Diagnosis and detection of deficiencies of micronutrients: minerals

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Determination of the presence or absence of a deficiency of one or more of the micronutrient minerals (usually described as trace elements) can be a complex problem, frequently requiring the integration of clinical, nutritional and biochemical data. Almost invariably, laboratory investigations are required and this short review describes the more common techniques applied for the various essential trace elements. Using a combination of techniques it is usually possible to determine, with confidence, whether an individual subject or small groups of subjects have a deficiency of a specific trace element, but simple reliable tests which can be used in population studies are still lacking for several key elements. This problem appears most acute for studies of chromium, copper and zinc, deficiencies of which may have important roles in the pathogenesis of a variety of human disorders.

Our ability to diagnose deficiencies of trace elements is dependent upon advances in our knowledge of their functions and biological roles. Thus, in general, contemporary investigators have assumed that the body status of a micronutrient can be adequately assessed by current criteria, but, as new research has revealed more subtle or less marked symptoms which can be related to a relative lack of the nutrient, this has prompted a reappraisal of the current efficacy of techniques. For example, it is generally assumed that the most common micronutrient deficiency, iron deficiency, can be readily detected by widely known clinical and laboratory criteria, but the recognition of more subtle roles and of transient deficiencies of the nutrient mean that more sensitive markers of marginal deficiency or markers of previous dietary history are still required.

The micronutrient minerals are usually classified under the title ‘trace elements’. These are generally defined as constituting less than 0.01% of body mass. A list of the essential trace elements and others which may eventually prove to be essential is given in Table 1. There is no general method by which deficiency of the different nutrients can be diagnosed.
Diagnosis and detection of deficiencies of micronutrients: minerals

Table 1 Essential elements for human nutrition

<table>
<thead>
<tr>
<th>Essential to man</th>
<th>Probably essential to man</th>
<th>Some evidence of essentiality in some animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>Arsenic</td>
<td>Bromine</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Lithium</td>
<td>Cadmium</td>
</tr>
<tr>
<td>Copper</td>
<td>Nickel</td>
<td>Lead</td>
</tr>
<tr>
<td>Fluorine</td>
<td>Silicon</td>
<td>Tin</td>
</tr>
<tr>
<td>Iodine</td>
<td>Vanadium</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molybdenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From Casey and Robinson and Gibson

and various different approaches need to be utilised. In common with methods for detection of vitamin deficiencies (see article by Bates in this issue), relevant information can be obtained from: (i) evaluation of dietary intakes in comparison with dietary reference values; (ii) examination of clinical signs; or (iii) evaluation of putative functional indices. In the majority of situations, analysis of biochemical indicators is required for definitive diagnosis. This short review will describe the author's views on the approaches used for the more commonly observed problems.

Recommended nutrient intakes

In many situations, the investigator will initially become aware of a potential abnormality in the status of a trace element by comparison of dietary intake data with recommended dietary reference values (e.g. such a procedure led to the recognition of a role for zinc deficiency in the pathogenesis of acrodermatitis enteropathica). Those UK Dietary Reference Values which are available for the essential micronutrients listed in Table 1 are given in Table 2. The reference nutrient intake (RNI) is given where this is available. This is the requirement intended to cover 97.5% of healthy people in the specified population group. For several elements, the committee felt that insufficient reliable data were available to produce an RNI and only a more broad category of 'safe intake' was recommended. However, such data must be treated with caution because they are only designed to be applicable to population studies and the difficulty in establishing these recommendations with acceptable precision for trace elements must be recognised. Thus, it is clear that, if a single subject is
Micronutrients in health and disease

Table 2 Dietary reference values for trace elements

<table>
<thead>
<tr>
<th>Element</th>
<th>Index</th>
<th>0–1 years old</th>
<th>1–10 years old</th>
<th>15–50+ years old</th>
<th>Pregnancy</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>RNI</td>
<td>0.3</td>
<td>0.7</td>
<td>1.2</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Iodine</td>
<td>RNI</td>
<td>0.06</td>
<td>0.11</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Iron</td>
<td>RNI</td>
<td>7.8</td>
<td>8.7</td>
<td>14.8*/11.3**</td>
<td>14.8</td>
<td>14.8</td>
</tr>
<tr>
<td>Selenium</td>
<td>RNI</td>
<td>0.01</td>
<td>0.03</td>
<td>0.075</td>
<td>0.090</td>
<td>0.090</td>
</tr>
<tr>
<td>Zinc</td>
<td>RNI</td>
<td>5</td>
<td>7</td>
<td>9.5</td>
<td>13</td>
<td>9.5</td>
</tr>
<tr>
<td>Chromium</td>
<td>SI</td>
<td>0.1–1.0*</td>
<td>0</td>
<td>0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoride</td>
<td>SI</td>
<td>0.05p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>SI</td>
<td>0.016*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molybdenum</td>
<td>SI</td>
<td>0.5–1.5*</td>
<td>0.05–0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values derived from UK Department of Health Report published in 1991. The original report contains a more detailed breakdown between population groups, the means of the data are reported here. Values are mg/day except for μg/kg/day and mg/kg/day.

RNI, reference nutrient intake; SI, safe intake.

• Value for women with average menstrual losses, high menstrual losses may necessitate iron supplementation; *value for young men (11–18 years).

found to have a dietary intake below those recommended, this can only provide a general indication of an increased likelihood of deficiency and further investigations must be undertaken in order to draw firm conclusions.

Tests for specific nutrients

This section provides a brief critical overview of those laboratory techniques which are in relatively widespread use for the diagnosis of specific trace element deficiencies.

Chromium

There is continuing debate about the biological role of chromium in mammalian systems following many years of investigations and speculation about a potential role in glucose metabolism and insulin activity. Marginal chromium deficiency has been reported in a number of population groups and, in these cases, the effect of chromium supplementation on glucose tolerance was the definitive procedure which identified the underlying relative deficiency. More generally, investigators have examined the chromium content of serum, erythrocytes, whole blood, urine or hair, although the precise relationship between whole body chromium status and the chromium concentration in any of these compartments has not been defined.
Obtaining accurate and precise analysis of chromium in biological materials has also previously limited the reliability of studies dependent upon measurements of chromium concentrations, with reported serum chromium values falling over 1000-fold in the last 30 years\(^4\). Thus, early publications in this area must be treated with caution.

**Copper**

Current laboratory techniques do not seem to be adequate for the diagnosis of marginal copper deficiency, although gross deficiencies are readily detected. During studies of experimental copper deficiency in man, it has become apparent that, with encroaching deficiency, clinical effects are seen, but conventional biochemical indicators of copper status gave unreliable data. Plasma copper concentrations may not drop significantly unless body stores are severely depleted\(^5\) and, additionally, they are influenced by non-nutritional factors, such as gender, age, hormones and infectious or endotoxin stress. Approximately 80% of the copper in plasma is present in caeruloplasmin, but measurement of this does not appear to offer significant benefits in comparison with plasma copper. Several workers have suggested that measurement of the activity of Cu,Zn superoxide dismutase (Cu,Zn SOD) may be a better index of body copper status (see Olivares & Uauy\(^6\)), but oxidative stress elevates Cu,Zn SOD activity even during copper deficiency\(^7\). Measurements of other copper-dependent enzymes also suffer from problems in interpretation/specificity of abnormal activities\(^5\)-\(^6\).

An example of the difficulty in interpretation of such biochemical measurements is the situation in elderly subjects. They habitually consume less than the recommended dietary intake for copper, and other trace elements\(^6\), but, paradoxically, plasma copper and caeruloplasmin levels are elevated in healthy elderly subjects, whereas indices of cellular copper (leukocyte copper) content are decreased with increasing age\(^8\). It has been suggested that isotopic studies of the size and turnover of body copper pools might aid in the diagnosis of copper deficiency\(^9\), but this requires verification.

**Iron**

The assessment of iron status has been the subject of numerous reviews and a number of biochemical and haematological techniques are available to provide relevant information in this area. Nevertheless, as previously stated, our increasing understanding of the manner in which marginal iron deficiency influences intellectual performance, etc.
Table 3  Effect of the different stages of iron deficiency on biochemical and haematological indicators of iron status

<table>
<thead>
<tr>
<th></th>
<th>Overload</th>
<th>Normal</th>
<th>Depleted stores</th>
<th>Iron deficiency</th>
<th>Iron deficiency anaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin</td>
<td>↑</td>
<td>N</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>↑↑</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>MCV</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↓</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

Data derived from Yip and Dallman\(^\text{10}\). MCV, mean corpuscular volume, N, normal value.

indicates the need to further develop our expertise in this area\(^1\). Various strategies for determining iron status in population groups have been proposed including the so-called ‘ferritin’ model used by the second US National Health and Nutrition Examination Survey which measured serum ferritin, transferrin saturation and erythrocyte protoporphyrin (cited in Yip & Dallman\(^\text{10}\)). Any subject having two or more abnormal tests was considered to be iron deficient. In routine assessment of individual subjects, iron deficiency is usually suspected on the basis of low haemoglobin concentration or haematocrit values, and, in mild anaemia, this is usually immediately followed by an examination of the response to iron therapy. The relationship between the various different measures and the stages of iron deficiency is shown in Table 3\(^\text{10}\).

**Manganese**

According to Keen and Zidenberg-Cherr\(^\text{11}\), reliable indicators for the assessment of manganese status have not been identified, although most studies in this area have examined either whole blood manganese or serum manganese concentrations. Experience of human studies is relatively limited, but plasma manganese concentrations are reduced in subjects consuming low manganese diets and slightly higher than normal following consumption of manganese supplements\(^\text{11}\). In an analogous manner to the situation reported for copper, measurement of the activity of the mitochondrial, manganese-containing superoxide dismutase has been suggested as a better index of body manganese status, but this appears to be sensitive to changes in oxidative stress even when there is no change in body manganese status\(^\text{12}\).

**Molybdenum**

Molybdenum has been recognised as having essential functions in mammals for almost 40 years\(^\text{13}\). It functions as a cofactor for various
enzymes involved in hydroxylation reactions, including aldehyde oxidase, xanthine oxidase/dehydrogenase and sulphite oxidase. Other possible functions have been proposed\textsuperscript{14}. Isolated syndromes related to a functional molybdenum deficiency\textsuperscript{15} and an acquired deficiency during prolonged parenteral nutrition\textsuperscript{16} have been described. Accurate determination of molybdenum levels in biological fluids and tissues is a complex problem with most reliable data now being obtained using relatively inaccessible techniques, such as inductively coupled plasma (ICP) emission spectrometry or inductively coupled plasma mass spectrometry (ICPMS). Analysis of the activity of the molybdenum-containing enzymes, therefore, remains the most practical and widespread method of choice for assessing functional molybdenum status in population studies.

**Selenium**

Interest in the assessment of selenium status has increased greatly in recent years with recognition of the importance of this nutrient in human health combined with evidence of a fall in dietary intakes in some European countries\textsuperscript{17}. The functional consequences of these reduced intakes are not well understood, but selenium is reported to have several biological roles, including as a constituent of: (i) several glutathione peroxidases (which act to detoxify lipid and other peroxides); (ii) iodothyronine de-iodinases (to produce the active thyroid hormone, tri-iodothyronine); and (iii) selenoprotein P (which may act to prevent oxidative damage). Other selenoproteins are undoubtedly present in mammals, but have not yet been characterised\textsuperscript{18}.

It appears that both blood selenium levels and the activity of the selenium-dependent enzyme, glutathione peroxidase, respond in a predictable way to a fall in the dietary intake of selenium and are thus suitable for detection of deficiency in population studies. Until recently, the difficulty of analysis of selenium levels meant that most such studies used the enzyme activity as a rapid simple measure of status, but the development of rapid analytical techniques using ICPMS and the increasing availability of this instrumentation may change this pattern.

**Zinc**

The reliable assessment of zinc status has been a problem for nutritionists for a considerable period of time (e.g. see Solomons\textsuperscript{19}). Although zinc is known to be a constituent of well over 200 enzymes, a relative lack of zinc does not appear to reliably and reproducibly influence the activity of any of these! Most investigators now use a combination of techniques to assess body zinc status, although serum or plasma zinc
Micronutrients in health and disease

Concentration are most commonly assessed. However, whilst serum concentrations of zinc undoubtedly fall during zinc deficiency, they are also known to fall in situations unassociated with zinc deficiency, such as following postoperative or endotoxin stress. Direct analyses of the tissue zinc content are also subject to conflicting interpretations, although Gibson argues that hair zinc analyses can provide valuable information in studies of children.

In an attempt to overcome these problems, we have developed stable isotope techniques to measure the size and turnover of rapidly exchanging pools of body zinc. Our data and that of others indicate that some subjects may be at risk of deficiency due to a reduction in the zinc available in rapidly exchangeable body pools and that kinetic measurements of the size of these pools can provide a clinically relevant assessment of zinc status. At the present time, such approaches appear to offer the optimum way of assessing zinc status, but their widespread application seems limited due to the cost and complexity of the isotope and analytical technology required. In the absence of other valid indicators, firm evidence for a pre-existing deficiency continues to require the demonstration of a positive response to zinc supplementation.

Conclusions

It is difficult to draw general conclusions about the efficacy of our current technique of the assessment of deficiencies of the micronutrient minerals. Most workers in the area have their own favourite techniques and biases for each nutrient. The previous section has attempted to identify both those techniques in which the author believes some confidence can be placed and those where caution must be exercised. Where analyses of multiple micronutrients are required for population groups the situation becomes very difficult, since only relatively few measures can realistically be undertaken. In a recent key paper, an experienced research group attempted to evaluate nutrient adequacy of iron, zinc, copper and selenium in a ‘free-living’ population group in the UK. They chose to examine the following: iron (using haemoglobin concentration, packed cell volume, erythrocyte count, MCV and serum ferritin); zinc (serum zinc concentration and alkaline phosphatase activity); copper (serum copper concentration and erythrocyte Cu,Zn, superoxide dismutase activity); and selenium (whole blood glutathione peroxidase activity). Their choice of analytes is entirely justified from current data and the analytical work involved in such surveys should not be underestimated. However, it is depressing to consider that, even after more than 30 years of research in these areas, the measures used for...
assessment of zinc and copper status can be severely criticised for lack of sensitivity or specificity and there are no practical alternatives which can be recommended. This exemplifies the need for much further work to devise and evaluate valid biomarkers in these areas. It is also clear that such information will only come from a clearer understanding of the physiology and biochemistry of these essential and fascinating nutrients.

**References**


