the uptake of glucose and thus reduces the amount of acid produced and secreted into the extracellular plaque fluid (Kanapka and Hamilton, 1971; Marquis, 1995). These effects attenuate the pH drop in plaque fluid that would otherwise occur and, hence, the severity of the acidic challenge to the enamel (Birkeland Charlton, 1976).

The effects of fluoride on the processes of enamel de- and remineralization in erupted teeth include: (1) a reduction in the acid solubility of enamel; (2) promotion of remineralization of incipient enamel lesions, which are initiated at the ultrastructural level several times each day according to the frequency of eating or drinking foods containing carbohydrates metabolizable by plaque bacteria; (3) increasing the deposition of mineral phases in plaque, which, under acidic conditions produced during plaque metabolism, provide a source of mineral ions (calcium, phosphate, and fluoride) that retard demineralization and promote remineralization; and (4) a reduction in the net rate of transport of minerals out of the enamel surface by inducing the reprecipitation of fluoridated hydroxyapatite within the enamel (Margolis and Moreno, 1990; Ten Cate, 1990). These various mechanisms underlying the protective effects of fluoride on the erupted teeth of children and adults require frequent exposures to fluoride throughout life in order to achieve and maintain adequate concentrations of the ion in dental plaque and enamel.’ (pg 290)

‘Many studies conducted prior to the availability of fluoride-containing dental products demonstrated that dietary fluoride exposure is beneficial, owing to its ability to inhibit the development of dental caries in both children and adults (Russell and Elvove, 1951). The results of most of these studies showed that the prevalence of dental caries in communities with optimal water fluoride concentrations (range 0.7 to 1.2 mg/liter, depending on average regional temperature) was 40 to 60 percent lower

than in areas with low water fluoride concentrations. ... Other studies have shown that the earlier children are exposed to fluoridated water or dietary fluoride supplements, the greater the reduction in dental caries in both the primary and permanent teeth (Hargreaves et al., 1988; Lewis, 1976; Stephen et al., 1987). The lack of exposure to fluoride or the ingestion of inadequate amounts of fluoride at any age places the individual at increased risk for dental caries.’ (pg 297)

Reference 2.0:

‘Fluoride is important both in protection and repair of enamel. It has been known for some time that fluoride can be incorporated into the structure of tooth enamel, making it stronger. People who received some fluoride in their diet (for example because it occurred naturally in their water supply) were found to have substantially lower numbers of decayed teeth than those who had little or no exposure to fluoride. As a result many community water supplies are now supplemented with small amounts of fluoride. Marked improvements in dental health are seen, especially among children... Regular use of properly formulated fluoride toothpastes has led to extraordinary reductions in the number of decayed teeth throughout most of the world where such toothpastes are available and used. Moreover, use of fluoridated toothpaste in areas where water fluoridation is present provides additional benefit in caries reduction (possibly because regular hygiene also reduces the number of bacteria present on the teeth). In addition, fluoride (at the concentration present in most toothpastes) inhibits the action of acidogenic bacteria, but whether this is part of the explanation for the unexpectedly large effects of the use of these toothpastes is still being researched.’ (pg 505)

Reference 3.23:

‘The essentiality of fluoride is debatable but since epidemiological studies have demonstrated in children an inverse relationship between the incidence of dental caries and their calculated intakes of fluoride, the element has been accepted as being beneficial to oral health ^{1,2}. Both topical and systemic fluoride replace hydroxyl moieties in enamel to form calcium fluoroapatite, which is less soluble in acid than is calcium hydroxyapatite, thus increasing resistance to demineralisation and improving mineralisation. Additionally, fluoride may have an antimicrobial effect on cariogenic oral microflora ². Ninety-five percent of systemic fluoride is in the skeleton and teeth. The concentration in bone increases with age and it has been suggested, but not proven conclusively, that fluoride may have a role in both the mineralisation of bone and the maintenance of peak bone mass ¹.’ (pg 22)

Reference 5.0:

‘About 99% of the body’s fluoride is found in calcified tissues (bone and teeth), to which it is strongly but not irreversibly bound... In healthy, young or middle-aged adults, approximately 50% of absorbed fluoride is retained by uptake in calcified tissues and 50% is excreted into urine. In young children, as much as 80% can be retained owing to the increased uptake by the developing skeleton and teeth.’ (pg 215)

'The function of fluoride appears to be in the crystalline structure of bones; fluoride forms calcium fluoroapatite in teeth and bones. The incorporation of fluoride in these tissues is proportional to its total intake. There is an overall acceptance of a role for fluoride in the care of teeth. The cariostatic action (reduction in the risk of dental caries) of fluoride on erupted teeth of children and adults is owing to its effect in the metabolism of bacteria in dental plaque (i.e. reduced acid production) and on the dynamics of enamel demineralization and remineralization during an acidogenic challenge. The ingestion of fluoride during the pre-eruptive development of teeth also has a cariostatic effect because of the uptake of fluoride by enamel crystallite and formation of fluorhydroxyapatite, which is less acid soluble than hydroxyapatite. When drinking water contains 1mg/l there is a coincidental 50% reduction in tooth decay in children. Fluoride (at relatively high intakes) also has the unique ability to stimulate new bone formation and, as such, it has been used as an experimental drug for the treatment of osteoporosis. Recent evidence has shown an especially positive clinical effect on bone when administered in a sustained-release form rather than in forms that are quickly absorbed from the gastrointestinal tract.' (pg 216)

Reference 6.3:

'Fluorine (F) forms calcium fluorapatite ($3\text{Ca}_3[\text{PO}_4]_2\text{CaF}_2$) in tooth and bone. It may have a role in bone mineralisation and it assists remineralisation of bone in pathological demineralising conditions and protects against dental caries. The addition of 1mg/kg (1 ppm) to the drinking water of a population results in the reduction of tooth decay in children by approximately 50 per cent^{1,2}. However no essential function has been proven in humans.' (pg 187)

ANNEX 4.27

Chloride

Source documents for reviewing chloride

Reference 3.23:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Reference 4.21:

Review of Sodium Chloride. Expert Group on Vitamins and Minerals. April 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm0108p.pdf>).

Reference 5.0:

Introduction to Human Nutrition. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.

Reference 6.3:

Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.

1) Water and electrolyte balance

Code	Proposed statement
<i>Cl1:</i>	<i>Chloride is necessary for normal water and electrolyte balance throughout the body.</i>

Reference 3.23:

'Chloride is the major extracellular and intracellular counter anion to sodium and potassium...' (pg 175)

Reference 4.21:

'Chloride is also important in maintaining the fluid balance ...' (pg 5)

Reference 5.0:

'Sodium cation is an active participant in the regulation of osmotic and electrolyte balances, while chloride anion is a passive participant in this regulatory system...' (pg 190)

Reference 6.3:

'Chloride (Cl) is the major extracellular and intracellular counter anion to sodium and potassium, with 70 % of the body burden in the ECF, and the remainder in the intracellular space, connective tissue and bone¹.' (pg 159)

2) Stomach acid

Code	Proposed statement
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<i>Cl2a:</i>	<i>Chloride is necessary for the normal composition of stomach acid, involved in digestion.</i>
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<i>Cl2b:</i>	<i>Chloride contributes to normal digestion.</i>
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Reference 4.21:

'Chloride ... is an essential component of the gastric juices.' (pg 5)

Reference 5.0:

Among the main functions of chloride anion are as dissociated hydrochloric acid in the stomach and in the chloride shift in the erythrocyte plasma membrane, where it exchanges with the bicarbonate ion.' (pg 190)

ANNEX 4.28

Phosphorus

Source documents for reviewing phosphorus

Reference 1.4:

Institute of Medicine Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington D.C. National Academy Press, 1997.

Reference 2.0:

Encyclopedia of Human Nutrition 2E. Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference 3.23:

Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).

Reference 4.26:

Review of Phosphorus. Expert Group on Vitamins and Minerals. August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/reviewofphosphorous.pdf>).

Reference 5.0:

Handbook of Nutrition and Food. Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

1) Bone and teeth

Code Proposed statement

P1: Phosphorus is necessary for the normal structure of bone and teeth

Reference 1.4:

'Eighty-five percent of adult body phosphorus is in bone.' (pg 146)

'Another process depleting the blood of its P_i is mineralization of nucleated bone and cartilage matrix. The amorphous calcium phosphate formed in the first stages of mineralization exhibits a Ca:P molar ratio of about 1.33:1, or very close to the molar ratio of Ca:P in adult ECF... ECF supports calcium phosphate deposition only in the presence of a suitable crystal nucleus. As a consequence, in nonosseous tissues, ECF [Ca^{2+}] and P_i concentrations will be essentially what can be measured in peripheral venous blood. However, at active bone-forming sites, ECF is depleted of both its calcium and phosphate. Osteoblast function seems not to be appreciably affected by ECF [Ca^{2+}], but like other tissues, the osteoblast needs a critical level of P_i in its bathing fluid for fully normal cellular functioning. Local P_i depletion both impairs

osteoblast function and limits mineral deposition in previously deposited matrix.’ (pg 151)

‘Estimates of optimal Ca:P intake ratios have frequently been based on the calcium and phosphorus needs of bone building. The molar ration of Ca:P in synthetic hydroxyapatite is 1.67:1; in actual bone mineral, usually closer to 1.5:1; and in amorphous calcium-phosphate (the first mineral deposited at the mineralizing site), 1.3:1 (Nordin, 1976).’ (pg 153)

‘Only limited quantities of phosphate are stored within cells, and most tissues depend upon ECF P_i for their metabolic phosphate. When ECF P_i levels are low, cellular dysfunction follows. At a whole organism level, the effects of hypophosphatemia include ... bone pain, rickets and osteomalacia...The skeleton will exhibit either rickets in children or osteomalacia in adults. In both, the disorder consists of a failure to mineralize forming growth plate cartilage or bone matrix, together with impairment of chondroblast and osteoblast function (Lotz et al., 1968). Phosphorus is so ubiquitous in various foods that near total starvation is required to produce dietary phosphorus deficiency.’ (pg 157)

Reference 2.0:

‘ Cellular uses of phosphates in intermediary metabolism are extensive. Typically P_i ions are converted to various organic phosphates (P_o). Practically all energetic steps utilize high-energy phosphate bonds (adenosine triphosphate or ATP) for the synthesis of organic molecules, ...to provide skeletal support and protection, for teeth, and for other mechanisms.’ (pg 1525)

‘Mineralized bone serves as an important store of P_i ions that can be retrieved from hydroxyapatite crystals through the action of parathyroid hormone on bone cells. Therefore, hypophosphatemia and phosphate deficiency are rare events in adults without other major complications, because of the large reservoir of P_i ions in the bone fluid compartment and in the mineral phase of bone.’ (pg 1525)

‘Some P_i ions that enter bone may enter bone cells, especially osteoblasts or lining cells, whereas other ions bypass the cells and go directly to the BFC, an extension of the blood-extracellular continuum. In the BFC, P_i ions in solution increase the P_i concentration (activity) which permits these ions to combine with calcium ions in excess of their solubility product (K_{sp}) and form mineral salts (precipitate) in bone extracellular tissue. The formation of hydroxyapatite crystals, i.e. mineralization, is essential for structural support and protection of internal organs from environmental trauma. The P_i ions are, therefore, essential for the formation of endoskeletons typical of most vertebrates except cartilaginous fish.’ (pg 1526)

‘The 5% or so of P_i in teeth is fixed and nonretrievable. The phosphates in bone tissue exist almost exclusively in the mineral phase, i.e. hydroxyapatite. The P_i ions in the bone crystals serve as an important reservoir for transferring ions to the blood compartment via PTH.’ (pg 1527)

Reference 3.23:

‘Between 80 and 85% (600-900 g) of phosphorus exists as phosphate in the calcium hydroxyapatite in the skeleton.’ (pg162)

‘Prolonged moderate hypophosphataemia leads to osteomalacia.’ (pg 162)

Reference 4.26:

‘Phosphorus... is needed for optimum bone health.’ (pg 6)

Reference 5.0:

‘Essential for bone formation and maintenance. Important in the development of teeth.’ (pg 88)

2) Cell membranes

Code Proposed statement

P2: Phosphorus is necessary for the normal structure of cell membranes, in the form of phospholipids.

Reference 1.4:

‘Structurally, phosphorus occurs as phospholipids, which are a major component of most biological membranes, and as nucleotides and nucleic acids.’ (pg 147)

Reference 3.23:

‘The residue is in soft tissues as phosphate, mainly as a component of proteins, phospholipids and nucleic acids;...’ (pb 162)

Reference 4.26:

‘Phosphorus occurs in phospholipids, a major constituent of most biological membranes, and as nucleotides (Food and nutrition Board, 1997).’ (pg 6)

Reference 5.0:

‘Maintenance in many metabolic functions, especially...phospholipids formation.’ (pg 88)

3) pH regulation

Code Proposed statement

P3: Phosphorus contributes to the normal regulation of pH levels in the body.

Reference 1.4:

‘ The functional roles include: (1) the buffering of acid or alkali excesses, hence helping to maintain normal pH;...’ (pg 147)

Reference 2.0:

‘Cellular uses of phosphates in intermediary metabolism are extensive. Typically P_i ions are converted to various organic phosphates (P_o)... In addition, phosphates circulating in blood have buffering activity.’ (pg 1525)

Reference 3.23:

‘Furthermore via the interconversion of HPO_4^{2-} and $H_2PO_4^-$ phosphorus contributes to extracellular and intracellular acid-base regulation.’ (pb 162)

Reference 4.26:

‘In addition to its role in supporting tissue growth, the functional roles of phosphorus include the buffering of acid or alkali excesses for the maintenance of normal pH as well as involvement in phosphorylation reactions for the activation of many catalytic proteins.’ (pg 6)

4) Energy metabolism

Code Proposed statement

P4: Phosphorus is necessary for normal energy production

Reference 1.4:

‘The functional roles include: ... (2) the temporary storage and transfer of the energy derived from metabolic fuels; and (3) by phosphorylation, the activation of many catalytic proteins.’ (pg 147)

Reference 2.0:

‘Cellular uses of phosphates in intermediary metabolism are extensive. Typically P_i ions are converted to various organic phosphates (P_o). Practically all energetic steps utilize high-energy phosphate bonds (adenosine triphosphate or ATP) ...’ (pg 1525)

‘Within cells the P_i ions are almost immediately used to phosphorylate glucose or other molecules...’ (pg 1526)

Reference 3.23:

‘5-20 mmol (0.2-0.6 g) is present intracellularly in a large variety of phosphorylated compounds (e.g. adenosine triphosphate (ATP), guanosine triphosphate, etc) which are needed for metabolic energy transfer and storage processes, enzyme activation and control.’ (pg 162)

‘Hypophosphataemia with intracellular depletion of phosphate is associated with muscle weakness and altered tissue oxygen tension, perhaps arising from defective synthesis of ATP and impaired delivery of oxygen to tissues as a consequence of depletion of red cell 2,3-diphosphoglycerate content.’ (pg 162)

Reference 4.26:

‘Phosphorus plays an important role in carbohydrate, fat and protein metabolism.... The energy that is required for most metabolic processes is derived from the phosphate bonds of adenosine triphosphate and other high energy phosphate compounds.’ (pg 6)

Reference 5.0:

‘As a component of nucleic acids (RNA and DNA), which are important in genetic transmission and control of cellular metabolism... Maintenance in many metabolic functions, especially energy utilization...’ (pg 88)

5) Tissue growth

Code	Proposed statement
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<i>P5:</i>	<i>Phosphorus is necessary for the normal tissue growth, such as muscle.</i>
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Reference 1.4:

‘...Since phosphate is not irreversibly consumed in these processes and can be recycled indefinitely, the actual function of dietary phosphorus is first to support tissue growth (either during individual development or through pregnancy and lactation) and, second, to replace excretory and dermal losses. In both processes it is necessary to maintain a normal level of P_i in the extracellular fluid (ECF), which would otherwise be depleted of its phosphorus by growth and excretion.’ (pg 147)

‘...during growth, soft tissue will be accreting phosphorus as well. On average, lean soft tissue growth accounts for about 1 mmol (31 mg) phosphorus for every 5 mmol (115 mg) added to bone (Diem, 1970).’ (pg 153)

‘There is a clear and absolute requirement for phosphorus during growth. At each growth stage, average net daily additions of bone and soft tissue mass can be approximated. Thus, the absorbed phosphorus intake can be readily estimated. In the mature adult, the requirement can be defined instead simply as the intake needed to maintain the plasma P_i within the normal range.’ (pg 159)

Reference 2.0:

‘Cellular uses of phosphates in intermediary metabolism are extensive. Typically P_i ions are converted to various organic phosphates (P_o). Practically all energetic steps utilize high-energy phosphate bonds (adenosine triphosphate or ATP) for the synthesis of organic molecules, to drive transport systems across cell membranes, to make muscles contract, to allow nerves to conduct impulses and transfer information, to convey genetic information...’ (pg 1525)

Reference 3.23:

‘The residue is in soft tissues as phosphate, mainly as a component of proteins, phospholipids and nucleic acids;...’ (pg 162)

Reference 4.26:

‘Phosphorus occurs in phospholipids, a major constituent of most biological membranes, and as nucleotides (Food and nutrition Board, 1997).’ (pg 6)

‘In addition to its role in supporting tissue growth, the functional roles of phosphorus include ...’ (pg 6)

Reference 5.0:

‘Important in building muscle tissue... Maintenance in many metabolic functions, especially...amino acid metabolism; protein formation.’(pg 88)

6) Breast milk

Code	Proposed statement
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<i>P6:</i>	<i>Phosphorus is necessary for normal breast milk.</i>
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Reference 1.4:

‘Currently no evidence supports that phosphorus requirements are increased during lactation. Apparently increased bone resorption and decreased urinary excretion of phosphorus (Kent et al., 1990), which occur independent of dietary intake of phosphorus or calcium, provide the necessary phosphorus for milk production.’ (pg 179)

Reference 2.0:

‘Human breast milk contains approximately 140 mg l⁻¹ of phosphate, compared with almost 340 mg l⁻¹ of calcium. These large quantities of ions typically cannot be entirely provided by absorbed P_i and calcium, and therefore the skeletal reservoir of mineral becomes a significant contributor to the P_i of milk during an extended lactation. Lactating mothers need adequate amounts of P_i and calcium to support the secretion of these ions in milk and subsequently to restore bone mineral lost during the peak period of a full lactation (6 months or longer).’ (pg 1526)

Reference 5.0:

‘Essential for normal milk secretion.’ (pg 88)

ANNEX 5

Reference List

Reference Group 1

US Institute of Medicine - Dietary reference intakes for vitamins and minerals:

- 1.1 *Institute of Medicine Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc.* Washington D.C. National Academy Press, 2001.
- 1.2 *Institute of Medicine Dietary Reference Intakes for Vitamin C, Vitamin E, selenium and carotenoids.* Washington D.C. National Academy Press, 2000.
- 1.3 *Institute of Medicine Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin and choline.* Washington D.C. National Academy Press 2000.
- 1.4 *Institute of Medicine Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride.* Washington D.C. National Academy Press, 1997.

Reference Group 2

- 2.0 *Encyclopedia of Human Nutrition 2E.* Editor-in-chief, Michele J. Sadler, editors, J.J. Strain, Benjamin Caballero. San Diego : Academic Press, c1999.

Reference Group 3

Reports of the European Scientific Committee on Food:

- 3.1 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Preformed Vitamin A (retinol and retinyl esters).* September 2002. (http://europa.eu.int/comm/food/fs/sc/scf/out145_en.pdf).
- 3.2 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Beta Carotene.* October 2000. (http://www.europa.eu.int/comm/food/fs/sc/scf/out80b_en.pdf).
- 3.3 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of vitamin D.* December 2002. (http://www.europa.eu.int/comm/food/fs/sc/scf/out157_en.pdf).
- 3.4 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin E.* April 2003. (http://www.europa.eu.int/comm/food/fs/sc/scf/out195_en.pdf).
- 3.5 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin K.* April 2003. (http://www.europa.eu.int/comm/food/fs/sc/scf/out196_en.pdf).

- 3.6** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B1.* July 2001.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out93_en.pdf).
- 3.7** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B2.* November 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80i_en.pdf).
- 3.8** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Levels of Nicotinic Acid and Nicotinamide (Niacin)* April 2002.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80j_en.pdf).
- 3.9** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Pantothenic Acid.* April 2002.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80k_en.pdf).
- 3.10** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B₆.* October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80c_en.pdf).
- 3.11** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Folate.* October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80e_en.pdf).
- 3.12** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Vitamin B₁₂.* October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80d_en.pdf).
- 3.13** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Biotin.* September 2001.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out106_en.pdf).
- 3.14** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Calcium.* April 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out194_en.pdf).
- 3.15** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Magnesium.* September 2001.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out105_en.pdf).
- 3.16** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Copper.* March 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out176_en.pdf).
- 3.17** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Iodine.* September 2002.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out146_en.pdf).
- 3.18** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Zinc.* March 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out177_en.pdf).
- 3.19** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Manganese.* October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80f_en.pdf).
- 3.20** *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Selenium.* October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80g_en.pdf).

- 3.21 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Trivalent Chromium.* April 2003.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out197_en.pdf).
- 3.22 *Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Molybdenum.* October 2000.
(http://www.europa.eu.int/comm/food/fs/sc/scf/out80h_en.pdf).
- 3.23 *Report of the Scientific Committee for Food (Thirty-first series) on 'Nutrient and energy intakes for the European Community' (1992).*

Reference Group 4

Draft reports of the UK Expert Group on Vitamins and Minerals:

- 4.1 *Revised Review of Vitamin A. Expert Group on Vitamins and Minerals.* April 2002.
(<http://www.food.gov.uk/multimedia/pdfs/reviewvita.pdf>).
- 4.2 *Review of Vitamin D – Revised Version. Expert Group on Vitamins and Minerals.* August 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm-00-11r.pdf>).
- 4.3 *Review of Vitamin E – Revised Version. Expert Group on Vitamins and Minerals.* July 2002.
(<http://www.food.gov.uk/multimedia/pdfs/evm-00-13r.pdf>).
- 4.4 *Revised Review of Vitamin K. Expert Group on Vitamins and Minerals.* April 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-01-09p.pdf>).
- 4.5 *Revised Review of Thiamin. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0014p.pdf>).
- 4.6 *Revised Review of Riboflavin. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/reviewriboflavin>).
- 4.7 *Review of Niacin – Revised Version. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-01-11r.pdf>).
- 4.8 *Review of Pantothenic Acid. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/panto.pdf>).
- 4.9 *Revised review of Vitamin B₆. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/EVM/00/19/P.pdf>).
- 4.10 *Review of Folic Acid. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0018p.pdf>).
- 4.11 *Revised review of Vitamin B₁₂. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/EVM/00/20/P>).
- 4.12 *Revised Review of Biotin. Expert Group on Vitamins and Minerals.* April 2002. (<http://www.food.gov.uk/multimedia/pdfs/biotin.pdf>).
- 4.13 *Revised review of Vitamin C. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/vitaminc.pdf>).
- 4.14 *Review of Calcium. Expert Group on Vitamins and Minerals.* March 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0112p.pdf>).
- 4.15 *Review of Magnesium. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-01-13.pdf>).

- 4.16 *Review of Iron – Revised Version. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm-01-12r.pdf>)
- 4.17 *Draft Review of Copper. Expert Group on Vitamins and Minerals.* May 2002. (<http://www.food.gov.uk/multimedia/pdfs/reviewofcopper.pdf>).
- 4.18 *Revised Review of Iodine. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0006p.pdf>)
- 4.19 *Revised Review of Zinc. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/reviewofzinc.pdf>).
- 4.20 *Review of Manganese – Revised Version. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm9922p.pdf>).
- 4.21 *Review of Sodium Chloride. Expert Group on Vitamins and Minerals.* April 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0108p.pdf>).
- 4.22 *Revised review of Potassium. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/potassium.pdf>)
- 4.23 *Revised review of Selenium. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/selenium.pdf>).
- 4.24 *Review of Chromium. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/reviewofchrome.pdf>).
- 4.25 *Review of Molybdenum. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/evm0009p.pdf>).
- 4.26 *Review of Phosphorus. Expert Group on Vitamins and Minerals.* August 2002. (<http://www.food.gov.uk/multimedia/pdfs/reviewofphosphorous.pdf>).

Reference Group 5

- 5.0 *Handbook of Nutrition and Food.* Edited by Carolyn D Berdanier...[et al.]. Boca Raton, Fla.: CRC Press, 2002.

Reference Group 6

Reports of the UK Committee on Medical Aspects of Food and Nutrition Policy:

- 6.1 *Nutrition and Bone Health: with particular reference to calcium and vitamin D.* Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of Food and Nutrition Policy. Report on Health and Social Subjects No. 49. DH: The Stationery Office, 1998.
- 6.2 *Folic Acid and the Prevention of Disease.* Report of the Committee on Medical Aspects of Food and Nutrition Policy. Report on Health and Social Subjects No. 50. DH: The Stationery Office, 2000.
- 6.3 *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.* Report of the Panel on Dietary Reference Values. Report on Health and Social Subjects No. 41. DH: The Stationery Office, 1991.
- 6.4 *Nutritional Aspects of Cardiovascular Disease.* Report of the Cardiovascular Review Group. Report on Health and Social Subjects No. 46. DH: The Stationery Office, 1994.

Reference Group 7

- 7.0** *Introduction to Human Nutrition*. Edited on behalf of The Nutrition Society by Michael J. Gibney, Hester H. Vorster and Frans J. Kok. Blackwell Science, September 2002.

Reference Group 8

Reports of the British Nutrition Foundation Task Force:

- 8.1** *Calcium*. The Report of the British Nutrition Foundation Task Force. The British Nutrition Foundation, 1989.
- 8.2** *Iron. Nutritional and physiological significance*. The Report of the British Nutrition Foundation Task Force. T.J. Press (Padstow) Ltd, 1995.

Reference Group 9

International Life Sciences Institute (Europe) Concise Monograph Series (1999):

- 9.0** *Calcium in Nutrition*. Michael Gurr. ILSI Europe Concise Monograph Series. 1999. International Life Sciences Institute.