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Abnormality of energy metabolism in the skeletal muscle of patients with Liver Cirrhosis and changes under administration of Glucose and Branched-Chain Amino Acids

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We assessed changes in skeletal muscle energy metabolism by 31P-magnetic resonance spectroscopy (31P-MRS) and oxygen supply by near-infrared spectroscopy (NIR), after exercise and after administration of glucose and a branched-chain amino acids (BCAA), in healthy volunteers and patients with liver cirrhosis. As for the patients with liver cirrhosis, 4 were classified in Child-Pugh Grade A and the other 4 in Grade B.
In patients with liver cirrhosis, the intramuscular pH and PCr index (PCr/PCr+Pi) were lower than in healthy subjects after exercise in the fasting state; the ⊿pH and ⊿PCr index were statistically siginificant ($p<0.05$), indicating a disorder of aerobic metabolism. NIR revealed normality with oxygen supply and suggested a decreased oxygen utilization efficiency; that is, failure of the TCA cycle. The ⊿pH improved slightly after the administration of glucose alone, while it was definitely low ($p<0.05$) after co-administration of glucose and BCAA, which acts directly on the TCA cycle, indicating improvement of skeletal muscle energy metabolism.

Key words : liver cirrhosis, skeletal muscle, BCAA, glucose

INTRODUCTION

Patients with liver cirrhosis have a declined efficacy of glucose use associated with a decreased food intake and hyperinsulinemia as well as hypoalbuminemia, which is so-called protein-calorie malnutrition [1]. It is suggested that patients with liver cirrhosis metabolically have a condition equivalent to starvation after three-day fasting in healthy people in the early morning before having meals due to the declined gluconeogenesis and glucose storage capacity of the liver [2]. Under such a condition, patients have accelerated catabolization of muscular protein, alanine in particular, and production of glucose from the glucose-alanine cycle [3]. Clinically, patients have a higher incidence of loss of muscular strength or easy fatigability and muscular convulsion as represented by calf cramps of lower extremity muscle probably due to the metabolic abnormality of skeletal muscle [4, 5]. In particular, easy fatigability in the patients with liver cirrhosis indicates disorder of the muscular energy metabolism.

Haida *et al.*, co-researchers, developed a method for measuring the intracellular pH of muscle and PCr index (PCr/PCr+Pi) using 31P-magnetic resonance spectroscopy (31P-MRS) and observing changes under exercise loading in the patients with chronic alcohol-related diseases. This followed the hypothesis that the cause of metabolic abnormality can be deduced based on the determination of energy metabolism abnormality and examination of the oxygenation kinetics of intramuscular hemoglobin/myoglobin

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using near-infrared spectroscopy (NIR) and the elucidation of the oxygen supply to the muscle [6]. In this study, we performed similar examinations in the patients with liver cirrhosis, who tended to chronically have a marked decline in hepatic metabolic functions and abnormality of skeletal muscular functions. Furthermore, glucose and BCAA (valine, leucine, isoleucine), which has been attracting attention for its physiological therapeutic effects, were administered to assess the pathological mechanism and the presence or absence of the effects to alleviate metabolic abnormality of the muscle in liver cirrhosis.

SUBJECTS AND METHODS

1. Subjects

The study was conducted in eight healthy volunteers (six males and two females; age 60.3±7.8) and eight patients with liver cirrhosis (four males and four females; age 67.4 ±6.8). The causes of liver cirrhosis included Type B in one patient, Type C in six patients and NBNC in one patient. Four patients were assessed according to the Child-Pugh Criteria [7] to be at Grade A 4 cases and Grade B 4 cases, respectively (Table 1). The subjects were all dextral and included no alcoholics. Written Informed Consents of patients were obtained after sufficient explanations about the objectives, methods and risks of the study.

2. Methods

2-1. 31P-magnetic resonance spectroscopy (31P-MRS)

31P-MRS used in the study was BEM-250/80 (PhotoEnergeics, USA) of 2.0 Tesra. According to the methods devised by Haida *et al.* [6], the subjects were positioned in the decubital position and placed inside the MRS magnet coil with the left upper arm abducted by 90 degrees.

The phosphorous spectrum in the flexor tissues of the forearm was measured using a 4-cm double-turned surface coil. Spectrum signals of creatine phosphate (PCr), inorganic phosphorus (Pi) and adenosine triphosphate (ATP) were obtained by accumulating spectrum signals at 3.0 sec×20 and the least square curve fitting (Fig. 1). The pH and PCr index in muscular cells were calculated based on PCr and Pi and the chemical shift value of Pi according to the formula devised by Tailor *et al.* [8].

PCr index=PCr/(PCr+Pi)

pH=6.75+log[ä−3.27)/(5.69−ä)]

(ä: chemical shift difference between PCr and Pi)

2-2. Near-infrared spectroscopy (NIR)

NIR used in the study was OM-100A (Shimazu, Japan). Changes in oxidized hemoglobin (oxy-Hb), deoxidized hemoglobin (deoxy-Hb) and total hemoglobin (total-Hb) were obtained based on the change in the absorption coefficient of the wavelength by the difference in the oxidization of tissue hemoglobin/myoglobin (Hb/Mb) [9, 10]. According to the method devised by Haida *et al.* [6], the edge of two glass fibers were fixed to the forearm flexor group at a distance of 5 cm from NIR and one of them was placed under the incident radiation while the other was placed for detection at a distance of 2 cm from the other fiber inside the 4.0-cm NMR surface coil.

2-3. Exercise loading protocol

The subjects were tested under at least 12-hour fasting. After a couple of measurements at rest, the subjects lifted a weight equivalent to 7 % of the left grip strength (kg)

Table 1 subject. According to the Child-Pugh criteria, 4 patients were in Grade A and the other 4 patients were in Grade B.

No.	sex/age	Cause	Ascites	Hepatic encephalopathy	Alb(g/dl)	T.bil(mg/dl)	PT(%)	NH_3(μg/dl)	Fischer's ratio
1	64/F	HCV	−	−	2.9	0.6	77	79	1.76
2	66/F	NBNC	−	−	3.1	2.0	65	52	2.02
3	67/F	HCV	−	+	3.4	2.1	58	122	0.68
4	56/F	HBV	−	−	3.3	1.2	74	57	2.22
5	68/M	HCV	+	−	2.8	1.2	64	46	1.70
6	65/M	HCV	−	−	3.2	0.7	74	70	2.27
7	75/M	HCV	+	−	3.0	1.2	71	99	1.52
8	78/M	HCV	−	−	3.4	0.7	74	48	2.31

using the gripping movement of the pulley and lever at the speed of once every three seconds for six minutes and spectral changes and times necessary to return to the baseline were monitored. Healthy subjects were tested only under fasting and patients with liver cirrhosis were tested (1) under fasting, (2) at completion + at one hour after administration of 200 Kcal glucose and (3) at one hour after the administration of 48 Kcal BCAA.

3. Statistical processing

Measured data were shown as mean ± S.D. Physical measurement data were tested by Student's t-test. As for 31P-MRS data, changes from the baseline (⊿pH=pH after loading - pH at rest, ⊿PCr index=PCr index after loading - PCr index at rest) were tested by analysis of variance (ANOVA). Changes seen after the administration of glucose and BCAA were compared to the spectrum taken under fasting and tested by ANOVA. As for NIR data, standard cases in healthy volunteers and in the patients with liver cirrhosis were comparatively assessed. In any case, differences at the level of 5 % were statistically significant.

RESULTS

1. MRS at rest

There was no significant difference in the intramuscular pH and PCr index at rest between healthy volunteers and the patients with liver cirrhosis as shown in 0 time of Figure 2 and 3.

2. MRS after exercise loading under fasting

In the patients with liver cirrhosis, intramuscular ⊿pH increased after exercising loading. After exercise loading, the maximal value changed significantly to 0.49 ± 0.16 compared to 0.20 ± 0.18 in the healthy volunteers. Significant change was seen for about ten minutes after exercise loading (Fig. 2).

The ⊿PCr index also increased significantly in the patients with liver cirrhosis compared to the healthy volunteers. The maximal value changed significantly to 0.55 ± 0.12 compared to 0.35 ± 0.19 in the healthy volunteers. Unlike ⊿pH, however, the value rapidly returned to the baseline equivalent to the level in the healthy volunteers after the completion of exercise loading (Fig. 3).

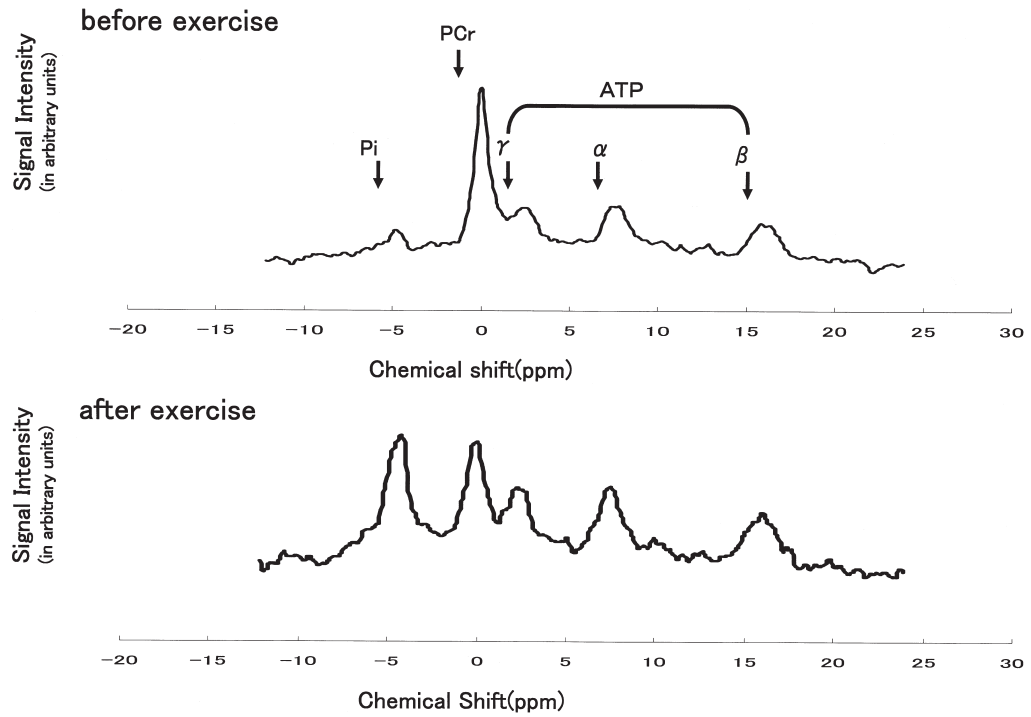

Fig. 1 Analysis of skeletal muscles energy metabolism by ^{31}P-MRS. PCr index=PCr/(PCr+Pi)
pH=6.75+log[(δ −3.27)/(5.69− δ)] (δ: Chemical shift difference between PCr and Pi)

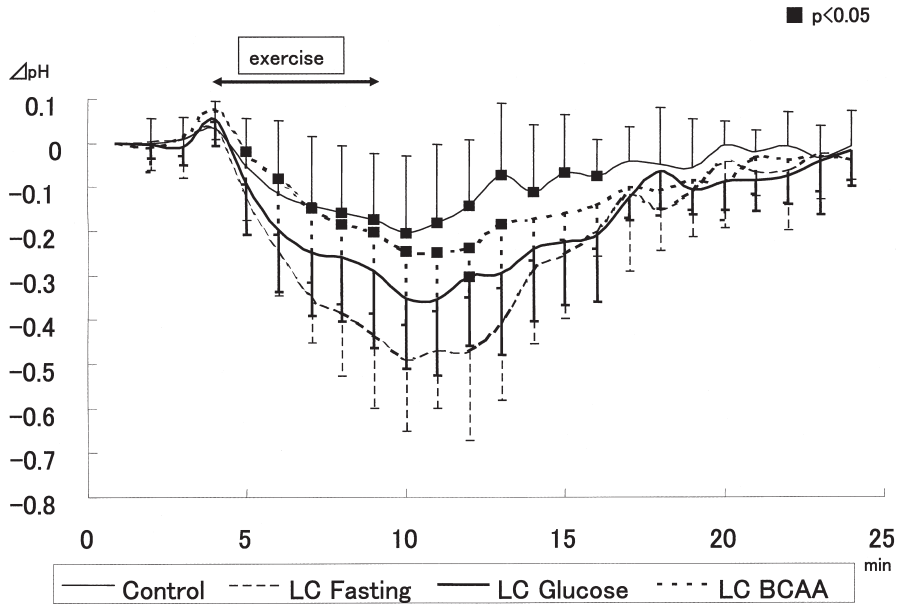

Fig. 2 Changes in ⊿ pH. The intramuscular ⊿ pH increased after exercise in patients with liver cirrhosis. It approximated the value in healthy subjects after administration of BCAA. After administration of glucose, the ⊿pH was intermediate between that in the fasting condition and that after administration of BCAA.

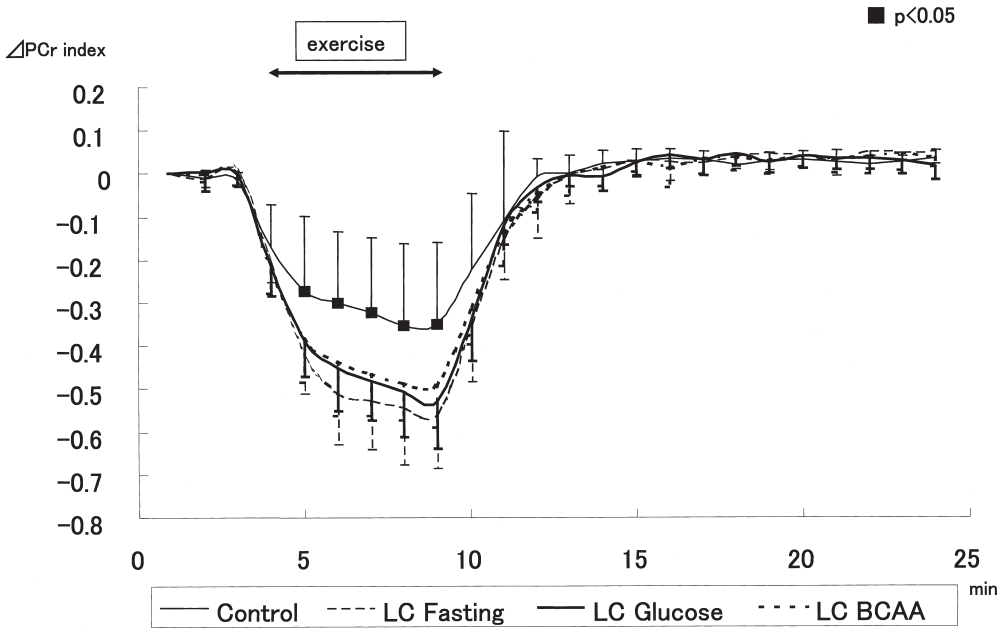

Fig. 3 Changes in ⊿PCr index. The ⊿PCr index, which increased after exercise loading, tended to decrease slightly more after the administration of glucose and administration of BCAA in this order compared to that obtained under fasting.

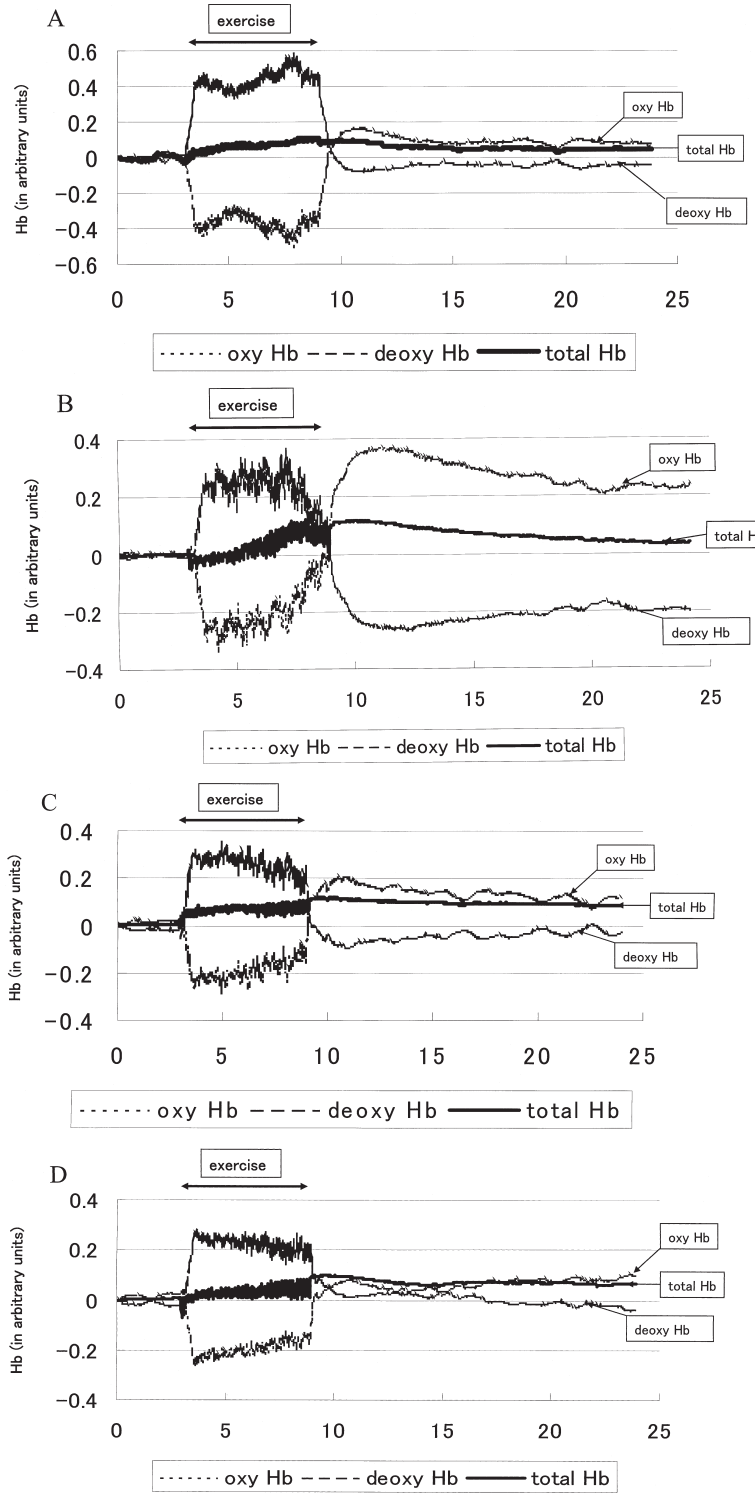

Fig. 4 NIR: (**A**) a patient in the control group (**B**) a patient with liver cirrhosis in the fasting condition (**C**) changes after administration of glucose (**D**) changes after administration of BCAA. In patients with liver cirrhosis, the oxy-Hb was higher and the deoxy-Hb was lower after exercise compared with those in healthy subjects. After administration of BCAA, the values of oxy-Hb and deoxy-Hb were similar to those in healthy subjects, while their variations were intermediate between values in the fasting condition and those in healthy subjects after administration of glucose.

3. Changes seen after administration of glucose and BCAA

Administration of BCAA reduced the difference in the changes in ⊿pH from the healthy volunteers as shown by the reduced maximal change of 0.25 ± 0.17 and the difference was significantly smaller than under fasting($P<0.05$). After the administration of glucose, ⊿pH was in between the data obtained under fasting and that obtained after the administration of BCAA (Fig. 2).

The ⊿PCr index, which increased after exercise loading, tended to decrease slightly more after the administration of glucose and administration of BCAA in this order compared to that obtained under fasting. After being relieved from exercise loading, the ⊿PCr index rapidly returned to the baseline after the administration of glucose and BCAA as seen in the healthy volunteers (Fig. 3).

4. Changes in NIR data

In the healthy volunteers, a marked decrease in oxy-Hb, drastic increase in deoxy-Hb and slight increase in total-Hb were seen after the commencement of exercise loading. After the completion of exercise loading, oxy-Hb and deoxy-Hb immediately reached the steady state. On the other hand, in the patients with liver cirrhosis, changes as seen in the healthy volunteers were observed under exercise loading, but a marked increase in oxy-Hb and marked decrease in deoxy-Hb continued after exercise loading compared to the healthy volunteers. As a result of the administration of BCAA, the patients with liver cirrhosis regained the condition almost equivalent to the condition of the healthy volunteers after exercise loading. After the administration of glucose, changes were in between the data obtained under fasting and in the healthy volunteers (Fig. 4).

DISCUSSIONS

Under exercise, a marked decrease in the intracellular pH and PCr index was seen in the skeletal muscle of the patients with liver cirrhosis. It is suggested that patients with liver cirrhosis have a lower ATP and PCr levels compared to healthy volunteers due to the reduction in muscular volume [11, 12]. Also in our measurements, patients with liver cirrhosis had a significantly lower arm muscle circumference (AMC) and grip strength compared to healthy volunteers. (Data is not shown) Therefore, the marked reduction in the PCr index indicates that successive muscular contraction consumes ATP and PCr relatively more markedly compared to the ATP and PCR pool despite the application of loading in proportion to the maximal grip strength. Besides, as there were neither differences among ages in forearm muscle metabolism by grasp movement of the hand [13] nor changes in vital signs and subjective symptoms before and after loading exercise on the subjects, it was considered that the results obtained were not affected by differences in the average ages.

The marked decrease in intracellular pH indicates the accumulation of lactic acid under exercise loading. The consumption of ATP energy under muscular movement immediately induces the operation of the creatinine cycle to compensate for the consumption of ATP energy. This results in the consumption of part of PCr, an increase in Cr and Pi and re-synthesis of ATP from ADP to maintain the ATP level. If the ATP level cannot be maintained, lactic acid production increases under anaerobic metabolism [6, 14].

Delayed recovery of pH after exercise loading indicates the persistent production of lactic acid at the stage of recovery or delayed washout of lactic acid probably because of (1) insufficient oxygen supply and impairment of washout of lactic acid due to circulatory disorder in the muscle and (2) because of aerobic metabolic impairment in the muscle if there is no possibility of circulatory disorder. To determine which mechanism (1) or (2) contributes to the delayed recovery of pH in the patients with liver cirrhosis, intramuscular oxygen kinetics were determined using NIR. The results indicate that patients with liver cirrhosis have poor consumption of oxygen, or in other words, aerobic metabolic impairment for some reason despite ample blood circulation and oxygen supply as substantiated by a slight increase in total-Hb and marked increase in oxy-Hb at the recovery stage from exercise loading.

Under aerobic metabolic impairment, the administration of glucose facilitates the production of lactic acid as a result of anaerobic glucose decomposition while having no effect on improving pH. In the experiment data, however, an improvement tendency, not a

significant difference, was seen compared to the condition under fasting. The decrease in the overshoot of oxy-Hb observed by NIR at the stage of recovery also indicates the consumption of oxygen, or in other words, a slight improvement of aerobic metabolism. As a result of the administration of glucose replenishing the glycogen stock consumed for the exercise under fasting, which is the so-called carbohydrate loading, glycogen stripping and activated Type I red muscles, aerobic metabolism may have been regained, but the mechanism is still unknown.

Meanwhile, branched-chain amino acid (BCAA: valine, leucine, isoleucine) is mainly metabolized in the skeletal muscle and serves as the energy source under exercise [15, 16]. When decomposed in the cells, actin and myosin release 3-methyhistidine and are excreted into urine. It is suggested that the administration of BCAA, which reduces urinary excretion [17, 18] and facilitates protein synthesis in the skeletal muscle [19], is useful for the maintenance of the skeletal muscle [20]. Meanwhile, BCAA is preferentially released from the liver and taken up by the skeletal muscle. In the skeletal muscle actively engaged in decomposition and synthesis of amino acids selectively catabolize BCAA. Leucine and isoleucine in collaboration with acetyl-CoA and valine in collaboration with succinyl-CoA form various intermediates, which are directly fed to the TCA cycle [20, 21]. It is suggested the catabolization is doubled or tripled under starvation.

The finding in the study that the administration of BCAA reduced the time necessary for the recovery of pH after exercise loading indicates a reduction in the production of lactic acid and in the percentage of anaerobic metabolism. Furthermore, a reduction in the decline in the PCr index, an increase in the oxy-Hb level and a decrease in the deoxy-Hb level at the recovery stage in NIR also suggest improvement of the efficiency of oxygen use and recovery of aerobic metabolism, which is high in the efficiency of ATP production.

Based on these findings, it is suggested that patients with liver cirrhosis have abnormalities of energy metabolism in the skeletal muscle, in particular abnormality of aerobic metabolism, and that the co-administration of BCAA, rather than glucose-only administration, is the key to alleviating these abnormalities.

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REFERENCES

1) Lautz HU, Selberg O, Körber J, Bürger M, Müller MJ: Protein-calorie malnutrition in liver cirrhosis. Clin Invest 1992; 70: 478-86.
2) Owen OE, Trapp VE, Reichard GA: Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. J Clin Invest 1983; 72: 5: 1821-32.
3) Goldberg AL, Chang TW: Regulation and signifiance of amino acid metabolism in skeletal muscle. Fed Pro 1978; 37: 2301-7.
4) Marchesini G, Bianchi G, Amodio P, Salerno F, Merli M, Panella C, Loguercio C, Apolone G, Niero M, Abbiati R, THE ITALIAN STUDY GROUP FOR QUALITY OF LIFE IN CIRRHOSIS: Factors Associated With Poor Health-Related Quality of Life of Patients With Cirrhosis. Gastroenterology 2001; 120: 1: 170-8.
5) Abrams GA, Concato J, Fallon MB: Muscle Cramps in Patients With Cirrhosis. Am J Gastroenterol 1996; 91: 7: 1363-6.
6) Haida M, Yazaki K, Kurita D, Shinohara Y: Mitochondrial Dysfunction of Human Muscle in Chronic Alcoholism Detected by Using 31P-Magnetic Resonance Spectroscopy and Near-Infrared Light Absorption. Alcohol Clin Exp Res 1998; 22: 3: 108-10.
7) Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646-9.
8) Taylor DJ, Bore PJ, Styles P, Gadian DG, Radda GK: Bioenergetics of Intact Human Muscle, A 31P Nuclear Magnetic Resonance Study. Mol Biol Med 1983; 1: 77-94.
9) Tamura M, Eda H, Takeda M, Kubodera T: NEW INSTRUMENT FOR MONITORING HEMOGLOBIN OXYGENATION. Adv Exp Med Biol 1988; 248: 103-7.
10) Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR: Validation of near-infrared spectroscopy in humans. J Appl Physiol 1994; 77: 2740-7.
11) Möller P, Bergström J, Fürst P, Hellström K: Muscle Biopsy Studies in Patients with Moderate Liver Cirrhosis with Special Reference to Energy-rich Phosphogens and Electrolytes. Scand J Gastroenterol 1984; 19, 267-72.
12) Jacobsen EB, Hamberg O, Quistorff B, Ott P: Reduced Mitochondrial Adenosine Triphosphate Synthesis in Skeletal Muscle in Patients With Child-Pugh Class B and C Cirrhosis. Hepatology 2001; 34: 7-12.
13) Kutsuzawa T, Shioya S, Kurita D, Haida M, Yamabayashi H: Effect of age on muscle energy metabolism and oxygenation in the forearm muscles. Med Sci Sports Exerc 2001; 33: 6: 901-6.

14) Chance B. Leigh JS, Clark BJ, Maris J, Kent J, Nioka S, Smith D: Control of oxidative metabolism and oxgen delivery in human skeletal muscle: A steady-state analysis of the work/ energy cost transfer function. Proc Natl Acad USA 1985; 82: 8384-8.
15) MacLean DA, Spriet LL, Huitman E, Graham TE: Plasma and muscle amino acid and ammonia response during prolonged exercise in humans. J Appl Physiol 1991; 70: 2095-103.
16) Wagenmakers AJM, Beckers EJ, Brouns F, Kuipers H, Soeters PB, Van Der Vusse GJ, Saris WHM: Carbohydrate supplementation, glyogen depletion, and amino acid metabolism during exercise. Am J Physiol 1991; 260: E883-90.
17) Marchesini G, Zoli M, Dondi C, Bianchi G, Cirulli M, Pisi E: Anticatabolic Effect of Branched-Chain Amino Acid-Enriched Solutions in Patients with Liver Cirrhosis. Hepatology 1982; 2: 420-5.
18) Yamauchi M, Takeda K, Sakamoto K, Ohata M, Toda G: Effect of oral branched chain amino acid supplementation in the late evening on the nutritional state of patients with liver cirrhosis. Hepatology Research 2001; 21: 199-204.
19) Tischler ME, Desautels M, Goldberg AL: Does Leucine, Leucyl-tRNA, or Some Metabolite of Leucine Regulate Protein Synthesis and Degradation in Skeletal and Cardic Muscle? J Biol Chem 1982; 257: 1613-21.
20) Louard RJ, Barrett EJ, Gelfand RA: Overnight Branched-Chain Amino Acid Infusion Causes Sustained Suppression of Muscle Proteolysis. Metabolism 1995; 44: 424-9.
21) Wagenmakers AJM: Muscle Amino Acids Metabolism at Rest and During Exercise: Role in Human Physiology and Metabolism. Exerc Sport Sci Rev 1998; 26: 287-314.