

## Impact of Supplementation with Branched Chain Amino Acids on Myocardium and Coronary in Regularly and Intensively Exercising Rats <sup>[1]</sup>

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### Abstract

This study was conducted to investigate the effects of branched chain amino acids (BCAAs) consumption on myocardium and coronary arteries, in rats subjected to regular and intense exercise. Eight-week old, 30 male rats were randomly divided into experimental- and control-groups. For a total of 8 weeks, every other day, both groups were subjected to a ladder-climbing exercise on a 1.5 m long ladder, with 2.5 cm steps interval, at 70 degrees angle. The experimental group, besides the standard diet, was additionally fed BCAA-supplement at a dose of 1.5 mg/g/day. This study showed that, the experimental group had more frequent atherosclerotic lesions compared to the control group (61.5% vs. 21.4%; P=0.034). Although creatine kinase was similar between the groups, creatine kinase - myocardial band isoform (CK-MB) was significantly higher in the experimental group compared both to control and baseline levels. This is the first study that examines the effects of consuming BCAA supplements on myocardium and coronary arteries in rats subjected to prolonged exercise. We demonstrated that continuous and long-term consumption of BCAA supplement in endurance exercises was associated with coronary atherosclerotic process and myocardial injury.

**Keywords:** Branched chain amino acids, Atherosclerosis, Endurance exercises, Myocardial injury

## Düzenli ve Yoğun Egzersiz Yaptırılan Ratlarda Takviye Olarak Tüketilen Dallı Zincirli Amino Asitlerin Miyokard ve Koroner Damarlar Üzerine Etkisi

### Öz

Bu çalışmada, düzenli ve yoğun olarak egzersiz yaptırılan ratlarda, dallı zincirli amino asit (BCAA) kullanımının miyokard ve koroner arterlere etkisi araştırılmıştır. Sekiz haftalık, 30 erkek rat rastgele deneysel ve kontrol gruplarına ayrıldı. Her iki grup, güneşli periyotla, 2.5 cm basamak aralığı olan, 1.5 metre uzunluğunda 70 derecelik açıyla duran bir merdiven üzerinde, toplam 8 hafta boyunca, tırmanma egzersize tabi tutuldu. Deney grubu, standart diyetle ek olarak BCAA takviyesi ile 1.5 mg/g/gün dozunda beslendi. Çalışma sonunda, deney grubunda, kontrol grubuna göre daha sık aterosklerotik lezyonları vardı (%61.5'e karşı %21.4; P=0.034). Ayrıca, kreatin kinaz, gruplar arasında benzer izlendiyse de, kreatin kinaz-miyokardiyal band izoformu (CK-MB) deney grubunda, hem kontrol, hem de başlangıç düzeylerine kıyasla belirgin olarak daha yüksekti. Bu, BCAA tüketen ve uzun süre egzersize tabi tutulan ratlarda, BCAA takviyesi tüketiminin miyokard ve koronerlere etkilerini inceleyen ilk çalışmadır. Bu çalışma zorlu egzersizle beraber, devamlı ve uzun süreli BCAA tüketiminin, koroner aterosklerotik süreç ve miyokardiyal hasar ile ilişkili olduğu gösterdi.

**Anahtar sözcükler:** Dallı zincirli amino asitler, Ateroskleroz, Zorlu egzersiz, Miyokardiyal hasar



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## INTRODUCTION

Leucine, isoleucine and valine are termed branched-chain amino acids (BCAA) due to their common side chain structural features and common catabolic pathways. BCAAs are essential amino acids in animals and must be acquired from external food. They act as the key building blocks for peptide synthesis <sup>[1,2]</sup>.

Although the vast majority of the energy source needed to contract skeletal muscles is obtained by aerobic metabolism of lipids and carbohydrates, BCAA oxidation contributes energy during endurance exercises <sup>[3]</sup>. This contribution can vary from 3 to 6% of total energy expenditure, depending on nutritional status and exercise intensity <sup>[4,5]</sup>.

Branched chain amino acid supplements have become attractive, to ordinary people as well as athletes due to their roles in protein synthesis and cellular metabolism; being an energy source in endurance exercise, improving sportive performance <sup>[6,7]</sup>, delaying the onset of central fatigue <sup>[8-10]</sup>, reducing the perceived soreness sensation of muscles <sup>[11]</sup>, and their ability in reducing muscle damage <sup>[12,13]</sup>.

Although BCAAs are essential for normal growth and function at cellular and organism levels, an excess amount of free BCAA, or their catabolic products resulting from defects in BCAA catabolic pathway, can be cytotoxic <sup>[14]</sup>. Previous studies have shown that feeding with high fat and BCAA supplements is associated with obesity-associated insulin resistance <sup>[15]</sup>, metabolic syndrome and impaired fasting glucose <sup>[16]</sup>.

Despite these metabolic effects, the exact effect of BCAA supplementation on coronary arteries and myocardium is poorly understood. In this study, we aimed to investigate the effects of BCAA supplementation on coronary arteries, myocardium and biochemical parameters, in rats subjected to prolonged intensive exercise.

## MATERIAL and METHODS

### Experimental Animals

Eight-week old, 30 male rats were included in the study. Male Wistar albino rats with specific pathogen-free conditions were purchased from Veterinary control institute, Erzurum, Turkey. The rats were randomly divided into 2 groups: experimental (BCAA supplementation) and control groups [15 rats each]. Rats were subjected to 12 h of dark/light cycling and survival was maintained at 20-25°C. For a total of 8 weeks, every other day, both groups were subjected to a ladder-climbing exercise with a ladder which had a length of 1.5 m and steps with 2.5 cm interval.

During the 8-week experimental period, one rat from the control group and two rats from the experimental group died; leaving 27 rats. At the end of the experimental

period, xylase 2.5 mg/kg and ketamine 80-100 mg/kg dose were applied to the rats and euthanized. Venous blood was collected from the rats for evaluation of biochemical and hematological parameters at the beginning and end of the study. At the end of the study, histopathological examination of myocardial and coronary samples of sacrificed rats was performed. We obtained the approval of the Animal Experiment Ethics Committee of Kafkas University (KAÜ-HADYEK/2016/004).

### Ladder-climbing Exercise

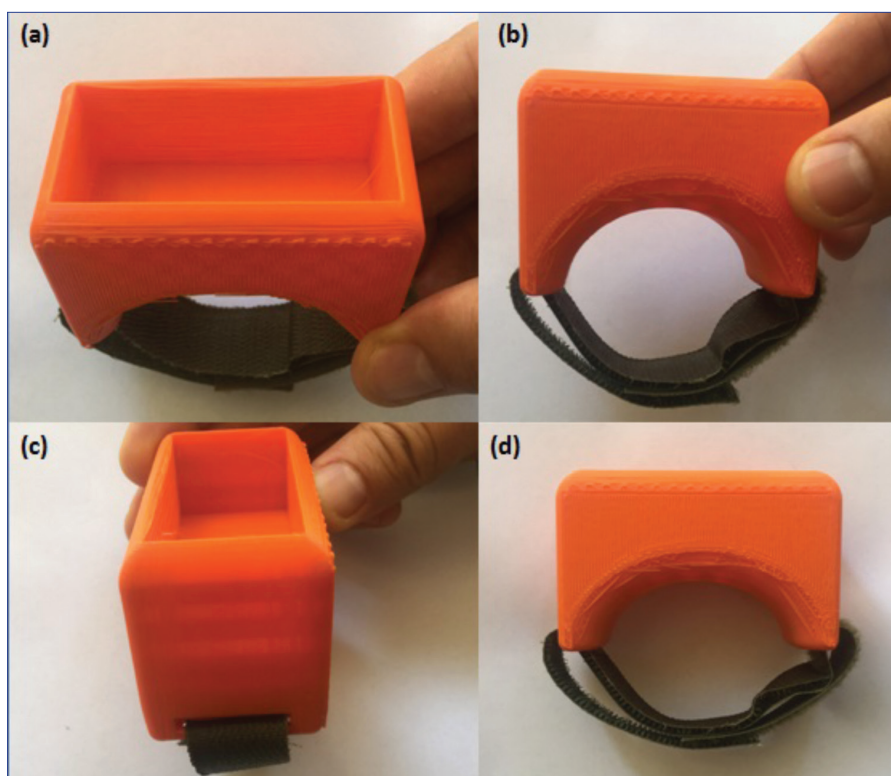
We modified the ladder-climbing exercise training program presented by Jung et al. <sup>[17]</sup>. The study and control groups were subjected to a climbing exercise with the aforementioned ladder placed at 70 degrees angle. In the first week, rats made climbing exercises without any load. From the second week onward, the rats were loaded with a saddle-shaped apparatus (produced by 3D printer technology in engineering Faculty of Kafkas University) (*Fig. 1*). The apparatus, which weighed approximately 25-30% of their body weight, was attached to their backs with a Velcro strip at the bottom of the ladder. After each climb, the rats were allowed to rest for two minutes and were taken to the bottom of the ladder again. If the climb was successful, the load on the back of the rats was increased in the next climb, and these loads were increased to 50%, 75%, 90% and 100% of their body-weight, respectively. Each rat was subjected to climbing exercise 8 times. The training session for a given rat was stopped when the rat refused to climb up the ladder after three successive touch/flick to his tail.

### Nutrition Protocol

The rats in the study and control groups were fed ad libitum with a standard diet containing 18.8% protein. The rats in the experimental group were additionally fed BCAA supplement at a dose of 1.5 mg/g/day <sup>[18]</sup> by gastric gavage. The BCAA supplement used in the study was in ratio of leucine:isoleucine:valine=4:1:1, and did not contain additional fat or carbohydrate.

### Sampling of Tissues and Pathological Examination

The sizes and weights of the sacrificed rats' hearts were macroscopically measured. The heart tissue was then transversally sliced at 2 mm intervals and the thickness of the ventricle was measured from the thickest wall. The specimens of all rats' hearts were individually placed in the tissue cassette. After the processing procedure, paraffin blocks were manually created. Prepared sections from the paraffin blocks with a thickness of 4 microns were stained with hematoxylin & eosin (HE) and examined by a light microscope. The presence of atherosclerotic lesions in the coronary vessels was assessed microscopically. The specimens were to detect any foamy macrophage; intra- and extracellular lipids; fibrous cap; intra-lesional hemorrhage; necrotic core; and micro calcification. Lesions



**Fig 1.** A saddle-shaped loading apparatus for rats. Views from different angles: (a), (b), (c), (d)

were evaluated according to the American Heart Association classification of human atherosclerotic lesions<sup>[19]</sup>. The rats were compared in terms of “presence of any-stage atherosclerotic lesion”, due to the number of rats being too small to compare the atherosclerotic stages separately. In addition, ventricular thicknesses were measured microscopically for better precision, besides already being measured macroscopically.

#### **Biochemical and Hematological Evaluation**

For determination of biochemical and hematological parameters of the serum; blood samples were taken before commencing, and at the end of the study period, in all cases. Blood was taken from the tail vein of each rat into coagulant tubes for biochemical analysis, and into ethylene diamine tetra acetic acid (EDTA) tubes for hematological analysis. Hematological (white blood cell, red blood cells, mean corpuscular volume and hematocrit) analysis was performed on a blood counter (VG-MS4e®, Melet Schloesing, France). The blood samples taken from each rat into coagulant tubes for biochemical analysis were centrifuged at 3000 rpm for 10 min, and the serum was harvested and kept at -20°C until the time of analysis. The biochemical parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, total cholesterol, urea, calcium, magnesium, phosphor, total protein, creatine kinase (CK), creatine kinase-myocardial band isoform (CK-MB), lactate dehydrogenase (LDH), amylase, lipase, Ferrum (Fe) and albumin)

were studied using a MINDRAY BS120® (Mindray Medical International Limited, Shenzhen, China).

#### **Statistical Analysis**

Statistical analyses were performed using the Statistical Package for the Social Sciences® (SPSS) version 22.0 (IBM, Chicago, Illinois). Continuous and categorical variables are expressed as mean±standard deviation and percentages, respectively. Differences in subject characteristics between experimental and control groups were analyzed using the *t*-test or Mann-Whitney U test for continuous variables, and the chi-square test for categorical variables. Dependent T-test and Friedman test were used to compare laboratory parameters of the rats between the beginning and the end of the study period.

## **RESULTS**

Thirty rats (15 experimental, 15 control) that underwent a ladder-climbing exercise training program were studied. The baseline biochemical and hematological characteristics of all rats are listed in *Table 1*. There was no significant difference in basal characteristics between the two groups except ALT and lipase. Both ALT and lipase levels were statistically higher in the experimental group compared to the control group (58.4±24.7 vs. 74.8±21.2; P=0.041 and 4.20 (3.40-4.40) vs. 4.65 (4.20-6.20); P=0.041, respectively).

During follow-up, one subject in the control group, and two subjects in the experimental group died, leaving 27 rats. At the end of the study, the experimental group had a more frequent atherosclerotic lesion compared to the control group (61.5% vs. 21.4%; P=0.034). Hematocrit, red blood cell (RBC) count, monocyte ratio, LDH and CK-MB levels were higher; mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were lower in the experimental group when compared with the control group (*Table 2*).

The rats with atherosclerotic lesion were evaluated and compared in terms of pathological features. The contents of the atherosclerotic lesions including foamy cell, fibrosis cap, and intra- and/or extra-cellular lipid content were noted (*Table 3*) (*Fig. 2*).

After the study period, changes in hematological and biochemical parameters of the rats were analyzed, and compared to the baseline. In the experimental group,

**Table 1.** Baseline biochemical and hematological characteristics of all rats, the control group, and the experimental group before the commencement of the study period, with *p* value

Parameters	All Rats (n:30)	Control Group (n:15)	Experimental Group (n:15)	P Value
WBC count (10 <sup>9</sup> /L)	6.76±2.58	7.02±2.68	6.47±2.54	0.813
Lymphocyte (%)	91.1±7.7	92.4±4.8	89.7±9.9	0.652
Monocyte (%)	3.40 (1.60-4.20)	3.40 (2.00- 4.20)	3.00 (1.17-4.80)	0.747
Granulocyte (%)	2.7 (0.8-7.80)	2.6 (1.0-9.20)	3.5 (0.7-7.80)	0.880
Hemoglobin (g/dL)	19.6±4.3	20.0±5.2	19.3±3.3	0.983
Hematocrit (%)	57.7±12.5	56.2±10.2	59.2±14.8	0.983
RBC (10 <sup>12</sup> /L)	8.90±1.74	8.71±1.47	9.10±2.03	0.747
MCV (fL)	64.7±2.3	64.4±2.1	64.9±2.7	0.652
MCH (pg)	22.0±2.2	22.7±2.0	21.4±2.3	0.290
MCHC (g/dL)	34.2±3.7	35.2±3.1	33.1±4.0	0.331
RDW (%)	10.6±1.2	10.5±1.2	10.7±1.1	0.914
MPV (fL)	5.3±1.1	5.0±1.4	5.5±0.4	0.201
PDW (fL)	7.3±2.2	7.4±2.2	7.3±2.2	0.813
ALT (U/L)	66.3±24.1	58.4±24.7	74.8±21.2	0.041
AST (U/L)	207.6 (166.2-261.6)	207.6 (166.2-261.6)	214.5 (156.3-291.60)	0.983
ALP (U/L)	480.7±146.2	493.5±150.6	466.9±145.8	0.591
Glucose (mg/dL)	138.61±43.14	131.63±43.84	146.09±42.67	0.146
Total Cholesterol (mg/dL)	46.46±14.08	49.93±17.15	42.75±9.03	0.451
Urea (mg/dL)	34.06±10.03	31.30±6.58	37.02±12.32	0.102
Calcium (mg/dL)	9.84±2.38	10.29±2.76	9.35±1.89	0.331
Magnesium (mg/dL)	7.22±2.67	7.35±2.49	7.08±2.93	0.621
Phosphor (mmol/L)	27.39 (18.20 - 30.69)	27.39 (6.56-29.52)	26.46 (20.60-33.72)	0.400
Total Protein (mg/dL)	6.6±1.0	6.7±1.0	6.5±1.0	0.505
CK (U/L)	3733.4 (1555.2-6210.6)	4195.2 (1555.2-5600.1)	3470.1 (1227.2-7841.6)	0.976
CK- MB (U/L)	630.2±245.2	643.8±203.6	615.7±290.5	0.914
LDH (U/L)	2259.5±951.4	2250.7±1200.4	2267.8±687.0	0.813
Amylase (U/L)	2390.2±446.2	2428.1±594.6	2349.5±211.4	0.477
Lipase (U/L)	4.20 (3.80-4.80)	4.20 (3.40-4.40)	4.65 (4.20-6.20)	0.041
Fe (µg/dL)	30.3±9.4	28.8±10.4	31.8±8.2	0.331
Albumin (mg/dL)	3.6 (3.3-3.90)	3.6 (3.3-4.20)	3.3 (3.3-3.60)	0.310

WBC: White blood cell; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red Cell Distribution Width; MPV: Mean Platelet Volume; Pct: Platelet Hematocrit; PDW: Platelet Distribution Width; ALT: Alanine amino transferase; AST: Aspartate amino transferase; ALP: Alkaline phosphatase; CK: Creatine Kinase; CK-MB: Creatine Kinase MB isoform; LDH: Lactate Dehydrogenase

white blood cell (WBC) count, granulocyte and monocyte ratio, RBC, MCHC, urea and CK-MB levels were found to be higher; while lymphocyte ratio, mean corpuscular volume (MCV), MCH, ALT, LDH, Fe and glucose levels were lower when compared with the control group. On the other hand, WBC count, granulocyte and monocyte ratio, MCHC, urea and CK-MB levels were higher; while lymphocyte ratio, MCV and ALP levels were lower in the control group (Table 4).

## DISCUSSION

This study aimed to determine the effect of BCAAs on coronary/myocardial histopathology and biochemical and hematological parameters, in rats subjected to intense

exercise. The main finding of our study is that BCAA supplementation was associated with atherosclerotic process of coronary arteries.

Recently, ordinary people as well as athletes consume diverse ergogenic aids, in increasing amounts to improve density of skeletal muscle and exercise performance [20]. Multiple explanations have been suggested regarding the increase in performance during endurance exercises due to consumption of BCAA supplements. It has been proposed that reduction in muscle damage leads to reduction in CK and LDH [12,13,21]. Several previous studies have observed a reduction in CK levels with use of BCAA, and hypothesized that one of the most important mechanisms by which BCAA works is to reduce muscle damage [12,21]. However, reduction in CK levels with the use

**Table 2.** Biochemical, hematological and pathological characteristics of all rats, the experimental group, and the control group, at the end of the study period, with p value

Parameters	All Rats (n:27)	Control Group (n:14)	Experimental Group (n:13)	P Value
WBC count (10 <sup>9</sup> /L)	10.32±3.06	10.62±3.36	10.00±2.79	0.302
Lymphocyte (%)	60.8±10.1	63.7±10.5	57.8±9.1	0.141
Monocyte (%)	13.0±3.8	11.2±2.8	15.1±3.7	0.002
Granulocyte (%)	23.7 (19.6-31.10)	23.0 (18.1-27.50)	26.7 (22.4-31.40)	0.239
Hemoglobin (g/dL)	20.5±4.5	19.5±6.0	21.5±1.8	0.054
Hematocrit (%)	54.5±13.1	49.3±16.3	60.1±4.0	0.003
RBC (10 <sup>12</sup> /L)	10.30±2.45	9.41±3.07	11.26±0.93	0.003
MCV (fL)	52.9±2.8	52.3±2.6	53.6±3.0	0.202
MCH (pg)	19.9±2.3	20.7±3.0	19.1±0.7	0.003
MCHC (g/dL)	37.8±4.6	39.7±5.3	35.8±2.4	0.009
RDW (%)	10.1±0.6	9.9±0.6	10.2±0.4	0.583
MPV (fL)	5.3±0.6	5.3±0.8	5.3±0.4	0.458
PDW (fL)	7.2±1.1	7.1±1.3	7.3±0.8	0.793
ALT (U/L)	52.2 (46.2-61.60)	52.1 (45.2-61.60)	53.2 (48.4-57.0)	0.685
AST (U/L)	180.8 (155.6-212.0)	159.1 (153.8-212.0)	191.0 (180.8-202.0)	0.061
ALP (U/L)	377.9±103.8	343.8±73.3	414.6±121.4	0.141
Glucose (mg/dL)	113.62±23.73	116.95±25.89	110.03±21.61	0.583
Urea (mg/dL)	51.03±9.09	53.38±12.11	48.49±2.56	0.867
Creatinine (mg/dL)	1.2±0.3	1.2±0.3	1.2±0.2	0.905
Calcium (mg/dL)	12.57±3.95	13.38±4.26	11.70±3.54	0.402
Magnesium (mg/dL)	3.28 (2.46-8.48)	3.11 (2.44-12.00)	3.52 (2.46-4.86)	0.793
Phosphor (mmol/L)	10.84 (5.34-12.72)	6.64 (5.34-11.10)	12.04 (10.84-13.88)	0.141
Total Protein (mg/dL)	7.0±0.6	7.1±0.7	7.0±0.3	0.458
CK (U/L)	1607.2 (1142.4-2200.6)	1168.6 (978.9-1652.2)	1746.6 (1465.2-2200.6)	0.081
CK- MB (U/L)	1282.9±374.1	1115.0±424.6	1463.7±199.1	<0.001
LDH (U/L)	1940.7±568.2	2178.3±284.9	1720.1±679.7	0.001
Amylase (U/L)	2370.5±720.5	2400.8±239.5	2337.8±1029.7	0.402
Lipase (U/L)	3.0 (2.6-3.40)	3.0 (2.6-3.40)	3.0 (2.8-3.20)	0.616
Fe (µg/dL)	22.0±5.9	22.3±7.0	21.7±4.5	0.867
Albumin (mg/dL)	3.5±0.3	3.5±0.5	3.5±0.2	0.550
RHW (gr)	1.5±0.2	1.5±0.2	1.6±0.2	0.076
LVWT (mm)	2.5 (2.0-2.88)	2.4 (2.0-2.80)	2.6 (2.0-2.95)	0.830
Presence of any-stage atherosclerotic lesion in any coronary artery, n (%)	11.00 (40.7)	3.00 (21.4)	8.00 (61.50)	0.034

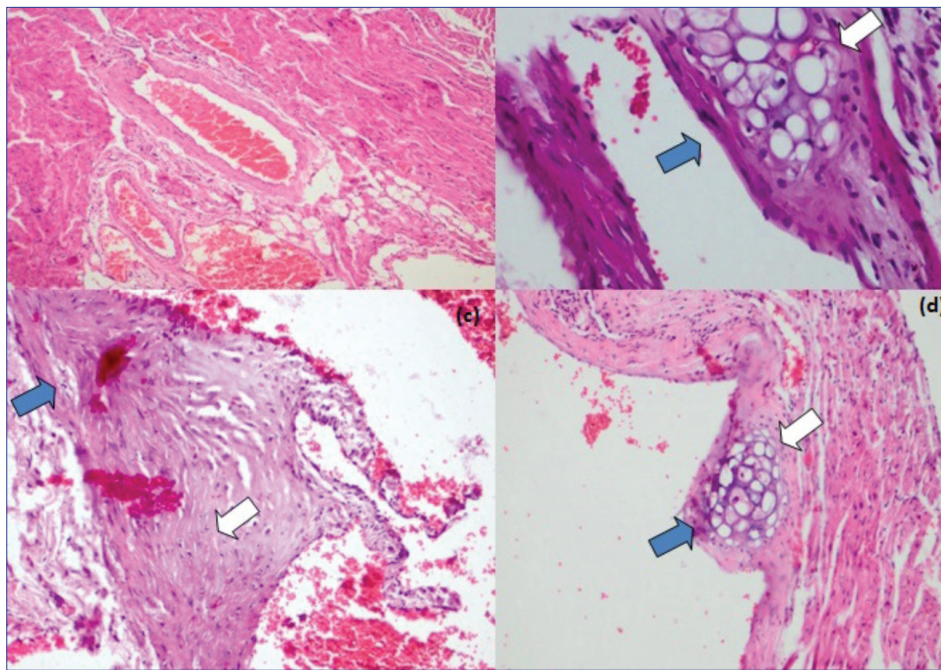
WBC: White blood cell; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red Cell Distribution Width; MPV: Mean Platelet Volume; Pct: Platelet Hematocrit; PDW: Platelet Distribution Width; ALT: Alanine amino transferase; AST: Aspartate amino transferase; ALP: Alkaline phosphatase; CK: Creatine Kinase; CK-MB: Creatine Kinase MB isoform; LDH: Lactate Dehydrogenase; RHW: Resected heart weight (g); LVWT: LV wall thickness

**Table 3.** Comparison of groups according to atherosclerotic features

Parameters	Control Group (n=14)		Experimental Group (n=13)		P Value
Any-stage atherosclerotic lesion, n (%)	3	(21.4)	8	(61.50)	0.034
Fibrous cap, n (%)	2	(14.3)	7	(53.8)	0.029
Intra- and/or extra-cellular lipids, n (%)	2	(14.3)	3	(23.1)	0.56
Foamy macrophage, n (%)	1	(7.1)	1	(7.7)	0.96

of BCAA is far from conclusive. A previously conducted study have found lower CK in BCAA-supplemented subjects, but when covariance analysis was performed, no

significant difference was detected between the BCAA-supplemented and non-supplemented groups [20]. Other previous randomized controlled studies have found no



**Fig 2.** Histopathological changes in the study groups. (a) Control group showing a natural view of normal coronary arteries (H&E staining; magnification 40x). (b) Extracellular lipid accumulation (white arrow) and fibrous cap (blue arrow), (H&E, 200x) in the experimental group. (c) The presence of intracellular lipid (white arrow) and foamy cells (blue arrow), (H&E, 200x) in the experimental group. (d) Extracellular lipid accumulation (white arrow) and fibrous cap (blue arrow), (H&E, 200x) in the experimental group

**Table 4.** Comparison of end-of-study laboratory parameters with baseline laboratory parameters

Parameters	Control Group			Experimental Group		
	Mean Difference	Std. Deviation	P Value	Mean Difference	Std. Deviation	P Value
WBC count (10 <sup>9</sup> /L)	-3.73214	4.88609	0.013	-3.52846	3.73307	0.005
Lymphocyte (%)	29.11	12.0	<0.001	31.78	15.03	<0.001
Monocyte (%)	-7.90000	3.23942	<0.001	-11.74077	4.61963	<0.001
Granulocyte (%)	-19.7857	9.7417	<0.001	-20.8538	11.6743	<0.001
Hemoglobin (g/dL)	0.5214	8.3398	0.819	-2.6462	3.9452	0.032
Hematocrit (%)	6.9357	20.6663	0.231	-1.3000	17.2770	0.791
RBC (10 <sup>12</sup> /L)	-0.68786	3.45281	0.469	-2.23615	2.51459	0.008
MCV (fL)	12.1143	3.8728	<0.001	11.4154	4.7663	<0.001
MCH (pg)	1.9143	3.9009	0.089	2.0923	2.4099	0.009
MCHC (g/dL)	-4.4643	6.3068	0.020	-3.0462	4.6205	0.035
RDW (%)	0.6857	1.5185	0.115	0.5692	1.2345	0.122
MPV (fL)	-0.2500	1.5659	0.561	0.2692	0.6447	0.158
PDW (fL)	0.1571	2.6938	0.831	-0.0462	2.3800	0.945
ALT (U/L)	4.3500	52.2047	0.760	21.2769	22.0222	0.005
AST (U/L)	-11.5286	279.7417	0.880	70.6538	172.9773	0.167
ALP (U/L)	176.5500	102.1896	<0.001	78.2615	183.1955	0.149
Glucose (mg/dL)	15.22571	51.63890	0.290	35.01462	38.98164	0.007
Urea (mg/dL)	-21.73071	15.58839	<0.001	-10.80692	13.16943	0.012
CK (U/L)	1889.1800	2893.6970	0.069	2815.1364	4246.5205	0.053
CK-MB (U/L)	-457.8929	351.0860	<0.001	-840.7231	349.0636	<0.001
LDH (U/L)	57.52	1267.76	0.726	480.72	922.11	0.006
Amylase (U/L)	11.0571	482.3316	0.933	24.6923	970.7561	0.928
Lipase (U/L)	0.61143	1.98005	0.269	4.10769	12.10086	0.244
Fe (µg/dL)	4.7357	9.3215	0.080	10.8615	6.7813	<0.001
Albumin (mg/dL)	-2.3286	7.8987	0.290	-0.0846	0.5352	0.579

WBC: White blood cell; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red Cell Distribution Width; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; CK: Creatine Kinase; CK-MB: Creatine Kinase MB isoform; LDH: Lactate Dehydrogenase

differences in CK levels between BCAA-supplemented and control groups [22,23]. In our study, we found no differences between BCAA-supplemented and non-supplemented groups.

While we did not observe any difference with respect to CK levels, between experimental and control groups, we did find LDH to be significantly lower in the experimental group. Consistent with previous data [23,24], we found no differences between the experimental and control groups with respect to glucose, creatinine, urea, albumin, total protein, and hemoglobin levels. In addition to those, we also found WBC count, the levels of ALT, AST, ALP, amylase, lipase, the concentration of calcium, magnesium and phosphor were similar between experimental and control groups.

Iron deficiency in endurance exercises has high prevalence, and it can be attributed to several factors, including inadequate dietary iron intake, exercise-associated iron losses, and reduced iron recycling [25]. At the end of our study, the iron levels were lower in both groups, though when compared to the baseline, only the drop in the experimental group was statistically significant. However, the level of hemoglobin in the experimental group was significantly increased compared to the baseline. The most likely cause of this is the production of  $\alpha/\beta$  globin protein in erythrocytes via essential amino acid-dependent mTORC1 (mechanistic target of rapamycin complex 1)/4E-BP (eukaryotic translation initiation factor 4E-binding protein) pathway [26].

Creatine kinase-myocardial band isoform is an enzyme found primarily in heart muscle cells and closely associated with myocardial injury. Several former studies have shown that, endurance exercise causes high levels of CK-MB [27-29]. These findings are in line with our study, which has shown a significant CK-MB elevation in both groups compared to the baseline. Interestingly, the rise in CK-MB in the experimental group, was significantly higher than the control group.

In the present study, myocardium and coronary arteries were examined histopathologically. Although increase in myocardial mass was observed in the experimental group, it was not statistically significant. This could be due to the elevation of local BCAA concentration. Increased BCAA concentration can lead to chronic induction of cardiac mammalian target of rapamycin (mTOR) activity which promotes cardiac hypertrophy via suppressing cardioprotective autophagy [14].

The adverse effects of BCAA supplements on coronary arteries were more pronounced. Significant histopathological changes such as subendothelial infiltration of macrophages, lipid accommodation, and formation of fibrous cap of the coronary atherosclerosis were observed in the experimental group. Several previous studies have

found that the BCAA levels were higher in patients with coronary artery disease and subsequent cardiovascular events. However, the mechanism by which the BCAA affected the heart was not clear in these studies [30,31]. We considered that mTOR is the most likely mechanism by which BCAA affects coronary arteries. Branched chain amino acids, especially L-leucine, are highly effective activators of mTOR signaling [32]. Mammalian target of rapamycin has various effects on vascular endothelium. In endothelium, it induces endothelial nitric oxide synthase (eNOS) uncoupling, endothelial senescence, and adhesion molecule expression, which all contribute to atherosclerotic vascular disease [33]. It has been demonstrated that the inhibition of mTOR by sirolimus, rapamycin, or lentivirus-mediated RNA interference suppresses atherosclerosis; decreases of macrophages throughout the atherosclerotic plaque; increases atherosclerotic plaque stability; and inhibits the mTOR linked proteins which are suggested to cause plaque destabilization [34-36]. Given the role of mTOR on destabilization and rupture of atherosclerotic plaques in addition to occurrence of atherosclerosis, the finding of CK-MB levels elevation in experimental group seems to be presumable.

In this study, we demonstrated that continuous and long-term consumption of BCAA-supplement in endurance exercises was associated with coronary atherosclerotic process and myocardial injury. Branched chain amino acid consumption may reduce muscle damage and induce improvement in hematological parameters.

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## CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

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