Carnitine Concentration of Red Blood Cells[1-3]

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ABSTRACT The presence of significant concentrations of carnitine in red blood cells is documented using an assay procedure that is described in detail. Red blood cells can be prepared for assay by simple techniques and stored frozen. The reproducibility of the assay procedure is within acceptable limits. Usefulness of the procedure in an experimental setting has been documented with an investigation of both plasma and red blood cell carnitine concentrations of rats of different ages. Earlier studies have demonstrated that plasma carnitine concentrations are two-fold higher in adult male rats than in adult female rats. In agreement with that data, the adult male rat has a red blood cell carnitine concentration which is two-fold higher than that of adult female rats. *Am J Clin Nutr* 1985;41:653–656.

KEY WORDS Carnitine, red blood cells, fatty acid oxidation

Introduction

Plasma and serum carnitine levels have been measured since the development of the first carnitine assay method—the Tenebrio bioassay (1). An increasing number of reports of altered plasma carnitine concentrations under various physiological conditions have appeared in the literature during recent years (2). In most of these studies carnitine has not been measured in red blood cells and there has not been a comparison of whole blood carnitine concentrations to plasma or serum carnitine concentrations (3). Our laboratory has presented preliminary data indicating that red blood cells do contain carnitine (4). The purpose of this report is to present data demonstrating that both rat and human red blood cells do contain carnitine and to describe an assay procedure for the determination of carnitine concentrations in red blood cells.

Methods

All animals were Sprague-Dawley rats which were maintained on a carnitine free diet since weaning (5, 6). The care and use of laboratory animals in these experiments followed the guidelines of the National Research Council. Rat blood was obtained by heart puncture from rats anesthetized with Nembutal and was immediately placed in a tube containing heparin. Human blood was obtained from partially used but not outdated blood units from a local blood bank.

Blood was separated into plasma and red blood cells and the red blood cells washed with saline in a Beckman TJ-6 refrigerated centrifuge (Beckman Industries, Palo Alto, CA) using a TH-4 rotor at 3000 rpm for 15 minutes. Unless otherwise specified, red blood cells in all experiments were washed twice with saline.

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

Plasma and red blood cell carnitine were determined by the following modification of the method of Cederblad and Lindstedt (7). Washed red blood cells were broken by addition of an equal volume of deionized water and freezing and thawing three times. Triplicate aliquots of L-carnitine standard (1.25–15 nmoles) and 100 μl of plasma or 200 μl of broken red blood cells were incubated in 0.1 N KOH at 50°C for 30 min in a final volume of 250 μl to hydrolyze acetylcarnitine and acylcarnitine. The samples were cooled and 750 μl of 0.6 M perchloric acid added to precipitate protein. After centrifugation, 500 μl of the supernatant was neutralized with 4 N KOH using phenol red as an indicator. These tubes were

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incubated overnight at 4°C and then centrifuged to remove the potassium perchlorate salt. The clear pink supernatant was used for the carnitine assay. Three solutions were used to make an ingredient mix. Solution I was [1-^{14}C]acetyl coenzyme A at a concentration of 3.2 μM and a radiospecific activity of 50 mCi/mmol. Solution II was 0.1 mM acetyl coenzyme A prepared in the following manner: 10 mg coenzyme A was dissolved in 500 μl of cold H_2O, neutralized with 100 μl of 1 M $KHCO_3$, mixed well with 200 μl of 0.1 M acetic anhydride and diluted to 80 ml with cold H_2O. Both Solution I and II were stored in aliquots at −20°C. Solution III was 1 M potassium phosphate buffer, pH 7.0, containing 2 mg/ml N-ethyl-maleimide to pull the reaction to near completion and thus make the standard curve a straight line. The ingredient mix consisting of 2 volumes of Solution I and 1 volume each of Solution II and Solution III was made immediately before use and stored on ice. The reaction tube contained 100 μl of carnitine standard and 100 μl of the ingredient mix. The reaction was started with 1 unit of carnitine acetyltransferase in a volume of 20 μl. After incubation at 37°C for 30 min, 200 μl of the reaction mixture was transferred to a 5 × 35 mm column of Dowex 2 × 8 (Cl^-). The column was drained directly into a polyethylene scintillation vial and was washed with two 0.5 ml aliquots of cold H_2O. Nine milliliters of ACS (Aqueous Counting Scintillant) was added and the vials counted in a liquid scintillation spectrometer. In recovery studies, known quantities of carnitine were added at the plasma and red blood cell hemolysate levels.

[1-^{14}C]Acetyl coenzyme A and ACS were purchased from Amersham Corporation (Arlington Heights, IL), carnitine acetyltransferase and N-ethyl-maleimide 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). Potassium bicarbonate, potassium hydroxide, perchloric acid, potassium phosphate, and acetic anhydride were all obtained from Fisher Scientific (Pittsburgh, PA).

Results

Removal of plasma from the red blood cell pellet is essential before red blood cell carnitine can be studied. However, it is important that the washing procedure effectively remove plasma from the red blood cell pellet without removing the carnitine. Blood was drawn and divided into six separate aliquots. Red blood cells were pelleted by centrifugation and washed with saline for up to 5 times. The carnitine concentration of the red blood cell, the plasma, and the first washing is given in **Table 1.** There was detectable carnitine in the first wash but not in any of the succeeding washes. The carnitine concentration of red blood cells decreased with one and two washings reflecting the removal of trapped plasma in the pellet. The carnitine concentration of red blood cells did not decrease when the number of washings was increased beyond two indicating that the red blood cell carnitine was not rapidly diffusing into the surrounding media. Therefore, red blood cells can be separated from plasma and washed with saline to remove any plasma trapped in the red blood cell pellet without washing out the carnitine within the red blood cell.

TABLE 1
Carnitine concentration of human plasma, washed red blood cells and washings

Treatment of RBC	RBC carnitine nmol/g hemoglobin	Plasma carnitine	Carnitine in 1st wash
		nmol/ml	*nmol/ml*
None	150	46.3	—
1 saline wash	130	45.8	3.1
2 saline washes	90	45.8	2.3
3 saline washes	90	45.3	1.7
4 saline washes	110	45.5	2.6
5 saline washes	110	47.0	2.8

Intraassay reproducibility was determined by preparing one of several aliquots of a human sample for assay as described in the methods section and assaying all the aliquots in triplicate on one day. Interassay reproducibility was determined by preparation of several aliquots of a human sample for assay as described in the methods section and assaying all the aliquots in triplicate on several different days. Data describing the interassay and intraassay reproducibility of carnitine concentration determinations on both plasma and red blood cells are presented in **Table 2.**

Previous data from our laboratory have shown that there is a progressive accumulation of carnitine in the plasma of the male rat from puberty to maturity with the end result that the plasma carnitine concentration of the adult male rat is approximately twice the plasma carnitine concentration of the adult female rat (8). In order to document the use of the red blood cell carnitine assay method in an experimental setting and to determine if a similar sex-linked relationship exists concerning red blood cell carnitine, male and female rats were maintained on a diet containing no carnitine but with adequate

TABLE 2
Human plasma and red blood cell carnitine intraassay and interassay reproducibility

	Plasma intraassay/interassay		Red blood cells intraassay/interassay	
Number of samples	10	20	14	19
Mean	34.7*	35.7*	198†	261†
Standard deviation	2.2*	3.3*	23†	16.2†
Coefficient of variation	6.3	9.2	11.6	6.2
Range	31.3–38.7*	30.7–44.2*	169–246†	219–289†
Means percent recovery	96	—	98	—

* Units = nmol/ml.
† Units = nmol/g hemoglobin.

precursors for carnitine biosynthesis. These rats were studied from 51 to 90 days of age. Data for these animals are listed in **Table 3**. The growth rate and blood parameters of the animals were normal. Once again, the male rats showed increasing plasma carnitine concentrations during development resulting in adult male plasma carnitine concentrations twice as high as the carnitine concentrations found in the plasma of adult female rats. This carnitine accumulation during development did not occur in the red blood cells of male rats. However, red blood cells of male rats contain significantly more carnitine than do red blood cells of females at all ages studied. In adult animals, the plasma carnitine concentration, the RBC carnitine concentration, and the whole blood carnitine concentration are all twice as high in the male as in the female. The percent of whole blood carnitine found in the plasma is approximately 70–80% and the percent of whole blood carnitine found in the red blood cell is approximately 20–30%.

Discussion

Red blood cells from both rats and humans were found to contain significant concentrations of carnitine. Using the described assay red blood cells can be prepared for assay with saline washes without removing the carnitine within the red blood cell. Recovery of added carnitine is within an acceptable range (96–98%) for both the plasma and red blood cell determinations. The coefficient of variation for both the intraassay and the

TABLE 3
Carnitine concentration of red blood cells of male and female rats at different ages

	51–60 Days male-female		61–70 Days male-female		71–80 Days male-female		81–90 Days male-female	
Number of rats	10	9	10	9	14	15	14	14
Body weight* (g)	162 ± 11	168 ± 10	259 ± 12	211 ± 10	295 ± 16	215 ± 6	353 ± 14	260 ± 9
Hematocrit* (ml%)	30 ± 2	30 ± 2	38 ± 2	38 ± 1	34 ± 2	36 ± 1	38 ± 1	39 ± 1
Hemoglobin* (g/dl)	10.2 ± 1.1	8.9 ± 0.7	12.4 ± 0.6	12.9 ± 5.0	12.4 ± 0.6	13.5 ± 0.3	14.7 ± 0.4	14.6 ± 0.5
Plasma carnitine* (nmol/ml)	38.7 ± 3.1	24.2 ± 1.7	41.0 ± 3.2	21.9 ± 1.1	42.9 ± 2.8	20.1 ± 0.8	56.4 ± 3.6	22.3 ± 0.9
RBC carnitine* (nmol/g hemoglobin)	92 ± 8	50 ± 4	67 ± 6	51 ± 5	71 ± 5	37 ± 3	64 ± 6	31 ± 2
Whole blood* carnitine (nmol/ml)	30.1 ± 2	19.3 ± 1	36.1 ± 3	19.1 ± 1	32.9 ± 1	20.0 ± 1	38.5 ± 1	17.1 ± 1
Percent of whole blood carnitine found in plasma	74	79	75	66	76	72	79	76

* = Mean ± standard error of the mean.

interassay data is greater for the red blood cell assay than for the plasma assay. However, as documented by the investigations using blood obtained from rats of different ages, both assay methods can be used in the experimental setting.

Our laboratory has demonstrated earlier that the adult male rat has a plasma carnitine concentration which is approximately twice the plasma carnitine concentration of the adult female rat (8). Data obtained with the red blood cell carnitine assay procedure described here indicates that the adult male rat red blood cell carnitine concentration is also two-fold higher than the red blood cell carnitine concentration of adult female rats. For all normal rats studied, the percent of whole blood carnitine found in the red blood cell is quite constant at 20–30%.

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References

1. Fraenkel G. Carnitine. In: Harris RS, Marrian GF, Thimann KV, eds. Vitamins and hormones. New York, NY: Academic Press, 1957:73–118.
2. Borum PR. Carnitine. In: Darby WJ, Broquist HP, Olson RE, eds. Annual reviews of nutrition volume 3. Palo Alto, CA: Annual Reviews Inc, 1983:233–59.
3. Mitchell ME. Carnitine metabolism in human subjects II. Values of carnitine in biological fluids and tissues of normal subjects. Am J Clin Nutr 1978;31: 481–91.
4. Borum PR. Carnitine in erythrocytes. Fed Proc 1979;38:352 (abstr).
5. Tanphaichitr V, Broquist HP. Lysine deficiency in the rat: concomitant impairment in carnitine biosynthesis. J Nutr 1973;106:80–7.
6. Borum PR, Broquist HP. Lysine deficiency and carntine in male and female rats. J Nutr 1977;107: 1209–15.
7. Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. Clin Chim Acta 1972;37:235–43.
8. Borum PR. Variation in tissue carnitine concentrations with age and sex in the rat. Biochem J 1978;176: 677–81.