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Abstract

Hepatic encephalopathy is one of the major complications in decompensated liver cirrhosis. The current study was conducted to clarify the mechanisms of zinc deficiency in liver cirrhosis and its involvement in hepatic encephalopathy via ammonia metabolism. Ten patients each with compensated or decompensated liver cirrhosis and 11 healthy volunteers were enrolled in the study. Serum zinc levels and its daily urinary excretion were measured, an oral zinc-tolerance test was performed to examine zinc malabsorption, and the effects of diuretics on zinc excretion and of zinc supplementation on ammonia metabolism in the skeletal muscle were studied. The mean serum zinc levels in patients with decompensated liver cirrhosis were found to be significantly lower than the levels in controls and patients with compensated liver cirrhosis. The serum zinc levels were inversely correlated with blood ammonia in the fasting state. In the oral zinc-tolerance test, the percent increase in serum zinc levels 120 and 180 min after ingestion was less in cirrhotic patients than in controls. A diuretic administration resulted in a significant reduction in serum zinc levels. An increased uptake of ammonia by and an increased release of glutamine from leg skeletal muscle after oral supplementation of zinc sulfate were evident. Taken together, zinc deficiency in decompensated cirrhotic patients appears to be due to low absorption and to high urinary excretion, for which excessive diuretic administration is, in part, responsible, and zinc supplementation might play an important role in the prevention of hepatic encephalopathy by activating glutamine synthetase.

KEYWORDS: zinc, ammonia, liver cirrhosis, hepatic encephalopathy

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Original Article

Effects of Zinc Deficiency / Zinc Supplementation on Ammonia Metabolism in Patients with Decompensated Liver Cirrhosis

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Hepatic encephalopathy is one of the major complications in decompensated liver cirrhosis. The current study was conducted to clarify the mechanisms of zinc deficiency in liver cirrhosis and its involvement in hepatic encephalopathy via ammonia metabolism. Ten patients each with compensated or decompensated liver cirrhosis and 11 healthy volunteers were enrolled in the study. Serum zinc levels and its daily urinary excretion were measured, an oral zinc-tolerance test was performed to examine zinc malabsorption, and the effects of diuretics on zinc excretion and of zinc supplementation on ammonia metabolism in the skeletal muscle were studied. The mean serum zinc levels in patients with decompensated liver cirrhosis were found to be significantly lower than the levels in controls and patients with compensated liver cirrhosis. The serum zinc levels were inversely correlated with blood ammonia in the fasting state. In the oral zinc-tolerance test, the percent increase in serum zinc levels 120 and 180 min after ingestion was less in cirrhotic patients than in controls. A diuretic administration resulted in a significant reduction in serum zinc levels. An increased uptake of ammonia by and an increased release of glutamine from leg skeletal muscle after oral supplementation of zinc sulfate were evident. Taken together, zinc deficiency in decompensated cirrhotic patients appears to be due to low absorption and to high urinary excretion, for which excessive diuretic administration is, in part, responsible, and zinc supplementation might play an important role in the prevention of hepatic encephalopathy by activating glutamine synthetase.

Key words: zinc, ammonia, liver cirrhosis, hepatic encephalopathy

Hepatic encephalopathy (HE) is one of the major complications in patients with advanced liver cirrhosis (LC), and abnormal ammonia metabolism is thought to be the major cause of the HE [1, 2]. After reports stated that zinc (Zn) supplements are effective in the treatment of HE in LC [3-5], the possible relationship between Zn and ammonia metabolism has been one of the most interesting aspects of HE research [6, 7].

It is well known that patients with LC show markedly diminished Zn levels in blood and a marked increase in urinary excretion of Zn [8-13]. Because Zn is secreted primarily into the intestinal tract via gastric, pancreatic,

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and bile juices, and its excretion in urine and perspiration is low [14, 15], it is not clear whether the increased urinary excretion affects the serum levels in LC patients. In particular, the effect of diuretics, which are frequently used in decompensated LC (DLC) patients with edema or ascites, is totally unknown. Impaired Zn absorption in patients with LC has also been considered the cause of Zn deficiency, though this hypothesis remains controversial [16-19].

Although Zn metabolism is related to the metabolism of ammonia by the activation of ornithine transcarbamylase (OCT) [6, 20-23], and through the inhibition of adenosine monophosphate (AMP) deaminase [24, 25], the effectsof Zn on glutamine synthetase [26, 27] have not yet been demonstrated.

In the current study, we carried out an oral Zn-tolerance test, and examined the effects of diuretics in order to unveil the precise mechanisms responsible for the fall in serum Zn levels. We also investigated the consequences of Zn supplementation in relation to ammonia uptake by skeletal muscle and glutamine release from the skeletal muscle in LC patient.

Subjects and Methods

Twenty patients with hepatitis B or C virus-related LC diagnosed by liver biopsy (10 compensated LC (CLC) and 10 DLC) were enrolled in the current study. None of the patients had a history of alcohol drinking. Twelve of the 20 patients had hepatocellular carcinoma (HCC) occupying less than 20% of the whole liver volume and being thought to not affect liver function. Patients who had a history of ascites, jaundice, and HE were regarded as being in DLC, and eight of ten DLC patients ingested diuretics during the current study. Eleven healthy volunteers from the hospital staff were taken as control subjects. Informed consent was obtained from each patient.

Fasting serum Zn, urinary Zn collected daily, and venous blood ammonia levels were measured in all cases. To examine Zn absorption from the gut, a single dose of Zn sulfate (2 mg/kg body weight) was given orally to 3 patients with LC and 5 controls, and serum Zn levels were measured before and 60, 120, and 180 min after administration. The effects of diuretics were analyzed in 5 CLC patients by examining alterations in serum Zn levels 30, 60, and 120 min after the ingestion of furosemide (20 mg). To examine the effects of chronic Zn supplementation on serum ammonia levels, 300 mg of Zn sulfate was given orally for 7 days to 3 DLC patients who had a history of HE, and blood ammonia and serum Zn concentrations were measured before and 3 and 6 days after Zn supplementation. In the other 4 LC patients and 2 LC controls, the femoral arterial-venous differences (artery concentration - the vein concentration) of blood ammonia and plasma glutamine and glutamic acid levels were determined before and after 3 days of Zn supplementation to analyze the effects of Zn on glutamine synthetase. Serum and urinary Zn levels were measured with a Hitachi 180-50 type atomic-absorption spectrophotomete (Hitachi Co. Ltd., Tokyo, Japan). Blood ammonia levels were measured by a microdiffusion method [28], and plasma glutamine levels were determined with an auto amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan). Statistical analyses of the differences between the groups were performed with the Student's t-test or Tukey-Kramer's test using the StatView-J5.0 program. Differences with a P value of less than 0.05 were considered significant.

Results

Fasting serum Zn levels in patients with DLC (56.3 ± 13.7 mg/dl, mean $\pm$ SD) were significantly lower than those in controls (84.9 ± 7.5 mg/dl, $P < 0.05$) and in CLC patients (75.0 ± 15.0 mg/dl, $P < 0.05$); there was no significant difference between LC patients with and without HCC (Fig. 1). There was a significant positive correlation between serum albumin, a Zn carrier protein, and serum Zn levels ($r = 0.71$, $P < 0.01$) (Fig. 2).

The total amount of urinary Zn excretion was 0.38 ± 0.14 mg/day in controls and 1.44 ± 0.77 mg/day in LC ($P < 0.0001$) (Fig. 3), and was inversely correlated with serum Zn levels in DLC patients ($r = -0.785$, $P = 0.211$, data not shown). Serum Zn levels significantly diminished 60 ($P < 0.05$) and 120 min ($P < 0.05$) after ingestion of the diuretic (furosemide) (Fig. 4).

In the oral zinc-tolerance test, the mean serum Zn levels before loading in controls (87.0 ± 4.7 mg/dl) were significantly higher than that in LC patients (56.7 ± 6.1 mg/dl). The levels of Zn 60, 120, and 180 min after Zn supplementation were clearly increased in all cases in both groups as compared with the levels before administration. However, the percentage increases at 60, 120, and 180 min were $186.3 \pm 17.9\%$, $176.0 \pm 13.1\%$, and $171.7 \pm 36.6\%$ in LC patients and $199.6 \pm 45.6\%$, $247.6 \pm 25.8\%$, and $219.2 \pm 23.4\%$ in controls, respectively,

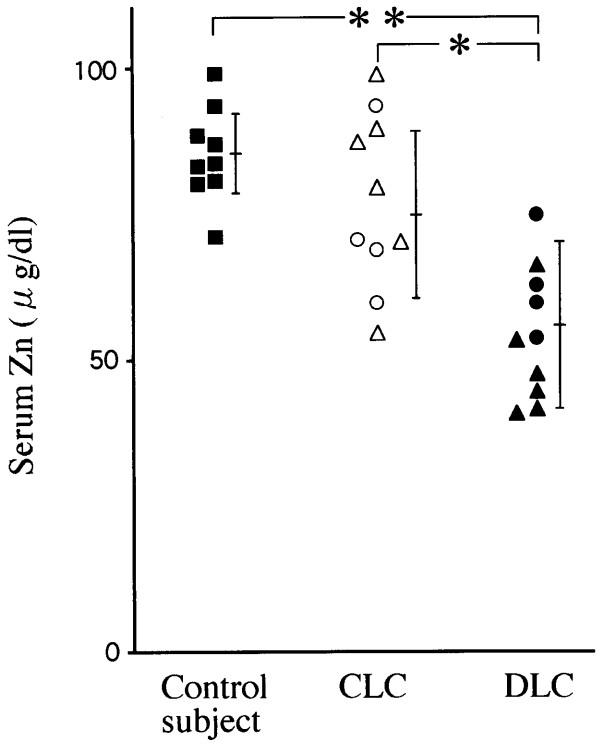

Fig. 1 Serum zinc levels in control subjects and patients with liver cirrhosis in the compensated or decompensated state. Mean levels in the decompensated cirrhotic patients were significantly lower than those in the control subjects and in the compensated cirrhotic patients. *, $P < 0.05$. **, $P < 0.01$. CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis. ■, control subjects; ○, compensated liver cirrhosis without hepatocellular carcinoma (HCC); ●, decompensated liver cirrhosis without HCC; △, compensated liver cirrhosis with HCC; ▲, decompensated liver cirrhosis with HCC.

with significant differences being observed at 120 ($P < 0.01$) and 180 min ($P < 0.05$) (Fig. 5).

There was a significant inverse correlation between fasting serum Zn and fasting ammonia levels in the vein ($r = -0.78$, $P < 0.05$) (Fig. 6). During the 7-days Zn supplementation, the serum Zn levels markedly increased, and the blood ammonia levels on the 3 rd and 6 th days decreased in all 3 patients with DLC (Fig. 7).

As an effect of Zn supplementation on ammonia metabolism in the skeletal muscle, glutamine release from skeletal muscle increased in all 4 LC patients who received Zn supplementation, and the uptake of ammonia by skeletal muscle also markedly increased in 3 of 4 patients. However, in one of 2 LC patients not receiving Zn supplementation, an increased uptake of ammonia and glutamine release was not observed (Table 1).

Discussion

The causes of low serum Zn levels in patients with severe LC are thought to be: I) inadequate protein intake, II) disturbed absorption from the gut, and III) excessive excretion in the urine. The recommended dose of Zn in the adult diet is approximately 10–15 mg per day. However, at our hospital, the Zn and protein content in the diet for CLC and DLC are 7.7 mg and 90 g, and 7.3 mg and 40 g per day, respectively, suggesting an inadequate Zn intake in LC patients. In the present study, we have attempted to clarify the involvement of the latter 2 factors in Zn depletion and the effects of Zn supplementa-

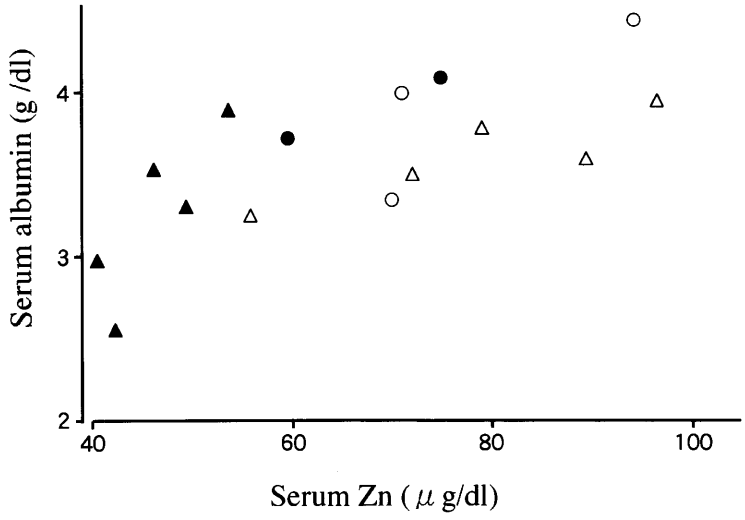

Fig. 2 Correlation between serum zinc and albumin levels in patients with liver cirrhosis. A significant positive correlation was found ($r = 0.71$, $P < 0.01$). Symbols are the same as those used in Fig. 1.

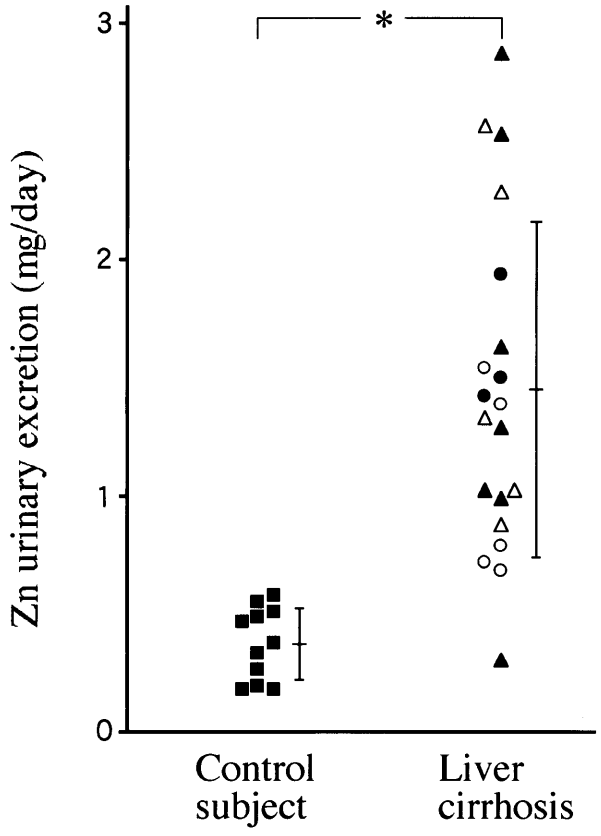

Fig. 3 Daily urinary excretion of zinc in control subjects and in patients with liver cirrhosis. The urinary excretion of zinc was significantly higher in cirrhotic patients than in the controls. Symbols are same as those shown in Fig. 1. *, $P < 0.0001$.

tion on ammonia metabolism in LC patients.

With respect to Zn absorption, the small increase in Zn levels 120 and 180 min after a single dose of Zn in LC patients in comparison with controls indicates Zn malabsorption from the gut in LC. These results in virus-related and nonalcoholic LC confirm previously reported findings that Zn malabsorption is not a unique phenomenon observed only in alcoholic LC [19, 29], as most studies have dealt with Zn reduction in alcoholic LC [8, 9, 30, 31]. Portal-hypertensive gastrocolopathy might be the primary causal mechanism in Zn malabsorption, as previously suggested [32-34].

Because more than 70% of Zn is excreted from the gut and only 5% of injected ^{65}Zn is removed by the kidney [14], the influence of urinary Zn excretion on serum levels seems to be low. Hyperzincuria is found in conditions associated with a hypercatabolic state such as surgery, burns, multiple injuries, bone fractures, diabetes mellitus, protein deprivation, and starvation [35]. In DLC, ascitic fluid and leg edema associated with hypoalbuminemia are common, and we usually employ various kinds of diuretics. We have noted that serum Zn levels are inversely correlated with its urinary excretion in DLC patients, and that these levels decrease according to the increase in urine volume after administration of a diuretic. This could be one of the causes of severe zinc depletion and could account for the development of HE after an excessive use of diuretics, although this mechanism has been reported to be related to hypo-

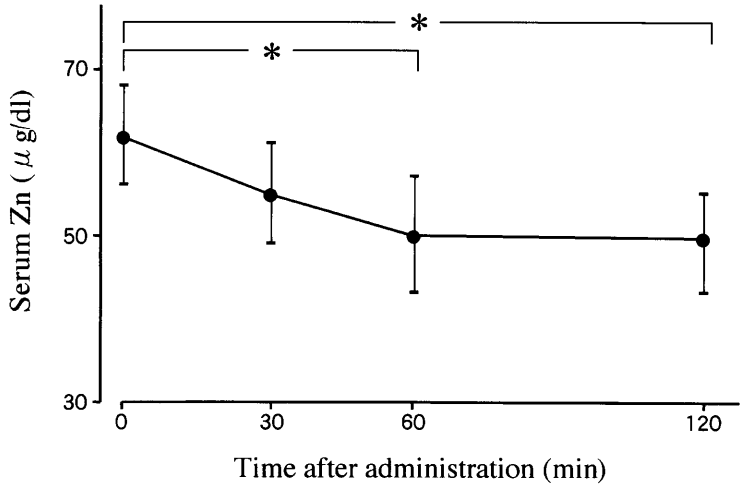

Fig. 4 Alterations of serum zinc levels in 5 patients with decompensated liver cirrhosis after ingestion of a diuretic (furosemide, 20 mg). Compared with the zinc levels prior to ingestion, levels 60 and 120 min after diuretic administration were significantly diminished. *, $P < 0.05$.

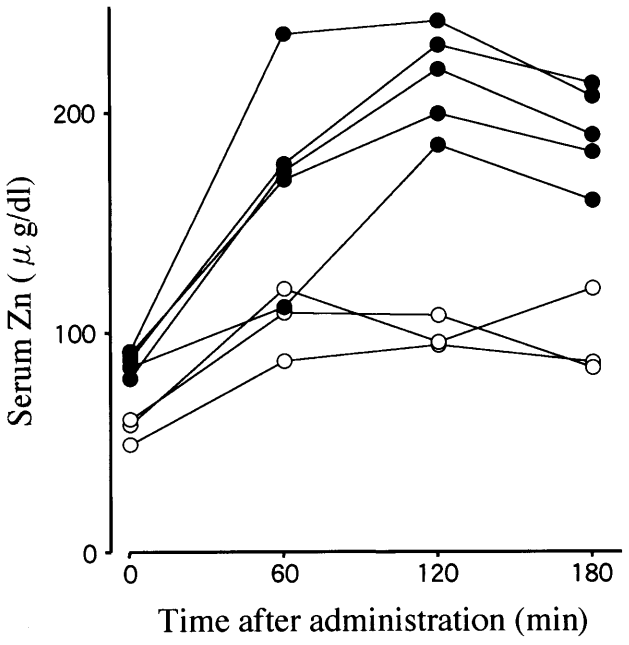

Fig. 5 Oral zinc-tolerance test results in control subjects and patients with liver cirrhosis. Percent increases in serum zinc levels 60, 120 and 180 min after a single dose of zinc sulfate (2 mg/kg body weight) in 5 control subjects (●—●) were 199.6 ± 45.6%, 247.6 ± 25.8%, and 219.2 ± 23.4%, and in 3 cirrhotics (○—○) were 186.3 ± 17.9%, 176.0 ± 13.1%, and 171.7 ± 36.6%, respectively. Significant differences in the percent increases between control subjects and patients with liver cirrhosis were observed at 120 ($P < 0.01$) and 180 min ($P < 0.05$).

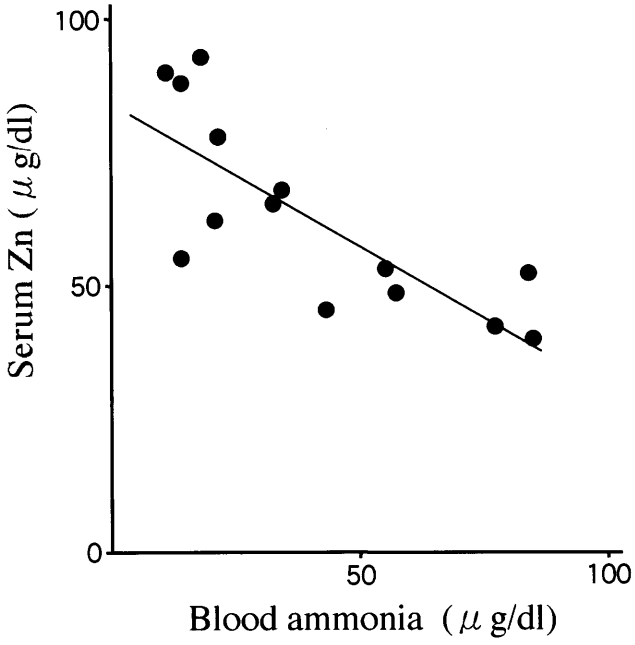

Fig. 6 Correlation between serum zinc levels and blood ammonia concentrations in patients with liver cirrhosis. A significant inverse correlation was found between the 2 parameters ($r = -0.78$, $P < 0.01$).

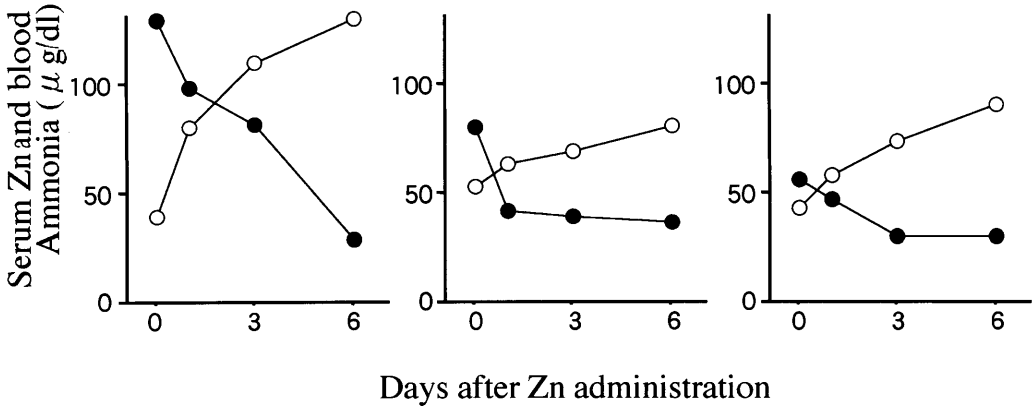

Fig. 7 Changes in serum zinc levels and blood ammonia concentrations in 3 patients with liver cirrhosis during oral zinc supplementation. Zinc levels (○—○) on the 3rd and 6th day of supplementation were higher than the levels before administration. In contrast, blood ammonia levels (●—●) decreased during the zinc supplementation.

Table I Femoral arterial-venous differences in blood ammonia and plasma amino acids before and after (3rd day) zinc sulfate supplementation (300 mg/day) in 4 patients with liver cirrhosis

	Zn (−)-control cirrhotics		Zn (+)-cirrhotics			
	Case 1 (B/A)	Case 2 (B/A)	Case 1 (B/A)	Case 2 (B/A)	Case 3 (B/A)	Case 4 (B/A)
Glutamine (nmol/ml)	−22/−63	−93/−87	−280/−330	−69/−156	−119/−126	−82/−85
Glutamic acid (nmol/ml)	+32/+24	+41/+39	+40/+30	+20/+11	+16/+16	+17/+17
Ammonia (μ mol/dl)	+9/+17	+20/+8	+10/+30	+13/+25	+45/+44	+43/+67

(B/A), (Before/After); −, release from skeletal muscle; +, uptake into skeletal muscle; Zn (−), no zinc supplementation; Zn (+), zinc supplementation for 3 days.

kalemia and to the passage of ammonia iron through the blood-brain barrier [36].

Symptoms of Zn deficiency, *e.g.*, delayed growth, deterioration of sexual function, dermatitis, anorexia, a decline in the sense of taste and smell, hair loss, delay in wound healing, and neuropsychiatric disorders, including stupor and depression, are well known [37-39]. Several clinical manifestations, including mental disorder in sickle cell disease, have also proven to be the consequences of Zn deficiency, and Zn supplementation corrects these symptoms [40]. Although these results suggest a direct effect of Zn depletion on mental disorders, our findings showed that serum Zn levels are inversely correlated with blood ammonia, which induces a mental disorder in LC patients, and that a decrease in blood ammonia is achieved in response to Zn ingestion.

Two major organs are involved in ammonia metabolism: the liver, in which ammonia is converted to urea via the urea cycle, and the skeletal muscle, where ammonia is released from aspartic acid by AMP deaminase [24] and where ammonia reacts with glutamic acid, resulting in a generation of glutamine by glutamine synthetase [26, 27]. In chronic hepatic insufficiency, it has been suggested that muscle plays an important role in disposing of ammonia and that a major fraction of ammonia taken up by muscle is converted to glutamine and released into the circulating blood [41]. Two mechanisms, a speeding up of the kinetics of urea formation in response to zinc supplementation in LC patients [3, 4, 7] and an inhibition of AMP deaminase with remarkable high affinity [24] have been demonstrated. In the present study, glutamine release from skeletal muscle increased in all 4 patients and the uptake of ammonia by skeletal muscle also markedly increased during Zn supplementation in 3 of 4 patients. These results may suggest the involvement of a third mechanism, whereby Zn activates glutamine synthetase and enhances ammonia metabolism in the skeletal muscle. A recent paper describing lowered glutamine synthetase activity in the testes from rats fed a low-Zn diet [42] probably supports our hypothesis. However, this idea needs confirmation in a larger group of patients, as we did not directly measure enzyme activity and we performed the examination in only 4 LC patients and 2 LC controls.

In conclusion, Zn depletion in DLC appears to be due to restricted dietary protein intake, malabsorption from the gut, and hyperzincuria, for which the ingestion of diuretics is partly responsible. Zn supplementation corrects the reduced serum Zn levels and decreases blood ammonia concentrations probably by activating muscle glutamine synthetase, resulting in the prevention of HE in DLC patients.

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