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Changes in arginase activity and AST enzyme levels in the cardiac and skeletal muscle and liver of lambs with white muscle disease

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Abstract: The aim of this study was to evaluate arginase activity and aspartate aminotransferase (AST) levels in the tissues of the cardiac and skeletal muscle and liver of lambs with white muscle disease (WMD). The cardiac and skeletal muscle and liver tissues were obtained from 8 lambs with WMD and 9 apparently healthy lambs. The diagnosis of WMD was made with the detection of hyaline degeneration upon histopathological examination. A significant increase in arginase activity in the cardiac and skeletal muscle and liver tissues and a significant decrease in AST levels in the cardiac muscle were detected in the WMD group compared to the control group. It was concluded that increased arginase activity in cardiac and skeletal muscle and liver may enhance polyamine synthesis for the repair of cardiac and skeletal muscle injuries in lambs with WMD and that AST in the cardiac muscle might decrease due to the cardiac muscle injury.

Key words: Arginase, aspartate aminotransferase enzyme, white muscle disease, lamb

1. Introduction

Nutritional myodegeneration, or white muscle disease (WMD), derived from a dietary deficiency of selenium and/or vitamin E, is characterized by cardiac and skeletal muscle degeneration with peracute to subacute form (1,2). The disease occurs during the first year of life, frequently before the age of 6 months (3). In the cardiac form, lambs with WMD show debilitation, weakness, a short clinical course, and an agonizing death, and in the skeletal form they often have cardiomyopathy and stiffness due to muscular fibrosis in the limbs (1). Significantly elevated serum aspartate aminotransferase (AST), creatinine kinase (CK), and lactate dehydrogenase (LDH) activities are determined during the acute phase of myodegeneration (1,4). White patches appear in the affected muscles (5,6), which resemble fish flesh (7). In addition, hyaline degeneration, necrosis, and calcification occur in myocardial and skeletal muscles (5,8).

Arginine plays a role in the regulation of muscle health by acting on protein synthesis, inhibition of proteolysis, and intracellular protein turnover (9). In addition, arginine functions as a precursor to nitric oxide (NO), polyamines, proline, glutamate, creatinine, and agmatine (10). The metabolism products of arginine, such as nitric oxide, citrulline, ornithine, and urea, are physiologically important with regard to tissue injury and repair, because the metabolite of NO can enhance tissue injury while the ornithine metabolite of arginine by arginase can increase tissue repair (11).

The role of arginase activity in tissue repair has not been investigated in WMD as a nutritional myodegenerative disease. Myodegenerative diseases may affect the muscle AST enzyme activity. Thus, this study evaluated the arginase activity and AST enzyme levels in tissues of cardiac and skeletal muscle and liver in WMD in lambs.

2. Materials and methods

2.1. Animal material

Eight lambs in agony, 2–3 months old, were presented to the Elazığ Veterinary Control Research Institute. The owner of the lambs stated that they presented signs of weakness and were unable to stand. The study material consisted of the cardiac and skeletal muscle and liver tissues of the dead lambs with WMD (WMD group) and the apparently healthy lambs of 3.5 months (control group) supplied from the Elkas slaughterhouse in Elazığ.

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2.2. Histopathological examination

A sample of cardiac tissue was fixed in 10% buffered formalin solution, subjected to routine processing, embedded in paraffin, cut into 5-µm thick sections, and stained with hematoxylin and eosin (HE). These sections were examined under light microscopy.

2.3. Enzyme analysis

Pieces of cardiac (right ventricle) and skeletal (gluteal muscle) muscle and liver tissues from the diseased and healthy lambs were excised, rinsed with 0.9% saline (0.9% NaCl), desiccated with filter paper, and kept frozen at –20 °C until analysis. Then 1 g of tissue samples was homogenized with distilled water (1/10, w/v) in a glass Potter-Elvehjem homogenizer in an ice bath. The mixture that was homogenized was centrifuged at 14,000 × *g* for 10 min in a cooled centrifuge. The supernatants were used as the enzyme source.

AST activity in the supernatants was measured with the Reitman and Frankel method (12) and 0.2 mL of the enzyme source was added to 1 mL of α-oxoglutarate-aspartate substrate solution. The mixture was kept at 37 °C for 1 h, after which 1 mL of dinitrophenylhydrazine-HCl was added. This mixture was left for 20 min at room temperature before adding 10 mL of NaOH. The absorbance of the resultant solution was measured against a blank (0.2 mL of distilled water instead of the enzyme source) at 505 nm in a Shimadzu UV 240 spectrophotometer. One unit of AST activity is an enzyme activity that produces 4.82×10^{-4} µmol glutamate at 37 °C and pH 7.5 in 1 min.

Arginase activity was determined by the measurement of urea produced with hydrolysis of L-arginine through arginase enzyme by the thiosemicarbazide diacetyl monoxime urea method (13). In this analysis, the enzyme source was diluted with $MnCl_2$ at the rate of 1:200 for liver and 1:5 for cardiac and skeletal muscle and was used as a new enzyme source with preincubation at 55 °C for 5 min. Tubes that contained 0.2 mL of the enzyme source, 0.4 mL of L-arginine, and 0.4 mL of carbonate buffer were incubated at 37 °C for 10 min. The reaction was ceased by adding 3 mL of acid reagent to the tubes after the incubation period. Thereafter, 2 mL of color reagent was added to the tubes, which were kept in a boiling water bath for 10 min. The tubes were then removed from the boiling water bath and cooled, and absorbance was recorded at 520 nm. One unit of arginase activity is an expression in mg protein of enzyme activity producing 1 µmol of urea from L-arginine for 1 h at 37 °C.

The measurement of protein was carried out according to the method of Lowry et al. (14), and bovine serum albumin was used as standard. One milliliter of alkaline copper reagent and 1 mL of the diluted enzyme sources were combined and incubated for 10 min at room temperature. Then 4 mL of Folin–Ciocalteu phenol reagent was mixed into the tubes, and the tubes were incubated at 55 °C for 5 min. The absorbance of the samples was recorded at 650 nm.

2.4. Statistical analysis

SPSS 12.0 was used. Differences between groups were evaluated by independent t test. Results were given as means ± SE. P-values of less than 0.05 were considered significant.

3. Results

Microscopic examination determined loss of striation and formation of degeneration and necrosis, characterized by a pink appearance, in the muscular fibers of the subendocardium. Focal calcifications were also recognized in some areas. In addition, infiltration of macrophages and mononuclear cells was detected in the interstitial area (Figures 1 and 2).

Arginase activity and AST levels in tissues of the cardiac and skeletal muscle and liver are given in the Table. A significant increase in arginase activity in the cardiac ($P < 0.001$) and skeletal ($P < 0.05$) muscle and liver ($P < 0.001$) tissues and a significant decrease in AST levels in the cardiac muscle ($P < 0.05$) were detected in the WMD group compared to the control group.

4. Discussion

Decrease in muscle enzymes and increase in serum enzyme activities may occur in the different muscular dystrophies (15). Various studies have reported that serum and/or plasma AST values increase in lambs with WMD (16,17). In this study, a significant decrease in AST levels in the cardiac muscle was found in the WMD group compared to the control group. Decreased level of cardiac enzyme AST accounts for cardiac muscular dystrophies, as noted microscopically, with loss of striation, myofibrillar (hyaline) degeneration, and necrosis characterized by the appearance of pink subendocardial muscular fibers.

Arginase activity is high in the liver for converting arginine to urea and ornithine, while in extrahepatic cells, such as muscle cells, macrophages, and endothelial cells, arginase activity is determined at low levels (18,19). Consistent with these reports, in this study arginase activity was found to be expressed at high levels in the liver and at relatively low levels in skeletal and cardiac muscle in the control group.

Ornithine, the biosynthetic precursor of proline and polyamines, is produced by arginase activity in hepatic and nonhepatic tissues (20). For example, it has been reported that in lactating mammary glands, liver arginase activity increases by about 25% to provide proline, which is necessary for milk production (21); myometrial arginase activity increases about 25-fold during pregnancy (22). Polyamine biosynthesis by arginase activity increases in a variety of tumors (23,24). In this study, a significant

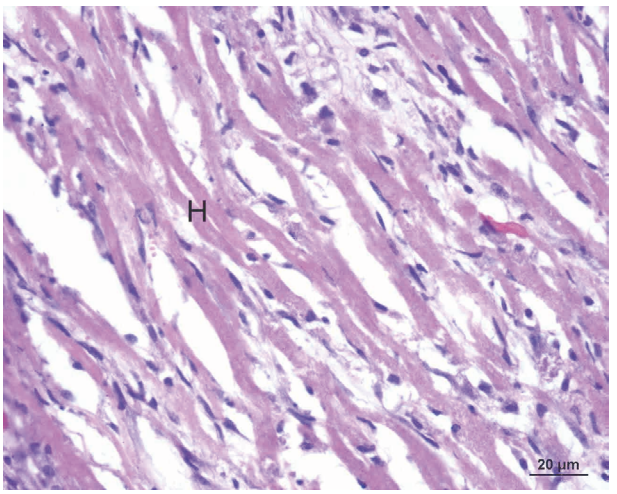

Figure 1. Infiltration of macrophages, mononuclear cells, and hyaline degeneration (H) in the cardiac muscle of lambs with WMD stained with H&E.

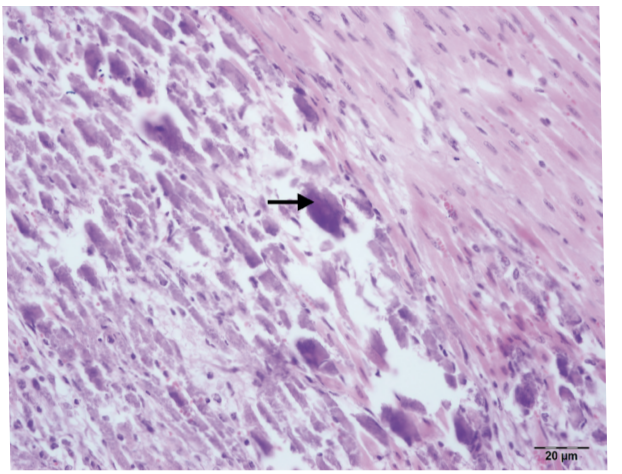

Figure 2. Focal calcifications (arrow) in the cardiac muscle of lambs with WMD stained with H&E.

Table. Arginase activity and AST levels in tissues of the cardiac and skeletal muscle and liver in lambs with WMD and in the control group.

Tissues	Groups	Arginase (U)	AST (U)
Cardiac muscle	Control	0.47 ± 0.10 (0.12–0.84)	28.50 ± 1.49 (20.64–34.56)
	WMD	3.24 ± 0.27[b] (2.11–4.37)	19.13 ± 2.0[a] (15.08–28.8)
Skeletal muscle	Control	11.31 ± 0.98 (8.16–16.02)	6.03 ± 0.56 (4.08–9.6)
	WMD	22.05 ± 2.33[a] (11.38–28.49)	4.56 ± 0.61 (1.88–7.24)
Liver	Control	127.25 ± 10.33 (94.33–187.5)	9.67 ± 0.80 (6.24–12.96)
	WMD	218.35 ± 7.32[b] (204.69–252.34)	9.20 ± 0.62 (7.2–12.05)

WMD: White muscle disease; AST: aspartate aminotransferase. Values are given as means ± SE. [a]: $P < 0.05$; [b]: $P < 0.001$. Minimum and maximum values are given in parentheses.

increase in arginase activity in the cardiac and skeletal muscle and liver tissues was determined in the WMD group compared to the control group. Several studies have reported that M2a macrophages express increased levels of arginase converts of arginine to polyamines (25,26); it has also been reported that arginine metabolism by arginase activity can cause a more profibrotic environment (27). In the degeneration of skeletal muscle, invasion of phagocytes and regeneration rapidly occurs, and in the degeneration of myocardial muscle, replacement fibrosis develops (28). In this study, it was assumed that increased cardiac and skeletal muscle and liver arginase activity can enhance polyamine synthesis for the repair of the injuries of cardiac and skeletal muscle in lambs with WMD.

Enhanced catabolism of arginine, usually by arginase activity, may result in arginine deficiency, and arginine deficiencies disrupt many cellular and organ functions (10). Arginine used as a dietary supplement has been reported to have beneficial effects in the event of short-term administration to mdx mice, a genetic model of Duchenne muscular dystrophy (29). Thus, short-term dietary arginine supplementation may be beneficial, in addition to selenium and vitamin E injection, in the treatment of WMD in lambs. Future studies may be conducted in order to evaluate the effect of short-term dietary arginine supplementation, in addition to selenium and vitamin E injection, on the treatment of WMD in lambs.

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