

mentation in pregnant women. Furthermore, the optimum amount and type of supplement should be investigated.

A supplement combining iron with vitamin A may be an effective option in areas where subclinical vita-

min A deficiency is still prevalent [15, 16]. Finally, the use of intermittent supplementation should be investigated in terms of general efficacy, subject compliance, supplement distribution, and cooperation of health personnel.

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# Anaemia and haemoglobin status: A new concept and a new method of assessment

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## Abstract

*Traditionally, anaemia has been determined and interpreted by the magnitude and severity of iron deficiency and the impact of intervention strategies. Internationally, it is defined as a state in which the quality and/or quantity of circulating red cells are reduced below a normal level. The body employs several mechanisms during the development of anaemia to maintain the oxygen supply to the tissues. Thus, applying any quantitative cut-off point as an indicator for anaemia may lead to misclassification, since haemoglobin concentration does not necessarily reflect the level of tissue oxygen supply. Ideally, an assessment strategy should be able to determine both the degree of haemoglobin oxygenation and the haemoglobin concentration at a tissue level. The Erlangen microlight-guide spectrophotometer is a non-invasive instrument that can assess both capillary blood oxygenation and relative haemoglobin concentration.*

## Haemoglobin and anaemia

The detection of anaemia is highly dependent on the procedure, since the quantity and quality of circulating erythrocytes are assessed by laboratory methods. The quantity of circulating red cells is defined by their amount per blood volume. The quality of erythrocytes may be evaluated by both their morphologic characteristics and their haemoglobin concentration. The two most commonly used indexes are mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) [1].

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## Misclassification of anaemia

The haemoglobin concentration provides information about both the quality and the quantity of erythrocytes that is accurate and relatively easy to obtain. Therefore, it is agreed internationally that blood haemoglobin concentration serves as the key indicator for anaemia [2], and a low concentration is the most common definition [3]. Different biological groups have different cut-off points for haemoglobin levels below which an individual is classified as anaemic (table 1) [4, 5]. However, the cut-off points describe only very approximately—and incompletely—the anaemia in a given individual. Based on response to iron supplementation, about 20% of women of a sample in Gothenburg, Sweden, were wrongly classified as anaemic (false positive) or non-anaemic (false negative) using the capacity to respond to iron with an increment of greater than 10 g/L in haemoglobin concentration [6].

In principle, there are several reasons for misclassification of anaemia. First, biological data for healthy individuals within a population show a normal distribution, which is also the case for the distribution of haemoglobin values [1]. Therefore, a certain number of healthy individuals always fall below a given cut-off point. The risk of wrongly classifying these people (false positive) will be smaller the farther the cut-off point is set to the left of the mean. In the case of a normal distribution of a non-anaemic population, about 2.5% lie below  $-2$  SD because of their genotypic characteristics. The number of these false-positive results plays only a minor role and therefore is not considered in practice. However, the farther the cut-off point is pushed to the extreme, the higher is the probability of false-negative results. This means that the use of a cut-off point for identifying or excluding patients will have a cost of lower sensitivity or lower specificity.

Second, genetic differences exist not only within but also between populations regarding haemoglobin levels. For example, the prevalence of low

TABLE 1. Haemoglobin cut-off points of anaemia in different population groups living at sea level

Population group	Haemoglobin concentration in blood (g/L)
Reference 4	
children 0.5-6 yr	110
children 6-14 yr	120
non-pregnant women	120
pregnant women	110
men	130
Reference 5	
children 0.5-4 yr	110
children 5-10 yr	110
girls 11-14 yr	115
boys 11-14 yr	120
adolescent girls	120
adolescent boys	130
women	120
men	135

haemoglobin concentrations is higher among African Americans than Caucasian Americans [7]. Thus, using the same cut-off point across different population groups may result in miscalculating the prevalence of anaemia.

Third, in addition to genetic constitution, the haemoglobin level may vary because of a person's biological constitution and the surrounding environmental conditions. Both may lead to different cut-off points. Some examples of variation among the recommended cut-offs are illustrated in table 1, as age, gender, and pregnancy are all considered when defining these values. Additional factors such as smoking and altitude may lead to an increase in the haemoglobin level of healthy individuals. Therefore, the US Centers for Disease Control (CDC) [8], for example, recommended additional cut-off values.

Furthermore, the alteration of a biological characteristic occurs gradually. However, the recommendations for cut-off points for biological and environmental differences are made gradual for practical reasons. Age groups can be subdivided into smaller groups [5], and the cut-off points differentiated by finer increments, such as 5 g/L rather than the 10 g/L used by WHO [4]. In the case of altitude, for instance, the CDC [8] goes even further and recommends a formula to calculate continuous cut-off points to reduce the possibility of misclassification.

### Homeostasis of decreased oxygen supply

It is well known that a reduced quantity and quality of erythrocytes and a decreased haemoglobin level lead to a deteriorated oxygen supply. However, the

body employs the following strategies to avoid a decreased oxygen supply at the tissue level:

- » High amount of oxygen per unit of blood
  - high number of erythrocytes (increased production, longer life-span);
  - high haemoglobin content in erythrocytes (increased MCV and MCH).
- » Increased amount of blood transported
  - high cardiac output per unit time to body tissue per unit time;
  - increased tissue perfusion (muscle work, intestinal digestion work, skin thermoregulation, etc.).
- » Efficient gas exchange between blood and tissue (decreased oxygen affinity of haemoglobin)
- » Low oxygen metabolism (decreased oxygen consumption)

The most important compensatory adjustment of a chronically insufficient oxygen supply is an increased rate of red cell production [9]. This phenomenon is well known in people living for extended periods at higher altitudes. After several weeks of residence high above sea level, the number of red cells in the bloodstream and, as a consequence, the haemoglobin level will be increased [10].

When the serum volume is unusually high, another mechanism employed by the body is to reduce the serum volume, causing an increased haematocrit. Thus, the amount of haemoglobin per pulmonary volume increases, allowing the body to transport more oxygen per heartbeat. As an example, haemoglobin values ordinarily rise after a woman gives birth, with a return of blood volume to normal non-pregnant values [11].

All these factors relate to the quantity and quality of red cells. Additional mechanisms, however, ensure an adequate supply of oxygen to the tissues. Increased energy consumption due to intensive muscle work, for example, leads to an elevated cardiac output. The cardiac output increases in a roughly linear fashion with increasing severity of anaemia [9]. Although the increase of cardiac output is an effective compensatory device, it is metabolically expensive [12, 13]. The overall consumption of oxygen may actually be 10 to 15 times higher in anaemia, a fact that contributes to the metabolic cost of cardiac and pulmonary overactivity [14].

Pathological changes in oxygen supply can lead to centralization. This means an increased peripheral resistance of blood flow to the skin [15], intestine, and kidneys [16]. Thus, central blood pressure will be stabilized, and the oxygen supply to central organs such as the brain and heart will be maintained [9]. A discrepancy between mean tissue and venous partial pressures of oxygen [17] indicates a heterogeneity of capillary flow through the organs (except the heart and kidneys). In fact, that may prove the existence of high-flow capillary channels in skeletal mus-

cle that serve a physiological function as a local reserve of oxygen. This may be considered a precautionary mechanism that ensures that oxygenated blood is immediately available through local distribution to prevent tissue anoxia.

Another important factor for an adequate oxygen supply is the oxygen affinity of haemoglobin in the red cells. This is usually expressed in terms of  $P_{50}$ , the oxygen tension at which haemoglobin is half-saturated. Decreasing the oxygen affinity (an increased  $P_{50}$  value) facilitates the delivery of oxygen to the tissues. This occurs in a number of situations, such as exposure to high altitude, blood loss, and anaemia [18]. Values determining the oxygen affinity of haemoglobin are temperature, pH, and the concentration of 2,3-diphosphoglycerate (DPG) in the erythrocytes. Higher temperatures, lower pH values, and an increased concentration of DPG all lead to decreased oxygen affinity [9].

Thus, decreased oxygen affinity of haemoglobin is one mechanism by which the body increases the oxygen supply to the tissues. It is one of the first mechanisms mobilized during anaemia.

### Definition of anaemia

The usual definition of anaemia refers only to the first of the above-listed strategies by which the body ensures an adequate oxygen supply to the tissues, namely, increasing the oxygen content of the blood by increasing the number and/or the haemoglobin content of the erythrocytes. As a consequence of this definition, the state of anaemia does not necessarily coincide with the body's ability to meet its tissue oxygen demands. As mentioned, however, several additional mechanisms regulate oxygen supply. It can be assumed that these mechanisms act synergistically and compensate for each other. For example, an efficient erythrocyte distribution can compensate for a low haemoglobin concentration.

Besides these factors, a major conceptual problem is seen in both the definition of anaemia and the selection of an adequate indicator. Internationally, there is no common, generally accepted definition of anaemia. Those most commonly used are structural descriptions, related to the qualitative and quantitative characterization of erythrocytes in the bloodstream. An example of such a structural definition is that anaemia is the state in which the quality and/or quantity of circulating red cells is reduced below normal level [19]. Other structural definitions consider the haemoglobin as a reference point; however, they do not mention the functional role of red cells and their effect related to anaemia.

Very few functional definitions of anaemia exist in the literature. One is that it is a disorder in which the

patient suffers from tissue hypoxia, the consequence of the low oxygen-carrying capacity of the blood [9]. Although functional definitions have the advantage of more flexibly covering the expressions of anaemia, here again they are limited to the quality of the oxygen-carrying capacity of the erythrocytes.

In addition to the transport of oxygen, erythrocytes may have other functional tasks in the body. If this is the case, an insufficient quantity and quality of red cells could consequently have several additional effects on the body's metabolism beyond the simple oxygen supply for tissue metabolism. As a consequence, anaemia would have side effects that are not fully consistent with the effect of inadequate haemoglobin.

Both definitions are driven by the concept that anaemia is caused by an inadequate quantity and quality of red cells. Thus, the authors of the definitions conclude that a haemoglobin concentration below that normally seen in healthy populations best characterizes anaemia. However, as previously explained, several other factors may influence the oxygen supply of body tissue. This shows that the definition is less objective or function-oriented, that is, influenced by its functional consequences, but more instrumental or indicator-oriented by the measurement to detect anaemia. Because the different mechanisms responsible for an adequate oxygen supply are interrelated, it may well be that the body tissues receive a sufficient amount of oxygen despite a haemoglobin level that is below normal. In such cases, iron supplementation is less effective than in cases of lower haemoglobin levels showing less effective compensatory mechanisms. Consequently, the following definition is suggested:

Anaemia is a disorder in which relative tissue hypoxia is caused by inadequate oxygen uptake, transport, distribution, and/or delivery.

In contrast to conventional definitions, the current assessment strategy is guided by methods that involve measuring the quality and quantity of red cells. As previously stated, the haemoglobin level is the most commonly used indicator, being both relatively easily and accurately measured. However, it provides information about only a part of the body's strategy to supply the tissues with adequate oxygen. It may well be that, in any given individual, a reduced haemoglobin level could be compensated for by other mechanisms.

### Assessment of functionally defined anaemia

More and more evidence suggests that both high iron stores and oxygen saturation may cause an increased

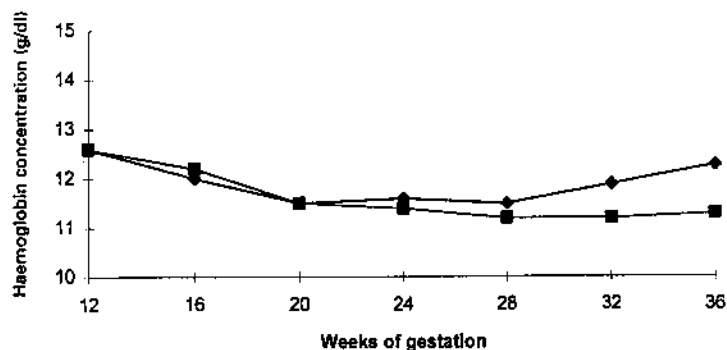


FIG. 1. Mean haemoglobin concentration in pregnant women treated daily with a placebo (■) or 200 mg ferrous iron (◆). (Modified from ref. 22.)

amount of free radical formation [20]. Thus, it is likely that the human body has developed mechanisms to regulate its oxygen supply other than those directly affecting the oxygen saturation. One indicator of this effect may be that during pregnancy, women experience lower haemoglobin levels, and it is nearly impossible to increase haemoglobin levels during the first 24 weeks of gestation, even with iron supplementation (fig. 1) [21–23]. Several physiological changes occur, such as increased cardiac output, increased blood volume, and increased DPG concentration in the red cells. These mechanisms maintain sufficient oxygen supply to the foetus [24].

It may be that free radicals harm foetal cells, having a particularly deleterious and dangerous impact during the first months of development. Thus, the body of the pregnant woman may have to develop alternative mechanisms to guarantee an adequate oxygen supply. Even though the woman may be anaemic by definition, her own relative oxygen supply may be met. It would be of interest to receive further information about other factors responsible for determining the oxygen supply to the tissues.

In newborn infants, increased cardiac output and increased DPG concentration play a role when the haemoglobin level is low. These mechanisms might compensate for the effect of the low level. As a result, it is difficult to determine whether a newborn is anaemic or not on the basis of haemoglobin cut-off points [25]. In summary, it may well be that the reduction in the haemoglobin level during different biological stages is purposefully driven to avoid negative side-effects.

In synthesis, several factors are responsible for a decreased haemoglobin level in the blood. The most prominent is iron deficiency. However, a lower haemoglobin level does not necessarily indicate anaemia, as several mechanisms support the oxygen sup-

ply in compensation. At the individual level, it is difficult to define a minimum haemoglobin level, since one mechanism may compensate for the task of another. Furthermore, it may well be that under certain biological conditions it is actually advantageous to reduce the level. Thus, a more comprehensive strategy of haemoglobin assessment is required to identify iron deficiency. Such a strategy must be capable of determining the oxygen supply according to the needs of the tissues. In particular, it should assess non-invasively both the haemoglobin concentration in different body tissues and the degree of oxygenation of the haemoglobin.

### The Erlangen microlight-guide photometer

The Erlangen microlight-guide photometer (EMPHO) is an apparatus designed to measure back-scattered light from living tissues *in situ*. It was developed at the Institute for Physiology and Cardiology of the University of Erlangen-Nürnberg [26]. It consists of four functional modules: the light source, the microlight-guide cable, the detection device, and the computing system [27] (fig. 2). Non-monochromatic light from a xenon arc lamp is transmitted by a central microlight-guide closely surrounded by a hexagon of six detecting fibres to the tissue surface. Both the illuminating fibre and the detecting microlight-guides are encased in a flexible rubber tube. At the measuring end they are glued together and inserted into a stainless steel cannula. At the other end they are attached to the optical instrument by means of special plugs. The six detecting fibres carry the light to a filter disc.

During measurement, back-scattered light is transmitted by the microlight-guides to the detection device. The filter disc, transparent only to a certain wave-

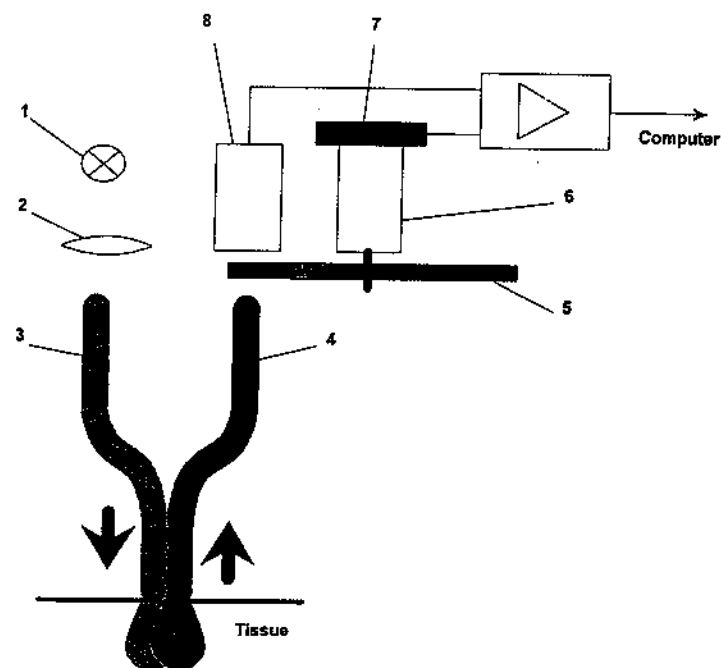


FIG. 2. Schematic drawing of the EMPHO detection device. 1. Xenon arc lamp; 2. lens system; 3. illuminating microlight-guide fibre; 4. detecting microlight-guide; 5. interference filter disc; 6. driving micromotor; 7. decoder wheel; 8. photomultiplier tube. (Modified from ref. 27.)

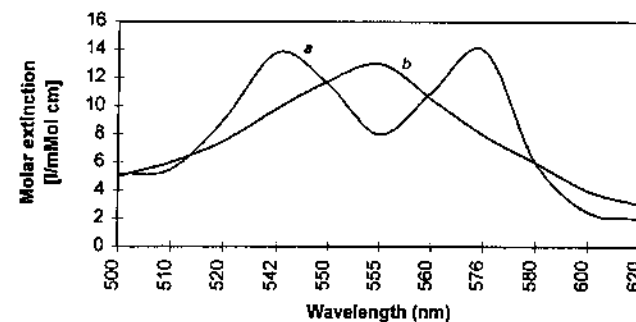


FIG. 3. Wavelength-dependent molar extinction of oxygenated (a) and deoxygenated (b) haemoglobin solutions. (Modified from ref. 26.)

length at a certain angle, monochromatizes the light. The intensity of the passing light is then determined using a photometer. By this method, 64 measurements are taken from wavelengths 502 to 628 nm, thus constructing a spectrum. As deoxygenated hae-

moglobin and oxygenated haemoglobin have two very different remission spectra (fig. 3), information about the haemoglobin concentration can be obtained. Specifically, EMPHO is able to measure both capillary blood oxygenation and relative hae-

moglobin content per volume of tissue at a very high speed (up to 100 spectra/second) and very low volume (five capillaries on average).

Oxygenated and deoxygenated haemoglobin can be regarded as two colours, bright red and crimson red. The shape of an unknown haemoglobin spectrum is compared with calculated blends of fully oxygenated (double-peaked) and deoxygenated (single-peaked) haemoglobin spectra. By an iteration procedure and successive comparison, the actual haemoglobin oxygenation is fitted. The result is the absolute haemoglobin oxygenation per volume of tissue.

The relative intracapillary haemoglobin concentration can be measured when the initial spectra are set to 100% and compared with the spectra of all subsequent measurements. By multiplying the haemoglobin oxygenation in the capillaries by the capillary haemoglobin concentration, the local intracapillary oxygen content may be evaluated [26]. This apparatus has been applied successfully to experiments with the rabbit eye [27], human skin [28], beating heart [29], foetal scalp [30], and gerbil brain [31].

Because of the low tissue volume measured by the EMPHO, some measurements will be closer to an arteriole and others closer to a venule. Therefore, a distribution of different oxygenation and haemoglobin concentrations per volume of tissue will be observed. Theoretically, a low haemoglobin concentration per unit blood volume will lead to values for

haemoglobin oxygenation and haemoglobin concentration per volume of tissue that are lower than normal. Thus, tissue hypoxia could be quite directly assessed.

Furthermore, by measuring different tissues, information about the redistribution mechanism may be obtained. On the other hand, the concentration of haemoglobin per volume of tissue might indicate the quantity and quality of erythrocytes, whereas the blood oxygenation provides information about the original blood oxygenation at a tissue level.

In summary, the evidence suggests that anaemia may have to be redefined to reflect truly its pathological effects. Furthermore, if oxygen supply-supporting mechanisms do indeed have predominant effects, it should be questioned whether haemoglobin per volume of blood is in fact an appropriate indicator of iron deficiency, as low values may be the result of choice rather than of iron deficiency.

Thus, future studies with EMPHO may provide information not only about the status of haemoglobin per volume of blood by determining haemoglobin per volume of tissue, but also about anaemia, which will make possible a judgement about the appropriateness of haemoglobin per volume of blood as an indicator of iron deficiency. The study of anaemia using new, non-invasive, tissue-accessing technology may therefore provide a better understanding of the indicators used to determine iron deficiency and may even provide more appropriate indicators.

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