THE COMPARISON OF FREE CARNITINE CONTENTS AMONG VARIOUS ANIMALS AND MYOFIBER TYPES

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Background

L-Carnitine (□-hydroxy-□-trimethylamino butyrate, MW: 161.21) is an essential constituent in mammalian tissues. It has a molecular weight similar to amino acids. L-Carnitine is derived from the diet (e.g. meat and dairy products) and by endogenous biosynthesis from essential amino acids (lysine and methionine) and a limited amount is produced by biosynthesis in tissues such as liver and kidney. This compound has an important role in the transport of long-chain fatty acids into the mitochondria matrix for energy-generating processes via □-oxidation. It has a widespread occurrence in nature. Plants contain a low amount of L-carnitine as compared with animal tissues (Bremer, 1983). In animals, the concentration varies widely depending on the species and type of tissues (Mitchell, 1978; Rinaudo et al., 1991).

Several methods for assaying L-carnitine in tissues have been published by various authors (Cederblad & Lindstedt, 1972; Deufuel, 1990; Marquis & Fritz, 1964; McGarry & Foster, 1976). Enzyme assays are commonly used to determine free L-carnitine contents in biological samples. However, when the analytical method is different, sample preparation or free L-carnitine contents would be considerably different. In addition, free L-carnitine contents in muscle are influenced by various factors (e.g. animal species, age, sex, anatomical muscle position, post-mortem time). Therefore, it is necessary to compare biological samples by the same analytical method.

Objectives

The aim of the present study was to examine the condition in order to adopt to the enzymatic method described by Marquis & Fritz (1964) from the cuvette to the microplate and to compare the free L-carnitine quantity between semitendinosus muscle tissues from various animals and avian muscle fiber types by the method modified in the present study and also the myoglobin content, which represents the avian muscle fiber types (e.g. white fiber, intermediate fiber, red fiber), was also determined.

Methods

Fowl, pig, cattle, horse, goat and deer were killed by bleeding or hunting. Muscle samples (*M. semitendinosus, M pectoralis profundus, M. pectoralis superficialis, M. sartorious, M. soleus*) were dissected from fresh carcasses. Free L-carnitine assay was carried out as described below: five grams of each minced muscle sample was homogenized in the 5-fold volume of 0.3 M perchloric acid and then centrifuged at 1,140 x g for 10 min. The supernatant was decanted, and the pellet was suspended in the 4-fold of the same solution and recentrifuged. The two supernatants were mixed, and added the same solution up to 50 ml. From this, 8 ml of acid-soluble fraction was transferred to a plastic tube, and neutralized with 1.6 ml of 1.2 M potassium carbonate. After centrifugation (8,385 x g, 10 min), the supernatant was filtered through a 0.45 □m filter, and then distilled water was added up to 10 ml. The resulting solution was used for enzyme assay described by Marquis & Fritz (1964). 100 µl of standard solution (0, 10, 20, 30, 40, 50 mg/l L-carnitine) or sample was added to 100 µl of solution A (0.93 mM DTNB, 0.55 mM Acetyl-CoA, 3.05 mM EDTA, 610 mM Tris-HCl buffer, pH 7.5) in a microplate and mixed carefully. The mixture was incubated at 37°C for 10 min, and the absorbance was measured at 415 nm. Then 50 µl of 0.118 mg/ml carnitine acetyltransferase (CAT, E.C. 2.3.1.7) was added to start the reaction. The solution was incubated at 37°C for 10 min, and the absorbance was measured at 415 nm. The difference between the two absorbances was calculated. Myoglobin assay was as follows: five grams of each minced muscle sample was homogenized in the 5-fold volume of distilled water and then centrifuged at 39,900 x g for 60 min. The supernatant was filtered through a 0.45 µm filter, collected into a 50 ml-flask and filled with distilled water up to 50 ml. The absorbance was measured at 525 nm. Myoglobin content was calculated using 0.7 of millimolar extinction coefficient and 17,000 of molecular weight.

Results and discussion

In this study, the conditions were determined in order to carry out the enzymatic assay (Marquis & Fritz, 1964) in the microplate by mixing three types of solutions (solution A, sample solution or standard solution, CAT). The final composition of mixed solution was 11.8 µg/ml CAT, 0.37 mM DTNB, 0.50 mM Acetyl-CoA, 1.22 mM EDTA, 244 mM Tris-HCl (pH 7.5). Two different concentrations were adopted by changing the volume of the three solutions. The error of the absorbance was less in the solution containing 100 µl of solution A, 100 µl of standard, 50 µl of enzyme than that of the solution containing 180 µl of solution A, 20 µl of standard, 5 µl of enzyme. The effect of calibration curve on the coefficient of determination was examined by changing the concentration of enzyme with the increasing concentration of L-carnitine. Increased concentration of L-carnitine up to 50 mg/l resulted the highest coefficient of determination, when CAT concentration was 23.6 µg/ml, and beyond this L-carnitine concentration the coefficient of determination was reduced remarkably. When CAT concentration was 11.8 µg/ml and L-carnitine concentration ranged between 20 mg/l and 70 mg/l, the coefficient of determination was higher than 0.994. Fifty-five calibration curves were made under the condition which each volume of three solutions were 100 µl of solution A, 100 µl of standard, 50 µl of CAT and final CAT concentration was 11.8 µg/ml. The correlation coefficient was 0.999 in microplate and this condition was used to analyze the sample.

The average values of free L-carnitine content in hen, broiler, pig, cattle, horse, deer, goat muscles wet weight basis were 5.6, 12.7, 15.1, 58.7, 83.0, 110.2, 213.1 mg/100 g, respectively. This showed that herbivores contain higher L-carnitine content in skeletal muscle than the other animals. Also there was a relationship between L-carnitine and myoglobin content. The higher L-carnitine content, the greater amount of red fiber found in the skeletal muscle. The correlation coefficient between L-carnitine and myoglobin was 0.994 and this indicated the close relationship as mentioned before. Also it is suggested that the oxygen of the myoglobin in the red fiber produces ATP via □-oxidation.

Pertinent literatures

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