Plasma carnitine compartment and red blood cell carnitine compartment of healthy adults

Peggy R Borum, PhD

ABSTRACT Carnitine is needed for a variety of important physiological functions in energy metabolism. Assessment of the carnitine status of an individual is compromised by limited data on the number of metabolic compartments of carnitine and their interrelationship, if any. Possible compartmentalization of carnitine in the blood of healthy adults was investigated because blood is one of the more readily available samples for the assessment of carnitine status. The data suggest that blood carnitine is partitioned into a plasma carnitine compartment and a red blood cell carnitine compartment, compartments that are separate and distinct metabolic compartments.

KEY WORDS Carnitine status, plasma carnitine, red blood cell carnitine

Introduction

Carnitine functions in a variety of critically important physiological processes that produce and maintain adequate levels of metabolic energy (1). During the past 15 y, a variety of different types of patients have been shown to be at risk for carnitine insufficiency (2). Although carnitine insufficiency can lead to severe physiological sequelae and even death, our understanding of the compartmentalization of carnitine in the human is limited. Blood is one of the more readily available samples for carnitine assessment of patients and was therefore used in our first investigation of the metabolic compartments of carnitine in humans.

The purpose of this study is to examine blood total carnitine concentrations in healthy adults and to evaluate the hypothesis that blood carnitine is partitioned into a plasma carnitine compartment and a red blood cell carnitine compartment, compartments that are separate and distinct metabolic compartments.

Methods

Samples of blood for the determination of normal values were obtained through the cooperation of the American Red Cross, Nashville, TN Chapter. A tube of blood with EDTA as anticoagulant was collected from each donor by the Red Cross for typing purposes. At prearranged Bloodmobile Collection Centers, the blood remaining after typing was kept on ice and transported to the laboratory within 2 h after being drawn. The age and sex of the donor were recorded.

Each blood sample was processed for carnitine analysis immediately upon receipt in the laboratory. The blood was separated into plasma and red blood cell fractions by centrifugation in a Beckman TJ-6 refrigerated centrifuge at 2000 × g for 15 min. The plasma was removed and frozen at −20°C until analysis. The buffy coat was removed and the red blood cells were washed twice with normal saline and frozen at −20°C until analysis. Plasma and red blood cells were assayed for total carnitine by the procedures described previously (3, 4).

Hemoglobin was measured using a Fisher Diagnostic Kit (Fisher Diagnostics, Orangeburg, NY) based on a modified Drabkin method (5). Hematocrits were determined using an International Micro-Capillary Centrifuge, Model MB, and an International Micro-Capillary Reader (International Equipment Company, Needham Heights, MA).

[1-14C]Acetyl coenzyme A and Aqueous Counting Scintillant were purchased from Amersham Corporation (Arlington Heights, IL), carnitine acetyltransferase from Sigma Chemical Company (St Louis, MO), L-carnitine from General Biochemicals, coenzyme A from P-L Biochemicals (Milwaukee, WI), and Dowex 2 × 8 from Bio-Rad Laboratories (Richmond, CA).

Data were analyzed using the VAX computer system in the Institute of Food and Agricultural Sciences at the University of Florida and the Northeast Regional Data Center of the State University System of Florida. The mean and standard deviation for each group were determined using standard statistical methods (6). The Mann-Whitney, Wilcoxon Rank Sum Test was used to test possible differences between means and the Spearman

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Table 1: Definitions of indices describing the plasma and red blood cell total carnitine compartments

<table>
<thead>
<tr>
<th>Index</th>
<th>Units</th>
<th>Method of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma carnitine (PC)</td>
<td>nmol/mL</td>
<td>Direct determination</td>
</tr>
<tr>
<td>Micromolar carnitine concentration of plasma aqueous phase (μMPC)</td>
<td>μM</td>
<td>(PC) ( \div (0.93) ) (nmol/mL) + (mLH2O/mL)</td>
</tr>
<tr>
<td>Red blood cell carnitine (RC)</td>
<td>nmol/g Hgb</td>
<td>Direct determination</td>
</tr>
<tr>
<td>Micromolar carnitine concentration of red blood cell aqueous phase (μMRC)</td>
<td>μM</td>
<td>([RC/wb] + [HCT/100] ( \div (0.73) ) ([nmol/mLwb] ( \div [mLrbc/mLwb] )) + (mLH2O/mL RBC)</td>
</tr>
<tr>
<td>Whole blood carnitine (wbc)</td>
<td>nmol/mL</td>
<td>(PC/wb) + (RC/wb)</td>
</tr>
<tr>
<td>Percentage of whole blood carnitine in red blood cells (RC/wbc)</td>
<td>%</td>
<td>([RC/wb] ( \div [wbc] )) \times 100</td>
</tr>
<tr>
<td>Partitioning factor</td>
<td>none</td>
<td>(μMRC) + (μMPC)</td>
</tr>
</tbody>
</table>

* wb = whole blood, RBC = packed red blood cells.

Correlation Coefficient was used to determine possible correlation between carnitine indices or between age and each carnitine index (7).

Results

Plasma carnitine compartment

The carnitine concentration in the plasma compartment is usually expressed in the literature in units of nmol/mL (Table 1). The mean, standard deviation, 5th percentile, and 95th percentile for the plasma carnitine concentration of samples obtained from 446 males and 444 females aged 17–65 y are listed in Table 2. Plasma carnitine concentrations are significantly higher in adult males than in adult females (Mann-Whitney, Wilcoxon Rank Sum Test two-tailed \( p = 0.0001 \)). No significant correlation of plasma carnitine with age is observed in adult males. However, the plasma carnitine concentrations of adult females do correlate with age (\( p = 0.0001 \)). Plasma carnitine concentrations in adult females aged < 40 y are significantly lower than the plasma carnitine concentration in adult females aged > 40 y (Mann-Whitney, Wilcoxon Rank Sum Test two-tailed \( p = 0.0001 \)). The mean plasma carnitine concentration of 335 females aged 17-40 y is 41.7 ± 10.3 nmol/mL (±SD) and the mean plasma carnitine of 108 females aged 41-65 y is 50.3 ± 11.8 nmol/mL (±SD). Plasma carnitine concentrations in adult females aged < 40 y are significantly lower than the plasma carnitine concentration in adult males aged 17–40 y and 41–65 y (Mann-Whitney, Wilcoxon Rank Sum Test two-tailed \( p = 0.0001 \)). In contrast, the plasma carnitine concentration in adult females aged > 40 y is not significantly different from the plasma carnitine concentration in adult males of either 17–40 y or 41–65 y.

Carnitine is a water-soluble compound. Because there has been no indication that any major fraction of the plasma carnitine compartment is bound to any high-molecular-weight ligand, the carnitine appears to be dissolved in the aqueous phase of the plasma and does not occupy the plasma space occupied by the colloidal proteins. Table 1 lists the equation used to calculate the micromolar carnitine concentration of plasma aqueous phase and the values for this index are listed in Table 2. This index expresses the carnitine concentration of the plasma compartment in units that allow direct comparison with the carnitine concentration in the red blood cell compartment. Because the micromolar carnitine concentration of plasma aqueous phase is obtained by simple multiplication of the plasma carnitine concentration, the differences in values obtained for males and females as well as the differences in values obtained for females of different ages are also observed for this index.

Red blood cell carnitine compartment

The mean, standard deviation, 5th percentile, and 95th percentile for the red blood cell carnitine concentration expressed as nmol carnitine/g hemoglobin for samples obtained from 348 adults aged 17–65 y are listed in Table 3. The mean values obtained for red blood cell carnitine are not statistically different in males and females and there is no significant correlation of red blood cell carnitine concentration with age in either males or females. Thus the red blood cell carnitine indices are presented for males and females together in Table 3. If it is assumed that the major fraction of carnitine in the red blood cell is dissolved in the aqueous phase of the cell and does not occupy the red blood cell space occupied by colloidal proteins or membranes, the micromolar carnitine concentration of red blood cell aqueous phase index can be calculated using the equation listed in Table 1. The value obtained in the

Table 2: Carnitine concentrations in the plasma compartment of a reference population aged 17–65 yr

<table>
<thead>
<tr>
<th>Index</th>
<th>Sex</th>
<th>No of subjects</th>
<th>Mean ± SD</th>
<th>5th Percentile</th>
<th>95th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma carnitine (nmol/mL)</td>
<td>Male</td>
<td>446</td>
<td>51.7 ± 10.8</td>
<td>34.5</td>
<td>69.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>444</td>
<td>43.8 ± 11.3</td>
<td>26.6</td>
<td>63.4</td>
</tr>
<tr>
<td>Micromolar carnitine concentration of plasma aqueous phase (μM)</td>
<td>Male</td>
<td>446</td>
<td>55.6 ± 11.6</td>
<td>37.1</td>
<td>74.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>444</td>
<td>47.1 ± 12.2</td>
<td>28.6</td>
<td>68.2</td>
</tr>
</tbody>
</table>
TABLE 3
Carnitine concentration in the red blood cell compartment for a reference population aged 17-65 yr

<table>
<thead>
<tr>
<th>Index</th>
<th>Sex</th>
<th>No of subjects</th>
<th>Mean ± SD</th>
<th>5th Percentile</th>
<th>95th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell carnitine (nmol/g hemoglobin)</td>
<td>Male and female</td>
<td>348</td>
<td>160 ± 68</td>
<td>79</td>
<td>270</td>
</tr>
<tr>
<td>Micromolar carnitine concentration of red blood cell aqueous phase (µM)</td>
<td>Male and female</td>
<td>389</td>
<td>61.8 ± 23.8</td>
<td>29.9</td>
<td>106.3</td>
</tr>
</tbody>
</table>

The adult reference population is listed in Table 3. This index expresses the carnitine concentration of the red blood cell compartment in units that allow direct comparison with the carnitine concentration in the plasma compartment. As seen with the red blood cell carnitine, the mean values obtained for the micromolar carnitine concentration of red blood cell aqueous phase are not different in males and females and there is no significant correlation with age in either males or females.

**Whole blood carnitine**

The whole blood carnitine index is expressed as nmol/mL whole blood and is calculated by the equation listed in Table 1. This index represents the summation of the plasma carnitine compartment and the red blood cell carnitine compartment. Whole blood carnitine concentrations (Table 4) are significantly higher in adult males than in adult females (Mann-Whitney, Wilcoxon Rank Sum Test two-tailed \( p = 0.0001 \)). A significant correlation of whole blood carnitine with age is found in both males and females (level of significance of Spearman correlation coefficient is 0.0127 for males and 0.0199 for females). For example, 10 males aged 17–20 y have a value of 47.1 ± 8.0 nmol/mL compared with 54.9 ± 12.3 nmol/mL for 17 males aged 51–60 y. Eighteen females aged 17–20 y have a value of 42.9 ± 8.4 nmol/mL compared with 48.7 ± 12.0 nmol/mL for 13 females aged 51–60 y.

**Partitioning of carnitine between the plasma carnitine compartment and the red blood cell carnitine compartment**

The percentage of whole blood carnitine in red blood cells is calculated using the equation presented in Table 1 and the values for this index are listed in Table 4. The values obtained are not different in males and females and there is no significant correlation with age in either males or females.

Because a ratio of two indices requires that the two indices be expressed according to the same baseline, the micromolar carnitine concentration of red blood cell aqueous phase index and the micromolar carnitine concentration of plasma aqueous phase index are the only two indices describing the two compartments of interest which can be used in a ratio expression. The ratio is termed the partitioning factor and is calculated according to the equation presented in Table 1. The reference values are presented in Table 4. The partitioning factor is significantly higher in females than in males (Mann-Whitney, Wilcoxon Rank Sum Test two-tailed \( p = 0.0017 \)). No correlation of the partitioning factor with age is found in either males or females.

**Interrelationship of the plasma carnitine compartment and the red blood cell carnitine compartment**

Figure 1 presents a plot of the red blood cell carnitine index vs the plasma carnitine index. The values for the two compartments demonstrate little correlation with one another (Spearman correlation coefficient of 0.22). A plot of the micromolar carnitine concentration of red blood cell aqueous phase index vs the micromolar carnitine concentration of plasma aqueous phase index (data not shown) also demonstrates little correlation between the two compartments with a Spearman Correlation Coefficient of 0.18.

TABLE 4
Indices describing the summation of and partitioning between the plasma compartment and the red blood cell compartment for a reference population aged 17-65 yr

<table>
<thead>
<tr>
<th>Index</th>
<th>Sex</th>
<th>No of subjects</th>
<th>Mean ± SD</th>
<th>5th Percentile</th>
<th>95th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood carnitine (nmol/mL)</td>
<td>Male</td>
<td>191</td>
<td>50.1 ± 9.8</td>
<td>34.1</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>156</td>
<td>44.0 ± 9.4</td>
<td>30.7</td>
<td>60.6</td>
</tr>
<tr>
<td>Percentage of whole blood carnitine in red blood cells (%)</td>
<td>Male and female</td>
<td>347</td>
<td>37 ± 9</td>
<td>22</td>
<td>53</td>
</tr>
<tr>
<td>Partitioning factor</td>
<td>Male</td>
<td>191</td>
<td>1.09 ± 0.41</td>
<td>0.52</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>156</td>
<td>1.28 ± 0.56</td>
<td>0.55</td>
<td>2.49</td>
</tr>
</tbody>
</table>
The present investigation reports the total carnitine concentration in plasma and red blood cells in healthy adult humans and addresses the question of whether blood carnitine is divided into two separate metabolic compartments.

The plasma compartment is usually described by the index plasma carnitine, which is lower in adult females aged < 40 y than the levels found in adult females aged > 40 y or in adult males of all ages. However, there are no significant differences in the values for the plasma carnitine index determined in females aged > 40 y and the values determined in adult males of all ages. Although estradiol levels were not determined in any of the individuals of the reference population, it can be assumed that the segment of the reference population consisting of females aged < 40 y would have higher estradiol levels than any other segment of the population. The data obtained from this human reference population is consistent with data obtained from rats, which demonstrated that in rats there is an inverse relationship between estradiol concentrations and plasma carnitine concentrations (13).

The red blood cell carnitine compartment is described by the index red blood cell carnitine and by the index micromolar carnitine concentration of red blood cell aqueous phase. The fact that the indices describing the red blood cell carnitine compartment do not differ between male and female subjects or with the age of the female subjects as do the indices describing the plasma carnitine compartment is consistent with the hypothesis that the plasma carnitine compartment is distinct from the red blood cell carnitine compartment. The function of carnitine in red blood cells has not been elucidated. The regulation of carnitine by estradiol, as seen in plasma, does not appear to occur in red blood cells. Blood carnitine is described by the whole blood carnitine index as the summation of the plasma carnitine compartment and the red blood cell carnitine compartment. The evidence for the hypothesis that the blood carnitine is partitioned into two metabolically separate compartments would be strengthened if values for indices describing the compartments were not significantly correlated with one another. The plasma carnitine index is not highly correlated with the red blood cell carnitine index and the micromolar carnitine concentration of plasma aqueous phase index is not highly correlated with the micromolar carnitine concentration of red blood cell aqueous phase. The observation that the plasma compartment (but not the red blood cell compartment) is affected by gender and age also supports the hypothesis that the plasma carnitine compartment is separate and distinct from the red blood cell compartment.

An alternate method of analysis of blood carnitine concentration is to examine the partitioning of carnitine between the two compartments. The percentage of whole blood carnitine in red blood cells is an indication of the partitioning of carnitine between the plasma carnitine compartment and the red blood cell carnitine compartment but is necessarily influenced by the hematocrit value, which may in turn be altered by a wide variety of factors. Calculation of indices used to describe both the plasma carnitine compartment and the red blood cell carnitine compartment in units of nmol/mL (μM) allow the partitioning of carnitine to be described using a ratio index. More precise values for the partitioning factor would be obtained if the aqueous fractions of plasma and red blood cells were measured directly in each individual rather than estimated using an average value (14). The partitioning factor is higher in females than in males which indicates that the partitioning of carnitine between the two compartments may be regulated metabolically.

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References

CARNITINE IN PLASMA AND RBC OF ADULTS