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A STUDY ON THE DISTRIBUTION OF DIFFERENT CARNITINE FRACTIONS IN VARIOUS TISSUES OF BOVINE EYE

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Abstract

The aim of the present investigation was to study the distribution of various carnitine fractions in different bovine ocular tissues. Different ocular tissues were homogenized and their carnitine content was determined. The carnitine fractions studied include short chain carnitine, long chain carnitine, acyl carnitine and free carnitine. All the four carnitine fractions were found to be present in all the ocular tissues studied. Iris contained the highest concentration short chain, long chain and acyl carnitine. However significant ($p < 0.05$) differences existed in long chain and acyl carnitine between iris and other tissues. Free carnitine was found in highest concentration in ciliary body which was significantly higher when compared to lens nucleus ($p < 0.05$). There was no significant difference in the carnitine fractions between aqueous and vitreous humor. These results show differential distribution of carnitine in bovine ocular tissues which may be involved in various functions besides fatty acid oxidation.

Key words: Bovine, ocular tissues, carnitine, fatty acid, energy, homeostasis.

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INTRODUCTION

L- carnitine (β –hydroxy-γ-trimethyl-amino-butyric acid) is a crucial component of activated fatty acid transport mechanism across the mitochondrial membrane (3). Carnitine facilitates oxidation of long –chain fatty acids, modulates the ratio of CoA to CoA-SH and is involved in trapping acyl residues from peroxisomes and mitochondria. Carnitine also participates in metabolism of branched chain amino acids and stabilizes cellular membranes. It is a free radical scavenger and has been reported to take part in control of nuclear transcription (3, 5). The primary sites for carnitine synthesis from 6-N-trimethyllysine are the liver and the kidneys, although the brain does have a small potential as well (11). The total content of carnitine in the human body is about 100 mmol (16 g) but it depends on the diet, muscle mass and age (3). Muscles contain 98% of the total carnitine while 1.5 and 0.5% of carnitine is found in liver and other tissues respectively (6).

The vertebrate eye is a complex sensory organ consisting of multiple, distinct tissues, each having its own unique biochemical composition, structure, and physiological function. The concentration of carnitine has been determined in ocular tissues of various animals (15, 1). The present study was therefore undertaken to determine the concentration of carnitine in different bovine ocular tissues.

MATERIALS AND METHODS

Sample preparation

Intact eyes from cows were obtained from a local slaughterhouse and placed into cold saline and transported to the laboratory at 4^0 C. Various ocular tissue collections were performed with the help of a magnifying lens. Ocular tissues were then separated and placed in appropriate containers. The tissues were homogenized in normal saline (10% W/V) using a stainless steel Omni – Mixer homogenizer (Omni International, Inc, Gainesville, VA, USA). The homogenates were then used for carnitine determination.

Extraction of carnitine

Free carnitine: 0.5 ml of homogenate in duplicate was deprotenized with 0.5 ml of ice- cold 1.2 M perchloric acid in the ratio 1:1 (V/V), allowed to stand at room temperature for 10 minutes. The sample was then centrifuged at 5000 x g for 10 minutes. The pellet was washed by suspending it in 0.5 ml of 0.6 M perchloric acid followed by centrifugation. The supernatants were pooled and used for estimation of free carnitine (FC) and total acid- soluble carnitine (TS) as described below. An aliquot of the pooled supernatant was neutralized with 1M KOH to bring the pH to 6.5 to 7.0. The neutralized samples were used to determine the concentration of free carnitine (FC) as described below.

Total acid-soluble carnitine: 0.5 ml of pooled supernatant was treated with 1.0 M KOH until the pH was alkaline, mixed and allowed to stand at room temperature for 30 minutes to ensure the release of free carnitine from its ester linkage of acyl carnitine (AC). After incubation, samples were neutralized with ice cold 1.2 M perchloric acid to bring the pH to 6.5 – 7.0. The concentration of acyl carnitine (AC) was calculated by subtraction of free carnitine (FC) from total acid – soluble carnitine (TS).

Determination of carnitine concentration

The concentration of carnitine in the homogenate was determined according to the method of Al-Kholaifi and Alhomida, 1999, (2). Briefly the assay mixture consisted of 10 µl of acetyl-CoA (15 mM), 10µl of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and 975 µl of the neutralized extract. The reaction was initiated by adding 5 µl of carnitine acetyltransferase (0.9 U). The absorbance was measured at 412 nm. The carnitine concentration was determined from the standard graph of carnitine.

Statistical Analysis

The statistical comparisons among various treatment groups were made by Tukey's multiple comparison test using InStat® package for personal computers (GraphPad TM Software, Inc., San Diego, USA) version 5. p values less than 0.05 were considered statistically significant.

RESULTS

Table 1 shows the concentration of different carnitine fractions in various tissues of bovine eyes. Short chain acyl carnitine was found in highest concentration in iris followed by cornea, lens cortex, lens nucleus, retina, sclera and ciliary body. However there were no significant differences in short chain acylcarnitine levels among different tissues. The concentration of long chain acyl carnitine was highest in iris followed by cornea which was followed by other tissues all of which had almost the same concentration. The differences in the concentrations of long chain acyl carnitine between iris and other ocular tissues were statistically significant ($p < 0.05$). Acyl carnitine was found in highest concentration in iris > retina > cornea, lens cortex, lens nucleus > ciliary body and sclera. The differences in the concentrations of acyl carnitine between iris and other ocular tissues were statistically significant ($p < 0.05$). Free carnitine was in highest concentration in ciliary body > sclera > iris > lens cortex > lens nucleus > cornea > retina. The differences in the concentrations of free carnitine between ciliary body and lens nucleus was statistically significant ($p < 0.05$). The concentration of total carnitine was highest in iris > lens cortex, retina, cornea > lens nucleus > sclera > ciliary body. However there was a statistically significant decrease in the concentration of total carnitine only between iris and ciliary body ($p < 0.05$).

Table 2 shows the concentrations of different carnitine fractions in ocular fluids of cow. Statistical analysis showed no significant differences ($p > 0.05$) between the various carnitine fractions in aqueous and vitreous humor.

Figures 1 and 2 shows the ratio of acyl to free carnitine

Table 1. Carnitine concentrations in various bovine ocular tissues.

Tissues	Short Chain Carnitine	Long Chain Carnitine	Acyl Carnitine	Free Carnitine	Total Carnitine
Iris	0.026 ± 0.01	0.011± 0.004	0.041± 0.001	0.018 ± 0.007 ^{ns}	0.054 ± 0.01
Sclera	0.011 ± 0.004	0.004 ± 0.001**	0.01 ± 0.002***	0.021± 0.01 ^{ns}	0.022± 0.005 ^{ns}
Cornea	0.015 ± 0.003^{ns}	0.006 ± 0.002*	0.02 ± 0.004**	0.006 ±0.001 ^{ns}	0.03 ± 0.002 ^{ns}
Retina	0.013 ± 0.01 ^{ns}	0.004 ± 0.001***	0.03 ± 0.006*	0.003± 0.001 ^{ns}	0.03 ± 0.01 ^{ns}
Ciliary Body	0.009 ± 0.001 ^{ns}	0.003 ± 0.00***	0.011 ± 0.002***	0.05 ± 0.02 ^{ns}	0.017 ± 0.00 *
Lens Cortex	0.014 ± 0.00 ^{ns}	0.005 ± 0.00**	0.02 ± 0.001***	0.009 ± 0.001 ^{ns}	0.03 ± 0.003 ^{ns}
Lens Nucleus	0.013 ± 0.003 ^{ns}	0.004 ± 0.00***	0.02 ± 0.003***	0.007 ± 0.001 *	0.02 ± 0.003 ^{ns}

Values are expressed as mean ± SD (n= 4) nanomoles of carnitine/mg of noncollagen protein.

$^{***}p < 0.001$ when compared to iris. Tukey's multiple comparison test.

$^{**}p < 0.01$ when compared to iris. Tukey's multiple comparison test.

$^{*}p < 0.05$ when compared to iris. Tukey's multiple comparison test.

^{ns}Non significant ($p > 0.05$) when compared to iris. Tukey's multiple comparison test.

Table 2. Carnitine concentrations in bovine ocular fluids.

Fluid	Short Chain Carnitine	Long Chain Carnitine	Acyl Carnitine	Free Carnitine	Total Carnitine
Aqueous Humor	0.022 ± 0.007	0.006 ± 0.002	0.03± 0.01	0.014 ± 0.001	0.04 ± 0.009
Vitreous Humor	0.03 ± 0.00 ^{ns}	0.01 ± 0.00 ^{ns}	0.04 ± 0.006 ^{ns}	0.03 ± 0.006 ^{ns}	0.07 ±0.02 ^{ns}

Values are expressed as mean ± SD (n= 4) nanomoles of carnitine/mg of noncollagen protein.

^{ns}Non significant when compared to aqueous humor ($p > 0.05$).

in different ocular tissues and fluids of bovine eye. The ratio of acyl to free carnitine was highest in cornea. There was a significant difference ($p < 0.05$) in the ratio of acyl to free carnitine between iris and sclera when compared to cornea. There was however no significant differences in the ratio of acyl to free carnitine between the ocular fluids ($p > 0.05$).

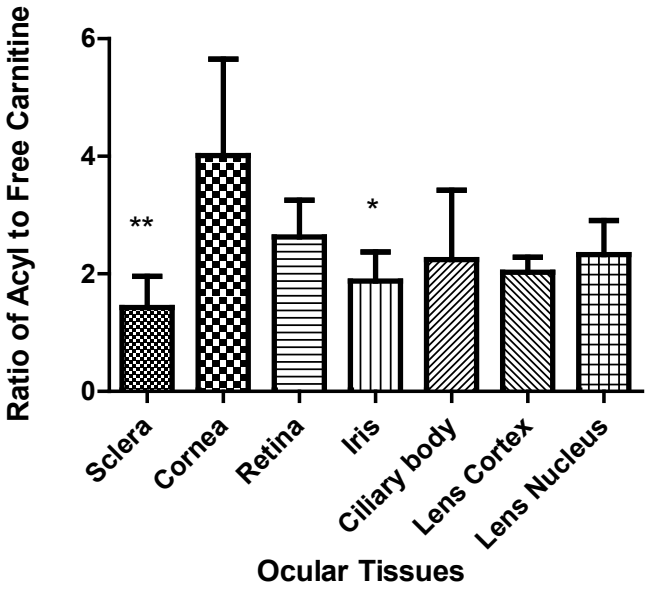

Figure 1. Ratio of acyl carnitine to free cartine in various bovine ocular tissues.
**P < 0.01 when compared to cornea. Tukey's multiple comparison test.
*P < 0.05 when compared to cornea. Tukey's multiple comparison test.

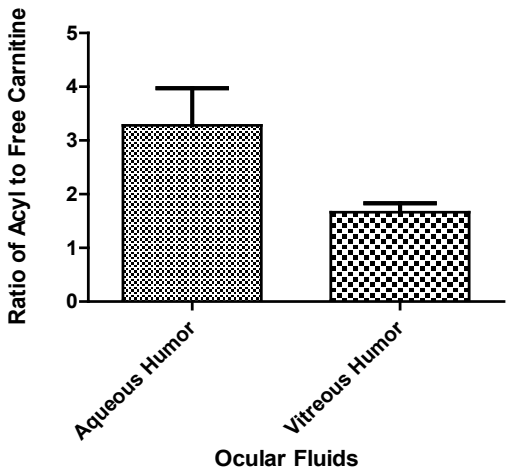

Figure 2. Ratio of acyl carnitine to free cartine in bovine ocular fluids.

DISCUSSION

Important components of vertebrate eye include retina, lens, and cornea which work in concert to bring photons of light into the eye, focus them correctly on the retina, and convert their energy into electrochemical signals that are conveyed to the brain where, ultimately, they are processed into a coherent visual image (7). The ocular surface consists of several integrated structures working together to ensure optimal functioning of the eye (10). The most relevant structures are the conjunctiva, which consists of a multilayered membrane containing mucin-producing cells (goblet cells) responsible for maintaining a lubricated ocular surface; the corneal epithelium, whose structure and anatomic composition are essential for the quality of vision; the tear film; and the lachrymal glands. L-carnitine (β-hydroxy-γ-N-trimethylamino-butyrate) is a polar molecule widely distributed in nature and in biological tissues. Functions of carnitine include its role in β- oxidation of fatty acids, oxidation of substrates like pyruvate and branched chain amino acids, maintenance of adequate levels of acetyl CoA for energy production etc. (14). L-carnitine ensures transfer of fatty acids to the mitochondria where they undergo oxidation. This process is associated with production of short-chain acylcarnitine which exits from the mitochondria or peroxisomes. L-carnitine ensures regeneration of coenzyme A and is thus involved in energy metabolism (9). Carnitine is present in tissues and body fluids in free and esterified form as short-chain and long-chain acylcarnitines. Total carnitine consists of the sum of free carnitine and all acylcarnitines. Animal studies have indicated differential distribution of carnitine in various ocular tissues (1, 15). In the present study iris was found to have the highest concentration of short chain, long chain acyl and total carnitine which is in accordance with earlier studies (13). The iris-ciliary body are two separate tissues, which are highly specialized anatomically and functionally. The ciliary body overlaps the iris and is tightly bound to it. The iris-ciliary body has been reported to have active lipid metabolism. The fatty acids are esterified and consist of long – chain saturated and unsaturated fatty acids. Studies of Pessotto et al., 1994 (15) suggest that carnitine plays an important role in those tissues of the eye where cells of a muscular nature are present and may represent, after esterification, an important energy reserve.

The ocular fluids viz., aqueous and vitreous humors were found to contain all the carnitine fractions and free carnitine. The vitreous humor has been reported to have active lipid metabolism (4) but there were no significant differences in the various carnitine fractions between the aqueous and vitreous humors. Similarly there was no significant difference ($p > 0.05$) between the ratio of acyl to free carnitine in aqueous and vitreous humors. The distribution of carnitine in the ocular tissues may reflect the metabolic state of the tissue in relation to its energy needs. The presence of carnitine in nonvascular tissues like lens and the ocular fluids like aqueous and vitreous humor may also indicate the role of carnitine in maintaining cell homeostasis (12). In these tissues carnitine levels may not correlate with their energy requirement or lipid metabolism but may be involved in cell homeostasis. For example, protein sites that are most readily susceptible to posttranslational modifications could be primary targets of the direct or indirect acetylation process induced by L-carnitine (16). The ocular lens is a unique tissue in the body: it is completely avascular, lacks innervation, and the vast majority of its constituent cells (called lens fiber cells) are devoid of organelles, essentially consisting of a plasma membrane encapsulating cytoplasm (10). Despite its primary role in lipid metabolism other functions of carnitine include sugar aerobic metabolism, oxidative phosphorylation, and osmosis etc. (13). Carnitine has also been reported to interact with membranes to change their physiochemical properties (8). Therefore carnitine may serve one or more of these functions in ocular tissues. In conclusion, we observed differential concentrations of various carnitine fractions in different tissues of bovine eye. These findings could help in understanding the biochemical and

physiological processes of ocular homeostasis.

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Other articles in this theme issue include references (17-44).

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