Measurement of Total RBC Volume Relative to Lean Body Mass for Diagnosis of Polycythemia

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Key Words: Polycythemia; Total RBC volume; Lean body mass; Obesity

Abstract

An elevated total RBC volume (TRCV) in milliliters per kilogram of body weight has been an essential criterion for determining whether a person is polycythemic. This may be misleading in obese subjects as the TRCV per kilogram of fat is only one-tenth that of the TRCV of the lean body mass (LBM). Various formulas based on surface area have been used to account for this difference, but they are not always reliable. Direct measurement of TRCV per kilogram of lean body mass was obtained originally in studies in which body composition was determined by the combined body density and total body water measurement method. This is impractical as a routine procedure, but simple-to-use instruments are now available for direct measurement of a person’s body composition and percentage of fat by impedance technology. Thus, the TRCV can be obtained by a direct measurement that discounts the effects of fat, and a graph has been designed to normalize the TRCV to milliliters per kilogram of LBM. The TRCV for men and women has been established as 36 mL/kg LBM; when it is more than 43 mL/kg LBM, a diagnosis of polycythemia can be made with confidence.

The entry point for the diagnosis of polycythemia vera is an elevated hematocrit.¹ However, the hematocrit is not a reliable predictor of total circulating RBC volume (TRCV).¹,² A major criterion for the diagnosis of polycythemia vera is an elevated TRCV, but there is confusion about what constitutes an elevated TRCV, and there have been differences of opinion on how to interpret a measured TRCV, particularly in obese people.³,⁴ The Polycythemia Vera Study Group in 1965 adopted an upper limit of normal of 36 mL RBCs per kilogram of body weight for men and 32 mL/kg for women when measured with isotopically labeled RBCs.¹ That the method selected for measurement of the TRCV was by isotopically labeled RBCs and not calculated from a measured plasma volume and the hematocrit corrected for the ratio of body hematocrit to venous hematocrit has been confirmed recently.⁵ The values (32 mL/kg and 36 mL/kg for women and men, respectively) selected were a consensus judgment based on the observed upper limit in several studies of a large number of healthy subjects. However, expressing results in milliliters per kilogram of body weight is likely to lead to difficulty in interpretation in obese subjects; the value obtained may be within the range considered normal as a result of greater than normal body fat content.

In an attempt to overcome this difficulty various formulas based on height and weight have been used to predict the TRCV. The Expert Panel on Radionuclides of the International Council for Standardization in Haematology⁶ has analyzed data obtained from several sources that contained height, weight, and blood volume information from which the Expert Panel concluded “that it was not possible to establish which formula could be recommended.” Consequently, the Panel developed 2 new prediction formulas, one for men and a second for women, based on body surface area.
The Expert Panel established 98% and 99% reference ranges for the blood volume in healthy subjects and proposed that these reference ranges for the blood volume be used to determine whether a person’s TRCV is normal or indicates anemia or polycythemia.6

There has been considerable discussion and confusion on the use of the various formulas. In this article, we describe a new approach based on direct measurements of the influence of body fat on the TRCV and propose a practical method to overcome the misinterpretation of measurements for obese subjects.

In 1955, Siri and Berlin7 reported that, based on body composition data determined from a combined measurement of body density and total body water, in the healthy person, there were 36 mL of RBCs per kilogram of lean body mass (LBM) and 4 mL of RBCs per kilogram of fat. In this context, fat is distinct from adipose tissue, which is a mixture of lipids, minerals, carbohydrates, proteins, and water, while the LBM is proteins, minerals, carbohydrates, and water and often is called the “fat-free body mass.” In a sense, the Siri and Berlin data were forerunners of the finding by Muldowney8 and by Hume and Goldberg9 that the TRCV was more highly correlated with total body water than with body weight. What was missing at that time was a simple way of measuring body fat content, and the subject was dropped since the methods for measuring body fat content were not applicable in the day-to-day practice of hematology.

The marked difference between the volume of RBCs that can be attributed to the LBM (36 mL/kg of LBM) and the volume (3-4 mL/kg of fat) that can be attributed to body fat content and the wide range (from 5% to 60%) of body fat as a percentage of body weight make it desirable to have a direct measure of body fat so as to derive a predicted TRCV in milliliters per kilogram of body weight for any particular person.

Various methods have been used to determine body fat content and the fat-free LBM. These include measurement of body density by the Behnke underwater weighing method10 from a determination of body water using deuterium,11 tritium,12 antipyrine,13 or urea14; the combined body density and body water method of Siri15 that measured body volume by helium dilution16; direct visualization of the ratio of body fat to tissues described as distinguishing total body fat mass from muscle tissue by magnetic resonance computerized tomography and soft tissue radiography17; and, more recently, by total body electric conductance.18-20 For a general review, see Lukaski.21 Most of these methods are impractical in a routine hematology laboratory or nuclear medicine department.

The way toward a practical method came when it was shown that different tissues could be distinguished from each other by their electric conductance.18 Bioelectric impedance is based on the fact that fluids and electrolytes behave as electric conductors, while cell membranes behave as condensers. When a constant low-level alternating current is applied, its transmittance and impedance are controlled by the different biologic structures. Since fat-free tissue has a much greater conductivity than fat, an estimate can be made of the fat-free proportion of the body. Evaluation of the calculation of the fat-free body mass (LBM) from impedance measurements showed a high correlation with calculation by body water and densitometry methods, with a significantly lower prediction error than anthropologic techniques, thus demonstrating its validity and reliability.22 This led to the development of methods based on the bioelectric impedance to measure the relative amounts of body fat and fat-free mass (LBM).19-21

The increasing awareness by the general public of physical fitness and obesity control has prompted the popularization of simple-to-use and relatively inexpensive instruments to measure body fat content using this principle, eg, Tanita Body Fat Monitor (Tanita, Tokyo, Japan) and Holtain Body Composition Analyzer (Holtain, Crosswell, Wales). The latter has been in use at the Hammersmith Hospital, London, England, for several years alongside prediction formulas in blood volume studies.

Methods

Six sets of data are known to us contain the information necessary to determine how body fat content will influence the interpretation of TRCV.7,8,23-25 These were based variously on body density, total body water, or the combined body density and body water measurements using the Siri equation and estimation of body composition by measurement of electrical conductivity. For each set of data, the regression between the measured TRCV in milliliters per kilogram of body weight and body fat content was determined using the statistical package in Microsoft Excel 97 (Microsoft, Redmond, WA).

The various methods for calculating body fat are as follows:

1. Body Fat = Body Weight – LBM, where LBM = total body water/0.73226
2. From the body density equations of Rathbun and Pace27
3. From the combined body water and body density method of Siri25
4. As determined by bioimpedance impedance measurements to differentiate between fat and LBM as measured by the Holtain body composition analyzer in the present study. Other bioimpedance measuring devices are commercially available.
The methods based on body density alone and body water alone use a constant of 0.732 for the fraction of the LBM that is water. While this number is used commonly, it is not usually recognized that in 30 human subjects, the mean was 0.714 with an SD of 0.027.28

**Results**

Figure 1 shows the relationship between TRCV and body fat as a fraction of body weight derived from 4 studies8,23-25 that used the body water or body density method and 1 study that used the combined body density–body water measurement method.7 Table II lists the calculated value of B (milliliters of RBCs per kilogram of LBM) from the 5 studies using body water or body density alone or in combination.

Table II
Total RBC Volume (TRCV) *

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Subjects</th>
<th>TRCV (mL/kg LBM)†</th>
<th>TRCV (mL/kg fat)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siri and Berlin7</td>
<td>46</td>
<td>34.0 (31.2-36.8)</td>
<td>2.4 (1.5-3.4)</td>
</tr>
<tr>
<td>Huff and Feller25</td>
<td>62</td>
<td>30.9 (28.8-32.9)</td>
<td>1.4 (0.7-2.0)</td>
</tr>
<tr>
<td>Allen et al23</td>
<td>81</td>
<td>36.5 (34.8-38.2)</td>
<td>3.9 (3.2-4.5)</td>
</tr>
<tr>
<td>Hyde and Jones24</td>
<td>23</td>
<td>37.6 (36.0-39.1)</td>
<td>3.6 (3.0-4.1)</td>
</tr>
<tr>
<td>Muldowney8</td>
<td>22</td>
<td>34.9 (34.1-35.6)</td>
<td>3.3 (2.9-3.8)</td>
</tr>
<tr>
<td>All 5 studies</td>
<td>234</td>
<td>34.7 (33.6-35.7)</td>
<td>2.8 (2.4-3.2)</td>
</tr>
<tr>
<td>4 Studies§</td>
<td>172</td>
<td>35.7 (34.6-36.8)</td>
<td>3.4 (2.9-3.7)</td>
</tr>
</tbody>
</table>

LBM, lean body mass.

* In milliliters per kilogram of LBM calculated from 4 studies of body water or body density and 1 study of the combined body water–body density method in healthy persons. Data are given as mean (95% range).
† Derived from intercept to y axis where fat is 0% of body weight.
‡ Derived from slope of regression line (see Figure 1).
§ Excluding Huff and Feller, as there are technical reasons to question the use of their data.

B is the intercept on the TRCV axis when the body fat content is extrapolated to zero. The slope of the regression line is the volume of RBCs per kilogram of body fat.

If, as Siri and Berlin7 assumed, the total circulating TRCV can be considered a 2-compartment system, 1 compartment attributed to the LBM and 1 compartment to body fat, then the TRCV in milliliters per kilogram of body weight takes the following form:

\[ Y = B - MX \]

where \( Y \) = TRCV in milliliters per kilogram of body weight, \( B \) = milliliters of RBCs per kilogram of LBM, \( M \) = milliliters of RBCs per kilogram of fat, and \( X \) = body fat as fraction of body weight. \( M \) has the value of 3.4 mL of RBCs per kilogram of body fat and is assumed to apply to healthy persons and to those who are anemic or polycythemic. \( B \) is 35.7 mL of RBCs per kilogram of LBM for healthy persons.

**Discussion**

The noninvasive methods for determining body fat are as follows:

1. From the body mass index (Quetelet index), which is a height and weight formula29
2. Dual absorption x-ray measurements30
3. Biologic impedance measurements18-20
4. Infrared absorption31

The body mass index is the least satisfactory of these approaches to a noninvasive estimate of body fat content (see Figure 1 in Gallagher et al32). Today, the availability of comparatively simple, inexpensive, and noninvasive devices for measuring body fat content makes it desirable to explore the usefulness of these instruments for measuring body fat content. This in turn makes it desirable to explore the usefulness of these instruments for calculating body fat content and using that information to interpret a TRCV measurement.

The devices have the potential of being particularly useful in the evaluation of patients with an elevated hematocrit and, in particular, for those nearing the limit separating the normal from an increased TRCV, an area in which interpretation has been difficult.

Figure 1, which shows the relationship between body fat content and TRCV expressed as milliliters per kilogram of body weight, can be used to determine whether a person has a normal TRCV or is anemic or polycythemic. For example, a person whose body fat content is 50% with a normal TRCV in milliliters per kilogram of LBM would have a TRCV of 19 mL/kg of body weight, whereas a lean person (fat = 10% of body weight) would have a TRCV of 33 mL/kg of body weight. If it is assumed that at any given level of body fat content the normal reference values would lie in
a reference interval of ±20% of the mean, the obese (fat = 50% of body weight) person would have an elevated TRCV when the measured value is 23 mL/kg or greater, whereas the TRCV is elevated in the lean person when it is 40 mL/kg or greater.

An example of the use of this method of evaluating a measured TRCV is shown in **Figure 2**, which shows the relationship between hematocrit and TRCV per kilogram of LBM in a series of patients studied at the Hammersmith Hospital by measuring the TRCV with chromium 51–labeled RBCs and body fat content by using the Holtain analyzer. This shows that in the region where the hematocrit is between 0.50 and 0.60, 21 (42%) of 50 patients had a TRCV when expressed as RBCs in milliliters per kilogram of LBM that was within the normal limits. Above a hematocrit of 0.60, all had an elevated TRCV. This finding is similar to what Berlin1 and Najean et al2 found for patients evaluated for entry in Polycythemia Vera Study Group protocols when the TRCV was expressed as milliliters per kilogram of body weight.

**Conclusions**

**Figure 3** can be used to obtain values for the TRCV normalized to its fat-free equivalent. Figure 3 is Figure 1 with the data points removed. For any given person when the percentage of fat has been determined, a line can be drawn vertically from the x-axis to the slope; where it intersects the slope, a horizontal line is drawn to the y-axis, which gives a reading of the normalized TRCV for that person. When the calculated TRCV per kilogram of LBM is greater than 120% of this reading, a diagnosis of polycythemia can be made with confidence. This is equivalent to more than 43 mL/kg of LBM, and it applies equally to men and women.

**References**


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