Table 2
Estimated abnormal rate of cut-off value (10th/90th percentile; healthy subjects) and standard value by NP-test (the data represents medians, with interquartile ranges)

Age (years)	Healthy subjects					Cirrhotic patients			Estimated abnormal	
	n	25th	Median	75th	90th	25th	Median	75th	%	*n*
NCT-A										
40–44	98	20.7	24.8	29.3	36.2	25.2	31.4	42.7	35.7	5/14
45–49	112	23.2	27.3	34.4	41.7	26.7	31.2	34.6	3.9	1/26
50–54	109	25.8	31.4	35.6	41.4	33.6	40.7	51.3	42.4	14/33
55–59	98	28.4	33.4	42.5	51.0	33.2	38.9	45.5	16.7	9/54
60–64	78	32.6	37.5	44.3	55.8	36.1	46.0	52.5	17.3	14/81
65–69	45	33.7	45.1	54.4	62.1	36.6	45.0	56.8	14.3	11/77
Total	540								18.9	54/285
NCT-B										
40–44	98	30.4	37.5	45.3	64.1	36.8	53.2	59.8	21.4	3/14
45–49	112	35.3	43.5	54.7	72.7	46.6	54.0	65.7	16.0	4/25
50–54	109	41.4	50.4	62.4	76.1	61.2	75.9	93.7	46.9	15/32
55–59	95	49.3	59.2	74.8	101.0	54.7	74.9	104.8	28.3	15/53
60–64	78	55.0	67.4	102.5	129.1	65.1	90.0	114.5	16.7	13/78
65–69	43	61.4	80.5	121.1	142.4	74.7	88.7	117.5	9.2	6/65
Total	535								21.0	56/267
FPT										
40–44	98	1.3	1.6	2.1	2.4	1.6	2.7	3.5	57.1	8/14
45–49	112	1.4	1.8	2.2	3.0	1.6	2.0	2.6	19.2	5/26
50–54	108	1.4	1.9	2.4	3.7	1.8	2.3	2.8	15.2	5/33
55–59	96	1.5	1.9	2.7	3.7	1.9	2.2	2.8	11.5	6/52
60–64	77	1.7	2.2	2.9	3.8	1.9	2.7	3.4	22.1	17/77
65–69	41	1.8	2.5	3.8	6.2	2.4	3.1	4.6	9.9	7/71
Total	532								17.6	48/273
DST										
40–44	96	20.0	25.0	27.0	31.0	17.0	21.0	28.8	30.8	4/13
45–49	110	17.0	22.0	25.0	29.0	20.5	22.0	24.5	4.2	1/24
50–54	105	16.0	19.8	22.0	26.0	14.0	16.5	19.5	43.8	14/32
55–59	91	13.0	16.3	20.0	23.0	13.0	17.0	21.0	21.7	10/46
60–64	74	11.9	14.0	17.0	21.0	12.0	15.0	19.0	23.9	16/67
65–69	41	9.6	12.0	15.0	18.0	12.0	14.0	17.0	1.6	1/64
Total	517								18.7	46/246
BDT										
40–44	98	9.1	10.6	12.4	14.9	10.9	16.0	17.9	57.1	8/14
45–49	112	10.3	12.9	16.0	18.6	12.3	14.4	17.5	22.2	6/27
50–54	109	11.3	13.9	16.7	19.7	12.5	15.6	20.0	27.3	9/33
55–59	99	11.9	14.6	20.7	27.1	13.4	17.8	23.5	11.1	6/54
60–64	79	13.9	17.5	21.5	25.8	15.2	20.6	27.3	25.6	21/82
65–69	45	15.7	20.3	24.8	[28.5	19.5	23.9	28.8	27.5	22/80
Total	542								24.8	72/290
RTT-A										
40–44	98	0.300	0.331	0.373	0.427	0.312	0.379	0.436	25.0	3/12
45–49	111	0.302	0.353	0.402	0.487	0.316	0.410	0.479	22.2	6/27
50–54	109	0.328	0.372	0.434	0.521	0.332	0.394	0.529	30.0	9/30
55–59	99	0.331	0.367	0.471	0.623	0.361	0.408	0.508	11.1	6/54
60–64	79	0.348	0.412	0.502	0.639	0.381	0.497	0.625	21.3	17/80
65–69	45	0.348	0.417	0.635	0.875	0.435	0.565	0.718	10.3	8/78
Total	541								17.4	49/281
RTT-B										
40–44	98	0.331	0.363	0.393	0.432	0.352	0.394	0.440	30.8	4/13
45–49	112	0.333	0.365	0.402	0.474	0.361	0.389	0.410	18.5	5/27
50–54	109	0.344	0.375	0.420	0.465	0.352	0.385	0.442	23.3	7/30
55–59	99	0.349	0.385	0.448	0.556	0.356	0.392	0.436	9.3	5/54
60–64	79	0.363	0.394	0.445	0.568	0.367	0.437	0.520	19.0	15/79

Table 2 (*Continued*)

Age (years)	Healthy subjects					Cirrhotic patients			Estimated abnormal	
	n	25th	Median	75th	90th	25th	Median	75th	%	*n*
65–69	45	0.379	0.420	0.561	0.812	0.416	0.486	0.586	5.1	4/78
Total	542								14.2	40/281
RTT-C										
40–44	98	0.352	0.392	0.445	0.508	0.386	0.445	0.599	30.8	4/13
45–49	112	0.386	0.427	0.490	0.627	0.373	0.419	0.466	14.8	4/27
50–54	109	0.385	0.424	0.499	0.647	0.382	0.436	0.519	3.3	1/30
55–59	98	0.388	0.422	0.513	0.700	0.385	0.444	0.529	11.1	6/54
60–64	79	0.398	0.468	0.572	0.670	0.397	0.455	0.601	16.5	13/79
65–69	44	0.395	0.444	0.590	0.903	0.445	0.518	0.632	1.3	1/78
Total	540								10.3	29/281

between alcoholic and non-alcoholic cirrhosis patients. Aging also showed profound effects on the test results. Weissenborn et al. [14] also reported that aging has significant effects on the NCT-A and NCT-B results. Neuropsychological tests have been reported to show learning effects or effects of increasing familiarity with the tests as they are repeated [6,20]. However, we noted no such effects with our system because it was designed so that only a short practice is conducted before the main tests. The level of education is also expected to affect the test results, but this effect was thought to be minimal on our test system because our test system is relatively simple and more than 90% of the population in Japan receives education for at least 12 years. Some of the liver cirrhosis patients enrolled in our study were taking drugs for hyperammonemia, but these drugs had no effects on the test results and abnormalities could be detected by each test.

Abnormal values for the tests included in our test system were determined based on the results obtained from healthy adult volunteers. The cut-off values were set at the 10th/90th percentiles, which are statistical outliers in healthy subjects, so that abnormal values were not overestimated. The incidence of abnormalities determined in liver cirrhosis based on these cut-off values was about 25% for each test, and 58.2% of the 292 liver cirrhosis patients showed deviations from the 10th/90th percentile cut-off values for at least 1 test. These percentages are lower than the 30–84% prevalence of SHE reported in liver cirrhosis patients [1–14]. Because our data were obtained from a large number of subjects in a multicenter clinical trial, however, the 10th/90th percentile cut-off values determined by us are expected to provide a simple method to screen SHE patients from liver cirrhosis patients. SHE is suspected if the results of any of the eight tests included in our test system are abnormal, but further studies are needed to determine if the diagnosis of SHE should be made only when two or more tests give abnormal values.

In general, SHE is diagnosed based on the results of multiple tests designed to assess performance cognition. Our test system also assesses multiple performance cognition functions (psychomotor function, attention, memory, and special cognition function) based on the results of eight tests. At present, there is no method in which test results are scored to make a diagnosis of SHE based on overall assessment of the test results. We were also unable to draw any definite conclusion from the scoring of the test results. Our results showed, however, that many liver cirrhosis patients who show abnormality in one test also show abnormality in other tests. In the future, it will be necessary to weight each test before scoring the abnormalities and making an overall assessment of the test results.

Recently, 11 types of neuropsychological tests conducted by Weissenborn et al. [14] and the CFF test [16] showed that low-grade HE could be diagnosed with great sensitivity and specificity, but their SHE extraction rate was similar to that obtained by us, and we do not believe that the diagnostic sensitivity can be enhanced by increasing the number of tests conducted.

Because the test results are affected by a number of factors such as the subject's physical condition, fatigue, sleeping time, experience operating computers, ocular disease, and testing environment, it is recommended that these factors be eliminated as much as possible when using our test system.

At present, there is no standard diagnostic method or criteria for SHE in Japan. The computer-aided quantitative neuropsychological test system developed by us converted two- or three-dimensional tests using paper and blocks into two-dimensional tests that can be conducted using a touch panel so that SHE can be easily diagnosed in daily clinical practice. Because the main objective of the present study was to computerize the tests commonly conducted to diagnose SHE, the test system developed by us was not compared to the conventional tests using paper and blocks. Further studies are needed to determine if our test system is superior to the conventional test methods.

Further prospective studies are also needed to determine, using the test system developed by us, the percentage of patients in whom SHE progresses to clinical hepatic encephalopathy by conventional coma grade, and determine the effects of SHE on quality of life by SF-36 (Short form 36) and the relationship between SHE and etiology of liver cirrhosis.

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Branched-chain amino acids as a protein- and energy-source in liver cirrhosis

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Abstract

Protein-energy malnutrition (PEM) is a common manifestation in cirrhotic patients with reported incidences as high as 65–90%. PEM affects largely the patients' quality of life and survival. Thus, diagnosis of and intervention for PEM is important in the clinical management of liver cirrhosis. Supplementation with branched-chain amino acids (BCAA) is indicated to improve protein malnutrition. As an intervention for energy malnutrition, frequent meal or late evening snack has been recently recommended. Plasma amino acid analysis characterizes the patients with liver cirrhosis to have decreased BCAA. Such reduction of BCAA is explained by enhanced consumption of BCAA for ammonia detoxication and for energy generation. Supplementation with BCAA raises in vitro the synthesis and secretion of albumin by cultured rat hepatocytes without affecting albumin mRNA expression. BCAA recover the impaired turnover kinetics of albumin both in rat cirrhotic model and in cirrhotic patients. Longer-term supplementation with BCAA raises plasma albumin, benefits quality of life issues, and finally improves survival in liver cirrhosis. Recent interests focused on the timing of administration of BCAA, since daytime BCAA are usually consumed by energy generation for physical exercise of skeletal muscles. Nocturnal BCAA seem to be more favorable as a source of protein synthesis by giving higher nitrogen balance. This minireview focuses on the basic and clinical aspects of BCAA as a pharmaco-nutritional source to control PEM in liver cirrhosis.

Protein-energy malnutrition (PEM) is a common manifestation in cirrhotic patients with reported incidences as high as 65–90% (Table 1) [1–3]. Protein malnutrition is usually represented by reduced serum albumin level (visceral protein) and by decreased skeletal muscle volume (muscular protein) in cirrhotics. The latter can be measured with conventional anthropometry as arm (or midarm) muscular circumference (AMC). Energy malnutrition is typically observed as an altered profile of thermogenesis such as reduced carbohydrate oxidation, increased fat oxidation, and subsequent decline of non-protein respiratory quotient. PEM affects largely the outcome of the patients by determining both their quality of life and survival (Fig. 1) (for recent studies, see [2] on protein malnutrition and [3] on energy malnutrition). Long-term prognosis after liver transplantation also depends on the patient's protein and energy nutritional state [4]. Thus, diagnosis of and intervention for PEM is an important issue in the clinical management of liver cirrhosis.

To improve protein malnutrition, efficacy of branched-chain amino acid (BCAA) supplementation is demonstrated [5,6]. Although there are still many criticisms against this nutritional intervention ([7–9], and for a recent comprehensive discussion, see [10]), ASPEN and ESPEN guidelines at least recommended the use of BCAA to improve hepatic insufficiency [11,12]. In several countries, parenteral BCAA formulae, enteral BCAA-enriched nutrient mixtures, or oral BCAA supplement is commercially available for cirrhotics. As an intervention for energy malnutrition, frequent meal or late evening snack has recently been recommended [11–15]. This article will particularly focus on the basic and clinical aspects of BCAA as a pharmaco-nutritional source to control PEM in patients with liver cirrhosis.

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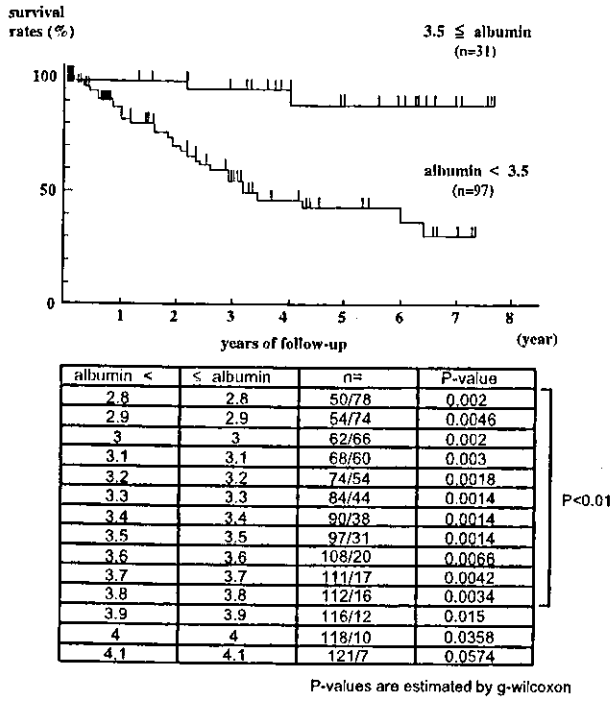
Table 1
Incidence of protein and energy malnutrition in patients with liver cirrhosis (constructed from [3])

Energy nutritional state	Protein nutritional state	
	Normal (%)	Malnourished (%)
Normal	13	25
Malnourished	12	50

Total number of patients = 128.



albumin <	≦ albumin	n=	P-value	
2.8	2.8	50/78	0.002	
2.9	2.9	54/74	0.0046	
3	3	62/66	0.002	
3.1	3.1	68/60	0.003	
3.2	3.2	74/54	0.0018	
3.3	3.3	84/44	0.0014	P<0.01
3.4	3.4	90/38	0.0014	
3.5	3.5	97/31	0.0014	
3.6	3.6	108/20	0.0066	
3.7	3.7	111/17	0.0042	
3.8	3.8	112/16	0.0034	
3.9	3.9	116/12	0.015	
4	4	118/10	0.0358	
4.1	4.1	121/7	0.0574	

P-values are estimated by g-wilcoxon

Fig. 1. Effect of serum albumin level on the survival of patients with liver cirrhosis. According to serum albumin at study entry, cirrhotic patients were stratified to those with hypoalbuminemia (<3.5 g/dl, $n = 97$) and those with normal albumin concentration (≥3.5 g/dl, $n = 31$). Only deaths due to hepatic failure were censored and survival rates were estimated by Kaplan–Meier method. $P < 0.05$ between two curves by the log-rank test (constructed from the study of [3]).

Mechanisms of branched-chain amino acid reduction in liver cirrhosis

Plasma amino acid analysis characterizes the patients with liver cirrhosis to have decreased BCAA [5]. There is a highly significant correlation between patients' plasma BCAA and albumin levels in liver cirrhosis, i.e., patients with low plasma BCAA have low serum albumin levels and those with high BCAA have high albumin [5]. Thus, in a similar manner to albumin as illustrated in Fig. 1, low plasma BCAA level or reduced Fischer's ratio (a molar ratio of BCAA to aromatic amino acids) also represents PEM and determines the survival of cirrhotic patients [5].

This reduction in plasma BCAA is brought about by the enhanced removal (or disappearance) of BCAA from the plasma of cirrhotic patients. The removal rate can be determined as a clearance of plasma BCAA and is demonstrated to be significantly higher in liver cirrhosis as compared with healthy control [16]. Such pronounced clearance of BCAA from plasma is explained partly by enhanced uptake and consumption of BCAA by skeletal muscles for ammonia detoxication [16] and for energy generation [17] (Fig. 2).

Hyperammonemia is a common manifestation of cirrhotic patients due to impaired hepatic capacity to detoxicate ammonia. Instead, skeletal muscles and, to a lesser extent, the brain clear blood ammonia by incorporating ammonia in the process of glutamine production from glutamate. The precursor glutamate requires BCAA for its synthesis. Thus, when exposed to hyperammonemia, skeletal muscles take up BCAA from the plasma to enhance their ability to degrade ammonia [16].

Thermogenesis in a physiological condition utilizes, of course, glucose as the most efficient energy source. In contrast, cirrhotic patients lose hepatic storage of glycogen due to liver atrophy and also get resistant to insulin in their peripheral tissues. Hence, in a cirrhotic condition, energy efficiency of glucose falls significantly, while that of BCAA rises, compensatorily, to 96% from physiological 45% [17]. Majority of such BCAA oxidation is supposed to occur in skeletal muscles and contribute to enhanced uptake of BCAA from plasma by skeletal muscles [17].

Mechanisms of branched-chain amino acid action in liver cirrhosis

Sequential effects of BCAA on albumin synthesis and secretion by hepatic parenchymal cells (HPCs), and subsequent whole body effects are summarized in Table 2. An important basic fact is that albumin mRNA is expressed solely in HPCs.

Supplementation with BCAA raises in vitro the synthesis and secretion of albumin by cultured rat hepatocytes without affecting albumin mRNA expression [18]. In an isonitrogenous condition, Fischer's ratio of the culture medium at 3–6 induces the most efficient synthesis and secretion of albumin by HPCs (plasma Fischer's ratio in normal subjects is 3–4). At Fischer's ratio below 3, the synthesis of albumin is suppressed to approximately 50% of normal condition, resulting in lower secretion of the protein. The synthesis rate is similar between Fischer's ratio of 3 and 30. However, intracellular breakdown of once-synthesized albumin (presumably preproalbumin) occurs above the ratio of 15 and again reduces the secretion of albumin by HPCs into culture media [18]. Such intracellular synthesis and degradation is supposed to be regulated at the rough surfaced endoplasmic reticulum (rough ER) level by BCAA. Possible molecular mechanism to explain this

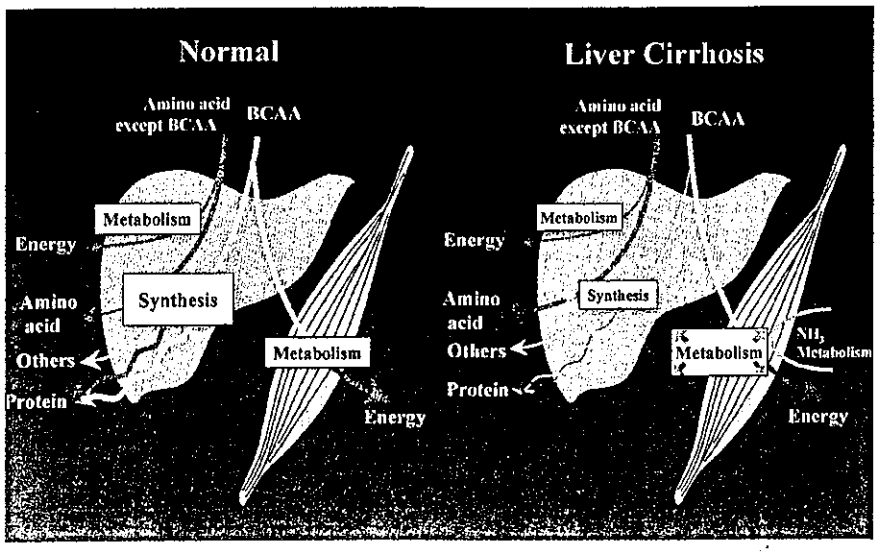


Fig. 2. Utilization of branched-chain amino acids (BCAA) by the liver and skeletal muscles in a physiological state (left panel) and in a malnourished condition with liver cirrhosis (right panel). In liver cirrhosis, increased amount of BCAA is consumed by skeletal muscles for ammonia metabolism and energy generation. Subsequently, the size of "Metabolism" pool in the muscle expands while that of "Synthesis" shrinks in liver cirrhosis as compared to those in "Normal" physiological condition.

Table 2
Sequential effects of branched-chain amino acids on in vitro and in vivo albumin synthesis and secretion

Time	Site	Effect	References
3 min	HPCs	Increased synthesis of preproalbumin	[18]
		(No effect on albumin mRNA)	[18] (HPCs), [21] (rat liver)
15–30 min	HPCs	Increased secretion of mature albumin	[18]
2 weeks	Whole body	Improved turnover kinetics of serum albumin	[19] (rat), [20] (human)
?	Whole body	Expansion of albumin pool	
2–6 months	Whole body	Rise in serum albumin level	[5,6] (human), [21] (rat)
2–4 years	Cohort	Improved survival of cirrhotics	[5,6] (human)
(6–8 months for rat)			[21]

Abbreviation: HPCs, hepatic parenchymal cells.

BCAA action is a current basic topic and will be briefly described in the next section.

Following increased secretion of albumin from HPCs, the impaired whole-body turnover kinetics of albumin, i.e., reduced synthesis and degradation rates and prolonged biological halflife, recover both in rat cirrhotic model [19] and in cirrhotic patients within 2 weeks of BCAA supplementation (Fig. 3) [20]. However, another 2–6 months supplementation with BCAA is required in liver cirrhosis to raise serum albumin (Table 2). Further continuance of BCAA supplementation benefits quality of life issues and finally improves survival [5,6,21].

Future questions (1)—energy metabolism

Recent interests focused on the timing of administration of BCAA, since daytime BCAA is usually consumed by energy generation for physical exercise of skeletal muscles in liver cirrhosis [17]. Nocturnal BCAA seem to be more favorable as a source of protein synthesis by giving higher nitrogen balance [22].

A remaining big question is whether or not energy supplementation should accompany nocturnal BCAA supplementation. Approximately 60% of the patients with liver cirrhosis is in energy malnutrition regardless of the presence or absence of protein malnutrition (Table 1). Usual energy source with ordinary BCAA content was enough, at least, to improve fuel metabolism in such cirrhotics [13]. Furthermore, Swart et al. and Chang et al. [23,24] showed that simple energy supplementation at night improved both nitrogen balance and fuel metabolism of cirrhotics. These results suggest that improved fuel supply saved amino acids from oxidation and directed them to protein synthesis. Theoretically, simultaneous supplementation with BCAA and energy might be ideal for patients with PEM. Thus, Nakaya et al. [15] used BCAA-enriched nutrient mixture, but demonstration of the specific overriding effect of BCAA has not been fully

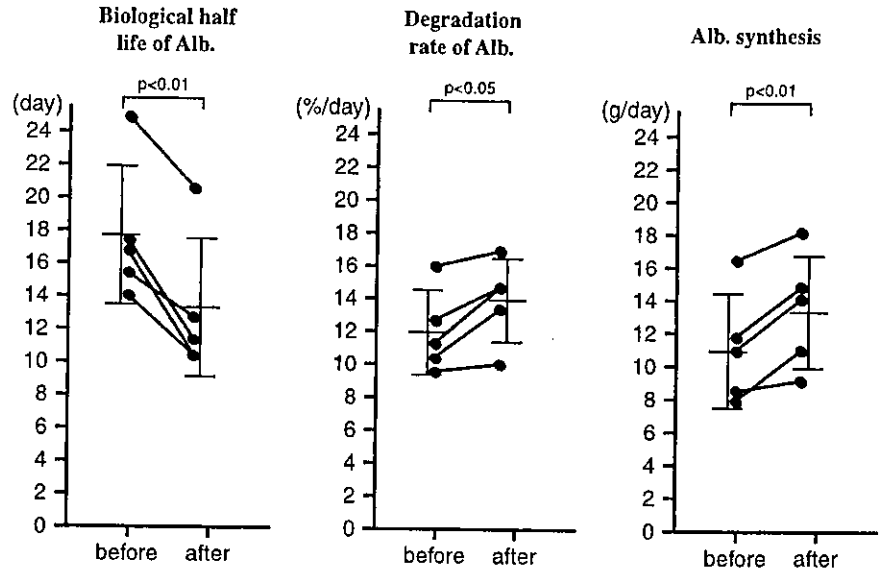


Fig. 3. Effects of 2 week-supplementation with branched-chain amino acids (BCAA) on albumin kinetics in cirrhotic patients [20]. Tracer experiment was carried out in five cirrhotic patients using iodinated human serum albumin before and after BCAA supplementation. Blood samples were drawn up to 30 days following intravenous injection of *I-albumin, serum disappearance curves of *I-albumin were constructed, and kinetic parameters were estimated by 4-compartment model analysis (from [20]).

materialized [25]. However, as suggested by Yamauchi et al. [14], it is possible that BCAA, in combination with energy, reduces muscle catabolism and, subsequently, exerts better protein sparing effect by 22:30 supplementation as compared with 19:00 supplementation. Further study will be required to determine what to provide at late evening to rescue PEM in liver cirrhosis. At present, it is safe to say that nutritional assessment of each patient will be demanded to prescribe BCAA and energy for order-made nutritional support of cirrhotics.

Future questions (2)—Molecular mechanisms of branched-chain amino acids: mTOR-dependent or independent?

Molecular mechanism of the action of BCAA, particularly that of leucine, is a focus of the recent research in this field. mTOR pathway and eIF4B pathway independently regulate protein synthesis in adipocytes, hepatocytes, and skeletal muscle cells under stimulation with leucine [26–29]. These observations suggest the use of leucine alone, instead of whole BCAA, to improve protein synthesis in several pathological conditions. This hypothesis may work when leucine or, ideally, a synthetic leucine analog is used at a pharmacological dose to activate transcription factors as mentioned above. However, administration of leucine alone at a nutrient dose will readily induce isoleucine depletion and worsen amino acid imbalance in liver cirrhosis. In contrast to protein synthesis, regulatory mechanism of protein breakdown by BCAA is still unclear. Anti-catabolic effect of leucine is recently reported to work through regulation of autophagy and lysosome-dependent proteolysis in myocytes [30]. This pathway is apparently mTOR-independent, but the responsible molecule has not been identified yet.

For the synthesis and breakdown of protein molecules, substantial amount of energy is simultaneously required in the cell [26]. This energy can be recruited endogenously or supplied exogenously in various forms as carbohydrate, fatty acid, and amino acid. When the endogenous pool of energy is not sufficient and the exogenous supply is not available, then the protein synthesis will not go on or the breakdown of body component will start to produce energy source, resulting in a worse nutritional state. Thus, correct estimation of energy requirement is essential when supplementing BCAA to improve protein malnutrition in patients. The most basic question is how much joule of energy is necessary to produce one molecule of albumin at the cellular level.

Conclusion

Experimental and clinical evidences support favorable effects of BCAA on protein malnutrition in liver cirrhosis. To raise the efficacy further, supplementation conditions including dose, time, and combinatory use of energy should be optimized by future studies.

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Activation of hepatic branched-chain α-keto acid dehydrogenase complex by tumor necrosis factor-α in rats

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Abstract

Tumor necrosis factor-α (TNFα) promotes oxidation of branched-chain amino acids (BCAA). BCAA catabolism is regulated by branched-chain α-keto acid dehydrogenase (BCKDH) complex, which is regulated by phosphorylation–dephosphorylation of the E1α subunit at Ser^{293}. BCKDH kinase is responsible for inactivation of the complex by phosphorylation. In the present study, we examined the effects of TNFα administration on hepatic BCKDH complex and kinase in rats. Rats were intravenously administered with 25 or 50 μg TNFα/kg body weight 4 h prior to sacrifice. The TNFα treatment at both doses elevated the activity state (percentage of the active form) of BCKDH complex from 22% to 69% and 86%, respectively, and the amount of phospho-Ser^{293} on the E1α subunit in each group of rats corresponded inversely to the activity state of BCKDH complex. The TNFα treatment of rats significantly decreased the activity as well as the bound form of BCKDH kinase. These results suggest that the decrease in the bound form of kinase is involved in the mechanism responsible for TNFα-induced activation of the BCKDH complex.

Keywords: Tumor necrosis factor-α; Branched-chain amino acids; Branched-chain α-keto acid dehydrogenase complex; BCKDH kinase; Phospho-Ser^{293} on E1α subunit; Bound form of BCKDH kinase; Free fatty acids

Critical illness is often associated with skeletal muscle proteolysis, increased muscle amino acid oxidation, and release of amino acids into the bloodstream. Such catabolic sequence arises from the systemic inflammatory response syndrome, which in turn is a result of the host response to severe infection, trauma, thermal injury, and cancer [1,2]. The mobilized amino acids are taken up by many tissues, particularly by the liver, where they are used for gluconeogenesis and for the production of acute-phase proteins. The principal mediators of these reactions are cytokines, particularly tumor necrosis factor-α (TNFα), which is produced by mononuclear phagocytes that cause hemorrhagic necrosis of certain tumors [3]. The synthesis of cytokines may be stimulated experimentally by administration of bacterial lipopolysaccharides or their toxins. However, the precise role and physiological significance of cytokines in protein and amino acid metabolism are only partially elucidated.

The effects of cytokines on the catabolism of branched-chain amino acids (BCAA), leucine, isoleucine, and valine, are of special interest because these amino acids have a crucial role in protein metabolism and also have a beneficial effect in the treatment of sepsis or trauma [4,5]. It has been reported that the oxidation of BCAA is enhanced under these physiological conditions [6].

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The first step reaction in the BCAA catabolism is reversible transamination catalyzed by BCAA aminotransferase to form the corresponding branched-chain α-keto acids (BCKA), and the second step is the irreversible oxidative decarboxylation of the BCKA by branched-chain α-keto acid dehydrogenase (BCKDH) complex. The BCKDH complex is a rate-limiting enzyme in the catabolism of BCAA and the regulation of the hepatic BCKDH activity has been intensively investigated in rats [7–12], because rat liver has a markedly high activity of the enzyme compared to those of other animal and human livers [13–15]. The activity of BCKDH complex is primarily regulated through a reversible phosphorylation (inactivation) and dephosphorylation (activation) of the E1 component of the complex by BCKDH kinase and phosphatase, respectively [16–18]. Especially, the kinase plays an important role in regulation of the activity state of the BCKDH complex [19]. BCKDH kinase phosphorylates the E1α subunit of BCKDH complex at two sites, termed sites 1 and 2. A site-directed mutagenesis study has demonstrated that phosphorylation at site 1 (Ser^{293}) abolishes the BCKDH activity, whereas phosphorylation at site 2 is silent [20].

There are a number of controversial and unsolved issues related to the effect of endotoxins and cytokines, especially TNFα, on protein and BCAA catabolism. The administration of TNFα has been reported to increase muscle proteolysis and BCKDH activity [21,22], while, in another study, TNFα did not increase muscle protein breakdown [23].

In the present study, we investigated the effects of TNFα administration on regulation of the activity state of hepatic BCKDH complex in rats. We have recently suggested that the BCKDH kinase bound to the complex (the bound form of the kinase) expresses the kinase activity, which is inversely correlated with the activity state of BCKDH complex [11,24]. Therefore, we addressed to examine the relationship between the activity state of the complex and the amount of bound form of the kinase in TNFα-treated rats.

Materials and methods

Materials. Protein A–agarose was purchased from Upstate Biotechnology (Lake Placid, NY). Lambda protein phosphatase was purchased from New England BioLabs (Beverly, MA, USA). Rat recombinant TNFα was supplied by Genentech (San Francisco, CA, USA). The broad-specificity protein phosphatase [25] and antiserum against the BCKDH E1 subunit [16] were prepared as reported previously. Specific antibody against phospho-Ser^{293} (pS293) on the BCKDH E1α subunit was obtained from Dr. Christopher J. Lynch (Pennsylvania State University College of Medicine, Pennsylvania, USA). Monoclonal antibody against BCKDH kinase was generated using recombinant BCKDH kinase by a standard protocol [26]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Chemicon International, (Temecula, CA, USA). All other reagents were of biochemical grade.

Animals. Female Wistar rats 9 weeks old were obtained from CLEA Japan (Tokyo, Japan). They were housed in the conventional animal room and had free access to water and laboratory chow CE-2 (CLEA Japan) for 1 week. On the final day of the experiment, rats (weighing 195–205 g) were deprived of food at 09:00 [27] and randomly divided into control and TNFα-treated groups. Rats in the TNFα-treated group were injected intravenously with rat recombinant TNFα at 25 or 50 μg/kg body weight in phosphate-buffered saline containing 1 mg/ml bovine serum albumin at 13:00. Rats in the control group were injected intravenously with equivalent amounts of the carrier solution. Four hours after injection (at 17:00), rats were anesthetized with pentobarbital (50 mg/kg body weight), blood was collected from the inferior vena cava with a syringe to prepare serum, and liver was rapidly removed, freeze-clamped at liquid nitrogen temperature, and stored at −80 °C until analyses. The experimental protocol was reviewed and approved by the Animal Care Committees of Gifu University School of Medicine and Nagoya Institute of Technology.

Assay of BCKDH complex and BCKDH kinase activity. Actual and total activities of the BCKDH complex were measured by the spectrophotometric assay as reported previously [28]. The total activity of BCKDH complex was obtained following dephosphorylation of BCKDH complex using a lambda protein phosphatase [28]. One unit of BCKDH complex catalyzed the formation of 1 μmol of NADH/min. The activity state of BCKDH complex was defined as the percentage of the actual activity to the total activity. The assay of BCKDH kinase was performed by measuring the ATP-dependent inactivation of BCKDH complex as described previously [28]. In this assay, the complex was fully activated using a broad-specificity protein phosphatase prior to the kinase assay [25]. Kinase activity is expressed as the first order rate constant of BCKDH complex inactivation.

Extraction of enzymes from rat liver and its immunoprecipitation. Rat liver extracts were prepared as reported previously [29]. Immunoprecipitation of BCKDH complex associated with the kinase in the extracts was performed using immunoadsorbents of protein A–agarose associated with polyclonal antibodies against the E1 component of the complex [24].

Immunoblotting and immunodetection. Immunoblotting analyses of the BCKDH E1α subunit [24] and pS293 on the subunit were performed as reported previously [30]. Immunoblotting analyses of the BCKDH kinase in both tissue extracts and immunoprecipitates were performed using the monoclonal antibody against the kinase as reported previously [31]. The kinase protein in the tissue extracts was measured as the total kinase in the tissue and that in the immunoprecipitates as the bound form of the kinase. Analysis of immunoreactive bands was performed on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from zippy. nimh. nih. gov. or on floppy disk from the National Technical Information Service, Springfield, Virginia, Part No. PB95-500195GEI).

Statistical analysis. All values are expressed as means + standard error (SE). Statistical analysis was performed by a one-way ANOVA and Fisher's PLSD test using StatView (Version 5.0) software (SAS Institute Cary, NC, USA). Difference with a P value less than 0.05 was considered significant.

Results

Concentrations of serum glucose, insulin, free fatty acids, and BCAA

Concentrations of serum glucose, insulin, and BCAA were not significantly different among three experimental groups, although serum glucose concentration

Table 1
Effects of TNFα on serum glucose, insulin, FFA, and BCAA concentrations in rats

Blood component	Control	TNF (25 μg/kg)	TNF (50 μg/kg)
Glucose (mg/dl)	166 ± 5	142 ± 12	131 ± 10
Insulin (ng/ml)	1.23 ± 0.1	2.03 ± 0.5	1.74 ± 0.4
FFA (mmol/L)	0.36 ± 0.05	0.58 ± 0.08*	0.53 ± 0.10*
Leucine (nmol/ml)	149 ± 5	164 ± 12	149 ± 12
Isoleucine (nmol/ml)	83 ± 3	88 ± 5	85 ± 5
Valine (nmol/ml)	190 ± 8	212 ± 12	208 ± 14

Values are means ± SE for six rats.
* Values are significantly different from control group ($P < 0.01$).

tended to be decreased by the TNFα treatment (Table 1). The concentration of serum free fatty acids (FFA) in both TNFα-treated groups was significantly higher than that in the control group but was not different between two TNFα groups (Table 1).

Activities of hepatic BCKDH complex and kinase in rats

The total activity of hepatic BCKDH complex was the same among three groups. However, the actual activity and the activity state were markedly higher in two TNFα groups than in the control group and tended to be, but not significantly, higher in the higher dose of TNFα when compared between two TNFα groups (Table 2), indicating that the treatment of TNFα at the dose of 25 μg/kg body weight is enough to significantly activate the hepatic BCKDH complex.

The activity of the BCKDH kinase was significantly lower (about half) in two TNFα groups than in the control group and tended to be lower in the higher dose of the TNFα when compared between two TNFα groups (Table 2), indicating an inverse correlation between the activity state of the BCKDH complex and the kinase activity.

Amounts of the BCKDH E1α subunit and pS293 on the subunit in rat liver

The amount on pS293 of the BCKDH E1α subunit was significantly lower in two TNFα groups than in the control group and tended to be, but not significantly, lower in the higher dose of TNFα when compared between two TNFα groups (Fig. 1A), being just inversely correlated with the activity state of the BCKDH complex. Meanwhile, no difference was observed in the amount of the BCKDH E1α subunit among three experimental groups (Fig. 1B).

Protein amounts of the total BCKDH kinase and the bound form of BCKDH kinase in rat liver

The total kinase and the bound form of the kinase were quantitated in the tissue extracts and the immunoprecipitates, respectively. In the analyses of the bound form of the kinase, the BCKDH E1α subunit was also measured and it was confirmed that the amount of the subunit in the immunoprecipitates was the same among three experimental groups (Fig. 2A). The amount of the bound form of the kinase was significantly lower in the two TNFα groups than in the control group (Fig. 2A). The tendency of the amount of the bound form of the kinase was the same as that of the kinase activity. On the other hand, no difference was observed in the total amount of the kinase in the liver extracts among three groups (Fig. 2B). GAPDH was also measured as an internal standard in the assay of the total kinase, and no difference was observed among three groups (Fig. 2B).

Discussion

TNFα is a polypeptide with a wide range of biological activities. Excessive systemic TNFα release is

Table 2
Total and actual activities, and activity state of BCKDH complex, and BCKDH kinase activity in rat livers

Enzyme	Control	TNF (25 μg/kg)	TNF (50 μg/kg)
BCKDH complex (U/g tissue)			
Total activity	1.16 ± 0.11	1.19 ± 0.08	1.18 ± 0.10
Actual activity	0.24 ± 0.02	0.82 ± 0.11*	1.01 ± 0.10*
Activity state (%)	21.6 ± 2.5	69.1 ± 7.6*	86.1 ± 4.4*
BCKDH kinase (mm^{-1})	1.05 ± 0.11	0.59 ± 0.03*	0.52 ± 0.07*

Values are means ± SE for six rats.
* Values are significantly different from control group ($P < 0.01$).

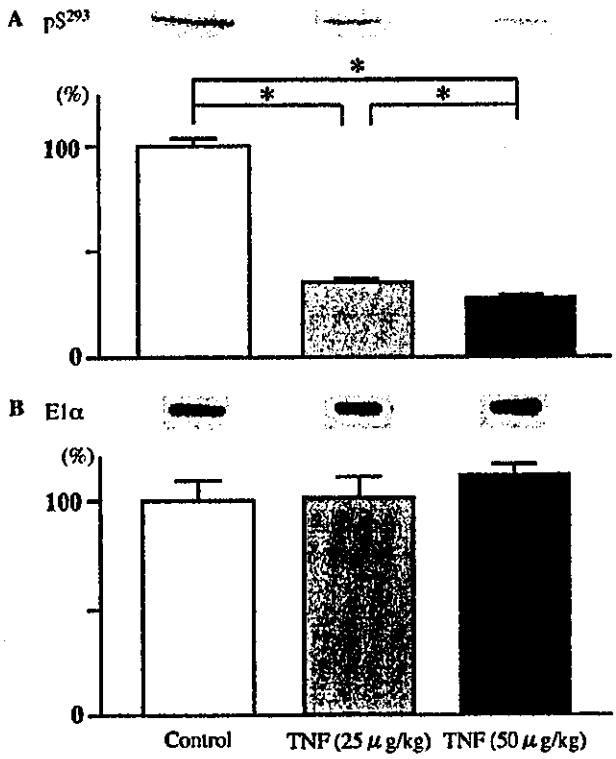

Fig. 1. Effect of TNFα on the amount of pS293 (A) and the BCKDH Elα subunit (B) in rat livers measured by Western blot analyses. The amounts of pS293 and Elα subunit in TNFα groups were expressed as percentages of the control group. All values are means ± SE for six rats. Values are significantly different from control group ($^*P < 0.01$). The typical images of band are shown above the graphs.

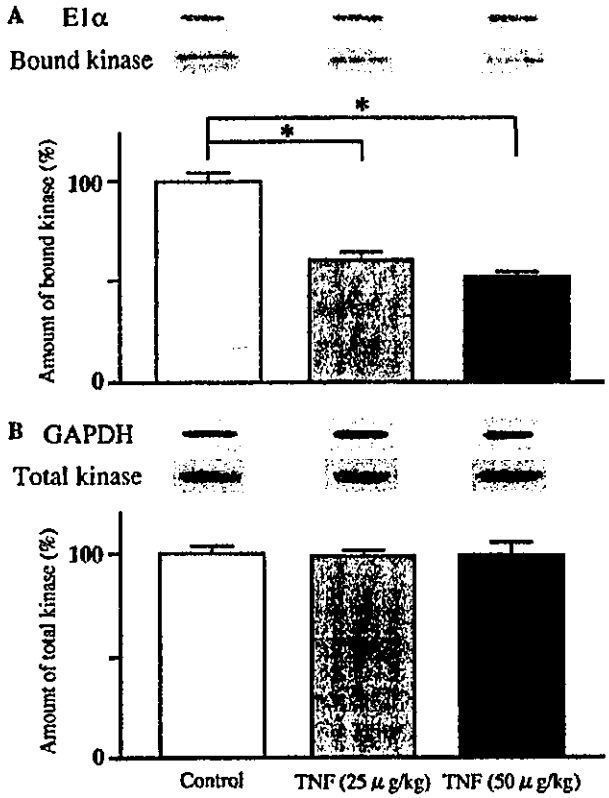

Fig. 2. Effect of TNFα on the amount of bound (A) and total (B) BCKDH kinase in rat livers measured by Western blot analyses. (A) In the analyses of the bound form of the kinase, the amount of the Elα subunit in the immunoprecipitates was also measured. (B) In the total kinase analyses, GAPDH was measured as an internal standard. The kinase amount in the TNFα groups was expressed as percentages of the control group. All values are means ± SE for six rats. Values are significantly different from control group ($^*P < 0.01$).. The typical images of band are shown above the graphs.

observed in infection, trauma, tissue injury, and cancer cachexia, and appears to induce a general catabolic state. In previous reports [32,33], TNFα induced fever and a transient neuroendocrine stress response, associated with increases in glucose (~10%) and FFA (~126%) turnover, and resting energy expenditure (~34%). The production of ATP by β-oxidation of fatty acids is much greater than that by glucose oxidation [34]. In the present study, the TNFα treatment tended to decrease the serum glucose concentration and significantly increased the concentration of serum FFA, suggesting that the TNFα-treated rats had the metabolic conditions similar to those reported previously.

TNFα also increases protein catabolism [1,35] and promotes the BCAA oxidation in association with activation of muscle BCKDH complex [21], the rate-limiting enzyme in the BCAA catabolism [6]. In the present study, we found that the activity state of hepatic BCKDH complex in rats was markedly increased by the TNFα treatment, whereas the total activity of the complex was not changed by the treatment. Furthermore, we found that the amount of pS293 on the BCKDH Elα subunit was just inversely correlated with the activity state of BCKDH complex. These findings indicated that the activation of the complex is attributed to a decrease in the inactive/phosphorylated form of BCKDH complex. The activity of BCKDH kinase, which is responsible for inactivation/phosphorylation of the complex, was decreased by the TNFα treatment, being compatible with the activation of the complex. We previously reported that the kinase exists as bound and free forms in rat liver and muscle, and that only the bound form may express its activity [11,24]. In the present study, we found that the bound form of the kinase was significantly decreased by the TNFα treatment. From these results, the decrease in the bound form of the kinase is involved in the mechanisms responsible for the activation of hepatic BCKDH complex by the TNFα treatment in rats.

An inverse correlation between the activity state of BCKDH complex and the kinase activity has been reported in many studies [19], and the present study also demonstrated such an inverse correlation. Therefore,

we support the hypothesis that the BCKDH kinase activity plays an important role in determining the activity state of BCKDH complex. However, the mechanism for the regulation of the BCKDH complex activity by dephosphorylation catalyzed by BCKDH phosphatase could not be ruled out. Unfortunately, information on the physiological role of the phosphatase is not available, although the phosphatase has been purified previously [18].

In our previous studies, female, but not male, rats exhibited a clear diurnal variation in the activity state of hepatic BCKDH complex [27]. There are bound and free forms of the BCKDH kinase in rat liver and the amount of the bound form of kinase is inversely associated with the activity of BCKDH complex in female rat liver [24]. It is likely that this short-term regulation of the activity state of BCKDH complex by changing the amount of the bound form of kinase may modulate the BCAA oxidation under a number of physiological conditions [11,24]. A present study clearly showed that the TNFα treatment significantly decreased the bound form of the kinase in association with the decrease in the kinase activity, thereby resulting in the activation of the complex in rat liver. The free form of the kinase, which appeared to be much greater in amount than the bound form in the liver [24], showed only a marginal increase by the TNFα treatment (data not shown). These findings strongly suggest that a control of the amount of the bound form of BCKDH kinase is an important short-term regulatory mechanism for determining the activity state of the BCKDH complex. Since the mechanism by which the bound form of the kinase is converted into the free form and vice versa has not been elucidated, detailed mechanisms for the action of TNFα against the kinase remained to be clarified.

It should be noted that many physiological conditions stimulating FFA oxidation such as starvation [9], exercise [9], and diabetes [36] promote the BCAA catabolism in association with activation of BCKDH complex. In the present study, the serum FFA concentration was elevated and the hepatic BCKDH complex was activated by the TNFα treatment. Furthermore, it has been reported that octanoic acid one of FFA is a potent inhibitor of the kinase [37]. These findings imply that FFA might be a modulator of the BCKDH kinase.

In conclusion, the present study demonstrated that the TNFα treatment activated the hepatic BCKDH complex in association with the decreases in the activity and the bound form of BCKDH kinase, suggesting that a control of the bound form of BCKDH kinase plays an important role in the short-term regulation of the activity state of hepatic BCKDH complex in rats. The mechanism responsible for such changes between bound and free forms of the kinase remains to be defined.

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第21章

肝硬変

市田隆文　訳

定義

肝臓全体に線維化と結節形成が解剖学的に認められる場合に肝硬変と定義される。この原因は多種多様であるが，最終像はすべて同じである。

線維化は肝硬変と同義語ではない。例えば，線維化といってもうっ血性心不全に起こる線維化は中心静脈域(zone 3)に認められ，閉塞性黄疸や先天性肝線維症(図 21.1 •)では主に門脈域(zone 1)に起こり，肉芽腫形成性肝疾患では中間域(zone 2)にそれぞれ観察されることより，これら線維化は真の肝硬変ではない。

また，線維化を伴わない結節形成として partial nodular transformation (PNT；図 21.1 •)も肝硬変とは定義づけられない。慢性肝炎と肝硬変の関連性は 19 章に記載されている。

肝硬変の生成

壊死に対する肝臓の反応は限られている。最も重要なことは肝小葉の虚脱とび漫的な線維性隔壁形成ならびに肝細胞の結節性再増殖である。肝硬変の病態はその要因に無関係に認められ，その形態様式はほとんど同じ形態をたどる。壊死は剖検時にはもはや明らかでないことがある。

線維化は肝細胞の壊死に続く病態である(図 21.2 •)。この線維化は zone 1 に interface hepatitis をもたらし，それが門脈-門脈域間の線維性架橋の形成につながる。そして，zone 3 にみられるこれら融合性壊死が中心静脈と門脈域をつなぐ bridging fibrosis を生じさせる。巣状壊死は限局性線維化と関連する。細胞死は肝臓の構築が改変されて結節を形成して生じ，そして肝硬変

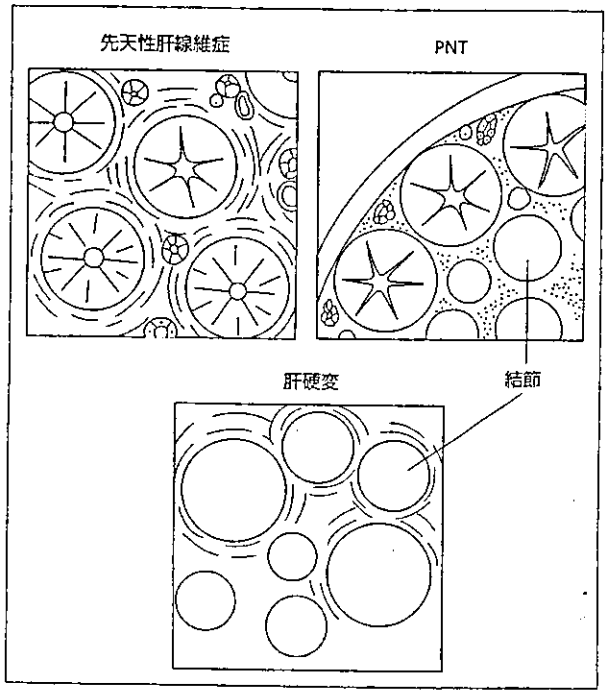

図 21.1　肝硬変は広範な線維化と結節形成で定義づけられる。先天性肝線維症は結節形成のない線維症である。PNT は線維化のない結節形成である

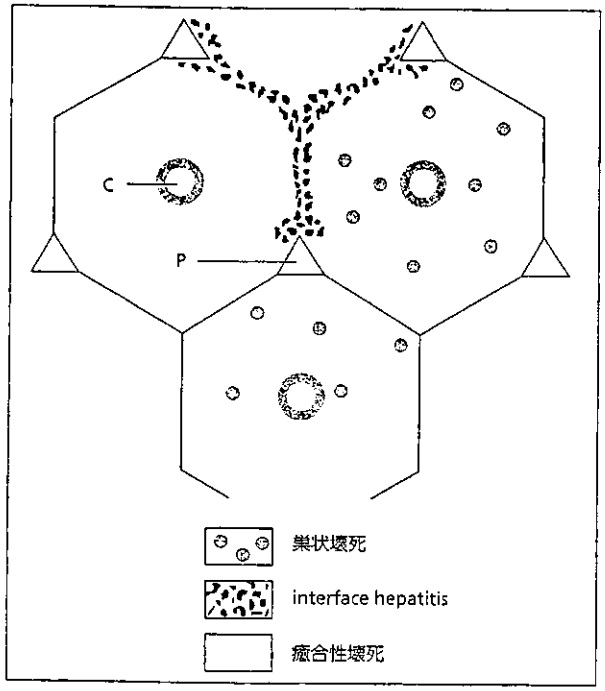

図 21.2　巣状壊死，interface hepatitis，癒合性壊死，門脈-門脈域間，門脈-中心静脈間で連携。C：中心静脈，P：門脈域(L.Bianchi 氏のご好意による)

が完成する。

肝類洞は門脈-中心静脈線維性架橋部の再生結節末梢部で，まだその形態を保ち続けている。門脈血流は機能を有している部分へ反れ，そのために結節中心部(zone 3)は血流不全となる。この形態はたとえ肝硬変の原因が除去されても続くようである。Disse 腔には異常な結合織が充填され，肝細胞との代謝交換に支障をきたすようになる。

新規の線維芽細胞は壊死に陥った肝細胞や細胆管増生部分から形成される。細胞成分を有さない隔壁が zone 1 や小葉内に形成されると，可逆的から非可逆的な線維化として進展していくこととなる。この線維性隔壁はそれぞれの原因によってその分布は異なる。例えば，ヘモクロマトーシスでは鉄の沈着は主に門脈域に起こり，アルコールによる場合では線維化は主に zone 3 を中心に認められる。

線維化 [9, 41]

正常の肝臓が線維化状態や肝硬変になるには肝星細胞やサイトカイン，さらにはプロテアーゼやその阻害物質が複雑に絡み合って生じるとされている。

この細胞外マトリックスの量的，組成的変化も特徴的である。通常みられる低濃度の基底膜が細線維からなるコラーゲンを含む高濃度の腸管型結合織に置き換わることがある。この変化は結合織の合成の亢進と分解の低下によるところが多い。

肝星細胞と隣接する類洞を構成する細胞や肝実質細胞との相互作用が認められ，同時にサイトカイン，成長因子，プロテアーゼ，その阻害因子などが細胞外マトリックスと複雑に相互作用を示している。線維性組織の形成にはこの細胞外マトリックスの増加のみならず，その取り除かれる変化にも関連している。すなわち，このことは細胞外マトリックスを分解あるいは阻害する多くの酵素のバランスに強く依存している(図 21.3 ●)。

この肝臓における線維形成と線維溶解の機構を理解することは，将来的に，この線維化を予防，もしくは線維を取り除くという治療方法を編み出すうえで大切なことになるかもしれない。

正常の肝臓は結合織として細胞外マトリックスを有している。それらはⅣ型コラーゲン(細線維性ではない)，フィブロネクチンとラミニンを含む糖蛋白質，ヘパラン硫酸を含むプロテオグリカンである。そしてこれらが Disse 腔に低濃度の基底膜を形成する。そして，肝障害が持続すると，これら細胞外マトリックスは 3 ～ 8 倍にも増加する。この細胞外マトリックスには，細胞性フィブロネクチン，ヒアルロン酸そして他のプロテオグリカンや糖含有物質と同様に，細線維形成性Ⅰ型コラーゲンとⅢ型コラーゲンを含む腸管型の高濃度の基底膜が認められる。その結果，類洞内皮細胞の篩板状構造を消失し，肝細胞の微絨毛を喪失し，類洞の毛細血管化をもたらし，血液と肝細胞の間の重要な代謝交換を阻害することとなる。

星細胞はリポサイトや脂肪摂取細胞，伊東細胞，ペリサイトとも呼称されている。この細胞が線維化に重要な細胞である。この細胞は Disse 腔に存在し，その細胞表面は肝細胞や類洞内皮細胞，さらに神経線維と接している。非活動期にはこの細胞はビタミン A を含む細胞内小滴を有している。身体が有するレチノイドの約 40 ～ 70 % をこの細胞が占めている。この細胞

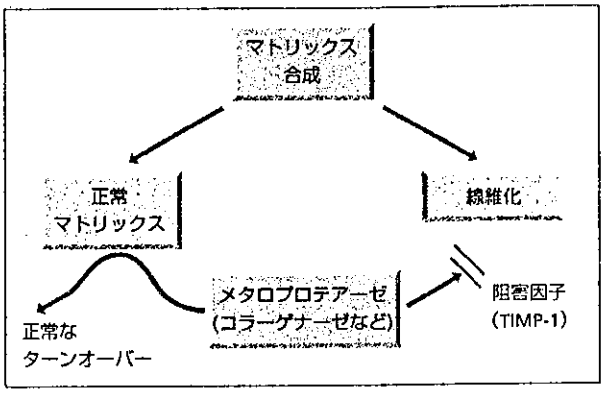

図 21.3 正常な結合織と異常な結合織の産生機序。TIMP：tissue inhibitor of matrix metalloproteinase

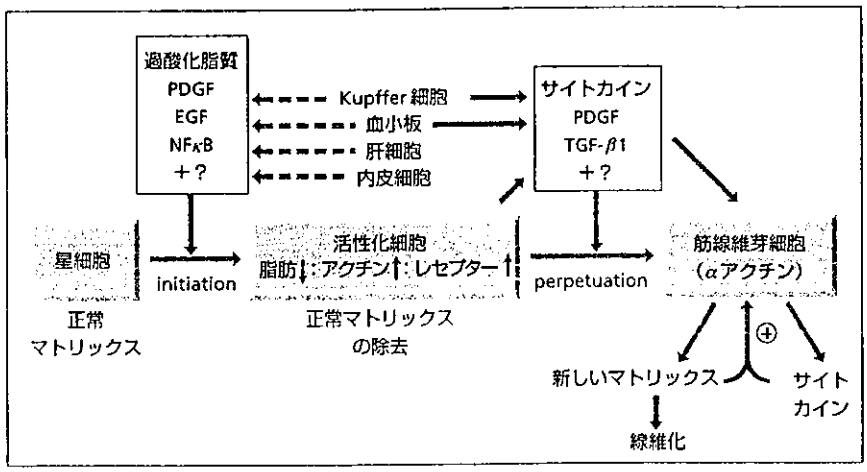

図 21.4 線維化における肝星細胞の活性化。筋線維芽細胞がおそらくコラゲナーゼ抑制物質を産生し，線維化を増強する

数は活動性の潜在能力，レチノイドの含有量や，細胞骨格フィラメントの表現などが異なるために，不均一に分布している。

星細胞は隣接する細胞が障害を受けた際に放出する因子によって活性化される(図 21.4 ●)。その因子として挙げられるのが，類洞内皮細胞，Kupffer 細胞，血小板からの TGF-β1，肝細胞からの過酸化脂質，血小板からの PDGF，EGF などである。それゆえ，通常は星細胞自ら導き出す因子によるオートクライン機構により活性化するが，その活性化の持続にこれら上述の因子によるパラクライン的機序も存在する。この転写因子としては活性化を調節する NFκB と STAT1 などである。

星細胞が活性化すると，レチノイド小滴は失われ，細胞数が増加し，細胞そのものが肥大する。そして，細胞内小器官の粗面小胞体が増加し，平滑筋に特異的な α-アクチンが出現してくる。そして，ある種のサイトカインや走化性因子ならびに細胞外マトリックスを放出しながら，細胞マトリックスを分解する酵素を産生する。星細胞が活性化している期間中にプリオン蛋白遺伝子を発現し，さらにプリオン蛋白質(PrP^C)の良性な細胞形成の合成能が誘導されている。このプリオン蛋白質の発現は正常の肝臓では認められず，慢性肝疾患において線維化より炎症の程度に相関して認められる[37]。

細胞外マトリックスは受動的な産物ではない。それぞれの蛋白質はそれぞれの領域を有し，インテグリンを含む細胞膜受容体を介して星細胞や他の細胞と密接な関連性を有している。そして，これらはコラーゲンの産生やメタロプロテアーゼの活性化に影響を及ぼす細胞内伝達経路を通じてその効果を媒介している[26]。

星細胞の増殖は肝障害時によくみられる現象である。PDGF が最も有力な分裂促進因子であり，それ以外の増殖刺激因子としてエンドセリン-1 (ET-1)やトロンビンならびにインスリン様成長因子 insulin-like growth factor が挙げられる。

星細胞は障害部位からの PDGF や monocyte chemotactic peptide (MCP-1)の放出に反応し，他の場所での増殖・遊走を通じ，障害部位に集まってくる。

類洞内皮細胞は肝障害の後，フィブロネクチンやⅣ型コラーゲンを含む細胞外マトリックスのいくつかの構成成分を産生するが，星細胞が優先的にマトリックスの遺伝子を発現している。そして，これら細胞が細胞外マトリックスの増加の源になっている。星細胞によるこの線維性マトリックスの生成は，TGF-β1，IL-1β，TNF，脂質過酸化産物，アルコールの代謝産物であるアセトアルデヒドなどの刺激がその要因である。

介在性マトリックスの増加も星細胞の活性化を刺激している。

マトリックスの合成と分解のアンバランスが肝臓の線維化の過程で重要な役割を演じている[9]。マトリックスの分解は matrix metalloproteinase (MMP)や tissue inhibitor of matrix metalloproteinase (TIMP)と MT1-MMP やストロメリシンなどの変換酵素とのバランスによるものである。これら物質がどこから産生されるかは明確ではないが，活性化された星細胞が MMP-2 とストロメリシンの主たる源であり，TIMP-1 と TIMP-2 の RNA を表現し，TIMP-1 と MT1-MMP を産生する[41]。Kupffer 細胞はⅣ型コラーゲン(MMP-9)を分泌する。肝障害中に生じる複雑なこれら変化の最終的な結果は正常基底膜のコラーゲンの分解を促進し，中間型コラーゲンの分解を抑制する。後者は MMP-1 (中間型コラーゲン)と関連性を有する TIMP-1 と TIMP-2 発現が増加することで説明されている。トランスジェニックマウスにおけるヒト TIMP-1 の過剰発現は四塩化炭素モデルで 7 倍も線維化を増加させることがわかっている[83]。実験肝障害で，その障害が改善される間は TIMP-1 や TIMP-2 の発現が減少し，最終的なコラゲナーゼ活性は線維化マトリックスを取り除く作用により増加している[31]。

テロメラーゼ欠損動物実験では，クロモゾームテロメアの短縮がみられることにより，四塩化炭素肝障害モデルでの肝硬変への進展を促進することが知られている[70]。すなわち，クロモゾームテロメアの維持は肝細胞の正常な増殖能力の中心的役割を演じていると考えられる。

活性化した星細胞(筋線維芽細胞)は平滑筋細胞の性状を示し，収縮する。これらは類洞を局所的に収縮させ，血流の調整に重要な役割を示している。収縮刺激物質は ET-1，アルギニンバソプレシン，アドレノメジュリンを含む。星細胞は一酸化窒素(NO)を産生し，これは ET-1 に対する生理的拮抗物質である。したがって，この収縮は ET-1 の増加と同様に NO の減少によるものである。

肝細胞障害に基づく肝線維化の程度は障害の原因やサイトカインに対する星細胞や Kupffer 細胞の反応と産生される増殖因子とのバランスなどさまざまである。障害の原因の除去で消散する軽度の線維化から，非可逆的な重度の瘢痕化および結節形成(肝硬変)まで，線維化のスペクトルは幅がある。同様に，門脈圧亢進症においても，星細胞の収縮などの可逆的変化から，類洞の毛細血管化や線維化による類洞の狭窄などの非可逆的な状態まで幅がある。

治療は原因を取り除くことと肝臓の炎症を制限することに努力を注ぐことで，この両者を同時に行うのが臨床医の役割である。さらに星細胞の活性化を抑制するか，活性化した星細胞を抑制するかは重要な研究領域である。活性化した星細胞アポトーシスを回復させることは，増加する細胞外マトリックスの源を取り除くうえで重要なことである[10, 31]。

サイトカインと肝増殖因子 [74]

線維化過程における役割に加えて，サイトカインは幅広い他の効果を有している。ホルモン様蛋白質として細胞の分化に関連し，細胞膜レセプターとの相互作用を通して生理的ホメオスターシスの維持，修復を行っている。そして，これらは肝臓そのものの内だけでなく，肝臓と肝臓外との交通，連絡にとっても重要である。サイトカインはさまざまなアミノ酸，蛋白質，炭水化物，脂質，ミネラルなどの中間代謝物を調節している。古典的なホルモンであるグルココルチコイドなどと密接に関連している。多くのサイトカインが，特異な前炎症的反応に加えて，増殖因子様の働きを示すのでサイトカインと増殖因子との間の区別にはいくらか不自然さがある。増殖因子やサイトカインはそれぞれ独立した作用は呈さない。

肝臓，特にKupffer細胞はTNF-α，IL-1，IL-6などの前炎症性サイトカインを産生する(図6.9●参照)。肝臓は循環しているサイトカインを浄化し，サイトカインの全身的作用を制限している。この全身性サイトカインのクリアランスがうまく作動しないと肝硬変における免疫学的変化が生じることとなる。そしてサイトカインは肝臓の再生を抑制することも知られている。

サイトカインの産生は腸管由来のエンドトキシンによる単球やマクロファージの活性を介して調節される。肝硬変におけるエンドトキシン血症は腸管の透過性亢進とKupffer細胞機能低下により助長される。Kupffer細胞は通常，肝細胞によるエンドトキシンの取り込みを防ぎ，解毒や排除を行っている。サイトカインの過剰産生は発熱や食欲不振などの肝硬変の全身性変化の一部と媒介する。脂肪酸合成はTNF-α，IL-1，インターフェロン-αにより増加し，その結果脂肪肝が誘発される。

IL-6，IL-1，TNF-αはCRP，アミロイドA，ハプトグロビン，補体B，α_1-アンチトリプシンなどの産生に関連した肝臓の急性蛋白合成を誘導する。

ウイルス肝炎や肝切除後の劇的な肝細胞の再生能力は，細胞表面の特異的なレセプターと相互作用する増殖因子によって開始されるものと推測されている。

肝細胞増殖因子 hepatocyte growth factor (HGF)は成熟肝細胞におけるDNA生合成の最も能力のある刺激物質であり，肝障害後の肝再生のトリガーである。これは星細胞を含む肝細胞のみならず他の組織や癌細胞によって産生される[13]。TGF-β1やグルココルチコイドと同様にIL-1αやIL-1βを含むいくつかの因子によってこのHGFの産生は調整されている。そしてこのHGFはメラニン細胞や造血系細胞を含む他の多くの細胞増殖を刺激している。

上皮細胞増殖因子 epidermal growth factor (EGF)は再生肝細胞で産生される。このEGF受容体は肝細胞膜の表面に高濃度にみられ，さらに核にも見出すことができる。EGFは再生機構が最も活発に起こる門脈域(zone 1)で多く取り込まれる。

TGF-αはEGFと約30～40％の割合でシーケンスの一致を認め，EGF受容体と結合することができ，肝細胞の複製が開始されることとなる。

TGF-β1はおそらく，肝細胞増殖の主たる抑制因子で，肝臓の再生期において非実質細胞に強く発現している。TGF-β1は正あるいは負の効果を現す。すなわち，実験系では細胞の種類と培養条件でどちらへも働く性質を有している。

TGF-βは培養肝細胞のアミノ酸取り込みに抑制的に働くが，EGFは刺激的に働く。

線維化のモニタ

結合織の代謝の代謝産物や蛋白質は血漿へ染み出るのでそれらを測定することができる。しかしながら，この測定結果は線維化全般を反映するために，肝線維化に特徴的な情報を得ることは困難である。

aminoterminal procollagen type Ⅲ peptide (PⅢ-P)はⅢ型コラーゲン線維の新生時にプロコラーゲン分子に開裂される。この血清PⅢ-P値と慢性肝疾患の肝線維化の程度とは相関性を示す[2, 35]。しかし，重なる点が多いために，個々の患者における単一の測定のみでは臨床的に診断の価値を認めない。特に，アルコール性肝障害の際にはこの血清PⅢ-P値は肝線維化をモニタする意味で有用性を認める[59]。しかしながら，血清PⅢ-P値の増加は線維化のみの進展よりは壊死，炎症をより強く反映しているものとされている。

他に多くの線維化マーカーのアッセイ系が研究されている。それはヒアルロン酸，TIMP-1 [42]，integrin-β1，YKL-40 [35]ならびにMMP-2 [55]などである。尿中のdesmosineやhydroxy-lysylpyridinolineはエラスチンやコラーゲン破壊に関連性があるマーカーで，肝線維化のマーカーとしても採用されている[2]。しかし，いまだにこれらの多くは一般に実験系での興味であって，臨床的には多くは用いられていない。それぞれの患者の線維化程度を知るうえで，これら線維化マーカーはいまだに肝生検を凌駕することは困難と考えざるをえない。

肝硬変の分類

形態学的分類

3種類の肝硬変の解剖学的種類があり，それらは大結節型，小結節型および両者の混合型である。

小結節型肝硬変は厚い規則性のある隔壁を有し，ほぼ大きさの揃った小さな再生結節で，すべての小葉が傷害を受けていることで特徴づけられる(図21.5●，21.6●)。この小結節型肝硬変はアルコール性，栄養不良性もしくは高齢者や貧血で再増殖能が損なわれた結果と考えられている。

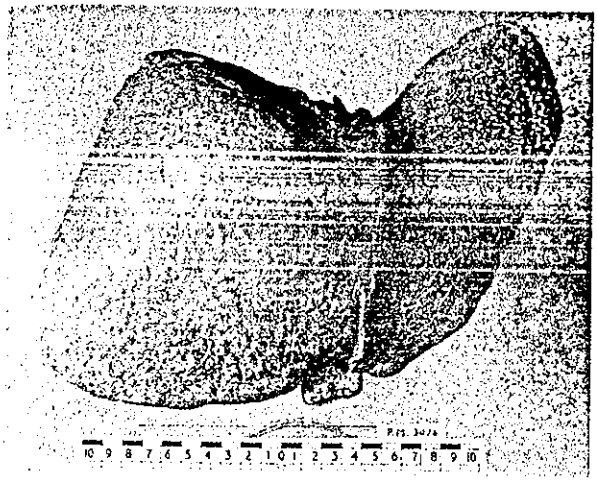

図 21.5 小結節型肝硬変の小さな結節

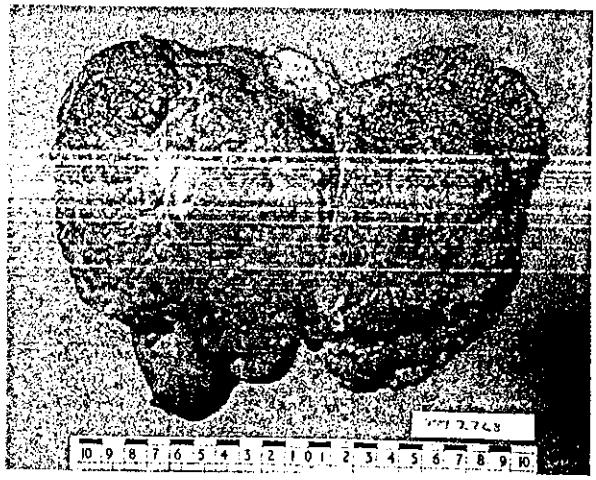

図 21.7 大結節型肝硬変の大きく歪んだ結節

図 21.6 小結節型肝硬変。全体的脂肪化。肝細胞は時に壊死に陥っている。線維性隔壁が肝臓を分けている(HE 染色，× 135)

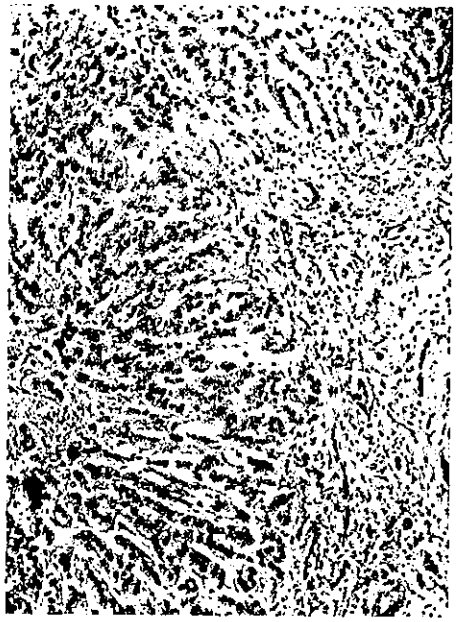

図 21.8 大結節型肝硬変。さまざまな大きさの肝細胞再生結節が細胆管増生所見などを含んださまざまな幅の線維束により分断されている。脂肪化はみられない。(HE 染色，× 135)

大結節型肝硬変はさまざまな大きさの隔壁と結節ならびに大きな結節の中の正常な小葉の存在などに特徴づけられている(図 21.7 •，21.8 •)。以前に生じた虚脱は 3 ～ 4 つの門脈域を含んだ線維性瘢痕に並列してみることができる。再生は大きな肝細胞と大きな核としてみられ，細胞索の厚さはさまざまであるのが特徴的である。

小結節型肝硬変での再生像は大結節型肝硬変や**混合型**の像として結果判断されることもある。時間がたつにつれ，小結節型肝硬変はしばしば大結節型肝硬変へ変換する。

原因(表 21.1 •)

1 B 型肝炎(デルタ肝炎を合併する場合も含む)および C 型肝炎

2 アルコール

3 代謝。例：ヘモクロマトーシス，Wilson 病，α_1-アンチトリプシン欠乏症，グリコーゲン病Ⅳ型，ガラクトース血症，先天性チロシン症，NASH (非アルコール性脂肪性肝炎)，腸管バイパス

4 肝内，肝外両方の遷延する胆汁うっ滞

5 肝静脈流出閉塞。例：静脈閉塞病，Budd-Chiari 症候群，収縮性心外膜炎

6 免疫異常。例：自己免疫性肝炎

7 毒素および治療薬。例：メトトレキサートやアミオダロン

8 Indian childhood cirrhosis

その他の可能性のある因子はこの後に表記する。

栄養障害については 25 章参照。

感染症：マラリア寄生症は肝硬変の原因とはならない。マラリアと肝硬変の併存はある特定の集団での栄養障害やウイルス肝炎を反映していると考えられる。

表 21.1 肝硬変の病因と特異的治療法

病因	治療法
ウイルス性肝炎(B，CおよびD)	？抗ウイルス
アルコール	禁酒
代謝	
鉄過剰	瀉血，デフェロキサミン
銅過剰(Wilson病)	銅キレート剤
α_1-アンチトリプシン欠損症	？移植
グリコーゲン病Ⅳ型	？移植
ガラクトース血症	牛乳および乳製品の摂取中止
チロシン血症	食事内チロシン抑制？移植
胆汁うっ滞	胆道閉塞解除 ？移植
肝静脈流出障害	
Budd-Chiari症候群	主静脈閉塞解除？移植
心不全	心疾患の原因治療
自己免疫性肝炎	プレドニゾロン
毒素および薬(例：メトトレキサート，アミオダロン)	原因を特定し中止する
Indian childhood cirrhosis	？ペニシラミン
原因不明	——

梅毒は新生児では肝硬変の要因となるが，成人では肝硬変にはならない。

住血吸虫症では虫卵が門脈域の線維性組織の反応を引き起こす。ある地域での肝硬変の併発は多分にC型肝炎ウイルスなど他の要因が関与しているものと推測される。

肉芽腫性変化：ブルセラ症や結核症，サルコイドーシスなどの肉芽腫は線維化とともに治癒する。結節状の再増殖は示さない。

特発性の肝硬変：原因不明であり，明らかに異種起源のグループである。その頻度は世界中で偏りがある。イギリスでは15～10％の頻度でみられるが，フランスやアルコール依存症の少ないアメリカの都会では少ない。特徴的な診断基準に従えばこの頻度はもっと下がるであろう。B型肝炎とC型肝炎の診断法が確立したので，多くのこれまで原因不明とされた肝硬変が肝炎後性の肝硬変となった。血清抗平滑筋抗体と抗糸粒体抗体の測定や肝生検は，その他のものを自己免疫性肝炎や原発性胆汁性肝硬変のカテゴリーに分離した。アルコール依存を否定するかアルコール摂取を忘れていた残りの一部はアルコール性肝硬変になるであろう。ほんの一握りの人が原因不明の肝硬変として残る。そしてその中のある者はNASHが原因と示唆される特徴をもっている[15，67]。

それぞれの肝硬変への機序はそれぞれの章で述べる。臨床病理学的特徴は肝硬変へ進展する"慢性肝炎"としてみても差しつかえないと思われる。

解剖学的診断

肝全体にみられる線維化を有する結節形成が肝硬変の診断根拠となる。腹腔鏡や開腹手術で結節形成を直接的に見ることで診断がつく。しかしながら，肝硬変の診断のために開腹手術は決してすべきではない。なぜならば，たとえ代償性肝硬変でも肝不全を引き起こすかもしれないからである。

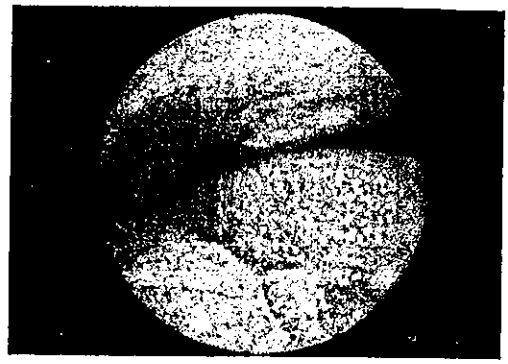

図 21.9 腹腔鏡で肝硬変の結節が観察される。左に観察されるのは胆嚢である

腹腔鏡は結節形成の観察を可能にし，また直接肝生検をすることも可能である(図21.9●)。

ラジオアイソトープ検査は肝臓での取り込みの減少や不規則な取り込み，脾臓や骨髄による取り込みを描出するが，肝硬変の結節形成は認識されない。

超音波検査は肝臓の表面の結節性を認識し(図5.5●参照)，門脈血流をみることに優れている[27]。尾状葉は右葉に比してやや肥大傾向にある。しかしながら，超音波検査は肝硬変診断には信頼性において劣っている。再生結節は局所病変としてみることができる[39]。これは経時的な画像検査やAFP測定により悪性腫瘍でないことが証明されていない限り，悪性腫瘍を考慮すべきである。

CT検査は肝硬変と合併症の診断に対して医療経済的に優れている(図21.10●)。肝臓の容積が測定でき，不規則な肝臓の表面をみることができる。良性の再生性結節はCTで描出することは困難である。脂肪化，鉄沈着による濃度の増加，占拠性病変はCTで容易に診断できる。造影剤の静脈注射後のCTでは門脈や肝静脈が描出され，脾腫を含む側副循環も観察され，門脈圧亢進症のより確実な診断を得ることができる。大きな側副循環路，例えば脾周囲，傍食道の血行路の描出は慢性の門脈体循環性脳症の診断に役立つ。腹水も診断できる。CTは経過を観察するうえで，多くの情報を得るために優れた手法といえる。選択された領域に直接生検も安全に行うことができる。

生検による肝硬変の診断は困難なこともある。鍍銀染色とコラーゲン染色は再生結節周囲の線維化の縁を染色するうえで必須である(図21.11●，表21.2●)。

肝生検像では，門脈域の欠落，異常な脈管の走行，門脈域での門脈が併走しない肝細動脈，線維性隔壁を有する結節の存在，異なった領域でのさまざまな大きさと様相の肝細胞，そして肥厚した肝細胞索などが補助診断として役に立つ所見である[72]。