Nutrition and Health
Series Editor: Adrianne Bendich

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Branched Chain Amino Acids in Clinical Nutrition

Volume 2

Humana Press

Nutrition and Health

Adrianne Bendich, Ph.D., FASN, FACN, Series Editor

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Preface

In man, the branched chain amino acids (leucine, isoleucine, and valine) are essential amino acids and thus must be obtained from dietary components. The branched chain amino acids are not only necessary for the synthesis of proteins but also have other metabolic functions and roles. For example, over several decades' evidence has supported the notion that branched chain amino acids, particularly leucine, are important in ameliorating or restoring metabolic imbalance. Studies in the 1970s showed that leucine promoted protein synthesis in muscle in vitro. Later, in the 1980s, it was shown that the branched chain amino acids stimulated protein synthesis in vivo. Subsequently, studies showed that branched chain amino acids could potentially be used clinically in ameliorating muscle catabolism. More recently the branched chain amino acids have been added to performance-enhancing supplements. Although this is a simplistic synopsis of historical events, it is now evident that the branched chain amino acids have a variety of functions. In simple terms the knowledge base associated with the branched chain amino acids have now been successfully harvested to enhance human health. Branched chain amino acids, like some other amino acids, have an almost ubiquitous function and are important in maintaining the cellular milieu of virtually every organ in the human body. For example, branched chain amino acids have roles in carbohydrate and lipid metabolism, insulin release and resistance, proteolysis, formation of keto acids, obesity prevention, and cancer. This does not mean to say that branched chain amino acids are the universal panacea. Indeed the administration of high amounts of branched chain amino acids may be toxic. The science of branched chain amino acids is complex and finding all the relevant information in a single source has hitherto been problematic. This is, therefore, addressed in *Branched Chain Amino Acids in Clinical Nutrition.*

The book has seven major Parts in two volumes.

Volume I

Part I: Basic Processes at the Cellular Level
Part II: Inherited Defects in Branched Chain Amino Acid Metabolism
Part III: Experimental Models of Growth and Disease States: Role of Branched Chain Amino Acids

Volume II

Part I: Role of Branched Chain Amino Acids in Healthy Individuals
Part II: Branched Chain Amino Acids: Status in Disease States
Part III: Branched Chain Amino Acids and Liver Diseases
Part IV: Branched Chain Amino Acid Supplementation Studies in Certain Patient Populations

Coverage includes the individual branched chain amino acids, amino acid ratios, essential amino acids, metabolism, amino acids cocktails, aminotransferases, tRNA, PPAR, uncoupling proteins,

insulin and insulin resistance, glucose and glycemic control, the hypothalamus, sirtuin, ammonia, cirrhosis, encephalopathy, apoproteins, maple syrup urine disease and oxidation disorders, mental retardation, fetal growth, skeletal and cardiac muscles, muscular dystrophy, amyotrophic lateral sclerosis, anorexia, obesity and weight loss, bladder carcinogenesis, tolerability, recovery, exercise, functional adaptations, psychomotor performance, whey protein, brain injury, obstructive pulmonary disease, ethanol oxidation, albumin, late evening snacks, organ transplantation, quality of life, and skin and radiotherapy. Finally there is a chapter on web-based material and additional reading.

Contributors are authors of international and national standing, leaders in the field, and trendsetters. Emerging fields of science and important discoveries are also incorporated in *Branched Chain Amino Acids in Clinical Nutrition.*

This book is designed for nutritionists and dietitians, public health scientists, doctors, epidemiologists, health care professionals of various disciplines, policy makers, and marketing and economic strategists. It is designed for teachers and lecturers, undergraduates and graduates, and researchers and professors.

London, UK

Rajkumar Rajendram
Victor R. Preedy
Vinood B. Patel

Series Editor Page

The great success of the Nutrition and Health Series is the result of the consistent overriding mission of providing health professionals with texts that are essential because each includes (1) a synthesis of the state of the science; (2) timely, in-depth reviews by the leading researchers and clinicians in their respective fields; (3) extensive, up-to-date fully annotated reference lists; (4) a detailed index; (5) relevant tables and figures; (6) identification of paradigm shifts and the consequences; (7) virtually no overlap of information between chapters, but targeted, interchapter referrals; (8) suggestions of areas for future research; and (9) balanced, data-driven answers to patient as well as health professional questions which are based upon the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapter authors. The international perspective, especially with regard to public health initiatives, is emphasized where appropriate. The editors, whose trainings are both research and practice oriented, have the opportunity to develop a primary objective for their book, define the scope and focus, and then invite the leading authorities from around the world to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

Branched Chain Amino Acids in Clinical Nutrition, a two-volume book, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, is a very welcome addition to the Nutrition and Health Series and fully exemplifies the Series' goals. The first volume of *Branched Chain Amino Acids in Clinical Nutrition*, "Cellular Processes, Genetic Factors and Experimental Models of Branched Chain Amino Acid Functions and Metabolism," is organized into three relevant parts. The ten introductory chapters in the first part, entitled "Basic Processes at the Cellular Level," provide readers with the basics so that the more clinically related chapters can be easily understood. The first chapter provides a broad-based perspective on the protein requirements for humans and describes the 20 amino acids that are classified as essential for humans to consume as these cannot be synthesized de novo in the human body. Tables and figures included describe major food sources of essential amino acids and specific food sources of branched chain amino acids. The second chapter describes the structures and functions of the three branched chain amino acids, leucine, isoleucine, and valine, that are classified as essential amino acids. We learn that these three branched chain amino acids make up approximately one third of all the amino acids in the body. The majority of the three amino acids are found in skeletal muscle where these function as both structural elements and stores for systemic nitrogen. The dietary requirements are approximately 40, 20, and 19 mg/kg of body weight/day of

leucine, valine, and isoleucine, respectively. Excellent sources of these amino acids are red meat, dairy, and soy protein-containing products. The typical Western diet provides sufficient protein to normally assure consumption of adequate levels of branched chain amino acids. The enzymes involved in the catabolism of these amino acids are described in detail and their locations within the body are reviewed with emphasis on their roles in muscle and the brain. The third chapter provides more detailed descriptions of the 15 enzymes responsible for the transamination and metabolism of the individual branched chain amino acids and describes the enzymatic reactions that share the same enzymes as well as those that differ in the metabolism of these amino acids. Peripheral as well as brain enzyme systems and shuttles are described in detail and illustrated in the included figures.

Chapter 4 describes in detail the role of isoleucine specifically in the functioning of certain adipocytes as well as its function in glucose metabolism. Laboratory animal studies are reviewed and the importance of isoleucine in the activation of liver and skeletal muscle free fatty acid uptake and oxidation is linked to a potential role in the development of obesity. The fifth chapter describes the unique metabolic activities of leucine. Leucine has been found to be a potent activator of the mammalian target of rapamycin (mTOR) pathway which is a critical nutrient-sensing pathway that governs cell metabolism, cell growth, and proliferation. Leucine also has a role in regulating insulin secretion and glucose utilization. The chapter includes an in-depth investigation of leucine regulation of several critical cellular processes in pancreatic β-cells, including metabolism, growth, proliferation, and insulin secretion, which ultimately influence overall glucose homeostasis. The multiple routes that are involved in acute regulation of insulin secretion are illustrated in the included figures.

Chapter 6 looks at the data from laboratory animal experiments that examine the effects of leucine and isoleucine on glucose metabolism and insulin regulation. These three chapters provide a comprehensive review of the roles of these amino acids in energy metabolism and suggest that this is an area where well-controlled clinical studies are needed. The seventh chapter provides additional detailed information concerning the importance of the hypothalamic metabolism of leucine in the brain and its effects on liver glucose production. The eighth chapter reviews new studies in cell culture and laboratory animals that are exposed to leucine and resveratrol. The rationale for this combination is that both leucine and resveratrol stimulate sirtuin-dependent pathways that are linked to enhancing longevity. The authors describe the synergistic actions of both substances on sirtuin-dependent downstream metabolic effects.

The next chapter in this part describes the role of branched chain amino acids in the metabolism of ammonia. This is a complex area of basic as well as clinical investigation and both are well described in this chapter. The author also describes the open questions concerning the interactions between muscle and liver metabolism of ammonia and the potential for unexpected effects when branched chain amino acids are given therapeutically. The last chapter in this part, Chapter 10, describes the use of a stable isotope form of leucine to calculate the in vivo rates of synthesis and catabolism of lipoproteins that are important in the determination of cardiovascular risk, survival of HIV, and other clinical manifestations of abnormal lipoprotein levels. This detailed chapter describes the calculations required to determine in vivo circulating HDL, VLDL, and LDL levels without exposing the patient to radiation.

Part II contains four chapters that review the inherited defects in branched chain amino acid metabolism and describes the resultant inborn errors of metabolism. The first chapter in this part focuses on genetic defects in the oxidative pathways involved in the breakdown of valine, leucine, and isoleucine. We learn that there are a total of 15 different enzyme reactions that are required for the breakdown of valine, leucine, and isoleucine. The chapter focuses on the 11 genetic defects and the enzymatic consequences of 11 of the pathways. Descriptions of the pathophysiological changes that are seen with each of the amino acid's associated genetic mutations in critical enzymes are described. Maple sugar urine disease is the most common manifestation of errors in the coding of the catabolic enzymes involved in the decomposition of the three branched chain amino acids and results in higher than normal levels of the amino acids in the blood and urine. Chapter 12 describes in detailed text and tables the 202 mutations described in 2013 that are associated with maple sugar urine disease. Because

of the rapid onset of severe brain damage in neonates with this disease, newborn screening is done on all infants in the USA and in many other parts of the world. Clinically, treatments involve the provision of a diet low in branched chain amino acids. There is ongoing research concerning the biochemical mechanisms associated with the clinical presentations of the many of the genetic defects associated with this disease. Chapter 13 describes other adverse consequence of genetic mutations specifically to the isoleucine degradation enzymes. The authors have documented cases of mental retardation, motor dysfunctions, behavioral disorders, and other abnormal manifestations of physical and mental functions in children and young adults that carry these mutations. The X-linked inborn errors of isoleucine degradation are described in detail and the author clearly indicates the need for further research. The next chapter examines the results of laboratory animal studies that look at the effects of a genetic defect in the metabolism of valine and the consequences to eating behaviors. The authors posit that the resultant valine deficiency specifically can affect satiety centers in the brain resulting in anorexia. Translating this research into clinical studies has not as yet occurred.

Part III: Experimental Models

Part III includes seven chapters that describe laboratory studies with experimental models of growth and certain disease states where branched chain amino acids have been examined to determine the metabolic role of these amino acids; in several chapters the administration of one or all of the branched chain amino acids has been shown to provide some improvement. Chapters 15 and 16 review the importance of protein and specific amino acids in the growth of the fetus (Chapter 15) and the neonate (Chapter 16). Intrauterine growth retardation is associated with a reduction in organ growth and permanent changes in organ metabolism and/or structure. As discussed by the author, intrauterine growth retardation (IUGR) may result from maternal undernutrition that can increase the risk of lifelong adverse health effects. Experimental models have provided data indicating that IUGR causes changes in islet cells, in the hypothalamic-pituitary-adrenal axis, and in the secretion of prolactin, progesterone, estradiol, and insulin, as well as in the glucose uptake by muscles, body fat content, and mitochondrial function. IUGR increases the risk of cardiovascular diseases, diabetes, and obesity in adult life. IUGR causes a reduction in organ growth and permanent changes in organ metabolism and/or structure. The experimental models have shown that leucine supplementation in models of low protein intake may not be as valuable as increase in total protein intake. The chapter includes excellent tables and figures. Chapter 16 provides unique data from a neonatal pig model on the role of leucine in muscle metabolism. The authors have used parenteral leucine infusions and show that a physiological rise in leucine enhances protein synthesis in skeletal muscle and cardiac muscle. They have also shown that leucine supplementation of a meal acutely stimulated protein synthesis in the neonatal pig model. Two chapters examined the importance of branched chain amino acids in models of obesity. Chapter 17 reviews the data from rodent models concerning the effects of branched chain amino acid status on insulin resistance and alterations in adipose tissue. Chapter 18 examines the data from models of high fat intake that have shown that leucine functions as a nutrient signal to coordinately regulate three major signaling pathways in the liver, skeletal muscle, and adipose tissue. Dietary supplementation of leucine significantly ameliorated the deleterious effects of consumption of a high-fat diet including obesity, hepatic lipid accumulation, mitochondrial dysfunction, and insulin resistance. The metabolic benefits of leucine supplementation included upregulation of genes related to mitochondrial synthesis of critical enzymes, increases in metabolic rates, and suppression of inflammation in adipose tissue.

The last three chapters in this part discuss different models that reflect the functions of branched chain amino acids. Chapter 19 describes research underway to understand the etiology of amyotrophic lateral sclerosis (ALS), an adult-onset neurodegenerative disease, also known as Lou Gehrig

disease, that is characterized by degeneration of neurons in the brain and spinal cord leading to progressive paralysis of respiratory and limb muscles. Both in vitro and laboratory animal studies are reviewed. There are indications of adverse effects of branched chain amino acids in certain of the models as a result of neurotoxicity that may be linked to oxidative stress. At present it is not known if these effects would be seen in humans, but caution is urged. The next chapter provides a unique perspective on the potential for leucine to enhance ethanol metabolism in the liver. The author describes experiments showing that leucine specifically (and not valine) accelerates ethanol clearance after acute ethanol administration by enhancing alcohol-metabolizing enzyme activities. Leucine treatment before alcohol intake also enhanced alcoholic enzyme activities and accelerates ethanol metabolism. It is well recognized that chronic alcohol intake leads to liver failure, such as hepatic inflammation and fatty liver, and induces liver cirrhosis. Accelerating ethanol oxidation may prevent liver failure. The last chapter in this part describes the development of a two-stage model of bladder cancer that involves the use of known cancer initiators and promoters. In this model, branched chain amino acid supplementation at very high doses compared to normal human dietary intakes enhanced tumor formation when certain basal diets were fed, but not with others. As indicated by the authors, "To date, however, there is no epidemiological data relevant to a relationship of dietary BCAA with the risk of bladder cancer."

Volume II

The second volume of *Branched Chain Amino Acids in Clinical Nutrition* concentrates on the role of these amino acids in healthy individuals, the effects of certain diseases on branched chain amino acid status, and finally, data from clinical studies that included therapeutic use of the amino acids in patients.

Part I: Role of Branched Chain Amino Acids in Healthy Individuals

The first part contains five chapters that begin with an in-depth examination of the safety of leucine supplementation in healthy adults. Often healthy adults use leucine supplements when they are exercising or attempting to build muscle. The next three chapters review the effects of leucine on muscle. The last chapter in the Part describes the potential for optimal surgery outcomes when amino acid ratios are used as an index of protein status. Chapter 1 describes the process used by the authors to determine the tolerable, and presumably safe, upper limit of intake of leucine in healthy adults. They define this value as the point at which the metabolic capacity to catabolize or oxidize the excess amino acid is exceeded because it represents the intake where the normal regulatory mechanisms are no longer sufficient to dispose of the excess. The amino acid intake corresponding to this inflection point does not represent a toxic intake level, but rather suggests that with increasing dietary intakes above this level the potential or risk for adverse events will increase. Also, amino acid intakes above this point are usually characterized by an increasing rate of accumulation in blood and excretion of the amino acid, and its secondary catabolites in urine. The figures included in this chapter help to illustrate these relationships. The experimental design used for leucine is reviewed and the authors report that with increasing intakes of leucine, a dose–response in leucine oxidative capacity was observed, with a breakpoint estimated at 550 mg/kg bw/day or 39 g/day for a 70 kg healthy adult. Simultaneous and significant increases in blood ammonia concentrations, plasma leucine concentrations, and urinary leucine excretion were observed with leucine intakes higher than 500 mg/kg bw/day. Thus, under acute dietary conditions, intakes greater than 500 mg leucine/kg bw/day may potentially increase the risk of adverse events, and is proposed as the tolerable upper safe intake (UL) for leucine in healthy adults.

The next chapter examines the requirements for protein and leucine intake in elite athletes to support skeletal muscle regeneration processes following endurance exercise in trained skeletal muscle. The authors present their rationale for adding free leucine to dietary protein to enhance the combined exercise-nutrient muscle response to the post-exercise regenerative processes and protein synthesis. Both the high dose (70 g whey protein/15 g leucine) and low dose (23 g whey protein, 5 g leucine) co-ingested with carbohydrate and fat over the first 90 min following intense cycling resulted in a proinflammatory transcriptome associated with increased leukocytes that reverted by 240 min to an anti-inflammatory signal in skeletal muscle. The other measurements also pointed to a positive effect of the post-exercise supplementation; however, in addition to the acute studies, long-term studies in trained athletes are needed. Chapter 3 reviews studies that used whey protein (from milk) plus leucine. The doses of L-leucine used in the studies reviewed varied from 2.24 to 7.5 g while the whey protein doses varied from 6.7 to 25 g. Outcomes also varied and included muscle responses post-exercise, in different aged populations and immune functions following exercise. The authors suggest that future studies assess dietary intakes and use consistent outcomes. The next chapter in this part reviews the data linking muscle atrophy in patients caused by several diseases to metabolic rationales for administering branched chain amino acids. The authors describe both animal models and patients with cancer-induced muscle loss (cachexia), glucocorticoid-induced muscle atrophy from Cushing's syndrome, as well as long-term therapeutic use of glucocorticoids, sarcopenia, and sepsis-induced muscle protein degradation. The final chapter, Chapter 5, describes the use of a ratio of branched chain amino acids to tyrosine as an index of presurgery health in patients with liver cancer; the index can also help to identify patients who would benefit with amino acid supplementation post surgery. Liver cancer patients undergoing surgical resection who had a low ratio before surgery had significantly more complications as well as more severe complications than patients with higher ratios. The authors suggest that liver cancer patients who have higher albumin levels (indicative of better liver function) and also have a higher ratio are at reduced risk for postsurgery complications. Patients with high albumin levels and low ratio may be the best candidates for supplementation with branched chain amino acids postsurgery.

Part II: Branched Chain Amino Acids: Status in Disease States

Part II contains five chapters that examine diseases of the heart, brain, and lungs and how these diseases affect the branched chain amino acid status of the patient. Branched chain amino acids, as discussed above, are critical for skeletal muscle integrity and are a major site of these amino acids' metabolism. Their role in cardiac muscle is the topic of Chapters 6 and 7. Chapter 6 examines clinical manifestations of genetic defects in branched chain amino acid metabolism as seen in propionic acidemia and methylmalonic acidemia that have been associated with dilated and hypertrophic cardiomyopathies. In addition to genetically related changes in branched chain amino acid metabolism, alterations in their metabolism are also seen in heart failure patients independent of a genetic cause. This is a new area of clinical research and a number of metabolic paths involving the catabolism of branched chain amino acids are hypothesized to potentially adversely affect cardiac tissue. Another new area that links branched chain amino acids with heart function, reviewed in Chapter 7, is the complex outcomes of mitochondrial cardiomyopathies (MCM) produced by mutational defects in energetic metabolism. Specifically, a mutation involving the branched chain amino acid valine has been identified and linked to impaired heart functions in the neonates that inherit this defect.

The next two chapters examine the effects of branched chain amino acids on brain functions, first as related to psychomotor skills and, in Chapter 9, their role in traumatic brain injury. With regard to psychomotor skills, we learn that these coordinated actions are used in everyday life, occupational work, and sport activities. Psychomotor performance depends mainly on cognitive function, attention,

concentration, and decision-making. Branched chain amino acids, especially leucine, can cross the blood–brain barrier and influence these functions through their involvement in the synthesis of neurotransmitters. Ingestion of small doses of branched chain amino acids has been shown to improve psychomotor performance. However, higher doses can exert negative effects on some brain functions and may impair psychomotor performance. New research is underway to better understand the biochemical changes in the brain following traumatic brain injury. Clinical research has reported that immediately following the brain injury, there are phase-dependent changes in plasma amino acids with a different profile in the acute, subacute, and rehabilitation phase. During the acute phase, decreased plasma branched chain amino acid levels are associated with increased plasma aromatic amino acid concentrations. There are numerous other changes in the brain and the authors of Chapter 9 indicate that currently, it is not known if repletion of the branched chain amino acids is of benefit immediately or will be of more value during a different phase of recovery. The last chapter in this section reviews the effects of chronic obstructive pulmonary disease (COPD) on branched chain amino acid status and the potential to enhance the strength of these patients with nutritional addition of these amino acids. The authors point out that COPD patients with low body mass index (BMI) and/or severe airflow limitations exhibit reduced branched chain amino acid profiles, but those with a normal BMI and/or moderate airflow limitations do not. Nevertheless, both muscle and plasma levels of the amino acid levels are directly related to the levels of muscle wasting seen in patients with COPD.

Part III contains five chapters that examine the influence of branched chain amino acids in patients with liver diseases. Chapter 11 describes the effects of liver disease on branched chain amino acid status and then reviews the use of the amino acids as therapeutic supplements in patients with liver cirrhosis, liver cancer, and additional adverse consequences of liver disease. In patients with liver cirrhosis, levels of branched chain amino acids are decreased in the blood and the levels of aromatic amino acids and methionine are increased. The authors examine in depth the adverse effects of these changes on brain and muscle biochemistry and by using global gene expression analysis, the authors provide critical data on the molecular mechanisms by which hepatic damage can be reversed following branched chain amino acid supplementation. The next chapter discusses the importance of the liver in the synthesis of albumin. Albumin is the major protein produced in the liver and albumin represents over 50% of the total plasma proteins. Plasma albumin level is a standard index of nutritional status, liver function, and/or pathophysiological conditions. One of the most important factors regulating plasma albumin levels is ingestion of a protein-rich meal. The authors indicate that in Japan, pharmacological supplementation of branched chain amino acids is used to improve hypoalbuminemia in patients with liver cirrhosis. The chapter focuses on the effects of amino acid supplementation, including branched chain amino acids, on the regulation of albumin synthesis and provides excellent figures to help the reader. Chapter 13 describes the effects of liver cirrhosis caused by hepatitis C and nonalcoholic fatty liver disease and the consequent protein energy malnutrition and muscle wasting that can accompany these serious liver diseases. The author indicates that these major metabolic defects result in a state of starvation during the overnight sleep time and provides clinical evidence of the benefits of a nighttime snack that includes branched chain amino acids. Based upon the preliminary findings, it is suggested that well-controlled studies be undertaken.

The next chapter reviews the importance of stabilizing the nutritional status in patients with end-stage liver disease requiring liver transplantation. Accurate nutritional assessment and adequate perioperative nutritional treatment are essential for improving outcomes after liver transplant. The overall survival rate in patients with low skeletal muscle mass was found to be significantly lower than in patients with normal/high skeletal muscle mass. The authors report that perioperative nutritional therapy including branched chain amino acids is useful for patients with sarcopenia, whose prognosis is poor without nutritional therapy. The final two chapters in this part review the importance of branched chain amino acid supplementation in the treatment of the post-liver surgery patient. In Chapter 15, we learn that post-transplant bacteremia is one of the most serious complications following liver transplantation. One potential avenue for prevention of bacteremia that is being tested is nutritional

support. It is known that in patients who have not been given supplementation prior to transplantation, serum levels of branched chain amino acids generally decrease and have been reported to be a risk factor for post-transplant bacteremia. This chapter reviews previous studies on the beneficial effects of branched chain amino acid supplementation in liver transplant patients: prevention of bacteremia, potential mechanisms of action, and avenues for future research. Chapter 16 examines the effects of liver surgery as a result of liver cancer and the data suggesting that branched chain amino acid supplementation may enhance the prognosis of these patients. The authors indicate that most liver cancers occur in patients with chronic liver disease. Advances in surgical technology and perioperative management have led to the standard use of hepatic surgical procedures for liver cancer and also metastatic liver tumors. Branched chain amino acid supplementation improves postoperative quality of life over the long term after hepatic resection by restoring and maintaining nutritional status and whole-body kinetics.

The last part in volume II examines the use of branched chain amino acid supplementation in several different patient populations that further attests to the growing interest in the clinical value of these amino acids. The final chapter provides an extensive and up-to-date listing of relevant websites and other resources. The first four chapters discuss the importance of these amino acids in patients who are losing muscle mass and may at the same time show symptoms of insulin resistance. Chapter 17 provides an overview of the most promising therapeutic uses of branched chain amino acids. Topics discussed include the effects of physical immobility in the elderly, cancer cachexia, and weight reduction in the obese patient. The common thread is the loss of muscle mass and the potential for leucine and/or branched chain amino acid-rich protein supplementation to reduce the muscle loss seen in these conditions. Chapter 18 reviews the data that link liver cirrhosis with insulin resistance. Insulin resistance increases in chronic liver disease and is a risk factor for the progression of liver disease, the potential for the development of liver cancer, and a decrease in long-term survival. The authors suggest that insulin resistance should be considered as an important therapeutic target in patients at any stage of chronic liver disease. They hypothesize that branched chain amino acids play a dual role in glucose metabolism in skeletal muscle, enhancing glucose uptake under normal insulin conditions while causing insulin resistance under high insulin conditions. The next chapter concentrates on the actions of leucine on glucose metabolism. Since its discovery, leucine has been shown to affect glucose homeostasis in liver, muscle, adipose tissue, and pancreatic β cells. In the β cells, leucine acutely stimulates insulin secretion by several mechanisms. These mechanisms can be modulated by factors that include, but are not limited to, the body weight of the patient, whether they are type 2 diabetics and whether or not they routinely exercise. Chapter 20 focuses on protein metabolism during insulin resistance and the effects of surgically implemented weight loss on branched chain amino acid status and requirements. Elevated plasma amino acid levels have been associated with obesity and insulin resistance and circulating branched chain amino acids have been identified as early biomarker predictors of diabetes risk in obese insulin-resistant subjects. The chapter includes detailed discussions of the results of bariatric surgery on branched chain amino acid levels and examines the data from twin studies to better understand the interactions between branched chain amino acid status and improvements in glucose control following acute, significant weight loss.

Chapter 21 examines the metabolism of branched chain amino acids within the skin and provides a unique perspective on the importance of these amino acids, especially leucine, in stimulating dermal collagen synthesis in wound healing. The chapter reviews the requirements for mixtures of amino acids to optimize skin collagen formation in the face of protein malnutrition, wound healing, and UV radiation and discusses the mechanism of action of these stressor to the skin. Chapter 22 reviews the potential for branched chain amino acid supplementation to reduce the muscle wasting seen in boys that have inherited the genetic disorder, Duchenne muscular dystrophy (DMD). The disease is characterized by progressive loss of muscle mass and accumulation of body fat. Steroids (e.g., prednisone) are the only treatment available at this time, but the drugs increase the accumulation of body fat and have other adverse side effects. The chapter provides a strong biochemically based rationale for

supplementing DMD patients with branched chain amino acids; however as yet, there are no large, well-controlled and patient-monitored intervention studies.

Part III, above, contained chapters that consistently reported a decreased branched chain amino acid status in patients with liver diseases including liver cancer. The next two chapters investigate branched chain amino acid supplementation in patients with serious liver diseases. Chapter 23 examines the potential for branched chain amino acid supplementation to reduce the adverse effects of radiotherapy used in patients with liver cancer. The chapter reviews the recent development of radiotherapeutic technologies that permit their application in this patient population. The authors inform us that most of the patients undergoing radiation therapy have chronic liver disease and frequently also have protein-calorie malnutrition. In addition, radiation can result in general fatigue, nausea, and vomiting that further aggravate the patients' nutritional status. Preliminary clinical investigations suggest that supplementation with branched chain amino acids during radiation therapy helped to reduce the loss of these amino acids and may be of benefit in maintaining albumin levels. Chapter 24 describes the condition called hepatic encephalopathy, which is a metabolic neuropsychiatric syndrome of cerebral dysfunctions due to severe chronic or acute liver disease. The manifestations of hepatic encephalopathy range from minor symptoms with personality changes and altered sleep patterns to deep coma. The chapter includes a detailed review of all clinical trials and meta-analyses that included studies where patients have been given branched chain amino acids for the treatment of hepatic encephalopathy regardless of route of administration. The combined evidence supported the use of oral branched chain amino acids as treatment for patients with hepatic encephalopathy based upon the results from several large, high-quality randomized controlled trials. However, further research is required to determine the optimal dose of these amino acids.

The above descriptions of the two volumes' 46 chapters attest to the depth of information provided by the 156 well-recognized and respected editors and chapter authors. Each chapter includes complete definitions of terms with the abbreviations fully defined for the reader and consistent use of terms between chapters. Key features of the two comprehensive volumes include over 250 detailed tables and informative figures, an extensive, detailed index, and more than 1,900 up-to-date references that provide the reader with excellent sources of worthwhile information. Moreover, the final chapter contains a comprehensive list of web-based resources that will be of great value to the health provider as well as graduate and medical students.

In conclusion, *Branched Chain Amino Acids in Clinical Nutrition*, a two-volume book, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, provides health professionals in many areas of research and practice with the most up-to-date, well-referenced volume on the importance of branched chain amino acids in maintaining the nutritional status and overall health of the individual especially in certain disease conditions. The volumes will serve the reader as the benchmarks in this complex area of interrelationships between dietary protein intakes and individual amino acid supplementation, the unique role of the branched chain amino acids in the synthesis of brain neurotransmitters, collagen formation, insulin and glucose modulation, and the functioning of all organ systems that are involved in the maintenance of the body's metabolic integrity. Moreover, the physiological, genetic, and pathological interactions between plasma levels of branched chain amino acids and aromatic amino acids are clearly delineated so that students as well as practitioners can better understand the complexities of these interactions. Unique chapters examine the effects of branched chain amino acid status and the effects of genetic mutations from pre-pregnancy, during fetal development and birth, and infancy through the aging process. The editors are applauded for their efforts to develop the most authoritative and unique resource in the area of branched chain amino acids in health and disease to date and this excellent text is a very welcome addition to the Nutrition and Health Series.

Adrianne Bendich, Ph.D., F.A.C.N., F.A.S.N.
Series Editor

About the Series Editor

Adrianne Bendich, Ph.D., F.A.S.N., F.A.C.N. has served as the "Nutrition and Health" Series Editor for over 15 years and has provided leadership and guidance to more than 120 volume editors that have developed the 60+ well-respected and highly recommended volumes in the series.

In addition to ***Branched Chain Amino Acids in Clinical Nutrition*** **volume I and volume II, edited by Rajkumar Rajendram M.D., Victor R. Preedy Ph.D., and Vinood B. Patel Ph.D**., **major new editions** in 2012–2014 include:

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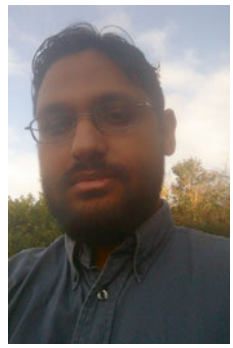

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Part I
Role of Branched Chain Amino Acids in Healthy Individuals

Chapter 1
Tolerability of Leucine in Humans

Rajavel Elango, Ronald O. Ball, and Paul B. Pencharz

Key Points

- Leucine supplements are popular especially among athletes, due to the perception that leucine promotes endurance and enhances athletic performance.
- Current reported leucine intakes in developed countries among healthy adults is approximately 200 mg kg day^{-1}.
- Currently no safe upper limits (UL) for leucine intake has been set.
- Recently using stable isotope labeled L-[1-^{13}C]-Leucine and graded test intakes of leucine (50–1,250 mg kg^{-1} day^{-1}), the upper limit to oxidize/metabolize leucine was tested in adult men.
- The oxidation of leucine measured as a rate of tracer oxidation plateaued with increasing intakes of leucine above 550 mg kg day^{-1}.
- Simultaneously increases in plasma ammonia concentrations, plasma leucine concentrations, and urinary leucine excretion were observed with leucine intakes above 500 mg kg day^{-1}.
- Taken together the data suggest 500 mg kg day^{-1} as a cautious estimate of the UL for leucine intake under acute dietary conditions.

Keywords Leucine supplements • Humans • Stable isotopes • Safe upper limits • Leucine oxidation

Abbreviations

α-KIC	Alpha- ketoisocaproic acid
ALT	Alanine amino transferase
BCAA	Branched chain amino acids

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BCAT	Branched chain aminotransferase
BCDH	Branched chain ketodehydrogenase
CPS1	Carbamoyl phosphate synthetase 1
DRI	Dietary Reference Intakes
EAR	Estimated average requirement
$F^{13}CO_2$	Label tracer oxidation
GDH	Glutamate dehydrogenase
NAGS	*N*-acetylglutamate synthase
NOAEL	No-observed-adverse-effect level
REE	Resting energy expenditure
UL	Tolerable upper intake level
VCO_2	Carbon dioxide production

Introduction

Use of dietary supplements, especially among athletes is increasing. It has been reported that 80–85 % of professional athletes consume nutritional supplements, including amino acids due to the perception that they enhance performance or recovery [1–3]. In the United States approximately 3.4 % of the general population uses amino acid supplements, 62 % on a daily basis [4]. Among the amino acids, the branched chain amino acids (BCAA-leucine, valine, and isoleucine) are popular as dietary supplements especially among strength training athletes. In particular, leucine as a supplement is widely consumed because it has been implicated to be the key amino acid involved in stimulating muscle protein synthesis [5–8]. Therefore it is perceived that leucine might improve athletic endurance, performance [9–16], and increase lean body mass, although some results [17] have been inconclusive. From a public health perspective dietary supplementation of leucine in excess of requirement may have adverse health effects, and therefore additional knowledge is necessary regarding the highest possible intake of leucine at which no adverse effect occurs. This chapter will present current dietary leucine intakes, define the concept of safe upper limits (UL), and summarize recent studies in animals and humans conducted to address leucine tolerability.

Current Leucine Intakes

In the recent Dietary Reference Intakes (DRI) report [18], based on distribution data from the 1988–1994 NHANES III, mean daily intake of leucine for all life stage and gender groups from food and supplements were reported to be 6,100 mg day^{-1}. Men 51 through 70 years of age had the highest intakes, at the 99th percentile for leucine, at 14,100 mg day^{-1} (~201 mg kg day^{-1}). Similar results have been reported from the UK adult National Diet and Nutrition Survey, ranging from a median to 90th percentile intake of 108–138 mg kg day^{-1}, respectively; the more recent UK survey [19] suggests a leucine intake of 200 mg kg day^{-1} in healthy adult men.

Defining Safe Upper Limits (UL) of Intakes for Leucine

The DRI's refer to the safe upper limits of a nutrient as the Tolerable Upper Intake Level (UL), and is defined as, "the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase." [18]

The term tolerable was chosen to avoid implying a possible beneficial effect. Instead, the term is supposed to suggest a level of intake that can, with high probability, be tolerated biologically.

With respect to the BCAA supplements, earlier reviews [20, 21] have summarized studies in athletes, normal adults, and patients with clinical disorders, and reported that intakes of 15–60 g day^{-1} (~200–850 mg kg^{-1} day^{-1} for a 70 kg man) of total BCAA did not result in adverse event outcomes for the parameters monitored. A significant problem with interpreting the reported human studies is the great variability in approaches; these experiments include supplements of all BCAA, at many different doses, different ratios among the BCAA in intake, different routes of infusion/feeding (intravenous versus oral), and different athletic training regimes. Therefore in the 2005 DRI report, no UL for BCAA, including for leucine, was set, and it was noted that there was a paucity of data from well-designed dose–response studies in both animals and humans.

Development of a Novel Approach to Define UL

Based on the recommendations in the DRI (2005) report [18], we developed a conceptual model [22], which will help design studies to identify the UL for amino acids. We reasoned that markers for identifying excess intake of an amino acid should have specific dose–response characteristics. In particular, variation with intake should display an inflection point that would identify the onset of the amino acid excess situation [22]. Previously, in neonatal piglets [23], an upper inflection point was observed in the dose–response curve for phenylalanine retention and $^{14}CO_2$ production from phenylalanine oxidation with graded phenylalanine intake. Once the maximum level of phenylalanine oxidation was reached then plasma phenylalanine levels, and retention, rose rapidly. Hence, it is reasonable that a suitable marker to define the upper limit of tolerance for a dietary amino acid would be the level of intake at which the maximum oxidation level was exceeded.

The above described response pattern for increasing phenylalanine intake should be observable for most amino acids and we proposed [22] that it can be applied to define the UL. When the amino acid intake is low, protein synthesis, oxidation, and excretion of the amino acid and related metabolites will be low (Fig. 1.1a). With increasing intake of the limiting amino, retention of the amino acid will increase as a result of increasing utilization of the limiting amino acid for protein synthesis and other required metabolic functions. Therefore a positive slope in amino acid retention will be observed. Once the requirement for the amino acid is met for protein synthesis and related functions, additional increments of the test amino acid will be primarily catabolized in proportion to the extra intake. This increasing catabolism in proportion to intake will occur because each additional increment in intake will be in excess of the requirements for metabolism, and will be oxidized. In this zone, the catabolic pathways are sufficient to deal with the excess intake and the excess amino acid is broken down and used for energy. For the range of amino acid intakes in this zone, a minimum slope or no slope in the retention of the amino acid will probably be observed, depending on the amino acid being tested. Further increases in amino acid intake will lead to a positive slope in the amino acid retention curve, due to increased retention of the amino acid in body pools. This increase in retention is a result of dietary intake exceeding the metabolic capacity to catabolize the amino acid in direct proportion to intake. This point, at which the metabolic capacity to catabolize or oxidize the excess amino acid is exceeded, can be regarded as one estimate of the UL because it represents the intake where the normal regulatory mechanisms are no longer sufficient to dispose of the excess (Fig. 1.1b). The amino acid intake corresponding to this inflection point does not represent a toxic intake level, but rather suggests that with increasing dietary intakes above this level the potential or risk for adverse events will increase. Also, amino acid intakes above this point are usually characterized by an increasing rate of accumulation in blood and excretion of the amino acid, and its secondary catabolites in urine (Fig. 1.1a, b).

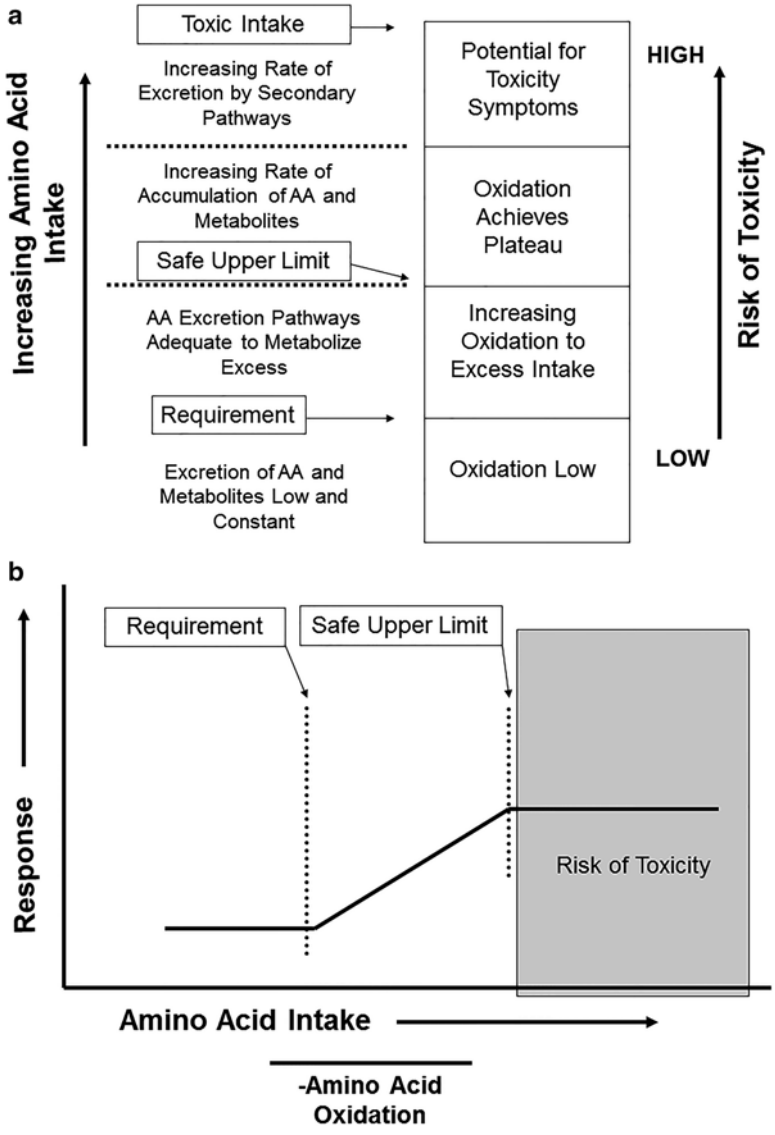

Fig. 1.1 An approach to define the safe upper limits of amino acids. (**a**) Schematic to describe body responses to increasing amino acid intake. (**b**) Patterns of response expected due to increasing intakes of amino acids. With increasing intakes of the amino acid, oxidation of the amino acid will increase. Once the "metabolic limit" to oxidize the excess amino acid is reached, amino acid oxidation will plateau and this inflection point will identify the upper tolerable intake limit (UL) for the test amino acid. As amino acid intake increases above the UL, the potential risk of adverse effects and toxicity may increase. *Source*: Adapted from J Nutr, Pencharz et al. *J Nutr.* 2008;138:1996S–2002S [22]

Leucine UL in Animals

Sakai et al. [24] used the above-described approach to identify an excess intake of leucine in rats. They identified the "metabolic limit" to catabolize leucine by measuring $^{13}CO_2$ production arising from graded leucine intakes ranging from 0 to 30 % of the diet. The maximum limit to oxidize excess

leucine was reached at 10 % of dietary intake; the oxidation achieved a plateau above the intakes of 10 % dietary leucine or 8.9 g leucine kg^{-1}. This inflection point was identified as the UL for leucine intake in rats [24]. They also reported that plasma leucine and other plasma amino acid concentrations were not significantly different among all the leucine intakes. Therefore, they were not able to identify accumulation of excess leucine and other metabolites as a potential biomarker, or surrogate marker. In an earlier study using a similar strain of rats and receiving a diet similar in composition, they observed significant growth inhibition in rats fed 15 % leucine or 12.4 g leucine kg^{-1}. This suggests that the inflection point, at which the maximum limit to oxidize excess leucine is reached, is an early marker to identify the potential for an adverse event (in this case – growth inhibition) and may identify the UL more appropriately.

Tsubuku et al. [25] in a controlled week-long experiment, with moderate or high amounts of protein in the diet, studied oral supplements of leucine excess in 4-week-old rats. They estimated ~3,500 mg kg^{-1} day^{-1} to be the no-observed-adverse-effect level (NOAEL), based on body weight, food consumption, and hematological measurements. Mawatari et al. [26] in 10-week-old female rats estimated that oral leucine at 1,000 mg kg^{-1} day^{-1} did not affect the outcome of pregnancy and did not cause fetal toxicity. Recently, Imamura et al. [27] under conditions of low-protein intake (6 %), suggested that 2 % leucine in the diet may be the NOAEL, using a novel gene marker panel. Thus, as concluded earlier by Baker [28] young experimental animals are able to tolerate a considerable dietary excess of leucine, when consumed in diets containing sufficient levels of protein and the other two BCAA.

Leucine UL in Humans

We hypothesized [29] that with increasing intakes of leucine above the estimated average requirement (EAR of 50 mg kg^{-1} day^{-1}) in adult men [30], the oxidation of leucine will increase and will reach a maximum, after which the leucine oxidation will achieve a plateau. This 'metabolic limit' to oxidize leucine may be used as a marker of an intake after which increasing intakes may result in increasing risk of adverse effects.

Five healthy young men participated in the study [29]. Each subject participated in a dose-escalation study design, where graded stepwise increases in leucine intake were provided on each study day. This study design was chosen to ensure that with each increasing dose of leucine intake, subject safety could be monitored. Each subject was initially studied at a leucine intake of 50 mg kg^{-1} day^{-1}. Following this baseline study subjects received increased dietary leucine in a graded stepwise intake of 150, 250, 500, 750, 1,000, and 1,250 mg kg^{-1} day^{-1} corresponding to the EAR, EAR ×3, ×5, ×10, ×15, ×20, and ×25 on separate study days. The study diets were adequate in protein (1 g kg^{-1} day^{-1}), energy (1.5 × resting energy expenditure, REE), and all other nutrients including carbohydrates and fats [29]. The study days were separated by a minimum of 2 weeks to ensure a sufficient washout period between the leucine excess study day diets. On each study day a baseline, and end of study blood and urine sample was collected for analysis of blood and urine biochemistry.

Leucine Oxidation

Oxidation of L-[1-^{13}C]leucine to $^{13}CO_2$ in breath ($F^{13}CO_2$) was measured with increasing intakes of dietary leucine. $F^{13}CO_2$ increased with increasing intakes of leucine until 500 mg kg^{-1} day^{-1}, after which oxidation remained at a plateau (Fig. 1.2a). Two-phase linear regression analysis [31, 32] identified a breakpoint at a leucine intake of 550 mg kg^{-1} day^{-1}, and this represents the maximum leucine oxidative potential in vivo in adult men [29]. Leucine oxidation (measured from plasma enrichment

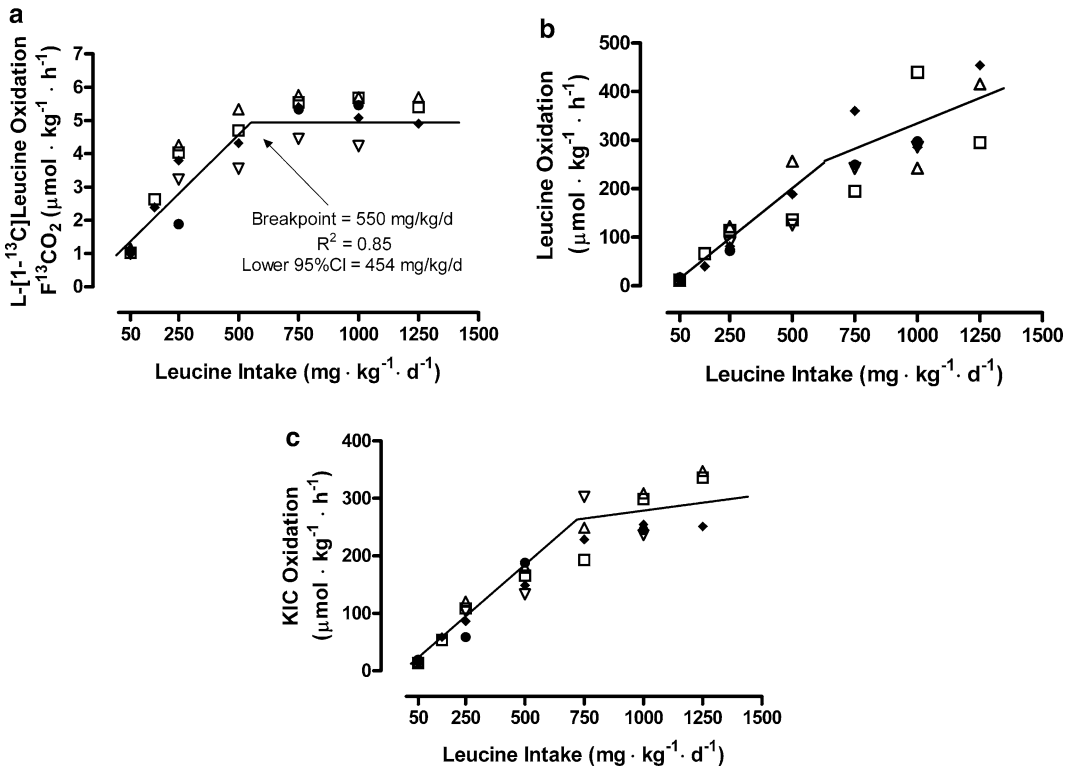

Fig. 1.2 Graded dietary excess leucine intake and metabolic oxidative capacity in healthy young men ($n=29$ observations). (**a**) Production of $^{13}CO_2$ from the oxidation of orally administered L-[1-^{13}C]-Leucine in young men ($F^{13}CO_2$). The oxidation of L-[1-^{13}C]leucine to $^{13}CO_2$, calculated as $F^{13}CO_2$, showed a significant response ($P<0.0001$) to increasing leucine intakes. Statistical analysis performed using mixed models ANOVA, followed by two-phase linear regression analysis. Using two-phase linear regression analysis, a 'breakpoint' in $F^{13}CO_2$ was identified at 550 mg kg^{-1} day^{-1} ($r^2=0.85$). The lower and upper 95 % CI was calculated to be 454 and 647 mg kg^{-1} day^{-1}, respectively. (**b**) Leucine oxidation. (**c**) KIC[1] oxidation. [1]*KIC, alpha-keto isocaproic acid. Source*: Elango et al. *Am J Clin Nutr.* 2012;96:759–767 [29]

of leucine) and KIC oxidation (a surrogate measure of intracellular leucine enrichment and oxidation) showed a dose response with increasing leucine intakes, which appeared to reach a plateau after 500 mg kg^{-1} day^{-1} although absolute leucine oxidation seemed to be increasing (Fig. 1.2b, c). Thus the metabolic capacity to dispose of excess leucine intake occurs between 500 and 700 mg kg^{-1} day^{-1} and the efficiency of leucine oxidation appears to have reached a plateau. Nevertheless, plasma and urinary biomarkers as discussed in the following sections displayed a simultaneous change that correlated with the 500 mg leucine kg^{-1} day^{-1}.

Plasma Ammonia and Biochemical Measures

There was a concomitant increase in blood ammonia concentrations (Fig. 1.3a, b) above normal values (<35 μmol/L) with significant increases in plasma leucine concentration (Fig. 1.4a) and urinary leucine excretion (Fig. 1.4b) in all subjects with intakes of leucine above 500 mg kg^{-1} day^{-1}, suggesting that

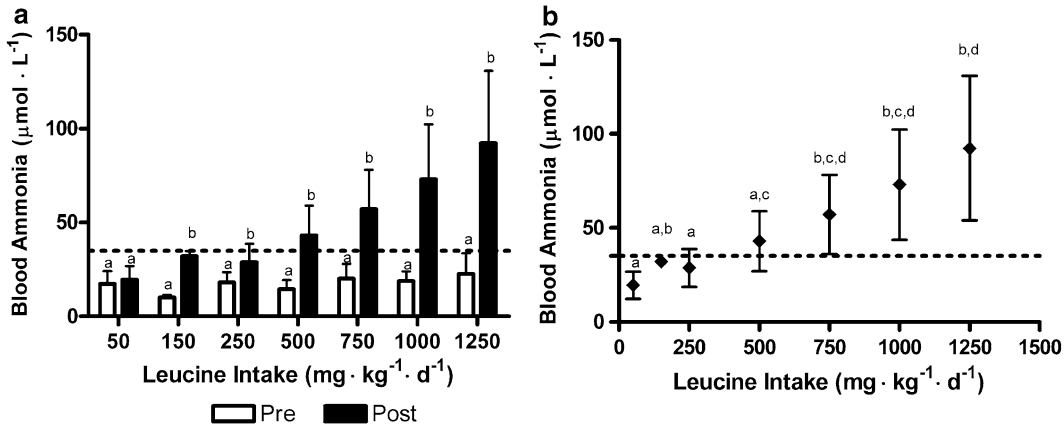

Fig. 1.3 Graded dietary excess leucine intake and blood ammonia levels. Values are means ± SD; $n = 2$ to 5 per mean; symbols with different superscript letters are significantly different, $P < 0.05$. Statistical analysis performed using mixed models ANOVA and post-hoc analysis using Tukey-Kramer's multiple comparison tests. *Dotted lines* indicate normal blood ammonia levels in young men (35 µmol/L). (**a**) Comparison of blood ammonia concentrations between fasted (pre) and end of study day (post) blood samples; (**b**) Blood ammonia concentrations at the end of each study day. *Source*: Elango et al. *Am J Clin Nutr.* 2012;96:759–767 [29]

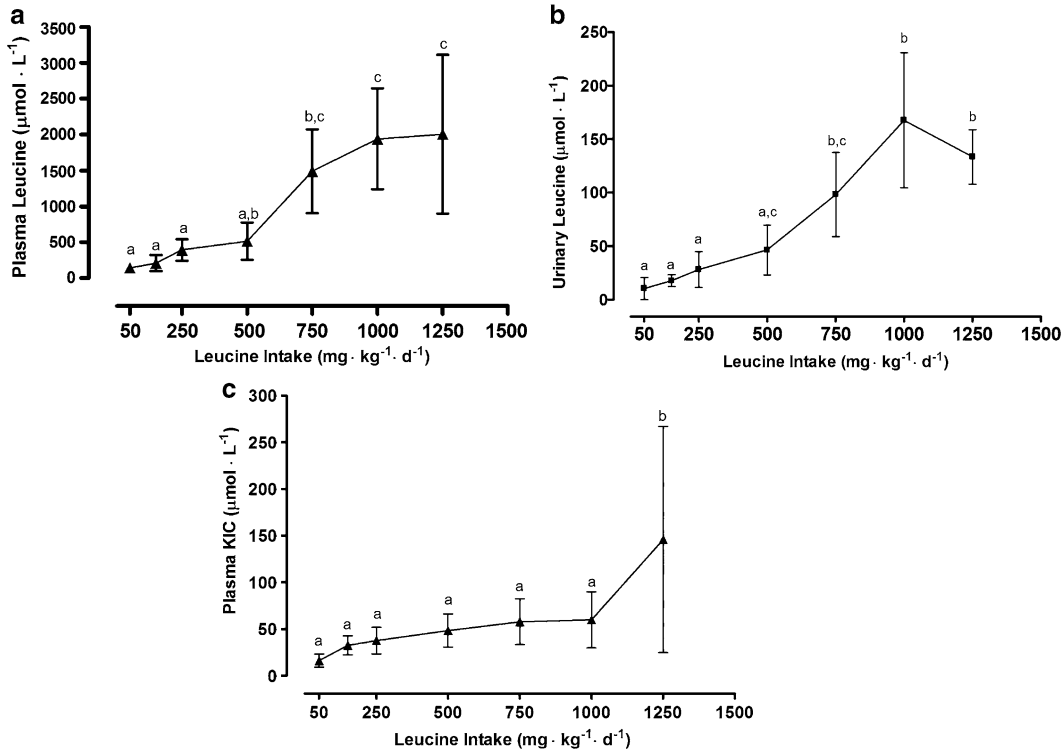

Fig. 1.4 Graded dietary excess leucine intake and plasma leucine concentrations, urinary leucine excretion, and plasma KIC concentrations. (**a**) Plasma leucine concentrations. (**b**) Urinary leucine excretion. (**c**) Plasma KIC[1] concentrations. [1]*KIC, alpha-keto isocaproic acid. Source*: Elango et al. *Am J Clin Nutr.* 2012;96:759–767 [29]

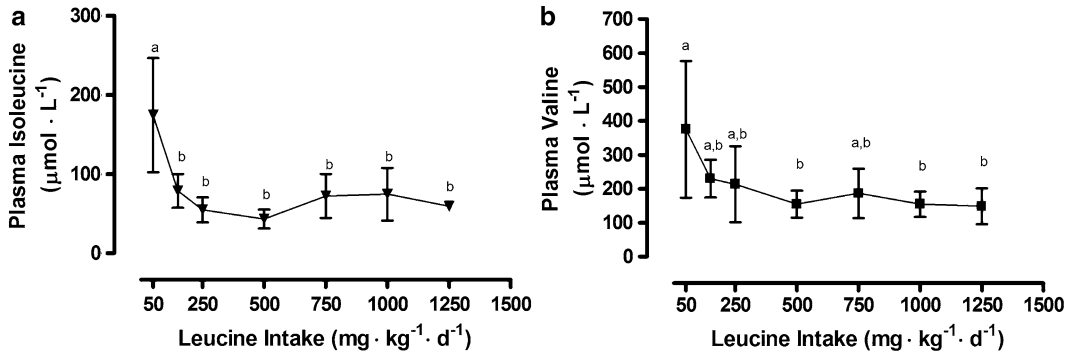

Fig. 1.5 Graded dietary excess leucine intake and plasma isoleucine, valine concentrations. (**a**) Plasma isoleucine concentrations. (**b**) Plasma valine concentrations. *Source*: Elango et al. *Am J Clin Nutr.* 2012;96:759–767 [29]

higher intakes might be harmful. Urinary excretion of KIC increased, although the increases were not significant until 1,250 mg kg^{-1} day^{-1} (Fig. 1.4c). Taken together the data suggest 500 mg kg^{-1} day^{-1} as a cautious estimate of the UL for leucine intake under acute dietary conditions [29, 33].

Plasma and urine samples were analyzed for other biochemical measures as potential markers of leucine UL. Although plasma ammonia concentrations significantly increased with increasing leucine intakes (Fig. 1.3a, b), no significant changes were observed for urea, creatinine, ALT, glucose, insulin, electrolytes (sodium, potassium, chloride), complete blood count including hematocrit, WBC, RBC, and hemoglobin [29]. Leucine has been suggested to act as an insulin secretagogue [34, 35], but we did not observe significant changes in plasma insulin due to increasing leucine intakes and plasma glucose remaining within the normal range of 3.3–6.1 μmol/L. Plasma leucine concentration increased significantly with leucine intakes greater than 500 mg kg^{-1} day^{-1}, and plasma valine and isoleucine concentrations decreased significantly with increasing leucine intakes (Fig. 1.5a, b), as reported previously [36–39]. The BCAA share a common catabolic pathway with the branched chain ketodehydrogenase (BCDH) controlling the irreversible catabolic step, which commits the carbon skeleton of the BCAA to the TCA cycle. Leucine concentrations have been shown to stimulate BCDH, as well as to compete with the other two BCAA for metabolism in vivo [40]; this phenomenon, referred to as BCAA antagonism, is well documented [36].

Potential Mechanism to Explain Plasma Ammonia Increase

Leucine has been shown to be an activator of glutamate dehydrogenase (GDH) [41], which results in alpha-ketoglutarate and ammonia production (Fig. 1.6). Leucine concentrations above 800 μM activates GDH to dispose of the surplus amino acids [42]; in the current study plasma leucine concentrations increased well above 2,000 μM, potentially leading to increased blood ammonia concentrations. Furthermore accumulation of isovaleryl CoA, a metabolite formed from leucine catabolism, has been shown to inhibit *N*-acetylglutamate synthase (NAGS) [43]. NAGS is an activator of carbamoyl phosphate synthetase 1 (CPS1), which regulates the urea cycle [43]. With inhibition of NAGS, CPS activation would not have occurred (Fig. 1.6), and possibly explains the increase in blood ammonia with no change in blood urea concentrations.

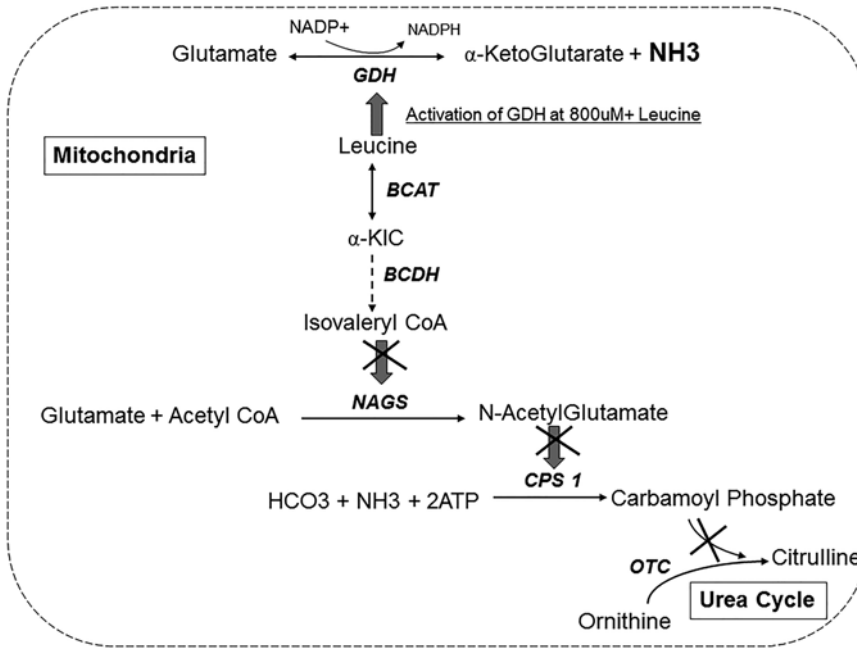

Fig. 1.6 Potential mechanism to explain hyperammonemia due to increasing leucine intakes. Leucine has been shown to activate glutamate dehydrogenase (GDH), which results in alpha-ketoglutarate and ammonia production [41]. In addition, catabolism of leucine yields isovaleryl CoA, a known inhibitor of *N*-acetylglutamate synthase (NAGS) [43]. NAGS activates carbamoyl phosphate synthetase 1 (CPS1), which regulates the urea cycle [43]. With inhibition of NAGS, CPS activation would not have occurred, and possibly explains the increase in blood ammonia with no change in blood urea concentrations

Leucine UL in Relation to Current Leucine Intakes

An analysis of the current habitual leucine intake was conducted in strength-training athletes who are chronic amino acid supplement users. The average protein intake [44] in these athletes was reported to be ~2 g kg^{-1} day^{-1}. Mean leucine content in food is ~15 % [45]. Therefore, for an athlete weighing 80 kg, the dietary leucine intake is 300 mg kg^{-1} day^{-1}. BCAA supplements available contain a maximum 1,800 mg/serving [29], with a suggested dose of 3 doses per day; this equals ~67.5 mg kg^{-1} day^{-1}. Therefore, the habitual total exposure of adults consuming 2 g protein kg^{-1} day^{-1} and this amino acid supplement is at ~367 mg kg^{-1} day^{-1}. These calculations reveal that most people, including athletes, consume less than the UL for leucine oxidation determined (500 mg kg^{-1} day^{-1}). However, there is probably a range in protein and leucine intake amongst athletes, with some consuming more than the recommended dose and thus at potential risk of adverse effects. The impact of chronic consumption of excess leucine by humans remains unknown, as the study was conducted with an acute dietary supply of leucine. A high chronic intake may either reduce the risk of adverse effects, by increasing the basal leucine oxidation rate, or increase the risk of adverse effects by gradual accumulation of metabolic events associated with excess intake, and such effects need to be confirmed in the future with long-term leucine supplementation, as recently recommended [46].

Conclusions

In summary, in the recent human study [29] with increasing intakes of leucine, a dose–response in leucine oxidative capacity was observed, with a breakpoint estimated at 550 mg kg^{-1} day^{-1} or 39 g day^{-1}. Simultaneous and significant increases in blood ammonia concentrations, plasma leucine concentrations, and urinary leucine excretion were observed with leucine intakes higher than 500 mg kg^{-1} day^{-1}. These results taken together with the recent animal data [27] suggest that under acute dietary conditions, as a cautious estimate, intakes greater than 500 mg leucine kg^{-1} day^{-1} may potentially increase the risk of adverse events, and could be proposed as the UL for Leucine in healthy adults [47].

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Chapter 2
Leucine-Protein Supplemented Recovery and Exercise

Andre R. Nelson, Leonidas G. Karagounis, and David S. Rowlands

Key Points

- Short-term (2–3 days) post-exercise protein and protein-leucine feeding relative to negligible-protein isocaloric controls substantially improved subsequent endurance performance in well-trained men under conditions of negative to neutral nitrogen balance (dietary protein insufficiency); however, effects on performance under neutral to positive balance conditions may be equivocal.
- Long-term (weeks-months) protein-carbohydrate supplementation following endurance training in deconditioned skeletal muscle improves whole-body maximal oxygen uptake (aerobic power), relative to isocaloric carbohydrate feeding.
- Protein and leucine food or supplements ingested following endurance exercise may support skeletal muscle regeneration and adaptive remodelling in trained muscle via substantially increased mTORC1 pathway activity and moderate to large (effect size) increases in skeletal muscle protein FSR, relative to non-protein controls.
- A metabolic-mitochondrial transcriptome was activated at 48 h by post-exercise protein feeding in trained human skeletal muscle. This, and evidence to show that chronic BCAA (mice) or whey protein (aged men) feeding plus exercise led to improved mitochondrial biogenesis and endurance performance supports a role for dietary BCAA and protein in the development of skeletal muscle respiratory capacity.
- New transcriptome data suggest post-exercise protein and protein-leucine supports an inflammatory, promyogenic molecular programme common to regenerative wound healing biology in trained skeletal muscle.

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Keywords Protein-leucine • Endurance • Exercise-performance • Skeletal muscle • Remodelling • Adaptation • Inflammation • Transcriptome • Translation

Abbreviations

ABCA5 6, 7	ATP-binding cassette sub-family A members 5–7
ACOX1	Acyl-CoA oxidase 1 palmitoyl
ACSL1	Acyl-CoA synthetase long-chain family member 1
ADAM2 12, 18, 21	A disintegrin and metalloprotease domain family
ADAMTS2 5, 8, 13	A disintegrin and metalloprotease with thrombospondin motifs family
ADFP	Adipose differentiation-related protein (perilipin-22)
ALOX5	Arachidonate 5-lipoxygenase
APOC1	Apolipoprotein C-I
ARSA and ARSB	Arylsulfatase A and B
bHLH	Basic helix-loop-helix
BTRC	Beta-transducin repeat containing (gene)
CACYBP	Calcyclin-binding protein
CASP1 3, 4, 10	Caspase 1 apoptosis-related cysteine peptidase 1, 3, 4, 10
CAV2	Caveolin 2
CD	Cluster of differentiation
CD36	Thrombospondin receptor
CD44	Cluster of differentiation factor 44
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21)
COX4I2 COX7B, COX7B2	Cytochrome c oxidase subunit IV isoform 2, VIIb, VIIb2
CPT2	Carnitine palmitoyltransferase 2
CROT	Carnitine O-octanoyltransferase
CTSC CTSH, CTSK, CTSL, CTSO, CTSZ	Cathepsin (CTS) family
CYP1B1 CYP27B1, CYP2U1, CYP46A1, CYP7A1	Cytochrome P450 (CYP) family
DGKZ	Diacylglycerol kinase zeta
DLAT	Dihydrolipoamide S-acetyltransferase
DUSP1	Dual specificity phosphatase 1
ENO1	Enolase 1
ECM	Extracellular matrix
E-box	Enhancer box
FABP1 and FABP5	Fatty acid-binding protein 1 and 5
FA-CoA	Fatty acyl coenzyme A
FBXL19	F-box and Leucine-Rich Repeat Protein 19
FBXO18	F-box protein helicase, 18
FBXO32	F-box protein 32
FBXW2	F-box and WD repeat domain containing 2
FSR	Fractional synthesis rate
GADD	Family of growth arrest- and DNA damage-inducible factors
GCK	Glucokinase (hexokinase 4)
HADH	Hydroxyacyl-CoA dehydrogenase
HK1,2	Hexokinase 1,2

HMGCS2	3-Hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)
HIF1α	Hypoxia-inducible factor alpha
IGF-1	Insulin-like growth factor 1
IGFBP3	IGF-binding protein 3
IL1β	Interleukin 1β
ISYNA1	Inositol-3-phosphate synthase 1
LASS4 and LASS5	LAG1 homolog ceramide synthase 4 and 5
LDHAL6A and LDHB	Lactate dehydrogenase A-like 6A and lactate dehydrogenase B
LOC15076	Hypothetical protein LOC150763
LPIN1	Lipin 1
LPL	Lipoprotein lipase
mTORC1	Mammalian target of rapamycin 1
MDH2	Malate dehydrogenase 2 NAD (mitochondrial)
MDM2	Mdm2 p53 E3 ubiquitin protein ligase homolog
ME3	Malic enzyme 3 NADP(+)-dependent mitochondrial
MMP9,13 19	Matrix metallopeptidase 9, 13, 19
mRNA	Messenger ribonucleic acid
MRPL19	Mitochondrial ribosomal protein L19
MyoD	Myogenic differentiation factor 1
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide
NDUFA4, NDUFA5, NDUFAB1, NDUFS6	NADH dehydrogenase (ubiquinone) 1 family (NDUF) members
NPC1L1	Niemann-Pick disease type C1 gene-like 1
OSBP2 and OSBPL9	Oxysterol-binding protein 2 and protein-like 9
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1- α
PPARγ	Peroxisome proliferator-activated receptor γ
PDK4	Pyruvate dehydrogenase kinase isozyme 4
PECI	Peroxisomal D3,D2-enoyl-CoA isomerase
PFKFB3	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
PKM2	Pyruvate kinase muscle isoform
PLA2G2A	Phospholipase A2 group IIA
PLIN	Perilipin
PLTP	Phospholipid transfer protein
PPM2C	Pyruvate dehydrogenase phosphatase catalytic subunit 1
RNF19	Ring finger protein 19A E3 ubiquitin protein ligase
RNF34	Ring finger protein 34 E3 ubiquitin protein ligase
RNF39	152 ring finger protein 39, 152
SCD	Stearoyl-CoA desaturase
SDHC-SDHD	Succinate dehydrogenase complex subunit C and D
SLCO2A1 and SLCO1B1	Solute carrier organic anion transporter family
SLC25A20	Solute carrier family 25 (carnitine/acylcarnitine translocase)
STARD4	StAR-related lipid transfer (START) domain containing 4
STAT1/3	Signal transducers and activators of transcription 1/3
SREBP1,2	Sterol regulatory element-binding protein 1,2
SULT1A1 and SULT1C1	Sulfotransferase family cytosolic 1A and 1C
SQSTM1	Sequestome 1
TGFB-1	Transforming growth factor beta 1
TGFBR2	Transforming growth factor beta receptor 2
TIMP1 2	Tissue inhibitor of metalloproteinases 1 and 2

TRIM5	Tripartite motif containing 5
UBE2C	Ubiquitin-conjugating enzyme E2C
UBE2D3	Ubiquitin-conjugating enzyme E2D 3
UBE2J2	Ubiquitin-conjugating enzyme E2 J2
UCHL1	Ubiquitin carboxyl terminal esterase L1
UGT2B28	Uridine diphosphate glucuronosyltransferase 2 family polypeptide B28
UQCRC2 and UQCRH	Ubiquinol-cytochrome c reductase core protein 2
UQCRH	Ubiquinol-cytochrome c reductase hinge protein
USP2 49, 50	Ubiquitin-specific protease 2, 49, 50
VO_2 max	Maximum volume of oxygen
YME1L1	Yeast mitochondrial escape 1 like 1
YY1	Yin-yang 1

Introduction

The endurance-trained state is arguably the natural expression condition of human skeletal muscle [1]. Early humans experienced environmental selection pressure and migration out of Africa, that would have favoured a high physical endurance capacity [2, 3]. Improved endurance capabilities in early humans likely facilitated scavenging and persistence hunting and the co-emergence and increased post-exercise consumption of readily digestible protein and calorie intake [4]. Therefore, scavenging, hunting and gathering a high-protein diet [5] coupled with long-durations of endurance exercise (up to 8 h) supported not only the high energetic and tissue amino acid requirements of the musculoskeletal system, but also the metabolic demands of an increasingly larger brain mass contributing to social, cultural and technological development [5]. Animals would have been consumed within the hours following hunting, tracking or gathering, which is also when nutrient delivery to the exercised tissue is best because of transient increases in muscle blood flow, insulin sensitivity and glucose and amino acid uptake [6, 7]. Disturbances to muscle homeostasis from regular endurance exercise coupled with post-exercise hyperaminoacidaemia from a protein-rich diet might, therefore, be the normal environmental cues for adaptive remodelling in human skeletal muscle [8].

Indeed, recent evidence from experimental models in contemporary men suggests that consuming whole protein and protein plus leucine following endurance exercise plays an important role in promoting several aspects of skeletal muscle endurance-exercise adaptation, including mitochondrial biogenesis and function, ECM and microvascular plasticity [8–11]. We proposed leucine as an effective nutritive agent to promote enhanced skeletal muscle recovery from exercise owing primarily to its potent stimulatory effect on the rate of protein synthesis [12]. Enriching low-protein meals with leucine can increase post-prandial skeletal muscle mTORC1-pathway signalling, protein synthesis rates [13], and expression of some myocellular genes [14]. Leucine also enhances the secretion of insulin, which governs the expression of hundreds of skeletal muscle genes coupled to transcriptional and translational regulation, energy metabolism, intracellular signalling, the cytoskeleton, ubiquitin/proteasome pathways and the immune response [15]. Therefore, skeletal muscle plasticity common to exercise adaptation may accrue in response to post-exercise protein- and leucine-rich nutritional inventions.

In this chapter, we will discuss the role for dietary protein and leucine ingestion in supporting skeletal muscle regeneration processes following endurance exercise in trained skeletal muscle. We present the rationale for adding free-leucine to dietary protein, and evidence that protein-leucine feedings might augment the combined exercise-nutrient muscle response by boosting post-exercise regeneration processes and protein synthesis, which could be a beneficial strategy for athletes undertaking strenuous daily exercise.

Enhanced Skeletal Muscle Recovery from Exercise Is Unlikely to Be Associated with Improved Glycogen Storage

Modern endurance athletes frequently engage in repeated bouts of fatiguing exercise during training or competition, and sometimes training several times per day. Glycogen is the most important fuel substrate for intense exercise. Intense and prolonged exercise substantially reduces the concentration of muscle glycogen but post-exercise carbohydrate consumption induces hyperglycaemia and hyperinsulinaemia, the latter enhancing myocellular glucose uptake and glycogen synthase activity, thereby enhancing glycogen resynthesis [16]. The post-exercise glycogen resynthesis rate can be maximized by ingesting >1.2 g carbohydrate·kg body mass^{-1}·h^{-1} [17] but when ingesting <1.2 g carbohydrate·kg^{-1}·h^{-1}, the addition of insulinogenic protein hydrolysates and amino acids (e.g. leucine) can also enhance resynthesis [18]. However, when protein was co-ingested with a high rate of carbohydrate (1.2 g·kg^{-1}·h^{-1}) and compared to an isocaloric no-protein control (1.6 g carbohydrate·kg^{-1}·h^{-1}), which was similar to the high rates of carbohydrate ingested in well-controlled performance studies [9, 11, 19], protein feeding had no effect on glycogen concentrations at 3 h or 48 h post-exercise [8]. Therefore, enhanced glycogen resynthesis is unlikely to be an important adaptive mechanism resulting from the addition of protein and amino acids to carbohydrate ingested post-exercise. Instead, dietary-protein stimulated, non-glycogen mechanisms, relating to improved cellular integrity, faster restoration of contractile function and the accrual of new and adaptive proteins, appear more likely [20, 21]. With a suspicion of the mechanisms involved, our laboratory conducted a series of performance- and mechanisms-focused studies to determine the impact of protein or protein-leucine feeding following exercise on protein synthesis and other molecular processes aligned with tissue regeneration [8, 9, 11, 19].

High-Protein and Carbohydrate Feeding Substantially Enhanced Subsequent Endurance Performance Relative to Isocaloric High-Carbohydrate Control

In the first of these investigations, Rowlands et al. [19] found that high-dose protein (0.7 g·kg^{-1}·h^{-1}) coingested with sufficient carbohydrate to ensure maximal glycogen resynthesis (1.4 g·kg^{-1}·h^{-1}) and some fat during 4-h recovery from 2.5-h intense cycling provided no clear benefit to performance of a repeated-sprint cycling test the next day (+15 h), relative to isocaloric low-protein, high-carbohydrate (0.1 and 2.1 g·kg^{-1}·h^{-1}, respectively) and fat feeding. This finding confirmed earlier work in a well-controlled isocaloric feeding model [22] suggesting that subsequent endurance performance is largely impervious to added post-exercise protein nutrition when recovery is short, relative to isocaloric controls with high (≥1.2 g·kg^{-1}·h^{-1}) rates of carbohydrate intake. Therefore, adding protein to high rates of carbohydrate ingestion produced no additional benefit to glycogen restoration or performance within a 4–24 h period. However, by day 4 (+60 h) there was a substantial 4.1 % mean improvement in repeated sprint power with the high-dose feeding [19]. Furthermore, this performance effect was associated with positive nitrogen balance during day 1 recovery compared to negative nitrogen balance with the control, indicative of greater body-protein accrual, and lower blood creatine-kinase concentrations. The latter finding is common to several other short-term performance recovery investigations with protein-carbohydrate feeding [22–24] and is interesting because it suggests improved myocellular membrane stability or attenuated structural damage with post-exercise protein feeding; however, other than suspected beneficial effects on whole-body or muscle protein turnover the amino acid mediated mechanisms responsible were not forthcoming.

A Rationale for Adding Leucine to Protein after Exercise: Amplify Insulin Secretion and Protein Manufacture without Providing Energy and Nitrogen in Excess of What Is Necessary to Enhance Skeletal Muscle Recovery

Long-term energy balance (chronic daily energy intake matching expenditure) is important to endurance athletes for the purpose of maintaining lean body mass and a high power-to-mass ratio. Overconsuming protein above that required to make nitrogen balance while maintaining a stable body mass inevitably reduces intake of other macronutrients; there is a risk that this may somewhat compromise glycogen availability for high-intensity training or competition, although effects may be negligible because the extra amino acids contribute to the effective carbohydrate pool via increased gluconeogenesis [25]. Planned caloric reduction and/or an increased training volume are methods periodically used to reduce body fatness and improve the power-to-mass ratio; tissue catabolism is increased during times of energy deficit, but can be minimized by adequate dietary amino acid provision [26]. Nevertheless, a criticism of the Rowlands et al. [19] design was the high protein-dose provided. Consumption of a high-protein diet (3.6 $g \cdot kg^{-1} \cdot day^{-1}$) relative to low or moderate protein (0.8 and 1.8 $g \cdot kg^{-1} \cdot day^{-1}$, respectively) impairs post-endurance exercise muscle protein synthesis [27] and increases tissue-protein breakdown and amino acid oxidation without an increase in the resting protein synthesis rate [28], suggesting that habitual high-protein feeding might be a less-than-optimal dietary strategy if tissue protein synthesis is an important mechanism of adaptation. For these reasons, adding free-leucine and lowering the protein-dose in a meal should retain potency of anabolic signalling with a reduced overall nitrogen and caloric load, and may also support other aspects of skeletal muscle plasticity to exercise via the insulin response and other pathway-associated gene expression.

With respect to the increased protein synthesis, the stimulatory effect of dietary protein on skeletal-muscle protein turnover is mediated largely by increases in the concentration of blood and muscle leucine, stimulating translation via enhanced intracellular mTOR-pathway signalling [12, 29, 30]. Recent evidence from EAA-cultured C2C12 myotubes indicates leucine has ~3 times the p70S6K- and rpS6-phosphorylating potency of other EAAs, whereas the effect of valine and isoleucine was insubstantial [29]. Furthermore, EAA signalling potency appeared limited to regulation of mTOR, p70S6K and rpS6 phosphorylation (translation initiation) rather than elongation via eEF2 and eIF2α [29]. Leucine increases expression of mRNAs for several myofibrillar proteins (myosin heavy chain-slow and heavy chain-fast and myosin light chain-1 and -3) via increased 4E-BP1 and S6K1 phosphorylation and mTOR-independent mechanisms [14] suggesting leucine-mediated mechanisms include effects on pre-translational events. Leucine may also impact protein turnover by lowering post-exercise proteolysis [31, 32].

The insulinotropic effect of leucine may also promote post-exercise tissue recovery. For instance, adding free-leucine (0.1 $g \cdot kg^{-1}$) to whey protein and carbohydrate (0.3 $g \cdot kg^{-1}$ and 0.7 $g \cdot kg^{-1}$, respectively) further increased insulin secretion over that induced by whey protein and carbohydrate or carbohydrate (0.7 $g \cdot kg^{-1}$) alone [33]. Leucine-induced insulin secretion increases muscle blood-flow and, therefore, nutrient delivery [34]. Insulin also affects protein turnover by reducing protein breakdown but has little effect on the rate of muscle protein synthesis, with or without amino acid provision [35]. Nevertheless, insulin has widespread effects on gene transcription, regulating ~800 genes in rested skeletal muscle [15]. In healthy humans, insulin infused at physiological concentrations acted synergistically with amino acids to increase mitochondrial ATP rate, mitochondrial-gene expression (NADH dehydrogenase subunit IV and COX subunit IV), mitochondrial protein synthesis and COX and citrate synthase enzyme activity [36, 37]. Thus, superimposition of leucine- and insulin-regulated mitochondrial biogenesis may combine to enhance some aspects of the skeletal muscle adaptive response to endurance exercise.

While leucine alone stimulates aspects of cellular growth, ingesting complete protein is still necessary to provide amino acid substrate for protein synthesis and to avoid a rebound reduction in the plasma concentration of isoleucine, valine [38] and glutamine (cellular glutamine efflux is coupled to leucine influx [39]) to support protein synthesis [13]. Whole proteins may also provide potentially beneficial bioactive peptide digestion products [40]. Next we asked whether a lower quantity of protein but with added free-leucine [19] could also elicit a worthwhile benefit to subsequent endurance performance and mechanisms associated with skeletal muscle recovery from exercise stress.

Post-exercise Protein-Leucine Supplementation Improved Subsequent Performance Under Nitrogen Stressed Conditions

Thomson et al. [9] reported a worthwhile benefit of post-exercise protein-leucine feeding to subsequent high-intensity endurance cycling performance. Leucine (0.1 $g \cdot kg^{-1} \cdot h^{-1}$) was added to whole protein, carbohydrate and fat (0.4/1.2/0.2 $g \cdot kg^{-1} \cdot h^{-1}$, respectively) ingested during the first 90 min of post-exercise recovery during 3 days of high-intensity cycling. A tightly controlled crossover design was utilised whereby the specific post-exercise effect of high-protein recovery feeding was isolated by providing the alternate supplement (isocaloric low-protein, carbohydrate and fat, 0.06/1.6/0.2 $g \cdot kg^{-1} \cdot h^{-1}$, respectively) at the opposite end of the day; this also enabled dietary protein intake to be clamped at 1.6 $g \cdot kg^{-1} \cdot day^{-1}$ with a controlled background diet, thereby removing total daily protein intake as a variable [9]. Average nitrogen balance (total amino acid nitrogen intake minus nitrogen loss) was marginally negative during the experimental period in both protein-leucine fed and control conditions, indicating that the benefit of protein-leucine to recovery performance occurred under mild nitrogen stress. Protein-leucine feeding was also found to lower the plasma creatine-kinase concentration and reduced perceived tiredness during repeated sprint performance tests.

From analysis of the confidence interval, we noted the possibility that the true effect of protein and protein-leucine feeding on recovery of endurance performance within several days could also be trivial [9, 19]. Indeed, when a protein-leucine, carbohydrate and fat supplement (20/7.5/89/22 $g \cdot h^{-1}$, respectively) or isocaloric carbohydrate/fat control (119/22 $g \cdot h^{-1}$) was administered for 1–3 h after exercise during a 6-day training block, and with the background diet comprising 1.9 g $protein \cdot kg^{-1} \cdot day^{-1}$ (protein-leucine) and 1.5 g $protein \cdot kg^{-1} \cdot day^{-1}$ (control) yielding a mildly positive nitrogen balance in both dietary conditions, subsequent high-intensity performance was not substantially affected by the protein-leucine recovery feeding [11]. And yet, protein-leucine feeding was still associated with reductions in creatine kinase of similar magnitude to that found earlier by our group [9, 19, 22] and others [23, 24]. These findings are important because they suggest a role for daily dietary nitrogen balance on the ergogenicity of post-exercise protein-leucine feeding in endurance athletes. The data indicate that protein-leucine supplementation in the immediate hours post-hard endurance exercise is most useful for aiding recovery when the remaining daily diet is mildly deficient in protein or amino acids, leading to a neutral-to-negative nitrogen balance physiological environment.

Long-Term Protein Supplementation: Do the Phenotypic Outcomes Match the Suspected Mechanistic Inference?

Although there are few well-controlled studies, chronic consumption of protein following endurance exercise training in healthy but previously untrained participants has been found to increase aerobic power (maximal oxygen consumption, VO_2max) indicating a benefit to endurance exercise

performance. Robinson et al. [41] found that chronic post-exercise protein-carbohydrate supplementation (20/55 g, respectively) in older men and women during 6 weeks of treadmill run training did not substantially impact long-term skeletal muscle protein synthesis rates relative to isocaloric carbohydrate (75 g), but resulted in a greater improvement in VO_2max (12.2 % ± SD 6.2 % versus carbohydrate 3.3 ± 8.7 %) [41]. In healthy young men and women, post-exercise chocolate milk consumption (mean intake of protein/carbohydrate: 0.94/0.31 g·kg^{-1}, respectively) following 4.5 weeks of aerobic cycle training improved body composition (lean-mass:fat-mass) and resulted in moderate effect-sized increases in absolute (~0.15 L·min^{-1}) and relative (~3 mL·kg^{-1}·min^{-1}) VO_2max versus isocaloric carbohydrate supplementation [42]. Although improvements in microvascular [8] and mitochondrial plasticity [10] associated with improved skeletal muscle growth and development in previously deconditioned skeletal muscle could account for the improvements in VO_2max, cardiovascular adaptations, such as, increased blood volume [43], might also contribute. Furthermore, these findings may not necessarily apply to well-trained athletes who already possess chronic hematological, microvascular and mitochondrial adaptations, warranting further research in trained endurance athletes on the potential impact of chronic post-exercise protein-leucine feeding on VO_2max and endurance performance.

Effect of Post-endurance Exercise Protein and Protein-Leucine Ingestion on Candidate Molecular Mechanisms Guiding Nutrient-Modulated Skeletal Muscle Regeneration

Accumulating new data provides insight into how elevated amino acid concentrations following post-exercise protein feeding may guide homeostatic regeneration and adaptive remodelling in skeletal muscle. Some insight has come from the study of protein turnover and candidate genes, but a greater leap forward in understanding of the post-exercise molecular programme guiding protein-leucine fed regeneration has arisen from inferences obtained from bioinformatic analysis of the transcriptome.

With regard to mixed muscle protein FSR, the mean effect size (ES) increase from adding protein to carbohydrate ingested post-endurance exercise, relative to isocaloric or carbohydrate-matched control conditions, ranges from small (ES 0.49) to large (ES 1.60) (Table 2.1). While there is good evidence to suggest that the mitochondrial fraction may contribute substantially to FSR after endurance exercise [44], Breen et al. [45] have recently reported that only myofibrillar FSR, but not the mitochondrial rate, increased in the first 3 h following endurance exercise in response to protein-carbohydrate feeding (Fig. 2.1). This suggests that either the mitochondrial fraction is less responsive in the recovery timeframe assayed, increases temporally more distal to exercise, or that the post-exercise mitochondrial protein synthesis rate is already maximally activated by endurance-exercise stimuli.

Increased blood amino acid concentrations, and in particular leucine, increase skeletal muscle FSR largely through activation of translation initiation via the mTORC1 signalling pathway [12]. mTORC1 activation has also been associated with stimulation of bHLH-motif transcription factors that bind E-box regions in the promoter regions of genes regulating ribosomal RNA synthesis via RNA polymerase gene expression [46, 47], and a number of other genes also associated with the myofibrillar adaptive response to contractile stimuli: STAT1 and STAT3 (regulating PPARγ) [48], HIF1α, SREBP1 and 2, and YY1 [49–51]. Transcription factor HIF1α upregulates the rate-limiting enzyme in glycolysis, pyruvate kinase M2 [51] and HIF1α and SREBP1/2 stimulate glycolysis and lipid biosynthesis, pentose phosphate pathway activity and anabolic cell growth and cell proliferation [50]. mTOR further controls transcription of mitochondrial components via its action on YY1-governed PGC-1α expression [49]. Regular protein-leucine nutritional mediation of the expression of mRNAs associated with ribosomal, metabolic, mitochondrial and myogenic functions could therefore promote increased functional protein expression and superior tissue remodelling when coupled with increased cellular protein FSR.

Table 2.1 Comparison of the effects of post-endurance exercise protein-carbohydrate ingestion relative to carbohydrate-control or placebo on mixed-muscle, myofibrillar, and mitochondrial protein fractional synthesis rates (FSRs) determined via tracer-incorporation

Reference Cohort	Exercise mode Duration: Intensity ± SD (%VO_2max/peak, if published)	Mean FSR ± SD%·h^{-1}	FSR Comparison ES ±90%CL[a] Inferences[b]
Howarth et al. [63] Six healthy men	Cycle 120 min: Variable intensity (50–80 %)	PRO: 0.088 ± 0.015 L-CHO: 0.066 ± 0.018 H-CHO: 0.060 ± 0.018	PRO vs L-CHO 1.31 ± 0.65; large increase likely PRO vs H-CHO 1.60 ± 0.80; large increase very likely
Harber et al. [64] Eight trained men	Cycle 60 min: 72 % ± 3 %	PRO: 0.129 ± 0.040 Placebo: 0.112 ± 0.028	PRO vs Placebo 0.49 ± 0.40; small increase likely
Breen et al. [45] Ten well-trained male cyclists	Cycle 90 min: 77 ± 3 %	Mitochondrial PRO: 0.082 ± 0.032 CON: 0.087 ± 0.051 Myofibrillar PRO: 0.057 ± 0.024 CON: 0.057 ± 0.023	Mitochondrial: PRO vs CON -0.10 ± 0.07; trivial difference almost certain Myofibrillar: PRO vs CON 1.32 ± 0.90; large increase likely
Lunn et al. [65] Six trained male runners	Run 45 min: 65 %	MILK: 0.110 ± 0.024 CON: 0.077 ± 0.024	MILK vs CON 0.99 ± 0.78; moderate increase very likely

All data are presented as mean ± SD; for data published as mean ± standard error of the mean (SEM), e.g. subject characteristics and fractional synthesis rate (FSR) [63–65]; or FSR [45], the SD was determined ($SD = SEM \times \sqrt{n}$)
Absolute VO_2max ($L \cdot min^{-1}$) data [63] were converted to relative VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) based on the published participant mean body mass
[a]Add and subtract this number by the mean effect to obtain the upper and lower 90 % confidence limits
[b]Inferences are based on the smallest important effect, defined as the pharmacokinetic threshold criteria of a 20 % difference. ES thresholds: <0.2 trivial, <0.6 small, <1.2 moderate, <2.0 large, <4.0 very large, >4.0 extremely large. Thresholds for assigning qualitative terms to chances of substantial effects: <0.5 %, almost certainly not; <5.0 %, very unlikely; <25 %, unlikely; <75 %, possible; >75 %, likely; >95 %, very likely; >99.5 %, almost certain

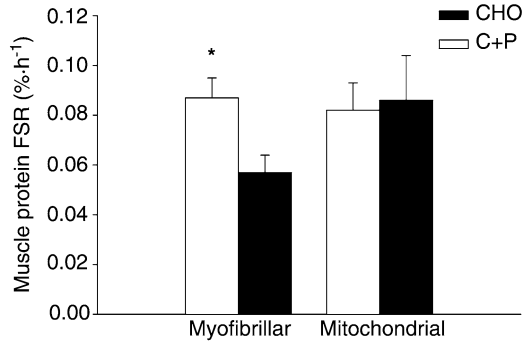

Fig. 2.1 Myofibrillar ($n = 10$) and mitochondrial ($n = 8$) fractional synthetic rate following protein plus carbohydrate (C + P; 25 g carbohydrate, 10 g whey protein) or carbohydrate only (CHO, 25 g) feeding immediately and at 30 min following 90 min of endurance exercise at 77 % maximal oxygen uptake in trained men [45]. Values are means ± SEM. Image obtained by permission from John Wiley and Sons, Boston, MA

Protein-Fed Post-Endurance Exercise Skeletal Muscle Transcriptome Describes Metabolic and Mitochondrial Gene Expression Occurring after Inflammatory Promyogenic Regeneration Responses

Recent transcriptome data [8] suggests no impact of protein nutrition on the metabolic-mitochondrial transcriptome until ~48 h post-exercise in men, at which time, PGC-1α and other mitochondrial gene expression were elevated in response to post-exercise protein feeding 2-day prior (Fig. 2.2). Mitochondrial respiratory capacity and endurance performance increased in endurance training mice chronic fed on branched chain amino acids [10]. Given the high mitochondrial protein content in muscle (~30 % [52]) and association of total mitochondrial volume with skeletal muscle respiratory capacity and endurance [53], boosting mitochondrial biogenesis by increasing BCAA availability might be a key functional adaptation to protein feeding coupled to endurance exercise. To provide an unbiased global picture of the impact of exercise on gene expression guiding remodelling, Mahoney et al. [54] used high-throughput gene microarray to show that a single bout of endurance exercise in lean healthy men stimulates the expression of genes involved in the oxidative stress response, electrolyte shuttling, transcription, proteolysis, cell growth and death and metabolism. Taking a similar novel discovery approach, our group determined the skeletal muscle transcriptome specifically induced in response to milk protein feeding following endurance exercise [8]. At 3 h following exercise, the gene expression profile was consistent with increased ECM protein expression and turnover, attenuated stress response and cell stability, activation of myocellular growth and development, modulation of cellular inflammatory and immunity and defence mechanisms [8]. With respect to immunity and defence, evidence for nutrition-modulated inflammatory leukocyte activity is interesting because neutrophils and macrophages are crucial for phagocytosis of damaged tissue and stimulation of successful skeletal muscle regeneration and myogenesis after exercise [55, 56]. Upregulated ECM protein turnover and regulated expression of matrix metallopeptidases and inhibitors (increased MMP9, MMP13, MMP19; decreased TIMP1, TIMP2; Fig. 2.3) suggested post-exercise protein feeding may also influence control over basement membrane degradation and ECM remodelling, facilitating recruitment of myogenic, myeloid, vascular and fibroblastic cells to damaged muscle [55], and open the interface for leukocytes, cytokines and myogenic growth factors [57].

In our 6-day performance study we found some evidence for modulated circulating-neutrophil function during recovery [58] and greater ECM protein turnover [11] lending further support for a role for protein-leucine ingesting during recovery in physiological support for athletes. Employing mass spectrometry-based metabolomics, we studied blood and urinary metabolites associated with whole-body amino acid and lipid turnover. Post-exercise protein-leucine feeding increased the mean 3-h recovery concentration of plasma amino acids (glycine, arginine, glutamine, leucine) and myristic acid metabolites (C14, myristoylcarnitine; and C14:1-OH, hydroxymyristoleylcarnitine) with neutrophil priming capacity, and reduced neutrophil superoxide production (NADPH oxidase activated respiratory burst). By day 6, however, the protein-leucine supplement reduced pre-exercise cortisol and acylcarnitine C16 (palmitoylcarnitine) during exercise, and increased post-exercise neutrophil superoxide production, relative to control. Although it is only one measure of neutrophil function, activation of the superoxide-generating NADPH oxidase complex is critical to neutrophil microbicidal capacity [59] and increased release of superoxide might benefit neutrophil cytotoxic activity, especially during intense exercise training.

Protein-leucine supplementation also increased urinary excretion of proline metabolites, including L-proline, hydroxyproline-derived proline, and glycylproline (Fig. 2.4). Hydroxyproline is a major component of collagen and plays a key role in collagen stability [4] and increased excretion is suggestive of greater ECM protein turnover. We provided 130 mg leucine·kg^{-1}·h^{-1} (free and protein bound) over 3 h following cycling. Increased post-exercise plasma C3 and C5 acylcarnitine concentrations indicate that this rate of leucine ingestion exceeds leucine degradation pathway enzymatic

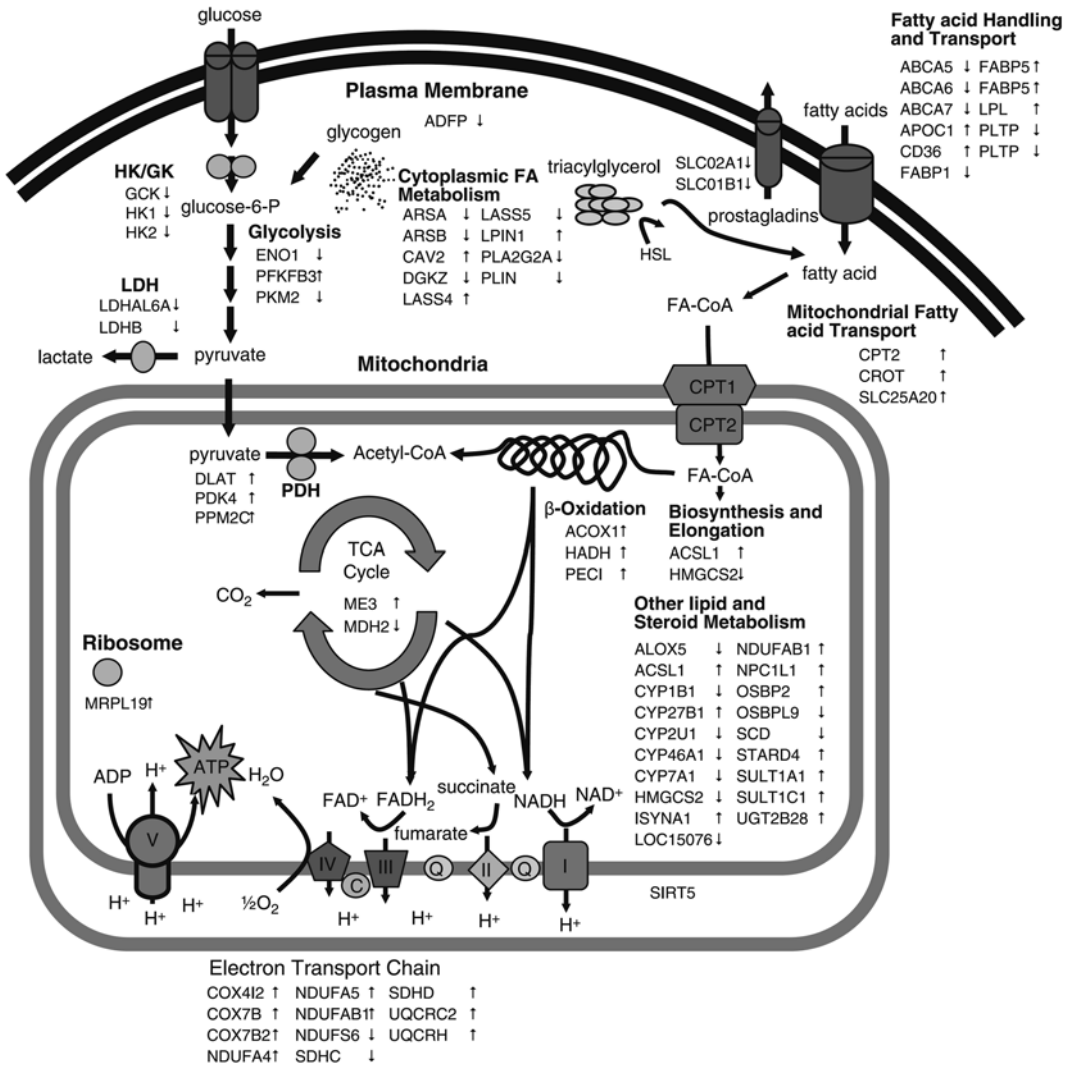

Fig. 2.2 Effect of post-endurance exercise protein versus control nutrition on expression of genes involved in the regulation of carbohydrate (glycolysis) and fatty acid transport, lipid and steroid metabolism in the skeletal muscle at 48 h post-exercise [8]. Gene expression *up arrow*/*down arrow*. Image obtained by permission from The American Physiological Society

activity after an acute bout of endurance exercise. Reduced urinary losses of BCAAs, proline, methionine and α-aminoisobutyrate (a product of both valine and pyrimidine metabolism) during subsequent exercise suggested reduced turnover or retention of these amino acids and metabolites, and more widespread alterations to concentrations of plasma and urinary amino acids and their metabolites at rest and during exercise by day 6 suggests adaptive processes in response to higher dietary intakes of leucine and protein.

With building evidence to show that post-exercise dietary protein and leucine intake modulates skeletal muscle gene expression and increases protein synthesis in a dose-sensitive fashion [13, 14, 60], we next studied the effect of three quantities of protein-leucine (control, zero protein-leucine; high dose, 70 g whey protein/15 g leucine; low dose, 23 g whey protein, 5 g leucine) co-ingested with carbohydrate and fat over the first 90 min following intense cycling on the skeletal muscle

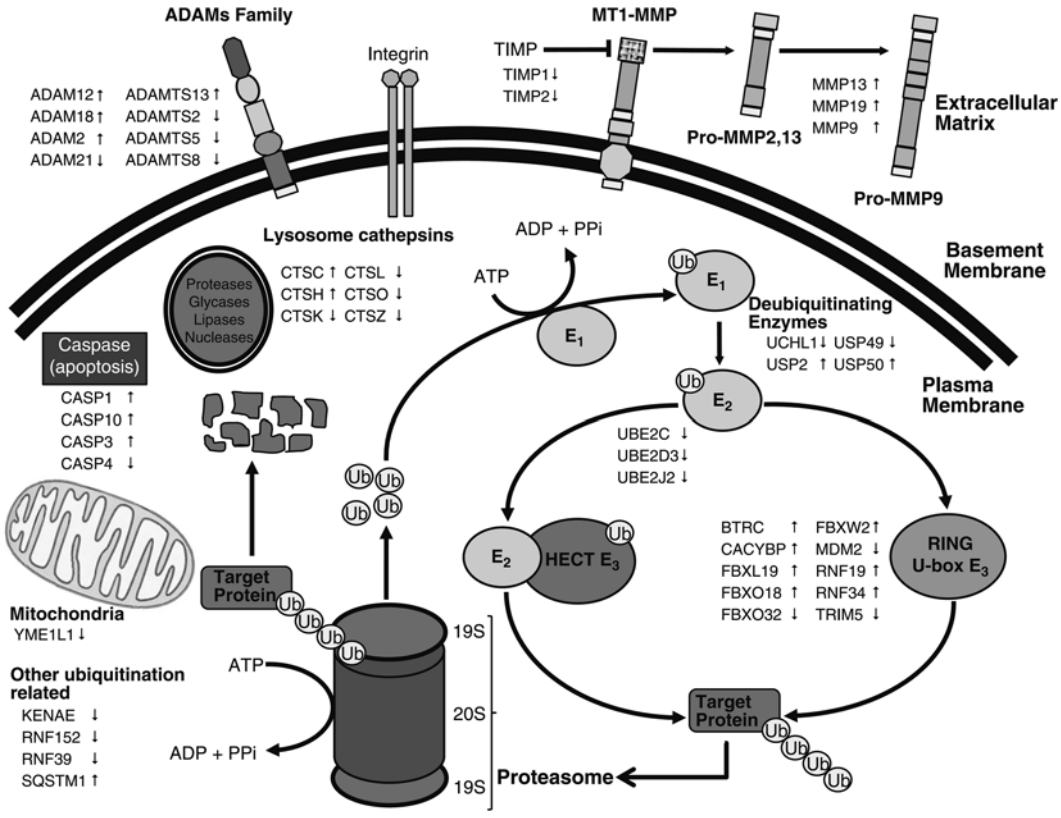

Fig. 2.3 Effect of post-endurance exercise protein versus control nutrition on expression of genes involved in protein modification and breakdown at 3 h post-exercise [8]. Summarized are genes classified under the molecular function ontologies: cysteine peptidase, metallopeptidase, and ubiquitin-specific protease activity. Gene expression *up arrow/down arrow*. Image obtained by permission from The American Physiological Society

transcriptome at 30 and 240 min post-exercise [61]. Bioinformatic interrogation of high-throughput gene microarray revealed a proinflammatory transcriptome associated with increased leukocyte migration most evident with the high-dose protein-leucine at 30 min into recovery, and reverting by 240 min to an IL-6-centered antiinflammatory promyogenic molecular programme with both protein-leucine quantities, relative to control [61]. The central hubs regulating increased leukocyte migration were IL1β and CD44 and connected immune-cell differentiation and connective tissue remodelling factors to construct a cell-growth regulatory network that included IGF-1 and IGFBP3, TGFB1 and TGFBR2, ECM function, remodelling, adhesion genes (e.g. decorin, biglycan, versican, tenascin, lumincan, connective-tissue growth factor) and others involved in macrophage activation and adhesion (CD86, CD44, CD163, CD14, CD68) (Fig. 2.5). Meanwhile, other modular hub gene regulation was consistent with myogenic or satellite cell activation (MyoD, myogenin) and cell cycle control consistent with cell cycle arrest and increased cell stability via CDKN1A, GADD45A/B/G and DUSP1. The transcriptome suggests protein-leucine feeding upregulated an early-phase myeloid-cell associated regeneration response reflecting wound-healing biology [56].

Inflammation may contribute to muscle repair and subsequent remodeling by stimulating resident and infiltrating immune cells (monocytes, macrophages and neutrophils) to cleanup cellular debris in muscle, stabilize membrane structure and the release of soluble factors to promote myogenesis and inflammatory resolution [56, 62]. Protein-leucine mediated restorative remodelling of muscle ECM and membrane stability may explain attenuated blood concentrations of muscle-membrane damage

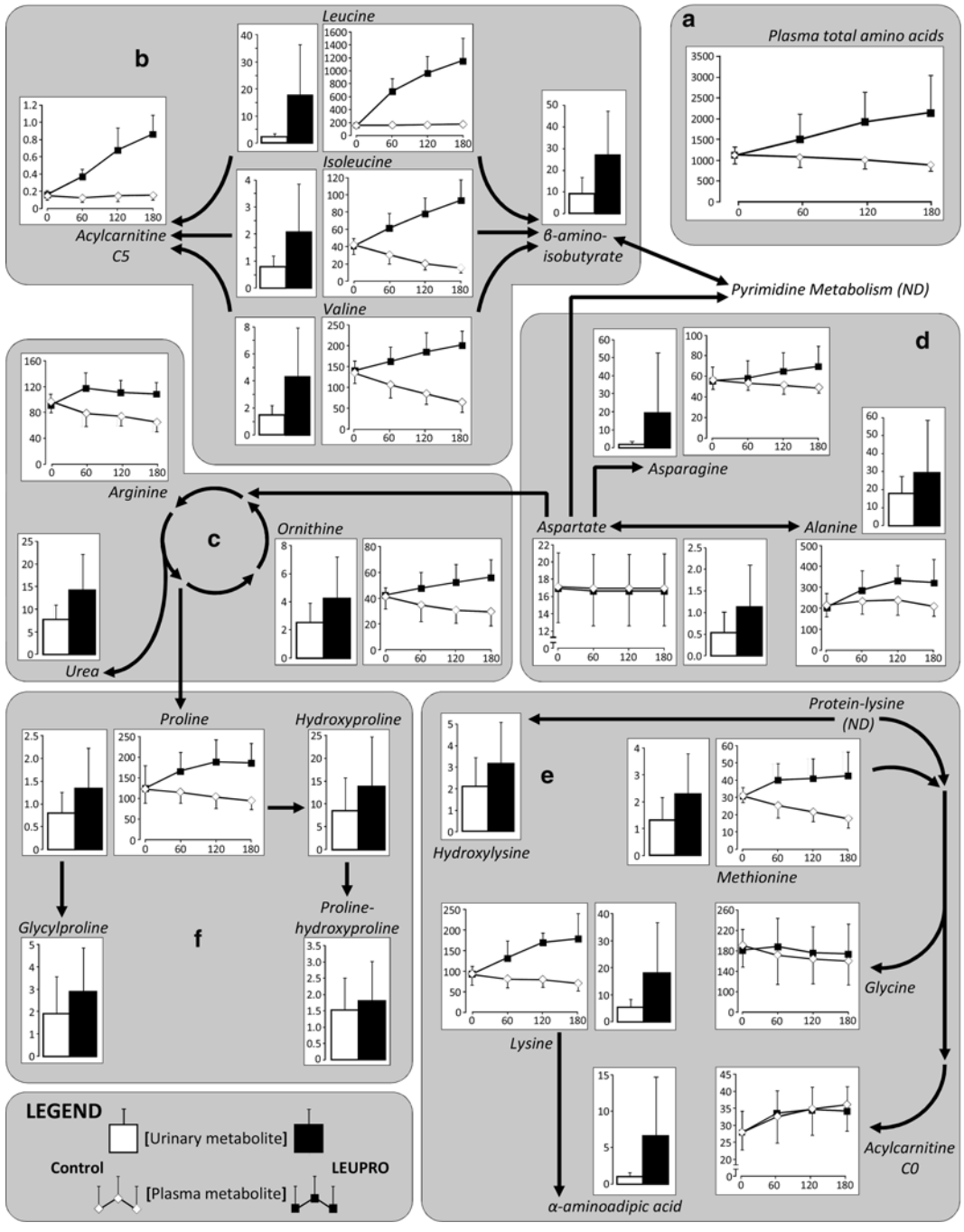

Fig. 2.4 Effect of 3-h protein-leucine or isocaloric control post-endurance exercise feeding on plasma and urinary metabolite concentrations during recovery from exercise on day 1 of a 6-day intense cycling protocol [11]. Shown are the responses of plasma essential and total amino acid concentration (**a**), and plasma and urinary concentrations of substrates and metabolites relating to the branched chain amino acids (**b**), the urea cycle (**c**), the metabolism of alanine and aspartate (**d**), the degradation of lysine (**e**) or the metabolism of arginine and proline (**f**). Data are means ± SD. Concentrations of plasma metabolites are in micromoles per litre and urinary metabolites in nanomoles per litre. ND, not determined. Image obtained by permission from Wolters Kluwer Health

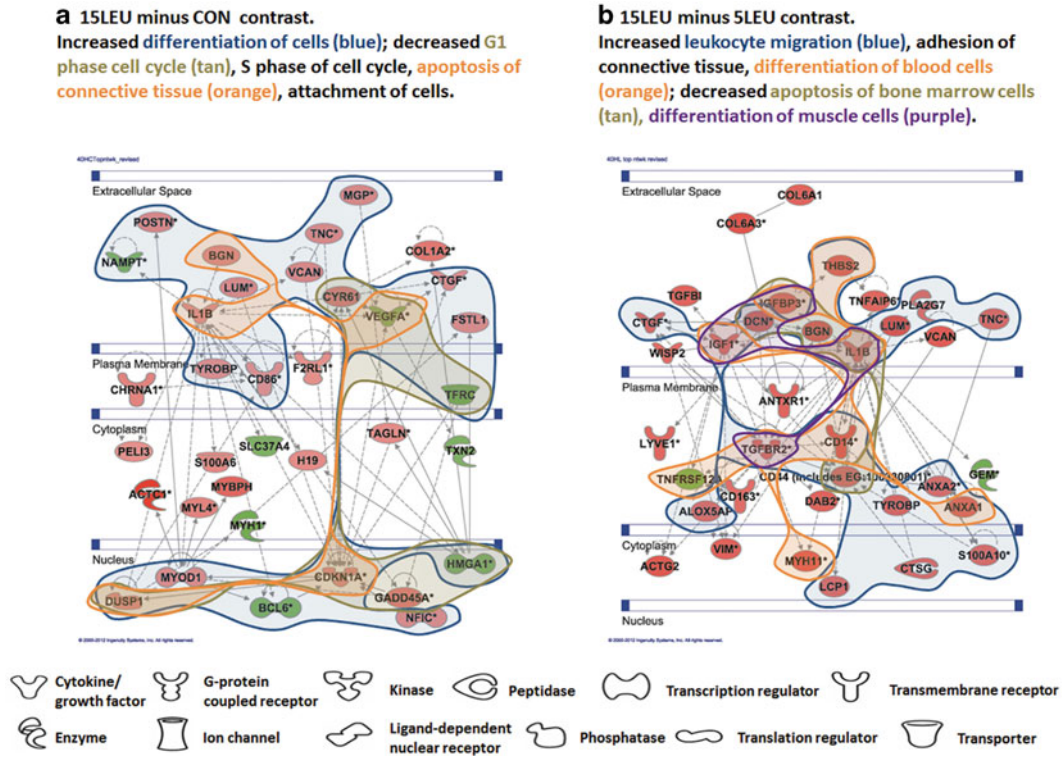

Fig. 2.5 Two top ranked Ingenuity Pathway Analysis (www.ingenuity.com) networks representing the transcriptome responding to protein-leucine quantity at 30 min into recovery from cycling exercise: (**a**) the high-dose (15LEU) vs control dose (CON), and (**b**) the 15LEU vs the low-dose (5LEU) contrast. Selected molecular function modules (coloured shading and labelling) are representative of the top-ranked within-network biology. Symbol colour indicates the direction of gene regulation: *green*, down-regulated; *red*, up-regulated. For brevity, full gene names and abbreviations are not included

marker in the blood (creatine kinase) previously observed in the days following post-exercise protein-leucine feeding [9, 11]. Combined with other evidence for increased protein synthesis [11, 13] and a myogenic transcriptome, these mechanisms could, in part, explain the reported improvement in performance following 3-days of ingesting similar quantities of protein and leucine after intense endurance cycling [9]. A summary of the working evidence for the principle molecular programme activated by protein and protein-leucine feeding following intense endurance exercise is shown in Fig. 2.6.

Conclusions

From early-human hunter-gatherers through to modern athletes, endurance exercise and post-exercise hyperaminoacidaemia has likely played an important role in human skeletal muscle health and performance. Protein-mediated mechanisms are likely to involve modulation of skeletal muscle protein turnover and gene expression to confer a functional benefit to endurance-exercise induced plasticity. Although high-dose protein feeding conferred a benefit to performance several days after initial exercise, long-term high-dose protein might overload nitrogen metabolism and negatively impact protein turnover; however, adding leucine to protein feeding amplifies insulin secretion, mTOR-signalling and protein synthesis with a reduced nitrogen load, relative to the same signalling potency with a

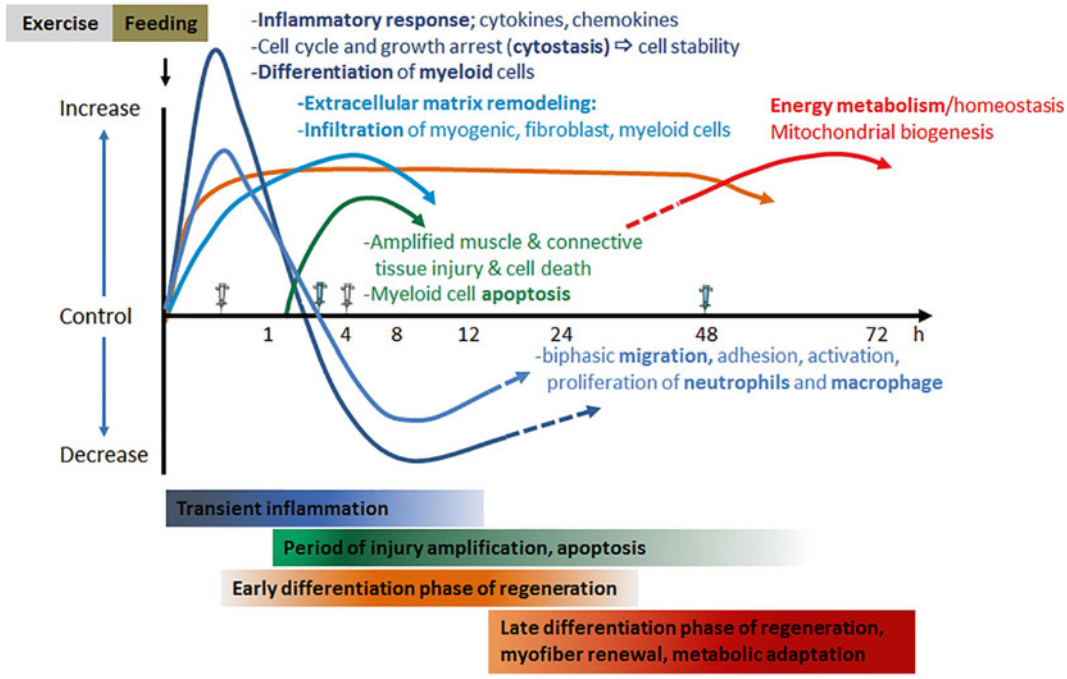

Fig. 2.6 Model for the effect of protein and protein-leucine feeding on modulation of the molecular programme regulating skeletal muscle regeneration in response to intense endurance exercise, constructed by inference from molecular functions obtained from the analysis of the transcriptome; needles indicate biopsy samples, blue from [8] and clear from [61]

higher dose of whole protein. The accumulated evidence from our recent investigations in multiday high-intensity cycling suggests that subsequent performance is enhanced with post-exercise protein and protein-leucine feeding when athletes are under mild nitrogen stress, but unaffected when in positive balance. Isotope and multiomic data analysis suggests that protein-leucine feeding modulates a proinflammatory, promyogenic skeletal muscle cellular response, and later may activate and support cellular growth and mitochondrial biogenesis. Long-term studies in sedentary populations suggest increased aerobic power with peri-training chronic protein feeding, but further research is required in trained athletes.

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Chapter 3
Use of Whey and Leucine on Muscle

Pablo Christiano Barboza Lollo, Jaime Amaya-Farfan, Carolina Soares Moura, and Priscila Neder Morato

Key Points

- Whey protein plus L-leucine increase the activation of cellular anabolic pathway.
- Whey protein plus L-leucine affect the glucose homeostasis.
- Hormonal response to co-ingestion of whey protein plus L-leucine is unique.
- Appetite and body composition can be altered by co-ingestion of whey protein.
- Whey protein plus L-leucine can activate anabolic mTOR pathway in heart and diaphragm.

Keywords Whey protein • Hydrolysates whey protein • Anabolism • Bioactive peptides • Protein supplementation

Abbreviations

mTOR	Mammalian target of rapamycin
GLUT-4	Glucose transporter 4
BCAAs	Branched chain amino acids

Introduction

Among the biological functionality of L-leucine is the ability to reduce catabolism, increase insulin levels and stimulate protein synthesis through activation of the mammalian target of rapamycin (mTOR) pathway and the S6K1 that might have been implicated in the stimulation of muscle protein synthesis; whey protein is a rich source of essential amino acids and can stimulate anabolism and beyond to supply amino acids that can stimulate release of insulin. The role of L-leucine as a regulator

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of muscle protein metabolism has been studied extensively but not the combined ingestion of L-leucine plus whey protein; the first study with this approach is from 2005 and at present at least 20 papers, in different experimental approaches (elderly, young, long-term supplementation, acute, humans, animals, muscles, heart, diaphragm), are published.

Another role of L-leucine is the modulation of appetite, like whey protein that also modulates several hormones that influence body composition. The mental and physical behavior, probably by changes in neurotransmitter levels [1] can be affected by L-leucine; details about the features of L-leucine, as well as the proposed mechanisms for such effects, are discussed in detail in other chapters of this book. Thus, supplementation with L-leucine is potentially capable of modulating muscle protein synthesis. As L-leucine is an important modulator of cellular protein synthesis, the ingestion of L-leucine together with a protein that provides the amino acids necessary for protein synthesis occurs as a nutritional strategy to enhance the anabolism. So many researchers interested in increasing the protein synthesis, tested this nutrition strategy, supplemented with L-leucine combined with a protein, as a signaling to cell anabolism plus amino acids for protein synthesis.

To maximize the strategy of muscle protein synthesis, it is necessary that protein intake with L-leucine contains all the essential amino acids in optimal proportions to the organism building its own proteins, beyond which, the protein chosen should be easily digestible and highly absorbable; precisely features that are characteristic to the whey protein. For these reasons, whey proteins are particularly appropriate to increase anabolism compared to just any other protein. Following this rationale, most studies that aim to increase protein synthesis combining supplemental L-leucine plus a protein, choose the whey protein as an excellent amino acid provider. The increased muscle protein synthesis may be useful in some situations, such as improvement of athletic performance, disease causing atrophy, aging or cachexia caused by cancer (not tested yet).

Whey Protein

Whey protein is a soluble protein that represents about 20 % of the dairy proteins in bovine milk, and has been acknowledged for its high nutritive value and digestibility [2] thus providing a fast supply of amino acids and peptides [3] and a unique hormonal response. With these features, the whey protein can stimulate a anabolism, what is attractive for situations of catabolic stress, like physical exhaustion [4], muscle mass loss [5] and immune deficiencies, amongst others [6]. Therein, the whey protein has been used as nutritional supplement and as ingredient of processed dairy food [7].

Milk and dairy products are inversely associated with a lower risk of metabolic disorders and cardiovascular diseases [8], and the whey protein can improve the metabolism by unknown mechanisms [9]; Morifuji et al. [10] reported an increase of fatty acid synthesis in the muscle, accompanied by a concomitant decrease in the liver, which was considered to be a positive effect on lipid metabolism in rats fed with whey protein. Additionally, the whey protein is associated with positive effects on the health, due to the production of hypertensive peptides [11], increase in skeletal muscle [12] and myocardial glycogen [13], increase in translocation of glucose transporter 4 (Glut4) to the plasma membrane [14], cellular glucose influx [15], heat shock proteins in exhausted rats [16] and decrease in the muscle damage indicators in soccer players [17] and appetite through modulation of satiety hormones [18, 19].

It is pertinent to note that the concentrations of BCAAs (branched chain amino acids) of whey protein, including L-leucine, are high (Table 3.1), the BCAAs represent around 21.2 % of the amino acids of whey proteins, or approximately 48 % of the essential amino acids. Cysteine is another amino acid in higher concentration in whey protein, up to fourfold higher than casein and soy (Table 3.1).

Both, BCAAs and cysteine are important amino acids to muscle mass; BCAAs and mainly L-leucine has been related to muscle anabolism [20], for more details see chapter about L-leucine and anabolism. Hack et al. [21] have shown that the regulation of the plasma cysteine level is disturbed in

Table 3.1 Amino acid composition of different milk proteins (in percent)

	Soya	Casein	Whey protein concentrates	Whey protein concentrates	Whey protein hydrolysates
L-alanine	3.95	2.68	5.03	5.02	4.91
L-arginine	7.80	3.53	2.62	2.66	2.32
L-asparagine	11.85	6.94	11.34	11.31	11.21
L-cystine	1.22	0.19	1.46	1.48	1.61
L-glutamic acid	20.46	22.11	18.52	18.55	18.07
Glycine	4.06	1.62	1.71	1.67	1.76
L-histidine	2.54	2.47	1.29	1.28	1.28
L-isoleucine	4.87	5.25	6.77	6.73	7.00
L-leucine	7.70	8.87	9.98	9.96	10.19
L-lysine	6.07	7.70	9.05	9.08	9.52
L-methionine	1.12	2.70	2.47	2.46	2.53
L-phenylalanine	5.36	4.53	2.81	2.84	2.79
L-proline	5.26	10.30	5.80	5.78	5.70
L-serine	5.47	5.45	5.22	5.21	5.06
L-threonine	3.75	4.14	7.52	7.54	7.43
L-tyrosine	3.75	5.32	2.83	2.85	2.79
L-valine	4.77	6.24	5.59	5.59	5.83

conditions with progressive skeletal muscle catabolism including cancer, HIV infection and old age. This composition and feature, of fast rate of protein digestion and amino acid absorption, make a whey protein a fast dietary protein source to supply essential amino acids, necessary substrates for protein synthesis and to stimulate anabolic pathways. The speed at which amino acids are absorbed has been indicated as a regulator of protein anabolism [22]. Additionally, the whey protein and L-leucine, separated or combined, has been proposed to increase the insulin levels, a hormone that increases the anabolism [23], because insulin concentrations can stimulate the cellular influx of amino acids and increase the muscle protein synthesis rate, additionally insulin is negatively correlated with whole-body protein degradation.

Postprandial plasma concentrations of BCAAs and incretins after a whey meal could explain the insulin release, because these amino acids are mediators of the insulinogenic response [24]. So the whey protein as supplement or food ingredient when consumed can stimulate the anabolism, mainly if associated with exercise, and improve the health in different fashions including the glucose homeostasis

Whey protein can be classified as, concentrate (a least 80 % of protein), isolate (a least 90 % of protein) and hydrolysates, normally obtained by enzymatic or acid hydrolysis of whey protein isolate. The whey protein isolates, concentrates or hydrolysates are high-quality and pure protein with minimal fat, carbohydrate and lactose [25].

Whey Protein Hydrolysates and Peptides

Hydrolyzing the protein alone could in itself produce peptides with biological activity and alter the function of the protein and metabolism. Bioactive peptides are specific protein fragments that are inactive within the sequence of the parent protein, but after the hydrolysis they are released and may exert various physiological functions [26]. So, it has been suggested, that the change of the physicochemical form in which the protein is presented to the organism could influence the general metabolism, probably as a result of the peptides that are produced during hydrolysis of the whey proteins [4, 13, 15].

Whey protein hydrolysates affect the metabolism by different fashions, and results in some bioactive peptides present in the hydrolysate with antioxidant capacity [27], immunomodulatory [28], opioid receptor binding properties [29], angiotensin converting enzyme inhibitory activity [30], antimicrobial [6] and increased translocation of glucose transporter [14] among others. Therein, whey protein hydrolysates designed for nutritional applications as supplements for athletes, enteral formulas and hypoallergenic infant formulae [17] have biochemical properties far beyond those expected from the facilitated digestion and absorption, such as cell signallers, thus modifying the metabolism and causing significant physiological changes.

Whey Protein Plus L-leucine

Probably the combination of whey protein and L-leucine involves some of the more anabolic nutrients known currently and are being studied most often for this purpose, to enhance anabolism (Fig. 3.1). We can see in Tables 3.2 and 3.3 a list with 18 studies that focus on consumption of L-leucine plus whey protein, of these, 14 studies aimed on the combination or indirectly on evaluation of the combination of whey protein plus L-leucine on anabolism. The other 4 studies aimed at examining the effects of whey protein plus L-leucine on health, especially on glucose homeostasis, because the whey proteins and L-leucine isolates are insulinotropic. Studies seek to change body composition, but those focusing on the loss of fat mass are a minority, assessing the power mix in appetite and metabolism.

Most studies are in humans, about 55 %, and so far, the research protocols used in humans are more acute or less than 1 week (69 % of total) perhaps for ease of controlling a group of people in a space for shorter time. In these ten studies with humans, it is observed that at least 8 evaluated directly or indirectly the anabolic potential of a combination of whey protein plus L-leucine. Of these ten studies, 5 showed some kind of anabolic advantage of whey protein plus L-leucine.

The anabolic effectiveness of whey protein plus L-leucine to carbohydrate alone seems clear. Farup et al. [31] investigated the effects of resistance exercise combined high-L-leucine carbohydrate plus whey protein hydrolysate group or a carbohydrate group, the high-L-leucine whey protein hydrolysate augments patellar and quadriceps cross-sectional area (CSA), this was the first study to test the tendon responses of whey protein plus L-leucine.

Tipton et al. [32] showed advantage of whey protein plus L-leucine against placebo (water flavored) in acute approach with no trained subjects supplemented post-exercise. But when the author compared with previous results of supplementation with whey protein alone [33] no difference between whey protein alone and whey protein plus L-leucine was observed. In elderly, an acute study of Koopman et al. [34] has shown no further elevated muscle protein fractional synthetic rate in elderly men on intake of L-leucine with carbohydrate and protein following physical activity. The authors comment that long-term intervention studies are warranted to address the efficacy of L-leucine supplementation to attenuate the loss of skeletal muscle mass with ageing. On the other hand, Katsanos et al. [35] in acute study with elderly, have shown that a high proportion of L-leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids mix whey-protein-based in the elderly. As Churchward-Venne et al. [36] pointed that a low dose of all amino acids of whey protein (6.25 g) plus L-leucine (3 g) was as effective as a large dose of complete whey protein (24.57 g) in stimulating postprandial myofibrillar protein synthesis, 1–3 h post-exercise recovery. The authors highlight that their findings do not support the current notion that the postprandial stimulation of muscle protein synthesis is directly proportional only to the rise in blood L-leucine, suggesting that intracellular amino acid availability can be important in the regulation of muscle protein synthesis, which increase the importance of whey protein and minimize the role of L-leucine.

Coburn et al. [37] were the first to test the anabolic effects of whey protein plus L-leucine in humans at long term, indicating that 8 weeks of L-leucine and whey protein supplementation enhanced the

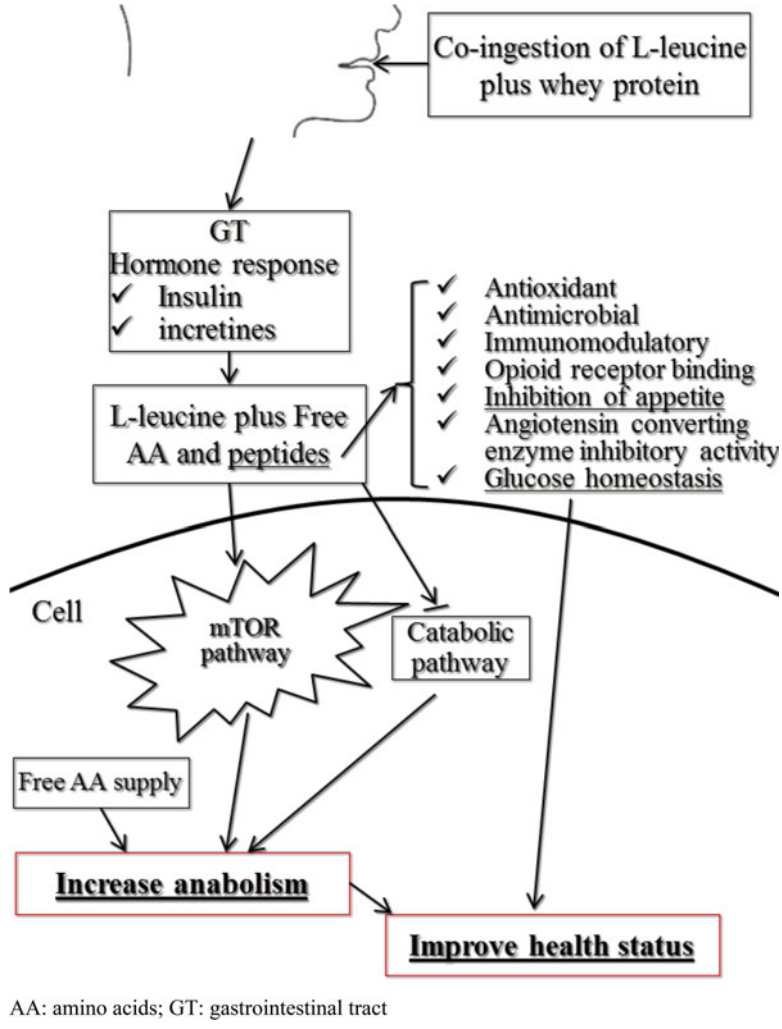

Fig. 3.1 Approach of effects of co-ingestion of L-leucine plus whey protein

acquisition of strength in the trained and untrained limbs against the control group, but the control group received only carbohydrate. Walker et al. [38] tested not only the anabolic effect of whey protein blend plus L-leucine, but also tested cognitive performance, once protein and branched chain amino acids may also improve cognitive performance during central fatigue. But no alteration on cognitive performance was observed. The authors commented that exercise stress experienced by participants may not have been severe enough to engender substantial central fatigue. In this study whey protein and L-leucine increased upper body strength and lean body mass; dietary supplementation with leucine and whey protein provided an ergogenic effect that enhanced the acquisition of strength [38].

Nelson et al. [39] focus not in anabolic effect of whey protein plus L-leucine, but in immunomodulatory effect post-exercise of this supplementation moisture. The authors observed that whey protein plus L-leucine supplementation during a 6-day block of intensified training appears to augment circulating neutrophil concentrations and respiratory burst activity, the performance was not clearly affected. The authors suggested that their data pointed that in well-trained males undergoing a period of intense exercise and fed a high-carbohydrate diet, L-leucine plus whey protein supplementation can attenuate basal serum cortisol, improving the immune system.

Table 3.2 Humans studies with whey protein plus L-leucine supplementation

Authors, year	Subjects, age	Aim	Duration	Main finds	Comments
Koopman et al. 2005 [44]	Untrained, 22.2 year	Determine the effect of whey protein plus L-leucine and carbohydrate in anabolic response after resistance exercise	Acute	Anabolic responses (protein breakdown rates were lower, and protein synthesis rates were higher) in group supplemented with carbohydrate plus protein and in group carbohydrate plus whey protein and L-leucine	The authors indicated that the supplemental L-leucine in combination with whey protein and carbohydrate likely represents an effective strategy to increase muscle anabolism following resistance exercise
Coburn et al. 2006 [37]	Untrained, 22.8 year	Examine the effects of resistance training in combination with a leucine and whey protein supplement on strength and muscle cross-sectional area	8 weeks	L-leucine plus whey protein supplementation may improve the increase of strength beyond that achieved with resistance training and a carbohydrate placebo	Maybe the leucine and whey protein supplement was administered to a group that was protein deficient compared to the PL, the subjects were randomly assigned to the groups
Katsanos et al. 2006 [35]	Untrained, 22.8 (young) to 66.7 (elderly)	Evaluate the effects of essential amino acid mixture (based in whey protein composition) with high content of L-leucine on muscle protein metabolism	Acute	A high proportion of L-leucine in a mixture of EAA[a] can improve the muscle protein synthesis in elderly	A high proportion of L-leucine reversed a attenuated response of muscle protein synthesis in the elderly
Frestedt et al. 2008 [45]	Untrained, 42.0 to 43.6	A specialized whey fraction (SWF[b]), high in leucine, was used as a dietary supplement to enhance weight loss	12 weeks	Subjects taking SWF showed a greater preservation of lean muscle and lost more body fat compared to control group	Subjects of control and treatment group lost a significant amount of weight with a 500 calorie reduced diet
Koopman et al. 2008 [34]	Untrained, 73 year	Evaluate the surplus value of the co-ingestion of whey protein hydrolysates plus L-leucine and carbohydrate following physical activity	Acute	Co-ingestion of leucine, carbohydrate and whey protein hydrolysates following physical activity does not further elevate muscle protein synthetic rate in elderly men	Long-term intervention studies are warranted to address the efficacy of leucine as a strategy to attenuate the loss of skeletal muscle mass with ageing
Tipton et al. 2009 [32]	Untrained, 29.8 year	Investigated the response of net muscle protein balance to ingestion of leucine plus whey protein in association with resistance exercise	Acute	The whey protein plus leucine in results in an anabolic response in muscle that is not greater than the previously reported response to whey protein alone	The anabolic pathways are fully stimulated by the amino acid content of the whey protein and (or) the exercise stimulus, additional leucine offers no further advantage in these young healthy volunteers
Walker et al. 2010 [38]	Military 26.9	Investigate the effects of whey protein and leucine supplementation on physical and cognitive performance and body composition	8 weeks	Whey protein and leucine increased the upper body strength and lean body mass, but no alteration on cognitive performance was observed	The exercise stress our participants experienced may not have been severe enough to engender substantial central fatigue

Churchward-Venne et al. 2012 [36]	Recreationally active, 22 year	Examine the role of L-leucine plus whey protein in the regulation of human myofibrillar protein synthesis, 1–3 h post-exercise recovery	Acute	A low dose of all amino acids of whey protein (6.25 g) plus L-leucine (3 g) was as effective as a large dose of complete whey protein protein (24.57 g) in stimulating postprandial myofibrillar protein synthesis, 1–3 h post-exercise recovery	L-leucine is potent in its ability to stimulate MPS, only a relatively small amount (0.75 g) is required to achieve a maximal stimulation of MPS when other EAA[a] are provided in larger quantities (~8.5 g)
Farup et al. 2013 [31]	Untrained, 78.1 year (elderly) and 23.9 (young)	Investigated the effects of maximal eccentric or concentric resistance training combined with high-leucine whey protein or placebo on muscle and tendon hypertrophy	12 weeks	High-leucine whey protein hydrolysate augments muscle and tendon hypertrophy following 12 weeks of resistance training—irrespective of contraction mode	The effect on muscle hypertrophy is augmented with a high-leucine whey protein hydrolysate
Nelson et al. 2013 [39]	Well-trained ciclists, 35 year	Examine the effects of whey protein and leucine ingestion post-exercise on neutrophil function and immunomodulators during a period of intense cycling	6 days	A immunomodulatory impact of post-exercise whey protein plus leucine supplementation during a 6-day block of intensified training appears to augment circulating neutrophil concentrations and respiratory burst activity	Altered plasma amino acid and acylcarnitine concentrations might partly explain post-exercise effect of whey protein plus L-leucine supplementation at immune system

[a]Essential amino acid
[b]SWF specialized whey fraction L-leucine rich

Table 3.3 In vivo, animal studies with whey protein plus L-leucine supplementation

Author, year	Species	Aim	Duration	Main finds	Comments
Rieu et al. 2007 [43]	Rats (old)	Evaluate the efficiency of leucine-rich whey protein fractions to stimulate muscle protein synthesis in old rats	30 days	L-leucine-rich proteins were efficient in improving muscle protein synthesis in old rats	Such supplements may be beneficial to improve muscle protein synthesis, and counteract, at least in part, the defect muscle protein synthesis observed in aging
Urschel et al. 2010 [47]	Horse	Determine whether whey protein or L-leucine addition to a glucose solution affects the post-gavage plasma insulin, glucose and amino acid responses	Acute	L-leucine but not whey protein augmented the serum insulin response to an oral glucose load	Post-exercise leucine administration may increase post-exercise rates of glycogen synthesis
Noatsch et al. 2011 [48]	Mice C57BL/6	Evaluate the effects of high-protein diets with dietary leucine supplementation on: energy homeostasis, body composition and UCP expression	14 weeks	Whey protein with or without leucine supplementation has no significant effect on energy homeostasis and UCP expression, but plasma cholesterol concentrations were reduced	Leucine plus whey protein is suggested to act alleviation of metabolic syndrome parameters in high-fat diet
Freudenberg et al. 2012 [49]	Mice C57BL/6	Investigate whether L-leucine supplementation is able to mimic the alleviating effects of high-whey-protein diets on metabolic syndrome parameters	20 weeks	L-leucine is able to mimic part but not all beneficial metabolic effects of high-whey-protein diets	High intakes of dietary protein or L-leucine can activate the mTOR pathway, but this does not happen in this study
Lollo et al. 2012 [42]	Rats	Investigate the dose–response effect of whey proteins plus L-leucine on the anabolic pathway mTOR in the diaphragm of sedentary and exercised rats	30 days	Whey protein plus L-leucine and exercise was able to activate the mTOR pathway of the diaphragm, maximal activation being obtained when the level of L-leucine addition was 4.5 % or 6 %	Significant increases in mass of the diaphragm were observed as a result of training and increases were also observed due to supplementation with L-leucine, although these did not reach statistical significance
Magne et al. 2012 [40]	Rats (old)	Explore protein synthesis during reloading, after immobilization, with leucine or milk proteins (whey protein and casein) supplementation	10-40 days	L-leucine supplementation failed in increase muscle mass gain. A high-protein diet, composed of one-half casein and the other half of whey protein, had a positive impact on muscle mass gain	This work emphasise a need of a synchronization between the stimulatory leucine signal and the availability of amino acid substrates for protein synthesis

Freudenberg et al. 2012 [49]	Mice C57BL/6	Evaluate the effects of high-fat diets containing either an adequate or a high amount of whey protein or supplemented with L-leucine corresponding to the leucine content within the high-protein diet	1 week	The positive effects of high-protein diets on metabolic syndrome associated traits are acutely due to effects on satiety possibly linked to amino nitrogen intake and on the subsequent suppression of liver lipogenesis without evidence for a specific leucine effect	Acute metabolic effects of high-protein as well as amino acid supplemented diets are strongly related to their effects on satiation and on the suppression of liver lipogenesis
Lollo et al. 2013 [41]	Rats	Investigate the dose–response effect of whey proteins plus L-leucine on the anabolic pathway mTOR in the heart of sedentary and exercised rats	30 days	In conclusion, the combination of L-leucine and whey protein has the potential to increase the mTOR pathway in the cardiac muscle without increasing the heart mass	Maybe a combination of whey protein plus L-leucine improve the physiological hypertrophy. Health parameter was not altered by supplementation

There has not been established an optimal dose in humans for L-leucine plus whey protein supplementation. The doses of L-leucine supplementation vary from 2.24 to 7.5 g while the whey protein doses vary from 6.7 to 25 g. The doses of human studies are compiled in Table 3.1.

Animal studies indicate that whey protein plus L-leucine can beneficially affect muscle protein anabolism. In animal studies, it is easy to assess the molecular effects of whey protein plus L-leucine activity in anabolic or catabolic pathways. The studies have shown that anabolic activities of cells are increased, not only in skeletal muscle [40] but also in tissues such as heart [41] and diaphragm [42], something that becomes very difficult to access in humans.

In animals, we found 8 studies combining whey protein plus L-leucine, 6 of these studies were in rodents, rats or mice and one with horses. All rodent studies lasted at least 1 week, going up to 20-weeks duration, thus allowing to evaluate the effects of medium- to long-term effects. In rodents, the L-leucine ingestion lead to activation of muscle protein synthesis, the meal with proteins containing a higher proportion of L-leucine increased the blood L-leucine concentration and an increase in muscle protein synthesis, but in long-time approach, this anabolic stimulus of L-leucine is not well reproduced (Table 3.4).

With this small number of studies, with different approaches, we cannot reach a conclusion about the effects of combining whey protein plus L-leucine. Once that the anabolic potential of the mixture is evidenced by the results according to different researchers [40–43] that demonstrate the mTOR activation or/and increased levels of insulin, but the muscle hypertrophy, cardiac or diaphragmatic not seem to be consistent in this set of studies. Perhaps the anabolic potential of the blend is balanced by an increase in catabolic pathways, or even a decrease in the sensitivity of the organism to exposure for a medium and long term to mixture of whey protein plus L-leucine. Further research is needed to elucidate this issue.

The Table 3.2 compiles the studies that tested the mixture whey protein plus L-leucine in vivo, attention is given to the main findings, as well as the objectives and duration of the different experiments (Table 3.5).

Conclusions

In general, there are few studies that tested the combination of whey protein plus L-leucine, the studies mostly seek observer anabolic capacity of this mixture in different situations (aging, post-exercise and disease). The combination of L-leucine plus whey protein should increase cellular amino acid availability results in increased muscle protein synthesis and positive net muscle protein balance following exercise [32] but this is not a consensus in long-term supplementation yet. L-leucine and whey protein supplementation enhanced the acquisition of strength [37, 38] in both studies with this approach. At present, only thesethree studies with human athletes or physically active individuals tested the combination of whey protein and L-leucine, there are still few studies that seek to evaluate the effects on physical performance (strength and central fatigue) and health (metabolic syndrome, immune system); we suggest that this point should be the focus in new experiments. Most studies seem to agree about obtaining some anabolic effect from the addition of L-leucine to whey protein, but null results have also been reported by a significant number of studies thus not permitting to reach a definite conclusion at present. We believe that future studies exploring whey protein plus L-leucine supplementation in disease-generating muscle atrophy, cachexia induced by cancer as well as its effects on central fatigue, immune system and catabolic pathways may elucidate the potential of mixing whey protein plus L-leucine and cellular mechanisms involved in the regulation of anabolic and catabolic organism that gets big load of anabolic nutrients, probably the combination of the most anabolic nutrients known. A limitation of some studies with humans and the present study is that no dietary analyses were conducted to determine protein intakes before or after administration of the L-leucine and whey protein; this should be avoided.

Table 3.4 Doses of whey protein and L-leucine in studies with humans

			Doses—all in grams			
Authors, year	Exercise	Duration	Whey protein	L-leucine	Control	Supplementation time
Koopman et al. 2005 [44]	Resistance training	Acute	0.16 g/kg[a]	0.03 g/kg	0.3 g/kg of carbohydrate	After resistance training session
Coburn et al. 2006 [37]	Unilateral resistance training	8 weeks	20	6.2	26.2 of maltodextrin	30 min prior to and immediately after each resistance training session
Katsanos et al. 2006 [35]	No exercise	Acute	6.7[b] g of whey protein with 26 % or 41 % of L-leucine		No control	Fasting state
Koopman et al. 2006 [46]	Simulated activities of daily living	Acute	0.2 g/kg/h[c]	0.1 g/kg/h	0.3 g/kg of carbohydrate	
Frestedt et al. 2008 [45]	No exercise	12 weeks	20[d]	2.24 of 20 g	Maltodextrin (isocaloric)	20 min before breakfast and one supplement 20 min before dinner
Koopman et al. 2008 [34]	30 min of a standardized resistance exercise	Acute	0×49 g carbohydrate/kg and 0×16 g/kg of a whey protein hydrolysate	The same, with addition of 0×03 g/kg leucine every h.	Carbohydrate plus whey protein	Post-exercise
Tipton et al. 2009 [32]	An intense bout of leg resistance exercise	Acute	16.6	3.4	Placebo (flavored water)	Post-exercise
Walker et al. 2010 [38]	Air Force training (endurance and resistance exercise)	8 weeks	19.7	6.2	Carbohydrate (isocaloric)	First dose 30–45 min before exercising and the second dose 30–45 min afterward.
Churchward-Venne et al. 2012 [36]	An acute bout of unilateral resistance exercise	Acute	25 or 6.25 of EAA[e]	3	12.55 EAA[e] plus 3 g of L-leucine	Post-exercise

(continued)

Table 3.4 (continued)

Authors, year	Exercise	Duration	Doses—all in grams			
			Whey protein	L-leucine	Control	Supplementation time
Farup et al. 2013 [31]	Maximal knee extensor training, one leg using eccentric and the other using concentric contractions	12 weeks	19.5 plus 19.5 of carbohydrate	2.77 of a dose	39 of carbohydrate	Half before and half after training
Nelson et al. 2013 [39]	Intense cycling	6 days	20[f]	7.5[f]	Carbohydrate (isocaloric)	1–3 h post-exercise

[a]Whey protein hydrolysate plus 0.49 g carbohydrate/kg (50 % as glucose and 50 % as maltodextrin)
[b]6.7 g of EAAs containing 1.7 g of leucine (26 % Leu; percentage of leucine found in whey protein), whereas subjects in the other group ingested 6.7 g of EAAs containing 2.8 g of leucine (41 % Leu)
[c]Whey protein hydrolysate plus 0.3 g carbohydrate/kg (50 % as glucose and 50 % as maltodextrin)—total time was 1 h
[d]Prolibra® is a rich L-leucine whey protein product
[e]Essential amino acid whey based
[f]Plus 89 g of carboidrate and 22 g of fat

Table 3.5 Doses of whey protein and L-leucine studies with animals

			Doses—all in grams			
Author, year	Exercise	Duration	Whey protein	L-leucine	Control	Supplementation time
Rieu et al. 2007 [43]	No exercise	30 days	Whey protein fractions[a, b]	10–14.5	Casein	In diet, all the time
Urschel et al. 2010 [47]	Endurance and Sprints	Acute	0.3 g/kg	0.3 g/kg	Glucose	Post-exercise
Noatsch et al. 2011 [48]	No exercise	14 weeks	20–50 %	4.5 %	20 % of whey protein	In diet, all the time
Freudenberg et al. 2012 [49]	No exercise	20 weeks	50 %	6 %	10 % of whey protein	In diet, all the time
Lollo et al. 2012 [42]	Endurance progressive protocol (treadmill)	30 days	17 %	3, 4.5 and 6 %	Casein	In diet, all the time
Magne et al. 2012 [40]	No exercise	10-40 days	16.6 % of casein[c]	4.45 %	Casein	In diet, all the time
Freudenberg et al. 2012 [49]	No exercise	1 week	10–50 %	6 %	No Leucine	In diet, all the time
Lollo et al. 2012 [42]	Endurance progressive protocol (treadmill)	30 days	17 %	3, 4.5 and 6 %	Casein	In diet, all the time

[a]β-Lactoglobulin (14.5 % leucine), Prolacta (13.4 %), α-lactalbumin (10.9 %), and casein (10 %)
[b]The authors carry out a pilot with whey protein, 144 g/kg of diet (Prolacta, Lactalis ingredients)
[c]Alanine was included in the control CAS+ALA diet to render the diets isonitrogenous. This amino acid has no effect on muscle protein metabolism. Valine and isoleucine were included in the CAS+LEU diet to prevent the fall of their plasma concentrations induced by leucine supplementation. CAS: casein; CAS+ALA: casein + alanine; CAS+LEU: casein + leucine

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Chapter 4
Branched Chain Amino Acids and Muscle Atrophy Protection

Yasuhiko Okimura

Key Points

- Branched chain amino acids (BCAAs) stimulate protein synthesis via mTOR complex 1 (mTORC1) in muscles.
- BCAAs inhibit protein degradation by suppressing the ubiquitin-proteasome system and lysosomal-autophagic system.
- mTORC1 is involved in the inhibitory action of BCAAs on muscle protein degradation.
- BCAAs protect against various muscle atrophies in animals.
- BCAAs protect against some muscle atrophies in humans.

Keywords Branched chain amino acid • Leucine • mTOR • mTORC1 • Ubiquitin • Autophagy • Disuse atrophy • Glucocorticoid • Sarcopenia

Abbreviations

BCAA	Branched chain amino acid
QOL	Quality of Life
IGF-I	Insulin-like growth factor I
PI3	Phosphatidylinositol-3 kinase
mTORC1	mTOR complex 1
mTOR	Mammalian Target Of Rapamycin
Raptor	Regulatory-associated protein of mTOR
GβL	G-protein β-subunit-like protein
PRAS40	Proline-rich PKB/Akt substrate 40 kDa
S6K	Ribosomal protein S6 kinase
4E-BP1	Eukaryotic initiation factor 4E-binding protein1
eIF4E	Eukaryotic initiation factor 4E
TSC1	Tuberous sclerosis 1 protein

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TSC2	Tuberous sclerosis 2 protein
Rheb	Ras homolog enriched in brain
GTP	Guanosine triphosphate
GDP	Guanosine diphosphate
GAP	GTPase activating protein
Vps34	Vacuolar protein sorting-associated protein 34
MAP4K3	Mitogen-activated protein kinase kinase kinase kinase 3
Rag	Ras-related GTP-binding protein
MAFbx	Muscle atrophy F-box
MuRF1	Muscle ring-finger protein 1
TNF-α	Tumor necrosing factor-α
EAA	Essential amino acid
CSA	Cross-sectional area
LC3	Microtubule-associated protein 1 light chain 3

Introduction

A variety of diseases and conditions, including sepsis, cancer, renal failure, excessive glucocorticoids and denervation and disuse of muscle, result in muscle atrophy. Muscle atrophy causes a decrease of mobility, increased susceptibility to injuries and reduced Quality of Life (QOL). In the absence of an underlying disease or condition known to cause muscle atrophy, muscle atrophy often occurs in elderly people. This condition, known as sarcopenia, decreases the QOL of elderly persons. The various types of muscle atrophy are due to increased protein breakdown, decreased protein synthesis or both. Muscle mass is maintained by the balance between muscle protein synthesis and degradation. Recent investigations have revealed the molecular basis of various types of muscle atrophy.

To prevent muscle atrophy, several types of intervention have been tried. Branched chain amino acid (BCAA) administration is one of the interventions because BCAAs have been reported to stimulate protein synthesis and attenuate protein degradation in muscles. BCAAs have been reported to ameliorate various muscle atrophies of humans as well as those of experimental animals. BCAAs are components of proteins that also function as signals that regulate cellular signaling pathways. The signaling functions of the BCAAs in muscle and the underlying mechanisms have been clarified due to advances in the understanding of the molecular mechanisms of BCAAs' activities and of muscle atrophy. BCAAs appear to function as signal regulators to protect against muscle atrophy. In this review, the regulatory functions of the BCAAs in cellular signaling are discussed first, and the effects of the BCAAs on several types of muscle atrophy in human and animals are described thereafter.

The Functions of BCAAs in Regulating Cellular Signaling

Underlying Mechanisms for Protein Synthesis

Skeletal muscle mass is maintained by the balance between muscle protein synthesis and protein degradation. The factors that stimulate muscle hypertrophy include IGF-I, nutrient intake and exercise. These factors also function to protect against muscle atrophy by stimulating protein synthesis and inhibiting protein degradation. The activities of these factors, including leucine, induce the activation of mTOR complex 1 (mTORC1), a Ser/Thr kinase signaling complex [1].

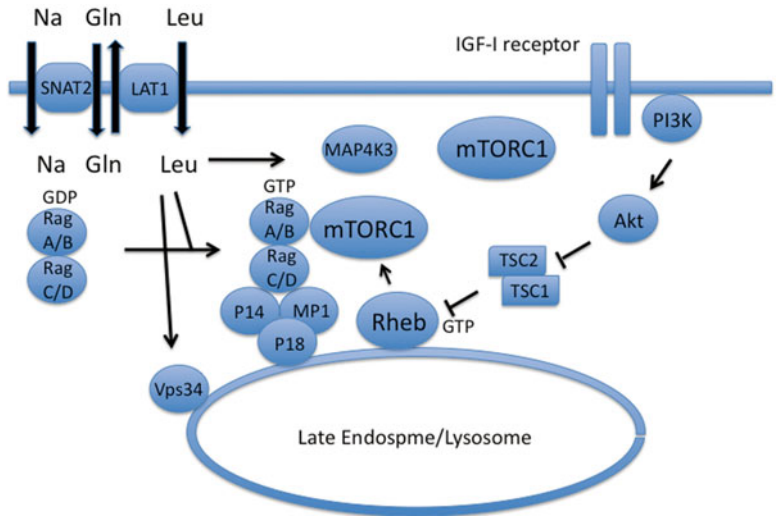

Fig. 4.1 Leucine activates mTORC1. The Rag family proteins RagA or RagB form a heterodimer with RagC or RagD. In an active heterodimeric complex, RagA or RagB is the GTP form and RagC or RagD is the GDP form. The Rag heterodimer interacts with Raptor, which is a component of mTORC1, and the interaction of the Rag heterodimer and Raptor is dependent on the level of GTP-bound RagA or RagB. The Rag heterodimer is located on lysosomal membranes. Leucine administration increases the level of active Rag heterodimers. As a result, mTORC1 translocates to the lysosomal membrane in an active-Rag-dependent manner. Rheb, which is also located on lysosomal membranes, is activated by growth factors through the inhibition of TSC1/2 activity. Activated Rheb stimulates mTORC1 activity on the membrane. Rag proteins do not stimulate mTORC1 kinase activity, but the activated Rag heterodimeric complex functions as a docking protein of mTORC1 to increase the association between mTORC1 and Rheb. Moreover, MAP4K3 is an amino acid-sensitive regulator of mTORC1 and leucine may stimulate the activity of the class III phophatidylinositol-3-kinase, Vps34, which stimulates mTORC1 signaling. These proposed mechanisms of leucine activity are different from those proposed for IGF-I, which stimulate the PI3K-Akt pathway and activate mTORC1 through suppressing TSC1/TSC2 activity

IGF-I is a well-known hypertrophic factor, and the mechanism by which it activates protein synthesis has been clarified. IGF-I is synthesized in various organs, although 70 % of the plasma IGF-I derives from the liver. The IGF-I produced in muscles plays an important role in maintaining muscle mass, and a decrease in the IGF-I content leads to muscle atrophy.

IGF-I phosphorylates and activates PI3 kinase, Akt and mTORC1 signaling pathway after binding to specific IGF-I receptors on the plasma membrane. mTORC1, which consists of mTOR, Raptor, GβL and PRAS40, plays a pivotal role in regulating muscle mass [2]. mTORC1 phosphorylates S6K at multiple sites. Activated S6K plays important roles in regulating protein synthesis and controlling cell size. mTORC1 also phosphorylates 4E-BP1, a well-known translation suppressor. 4E-BP1 binds to eIF4E, which recognizes the 5′-end cap of eukaryotic mRNAs. After it is phosphorylated, 4E-BP1 dissociates from eIF4E and then the eIF4E-dependent translation begins. mTORC1 is thought to activate protein synthesis through these two systems [3].

TSC1, TSC2 and Rheb are located in the upstream pathway of mTORC1 activation by IGF-I stimulation. Rheb is a Ras-related GTP-binding protein and the GTP-bound form of Rheb is active, whereas the GDP-bound form is inactive, as is generally found for G-proteins. Active Rheb interacts with mTOR and stimulates mTORC1 kinase activity. TSC1 binds to TSC2 to form a functional complex. TSC1 stabilizes the TSC1/TSC2 complex. Because TSC2 has a GTPase-activating protein (GAP) domain at its C-terminal, the TSC1/TSC2 complex converts active Rheb to the inactive form, thus functioning as a negative regulator of mTORC1. Akt phosphorylates TSC2 and suppresses the function of the TSC1/TSC2 complex, thereby stimulating mTORC1 activity indirectly. In this way, IGF-I activates mTORC1, which in turn stimulates protein synthesis [3] (Fig. 4.1).

Mechanisms Underlying BCAAs' Role in Protein Synthesis

It is well known that BCAAs stimulate protein synthesis in muscle. As early as the 1970s, Fulks et al. showed that BCAAs stimulate protein synthesis and inhibit protein degradation in the isolated rat diaphragm [4]. Buse et al. also found that a mixture of the three BCAAs stimulated the incorporation of ^{14}C-lysine into proteins in the isolated rat diaphragm. When tested separately, leucine was effective, but valine was ineffective and isoleucine was inhibitory. Leucine's stimulatory effect was observed in the presence of actinomycin D or cycloheximide, suggesting that neither RNA synthesis nor protein synthesis are required for the stimulatory effect [5].

Leucine has been reported to also stimulate protein synthesis in vivo. Orally administered leucine stimulates protein synthesis in the gastrocnemius and plantaris muscles of rats. The stimulatory effect is associated with increased phosphorylation of 4E-BP1, consequently inhibiting the formation of 4E-BP1-eIF4E complexes and stimulating translation. The stimulatory effect is also associated with increased phosphorylation of S6K. Rapamycin, a mTOR inhibitor, inhibits protein synthesis in leucine-treated rats. Additionally, rapamycin prevents the stimulatory effects of leucine on the availability of eIF4E for binding to eIF4G and inhibits the leucine-dependent phosphorylation of S6K1.

Although leucine, similar to IGF-I, stimulates protein synthesis through the phosphorylation of S6K and 4E-BP1 via mTORC1 activation, the pathway upstream of mTORC1 is different from the pathway that is activated by IGF-I. One mechanism proposed for the regulation of mTORC1 activity is the activation of the class III phophatidylinositol-3-kinase, vacuolar protein sorting-associated protein 34 (Vps34) [6]. Vps34 differs from the class I phophatidylinositol-3-kinase, PI3K in its substrate specificity and salt requirement in in vitro assays, in its binding partners and in its primary structure. Gulati et al. reported that amino acids induced an increase in the intracellular Ca^{2+} ($[Ca^{2+}]$ i). The rise of $[Ca^{2+}]$ i increases the direct binding of Ca^{2+}/calmodulin to an evolutionarily conserved motif in Vps34 that is required for the kinase activity and increased mTORC1 signaling [7].

Mitogen-activated protein kinase kinase kinase kinase 3 (MAP4K3) also has been reported to be an amino acid-sensitive regulator of mTORC1. Loss of the function of MAP4K3 in mammalian cells reduces mTORC1 activity, while a gain of the function of MAP4K3 increases mTORC1 activity, and the kinase activity of MAP4K3 is regulated by amino acids [8].

In addition, the Rag small GTPase family has been reported to play an important role in the spatial regulation of mTORC1. The Rag family consists of RagA, B, C and D. RagA or RagB forms a heterodimer with RagC or RagD. In an active heterodimeric complex, RagA or RagB is the GTP form and RagC or RagD is the GDP form. The Rag heterodimer interacts with Raptor, a component of mTORC1, and this interaction is dependent on the levels of GTP-bound RagA or RagB. Amino acid deprivation reduces the level of GTP-bound RagB. Knockdown of RagA/B suppressed the activity of the mTORC1 pathway, whereas constitutively active RagA/B rendered mTORC1 resistant to amino acid deprivation. Rag GTPases, unlike Rheb, do not stimulate mTORC1 kinase activity. A different indirect activation mechanism for mTORC1 function is supported by experimental data [9]. In this scheme, Rag heterodimers are located on the lysosomal membrane, amino acids increase the level of active Rag heterodimers, mTORC1 translocates to the lysosomal membrane in an active Rag-dependent manner, and the Rheb located on the lysosomal membrane is activated by growth factors via inhibition of TSC1/2 activity and finally, activated Rheb stimulates mTORC1 activity on the membrane. This scheme is interesting because it entails the convergence of growth factor signals and nutritional signals (Fig. 4.1).

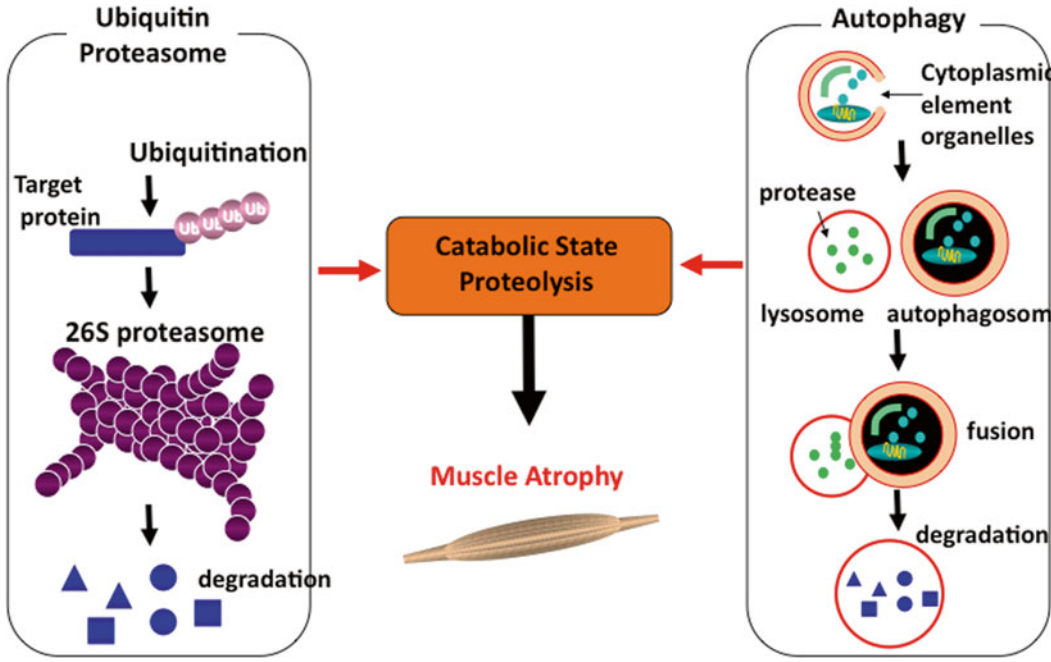

Fig. 4.2 Protein degradation is regulated by two major systems in skeletal muscles. The ubiquitin-proteasome system and the lysosomal-autophagic system stimulate protein degradation and lead to muscle atrophy

Inhibitory Effect of BCAAs on Muscle Protein Degradation

Protein degradation in skeletal muscle was regulated by two major systems. One is the ubiquitin-proteasome system and the other is lysosomal-autophagic system. In both systems, mTORC1 activation is involved in the inhibitory effect of BCAAs on protein degradation (Fig. 4.2).

The Ubiquitin-Proteasome System

Gene expression profiling studies have revealed that the levels of ubiquitin A and B, proteasome subunit proteins and ubiquitin ligases were increased in various types of muscle atrophy, indicating that the ubiquitin-proteasome system was activated [10, 11]. In the ubiquitin-proteasome system, proteins are ubiquitinated by a series of pathways involving E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzymes) and E3 (ubiquitin ligase), and the ubiquitinated proteins are degraded by the 26S proteasome. Different E2–E3 pairs function in the degradation of different proteins. During skeletal muscle atrophy, the expression of two muscle-specific ubiquitin ligases, atrophy gene-1/muscle atrophy F-box (atrogin-1/MAFbx) and muscle ring-finger protein 1 (MuRF1), were increased [10, 11]. Atrogin-1 is reported to be induced 8- to 40-fold in muscle atrophy resulting from fasting, diabetes, cancer and renal failure, up to threefold by hindlimb suspension, immobilization and denervation, and up to tenfold in a cachectic or dexamethasone-administration model [10]. Atrogin-1 promotes the degradation of MyoD, a key muscle transcription factor, and of eIF3f, an important activator of protein synthesis [12, 13]. MuRF1, which was initially found associated with myofibrils [14], ubiquitylates myosin-binding protein C and myosin light chains 1 and 2 in myofibrils and may play an important role in myofibrillar protein degradation [15]. The physiological importance of these

ubiquitin ligases was demonstrated in studies of mice with these enzymes knocked out. Bodine et al. reported that denervation-induced muscle atrophy was reduced in both atrogin-1/MAFbx and MuRF1 knockout mice [10].

The Lysosomal-Autophagic System

Autophagy is a process responsible for the bulk degradation of intracellular materials that is evolutionarily conserved in all eukaryotes and is activated by a variety of conditions including nutrient deprivation, infection and cancer. In autophagy, cytoplasmic components are engulfed by autophagosomes and delivered to lysosomes/vacuoles for degradation [16]. It has been reported that autophagy plays a crucial role in various organs, including skeletal muscles. A study that used electron microscopy showed that autophagosome formation was activated in denervated muscle [17]. Recently, microtubule-associated protein 1 light chain 3 (LC3), the mammalian homologue of yeast Atg8, was recognized as a protein marker of autophagosomes [18]. In a starvation condition such as that caused by food deprivation, LC3 expression is increased in muscle in accordance with autophagosome formation [19]. Autophagy is activated by food deprivation, denervation and disuse in rat skeletal muscles. When autophagy is activated, the conversion of LC3-I, the cytosolic form of LC3, to LC3-II, the autophagosome membrane-bound form of LC3, is increased. Sugawara et al. showed that leucine reversed the reduction of the weight of gastrocnemius muscle and reversed the increased expression of LC3-II that was induced in rats by 7 days of a protein-free diet [20]; however, Maki et al. did not observe a significant decrease in the LC3I/II ratio by BCAA in the hindlimb-suspension (HS) rat model [21].

Calpain Pathway

The calpain pathway is also involved in the myofibrillar proteolysis [22]. Calpains are a family of intracellular, non-lysosomal, Ca^{2-}-dependent cysteine proteases. A number of studies have focused on the role of calpains in conditions that induce muscle atrophy. Ca^{2+-} is the most important activator of calpain. During sepsis accompanied by muscle atrophy, $[Ca^{2+}]$ i are increased and calpain expression is upregulated in skeletal muscle. In addition, calpain activity is increased in skeletal muscle[23]. Other catabolic conditions also involve increased calpain activity. Glucocorticoids are reported to increase calpain activity in skeletal muscle, resulting in proteolysis of the myofibrils [24].

Amino Acid Transporters That Regulate Muscle Atrophy

mTORC1 is involved in the protective effect of leucine against muscle atrophy. However, it was unclear whether the extracellular leucine or the intracellular leucine plays a role in the protection against muscle atrophy. Recently, an amino acid-transport system was reported to be involved in the activity of BCAAs. Among the various glutamine transporters, the predominant one in muscles is sodium-dependent neutral amino acid transporter 2 (SNAT2). Evans et al. showed that selective inhibition of SNAT2 resulted in the depletion of intracellular glutamine in L6 myoblasts. Interestingly, the selective inhibition of SNAT2 leads to the decrease of other amino acids, including leucine. Leucine is not regarded as a SNAT2 substrate, suggesting that SNAT2 exerts indirect effects on leucine [25]. They postulated that the glutamine influx into the cytosol via SNAT2, which is coupled to the electrochemical gradient of Na across the plasma membrane, leads to the accumulation of a high concentration of glutamine in the cytosol. The subsequent efflux of the accumulated glutamine via plasma-membrane amino acid exchangers such as System L then drives the secondary accumulation

of other amino acids in the cell, such as leucine, which are poor substrates for SNAT2. In accordance with this result, SLC1A5, a high-affinity neutral amino acid transporter, and SLC7A5, an amino acid exchanger of glutamine and leucine, function in HeLa cells to transport leucine and subsequently stimulate mTORC1, suggesting that this system may exist in a variety of tissues [26].

Protective Effect of BCAAs Against Muscle Atrophy in Humans and Animals (Fig. 4.3)

Protective Effect of BCAAs Against Disuse Atrophy

Lack of muscle movement, such as during bed rest, cast immobilization or limb-suspension, causes muscle atrophy in humans. Microgravity also causes disuse atrophy, which is one of the problems that must be addressed in long-term spaceflight [27]. Disuse atrophy was shown to primarily affect slow-twitch, anti-gravity muscles such as the soleus and adductor longus muscles, resulting in muscle wasting, increased fatigue and decreased contractile function [28].

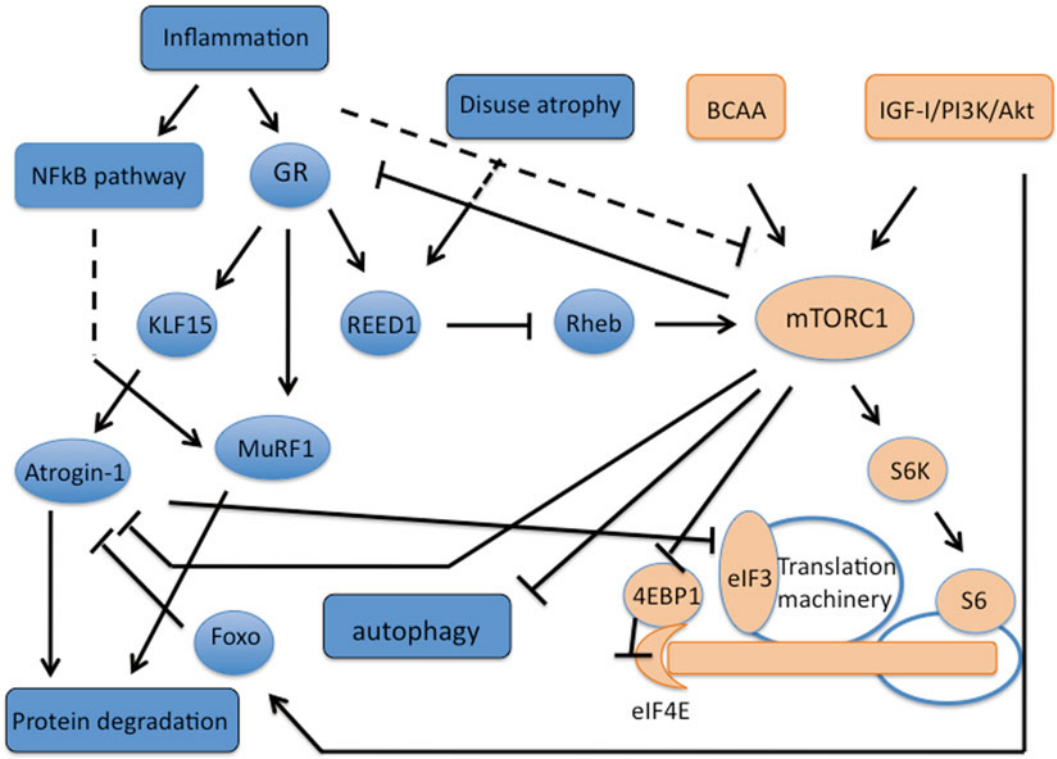

Fig. 4.3 Cell signaling pathways that regulate the synthesis and degradation of muscle proteins. Various diseases and conditions cause muscle atrophy. Excessive glucocorticoids increase REDD1 expression, which in turn inhibits Rheb function, reducing mTORC1 activity. Decreased mTORC1 activity leads to a decreased level of protein synthesis and increased expression of atrogin-1. GR directly and indirectly stimulates MuRF1 and atrogin-1 to activate protein degradation. Inflammation stimulates the NFkb pathway and activates MuRF1. Glucocorticoid excess may be involved in inflammation-induced muscle atrophy. REDD1 is also increased in disuse atrophy and involved in muscle atrophy. mTORC1, which is activated by IGF-I/PI3K/Akt pathway and by BCAAs, plays a pivotal role in stimulating muscle protein synthesis and inhibiting muscle protein degradation

HS and cast immobilization of experimental animals are often used to produce disuse atrophy models. The HS rat is a well-described animal model used to investigate the effects of decreased muscle activity [29]. Pronounced atrophy of the soleus muscle occurs in response to HS, accompanied by a decrease in cross-sectional area of the muscle fibers [30]. Kelleher et al. reported that mTORC1 activity as well as protein synthesis is attenuated during unilateral hindlimb immobilization of rats. The attenuation of mTORC1 activity was associated with increased expression of Regulated in Development and DNA Damage Responses-1 (REDD1), although the upstream signals of mTORC1 that are stimulated by Akt and ERK1/2 were preserved [31]. REDD1 has been reported to be a negative regulator of mTORC1 induced in response to hypoxia, stress and food deprivation that is responsible for the attenuation of mTORC1 activity [32].

Enhanced protein degradation is also observed in disuse atrophy. Both the ubiquitin-proteasome system and the lysosomal-autophagic system are activated. This response is not surprising because mTORC1 suppresses protein degradation systems, as described above. Maki et al. reported that 14 days of hindlimb suspension in rats caused muscle atrophy as evaluated by the cross-sectional area (CSA) of muscle fibers and that the muscle atrophy was associated with increased levels of atrogin-1 and MuRF1 proteins in the muscles [21]. Bodine et al. reported that atrogin-1 and MuRF1 are expressed 1 day after a limb is immobilized and peaked 3 days later [10]. However, Maki et al. showed that the atrogin-1 and MuRF1 protein levels are still higher in the muscles of HS rats than those of control rats on the 14th day of HS.

In the disuse atrophy of animal model, BCAA administration was reported to be effective in protecting against muscle atrophy. BCAA ingestion partially but significantly reversed the reduction of the CSA of atrophied muscle and reduced the expression of atrogin-1 and MuRF1 proteins [21] (Fig. 4.4). These results suggest the possible efficacy of BCAA treatment of disuse atrophy through the down-regulation of atrogin-1 and MuRF1 expression. In contrast, Bajotto et al. reported that BCAA supplementation of the diet did not prevent the muscle atrophy induced by HS for 6 days, although it reduced the loss of myofibrillar proteins [33].

mTORC1 appears to be involved in the inhibitory action of BCAAs on atrogin-1 expression. Herrnigtyas et al. showed that rapamycin, an mTORC1 inhibitor, reversed leucine-induced suppression of atrogin-1 expression, but the PI3K inhibitor wortmannin did not [34]. Although atrogin-1 expression is reportedly regulated by IGF-I via the activation of Foxo3, which is a downstream molecule of the PI3K-Akt pathway, the pathways by which leucine and IGF-I affect atrogin-1 expression are different.

Trials of BCAA administration have been conducted to reduce disuse atrophy in humans. However, the efficacy of BCAA administration in humans is not as clear as that found in animal experiments. Some reports indicated that BCAA treatment had inhibitory effect on disuse muscle atrophy. Paddon-Jones et al. showed that supplementation with 15 g of essential amino acids (EAA), including 3.1 g of leucine increased the synthetic rate in the postprandial period in patients on bed rest for 28 days. However, the lean leg mass was not changed and muscle strength was reduced [35]. Trappe et al. reported that muscle mass decreased by 4 % in a leucine supplementation group compared with a control group [36]. The differences in the effects of BCAA or leucine administration on humans and rats may be due to the species difference because BCAA metabolism is reported to differ in the two species.

Protective Effect of BCAAs Against Cancer-Induced Muscle Atrophy

Cancer cachexia induces anorexia, host protein wastage and metabolic abnormalities. Cancer cachexia is also associated with a poor response to therapy and increased susceptibility to treatment-related adverse events, as well as a poor outcome and QOL [37]. Muscle atrophy is one of the abnormalities of cancer cachexia. Various factors, including reduced dietary intake, increased oxidation of BCAAs,

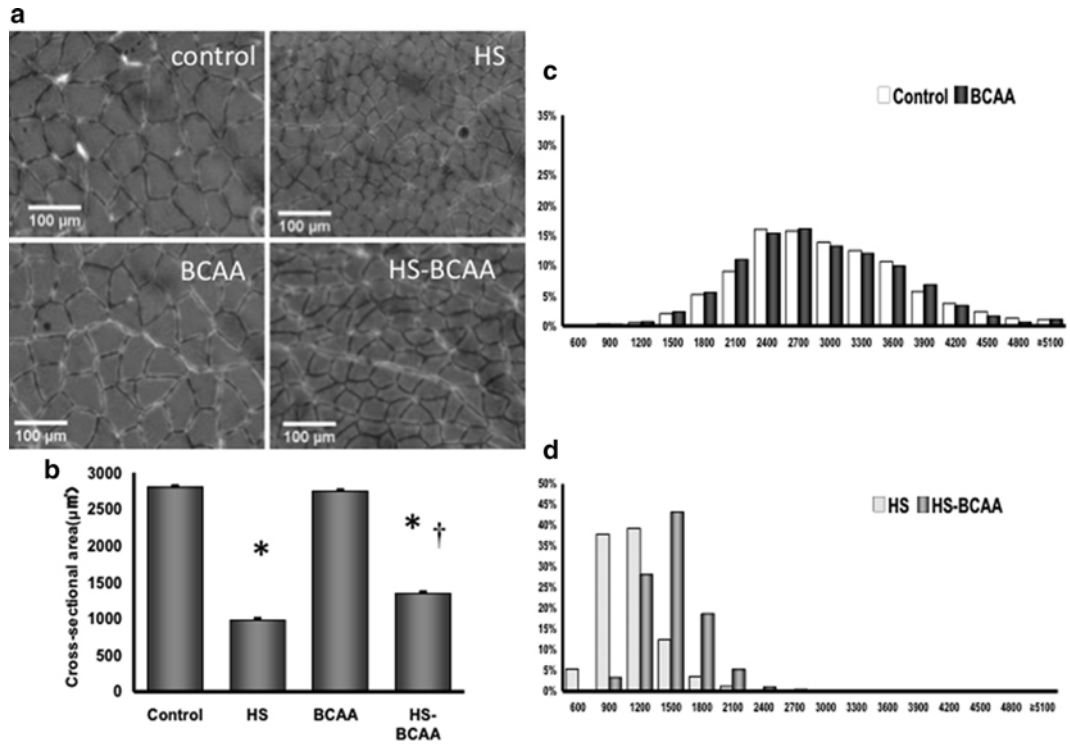

Fig. 4.4 BCAAs partially reverse hindlimb suspension-induced muscle atrophy. Male 6-week-old SD rats were treated with hindlimb-suspension (HS), oral administration of BCAAs (600 mg/kg) or both. After 14 days of treatment, the soleus muscles were excised and examined using hematoxylin-eosin staining (HE). (**a**) HE-stained section of soleus muscle at 400× magnification. (**b**) BCAA treatment partially but significantly reversed the reduction in the fiber cross-sectional area (CSA) induced by HS. (**c**) Distribution of the CSAs of soleus muscle fibers in the treatment groups is shown. The *X*-axis represents the CSAs in 300 μm^2 subgroups. The *Y*-axis indicates the percentage to total fiber numbers. There was no difference in the distributions of the CSAs of the control and BCAA-treatment groups. (**d**) In contrast, in the HS group, the distribution of the CSAs was shifted toward smaller values and this trend was partially reversed by BCAA-treatment. Nutr Res. 2012;32:676–83, with permissions

inflammatory cytokine production, elevated glucocorticoid levels and reactive oxygen species contribute to cancer-induced muscle atrophy. These factors participate in other diseases and states causing muscle atrophy and are not specific to cancer-induced muscle atrophy.

Administration of BCAA or their metabolites was shown to be an effective treatment for muscle atrophy in animals that were implanted with cancer cells [38]. In humans, supplementation with arginine, glutamine and β-hydroxy-β-methyl-butyrate, a metabolite of leucine, resulted in a significant increase in the fat-free mass in patients with solid tumors, whereas the supplementation was not effective in the control group [39]. BCAA administration could be beneficial to cancer patients experiencing muscle atrophy.

Protective Effect of BCAAs Against Inflammation-Associated Muscle Atrophy

Increased catabolism in muscles is often observed in inflammatory diseases. If the catabolism is mild, it may benefit the organism because it provides amino acid substrates to support hepatic gluconeogenesis and acute-phase protein synthesis. However, if severe inflammation such as sepsis is protracted,

muscle protein degradation is increased, leading to the loss of muscle protein, loss of muscle strength and decreased ambulatory ability. In sepsis, muscle atrophy is due to the failure of muscle to balance protein synthesis with increased protein degradation, although the relative contribution of protein synthesis and proteolysis to the catabolic state may depend on the etiology. Vary and Kimball demonstrated that sepsis preferentially targeted protein synthesis in fast-twitch but not slow-twitch muscles and that sepsis-induced inhibition of protein synthesis resulted from a restraint in peptide-chain initiation [40].

In sepsis, the plasma levels of inflammatory cytokines, including TNF-α are increased and muscle protein synthesis is attenuated. Infusing TNF-α into rats decreased the phosphorylation of mTOR, 4E-BP1 and S6K and the binding of eIF4E to eIF4G in their muscles, which is a similar event observed in rats with sepsis. Pretreatment of septic rats with TNF-binding protein, which suppresses TNF-α activity, ameliorated the reduced binding of eIF4E to eIF4G as well as the reduced phosphorylation of 4E-BP1, S6 and mTOR. These results indicate that TNF-α is a major cytokine that causes muscle atrophy in septic conditions [41].

TNF-α or other inflammatory cytokines activate the NFκB signaling pathway. NFκB is sequestered in the cytoplasm in an inactive state by inhibitory IκB proteins. TNF-α stimulates the activity of the IκB kinase (IKKβ) complex and phosphorylates IκB. Phosphorylated IκB is ubiquitinated and degraded in proteasomes. As a result, NFκB is released from the IκB-NFκB complex and is translocated to the nucleus where it activates the expression of the NFκB-regulated genes. Quadriceps muscle biopsies from severely cachectic patients with chronic obstructive pulmonary disease (COPD) and chronic heart failure are characterized by activated NFkB signaling.

The IκB-NFκB pathway stimulates MuRF1 expression in muscle. Muscle-specific activated-IκB transgenic mice exhibit muscle atrophy and MuRF1 expression was increased in their muscles. Interestingly, when muscle-specific activated-IκB transgenic mice were crossed into a MuRF1$^{-/-}$ background, a significant reduction of muscle-mass loss was observed in the offspring, indicating that NFkB activates the expression of MuRF1, which in turn stimulates muscle atrophy.

BCAA administration may not be effective in sepsis-induced muscle atrophy. As described earlier, oral administration of leucine increases the phosphorylation of 4E-BP1, S6K1 and mTOR. An intraperitoneal injection of lipopolysaccharide (LPS) abrogates the leucine-induced phosphorylation of 4E-BP1, S6K1, ribosomal protein S6 and mTOR. Pretreatment with the glucocorticoid receptor antagonist RU486 was unable to prevent the LPS-induced leucine resistance. In contrast, LPS administration did not prevent the ability of pharmacological levels of IGF-I to phosphorylate 4E-BP1 [42].

Protective Effect of BCAAs Against Glucocorticoid-Induced Muscle Atrophy

Cushing disease and Cushing syndrome are characterized by the signs and symptoms of excessive glucocorticoids. Muscle atrophy is one of the symptoms of excessive glucocorticoids. Furthermore, to treat various diseases, in particular autoimmune diseases, glucocorticoid is often administered and the administration is generally over a prolonged period. Thus, the incidence of muscle atrophy due to treatment-related excessive glucocorticoid is much higher than that of Cushing disease and Cushing syndrome. Glucocorticoid production is increased in a variety of inflammatory conditions in addition to the overproduction of inflammatory cytokines. Inflammation-induced muscle atrophy is partially influenced by excessive glucocorticoids.

Glucocorticoids stimulate protein degradation and suppress protein synthesis. The type 2 fibers are predominantly affected in glucocorticoid-induced muscle atrophy compared with type 1 fibers. This selectivity is explained by the abundant expression of glucocorticoid receptors by type 2 fibers. However, Yamamoto et al. showed that dexamethasone treatment decreased the CSA of muscle fibers

and that oral administration of BCAAs inhibited this decrease in the CSA even in soleus muscles, in which type 1 fibers are predominant [43]. In addition to the histological changes it caused, the expression of atrogin-1 and MuRF1 mRNA were induced by dexamethasone.

The glucocorticoid responsive element (GRE) is located in the MuRF1 gene. The GRE is highly conserved in the mouse, rat and human along with the immediately adjacent FOXO binding element. The MuRF1 promoter is responsive to both dexamethasone and FOXO1. Co-expression of the glucocorticoid receptor and FOXO1 leads to a dramatic synergistic increase in MuRF1 gene expression. In contrast, GRE is not present in the atrogin-1 gene.

Glucocorticoids directly stimulate the expression of KLF15, which in turn binds to the atrogin-1 gene and activates its expression [44]. In addition, KLF15 upregulates the gene expression of branched chain aminotransferase 2 (BCAT2), a mitochondrial enzyme that catalyzes the first reaction in BCAA catabolism, accelerating BCAA degradation in skeletal muscles [45]. Furthermore, KLF15 activates MuRF1 gene expression [44].

Glucocorticoids increase the expression of the REDD1 and myostatin genes [44]. REDD1 inhibits mTOR activity via sequestration of 14-3-3 and increasing the activity of TSC1/2 [46]. A constitutively active mutant of Rheb, which autonomously activates mTOR, represses dexamethasone-mediated gene activation, and mTOR blockade using rapamycin significantly enhances the dexamethasone-induced expression of a number of the glucocorticoid target genes. These results indicate the interaction between glucocorticoids and mTOR. Glucocorticoids inhibit mTOR activity and mTOR reduces glucocorticoid action.

BCAAs activate mTORC1 and inhibit glucocorticoid functions. BCAAs suppress the dexamethasone-induced atrogin-1 and MuRF1 gene expression in muscles and significantly reverse the dexamethasone-induced decrease in the CSA of muscle fibers [43], suggesting the possibility that the ubiquitin-proteasome system is involved in the suppressive effect of BCAAs on glucocorticoid-induced muscle atrophy (Figs. 4.5 and 4.6).

The lysosomal-autophagic system is also involved in glucocorticoid-induced muscle atrophy. Dexamethasone administration increases the conversion of LC3-I, the cytosolic form of LC3, to LC3-II, the autophagosome-membrane-bound form of LC3, in rat soleus muscles. The conversion of LC3-I is an indicator of autophagy and this conversion is blocked by BCAAs. These findings suggest that BCAAs might decrease the lysosomal-autophagic-mediated protein degradation in skeletal muscle (Fig. 4.6).

Protective Effect of BCAAs Against Sarcopenia

Sarcopenia is the loss of muscle mass that is associated with aging. The loss of muscle mass, which predominantly affects type 2 fibers, results in reduced mobility, reduced autonomy and increased susceptibility to injuries. Therefore, sarcopenia affects the QOL of elderly people and counteracting sarcopenia is an important goal in countries where the population of elderly people is increasing. The European Working Group on Sarcopenia in Older People proposed a diagnostic procedure, but has not achieved consensus on the cut-off points indicating sarcopenia. Although the etiology of sarcopenia is not entirely clear, the decrease of physical activity, loss of motoneurons, increase in the levels of inflammatory cytokines and the decreased levels of hormones, such as sex steroids and growth hormone, appear to affect the time of onset and the extent of sarcopenia.

There are many reports concerning BCAA administration for the treatment of sarcopenia. Kim et al. tested the effect of a 3-month treatment with a leucine-rich essential amino acid supplementation (AAS), exercise or both on muscle mass and function in women aged 75 or older. The leg muscle mass was increased in the exercise+AAS group and exercise group, and the knee extension strength

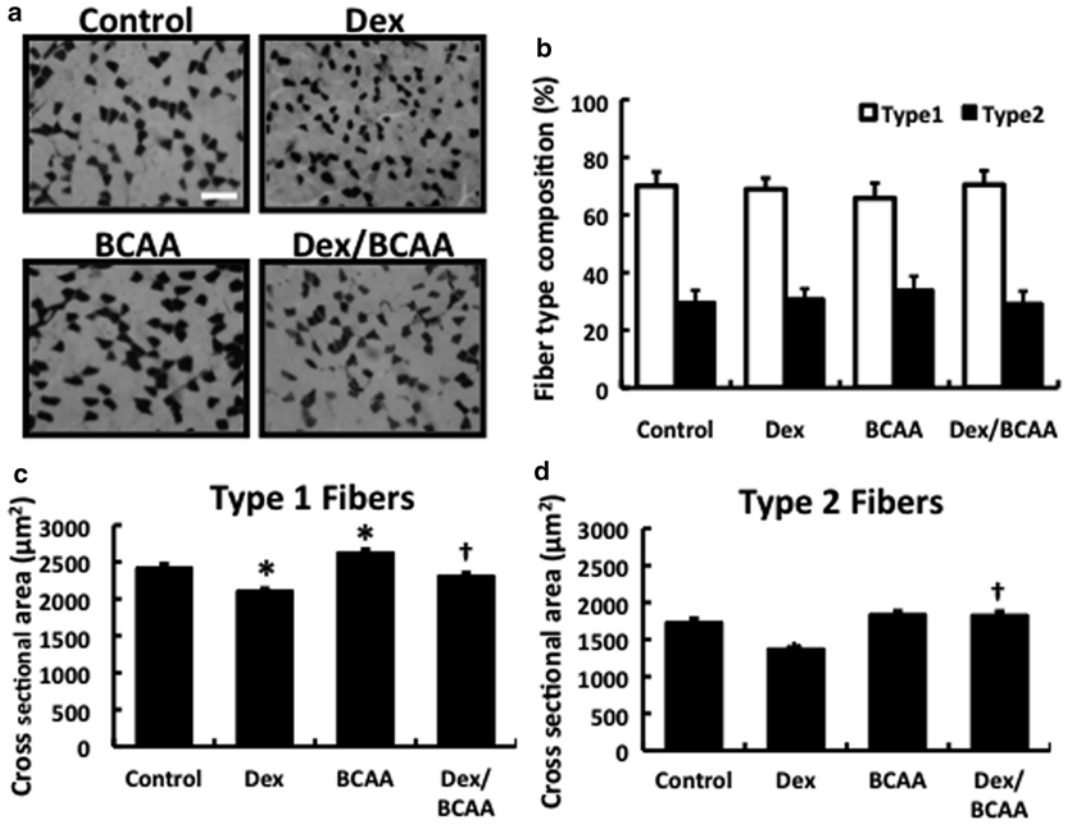

Fig. 4.5 BCAAs reverse dexamethasone-induced muscle atrophy. Dexamethasone (Dex, 600 μg/kg) was intraperitoneally injected into 8-week-old male rats once a day for 5 days. BCAAs (600 mg/kg) were orally administered for 5 days. (**a**) Soleus muscles were collected and examined with ATPase staining (pH 10.7). With this stain, type 1 fibers stain light, and type 2 fibers are dark. Scale bar: 200 μm. (**b**) Neither BCAA nor Dex affected the composition of type 1 and type 2 muscle fibers. (**c**) Dex administration significantly decreased the mean cross-sectional area (CSA) of type 1 fibers in soleus muscles. BCAA, when administered alone, showed an increase in the mean CSA and a protective effect against the Dex-induced decrease in mean CSA. (**d**) BCAA reversed the Dex-induced decrease in mean CSA of type 2 fibers. The response to Dex or BCAA administration in type 2 fibers showed a similar tendency observed in type 1 fibers, but not significant. *, $P<0.05$ vs. control group; †, $P<0.05$ vs. Dex-treated group. Muscle Nerve. 2010;41:819–27, with permissions

increased only in the exercise+AAS group [47]. In contrast, leucine supplementation for 3 months did not augment the skeletal muscle mass or strength in healthy elderly men [48]. These results are consistent with the report that dietary protein supplementation is required to enable muscle mass gain during exercise training of frail elderly people [49]. A recent systematic review concerning the effect of nutritional supplementation on muscle mass in sarcopenia also concluded that the positive effects of nutritional supplementation increase when associated with physical exercise [50].

Conclusions

The functions of the BCAAs in muscle and the underlying mechanisms have been clarified due to advances in the understanding of the molecular mechanisms of BCAAs' actions. BCAAs protect against various types of muscle atrophy via stimulating protein synthesis and suppressing protein degradation. The key molecule is mTOR in these actions of BCAAs and mTOR plays a pivotal role

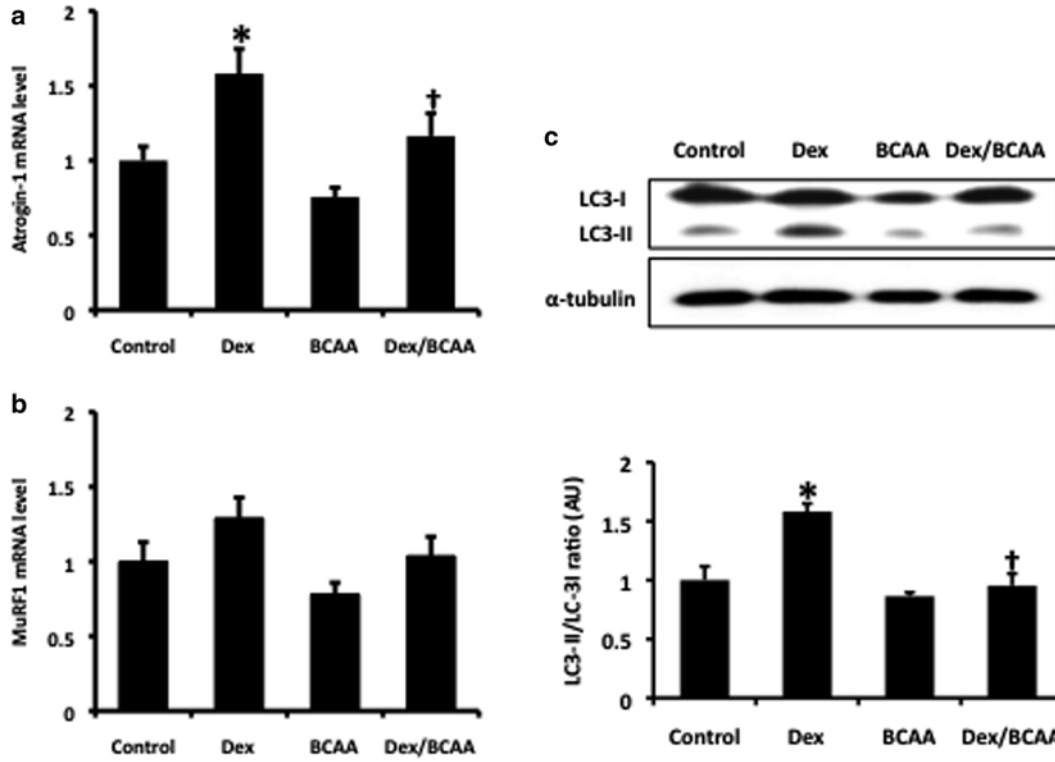

Fig. 4.6 BCAAs reverse the dexamethasone-induced increases in atrogin-1 mRNA expression and the LC3 conversion ratio. Dexamethasone (Dex, 600 μg/kg) was intraperitoneally injected into 8-week-old male rats once a day for 5 days. BCAAs (600 mg/kg) were orally administered for 5 days. (**a**) Dex stimulated atrogin-1 mRNA expression in soleus muscles. The Dex-induced expression of atrogin-1 mRNA was significantly attenuated by BCAA administration. (**b**) BCAAs and Dex had similar effects on MuRF1 mRNA expression, but they were not significant. (**c**) The LC3 conversion ratio (LC3-II/LC3-I) was calculated. Dex administration increased the conversion of LC3-I to LC3-II. BCAA administration completely reversed the Dex-induced effect on the LC3-I to LC3-II conversion. *, $P<0.05$ vs. control group; †, $P<0.05$ vs. Dex-treated group. Muscle Nerve. 2010;41:819–27, with permissions

in the protective action of BCAAs. In vivo animal experiments have shown the effectiveness of BCAA in protecting against muscle atrophy, although its effectiveness is not always observed in humans. Oral administration of BCAAs appears to have the potential to protect against muscle atrophies in human.

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Chapter 5
Role of Branched Chain Amino Acids in Cellular and Organ Damage: The Prognostic Significance of the Preoperative Branched Chain Amino Acid to Tyrosine Ratio

Toru Mizuguchi, Tohsihiro Mitaka, and Koichi Hirata

Key Points

- BTR is correlated with Fisher's ratio as an indicator of liver function.
- BTR could predict postoperative complication of the patients who would have initial hepatectomy for HCC.
- BTR could be a prognostic factor in HCC patients who undergo hepatectomy.
- BTR-ALB classification can be used to identify unique subgroups that are at risk of early recurrence.
- BTR-ALB classification identify putative target of adjuvant nutritional therapy for preventing early HCC recurrence.

Keywords Branched chain amino acid (BCAA) • BCAA to tyrosine ratio (BTR) • Prognosis • Complication • Biomarker

Introduction

Amino acid ratios are indicative of the systemic balance between protein metabolism and catabolism [1, 2]. The liver is heavily involved in protein and energy production, and hence, consumes more amino acids than any other organ in the body [3]. Consequently, when the liver is damaged by viral infection, alcoholic abuse, or fat deposition, amino acid metabolism also deteriorates [4]. Thus, the question arises as to whether particular amino acid ratios reflect the severity of liver damage.

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death in the world [5, 6]. Most cases of HCC are associated with viral infection, with hepatitis B virus being more common in Eastern countries and hepatitis C virus (HCV) being more prevalent in Western countries [7]. HCC most commonly develops in patients with progressive chronic hepatitis or cirrhosis, and the incidence of de novo liver carcinogenesis tends to increase with the severity and activity of fibrosis.

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The surgical indications for HCC include a tumor that is limited to the liver without multiple systemic metastases or severe portal hypertension, and good liver function [6], although exceptional cases such as those involving single systemic metastases might also be recommended for surgery. Therefore, previous studies of HCC patients who underwent surgery tended to involve patients with similar oncological backgrounds and liver function levels. However, this poses the question of how similar the liver functions of such patients are and how their liver function should be evaluated.

The systemic amino acid (AA) balance has been studied in various clinical settings [3, 8, 9]. Although systemic homeostasis is maintained under physiological conditions, surgical stress and tumors can disturb the balance between metabolism and catabolism [10]. Therefore, patients' AA profiles might be unstable in such conditions. On the contrary, the AA profile might be a useful biomarker for evaluating liver function and tumor grade under stable physiological conditions [1].

This chapter examines the relationship between preoperative amino acid ratios and liver function in HCC patients. In addition, we discuss the clinical value of amino acid ratios in HCC patients who elect to undergo surgery. Furthermore, we comment on the relationships between amino acid ratios and prognosis in HCC patients. As a result, we demonstrate that the branched chain amino acid to tyrosine ratio (BTR) prior to hepatectomy is a useful biomarker.

Fischer's Ratio and Branched Chain Amino Acid to Tyrosine Ratio (BTR)

When liver function deteriorates, protein productivity also decreases. In this situation, amino acid uptake by the liver is decreased, whereas branched chain amino acid (BCAA) uptake by the muscles is increased to compensate for the lack of energy production [11, 12]. In addition, aromatic amino acid (AAA) levels remain stable, which results in them accounting for a greater proportion of total AA. The ratio of BCAA to AAA is known as the Fischer's ratio and is increased in acute and fulminant liver failure (Fig. 5.1a) [12, 13]. In fact, the Fischer's ratio was found to decrease with the progression of hepatic encephalopathy; i.e., according to alert status (2.85 ± 0.18), encephalopathic status (1.83 ± 0.19), and coma status (1.48 ± 0.15) [14]. However, various experimental instruments, such as those required for ion-exchange chromatography, high-performance liquid chromatography, or gas chromatography, are needed to calculate it [15]. In addition, the Fischer's ratio is relatively complicated and time consuming to determine and so is rarely used in clinical practice. In contrast, the branched chain amino acid to tyrosine ratio (BTR; Fig. 5.1b) can be determined with a basic enzymatic method, which only requires the use of a spectrophotometer or fluorometer [15]. Accordingly, the BTR can be determined with automated clinical chemical analyzers, making it a suitable parameter for monitoring the severity of liver disease [8]. The correlation between the Fischer's ratio and the BTR is very high [15, 16]; therefore, the BTR has been widely adopted for clinical use [17]. In fact, the changes in the BTR after hepatectomy have been studied to see whether the administration of BCAA results in improvements in the BTR [18].

BTR as an Indicator of Liver Function

The BTR has been shown to decrease in liver conditions such as acute liver failure, chronic hepatitis, and liver cirrhosis, and it has also been found to be associated with the severity of liver disease [8, 16, 19]. In a previous study, we found that the mean BTR of the normal liver and the livers of patients with chronic hepatitis and liver cirrhosis were 6.41 ± 1.18, 5.83 ± 1.65, and 4.92 ± 1.42, respectively (Fig. 5.2). In addition, it was demonstrated that the BTR was correlated with prothrombin time (PT), albumin and bilirubin levels, and other parameters in liver conditions of varying severity other than HCC [16, 19]. We also reported the BTR of patients who initially underwent hepatectomy for HCC [19]. Consistent with the abovementioned reports [8, 16], our results (Fig. 5.3) showed that the BTR

Fig. 5.1 The formulae for the Fischer's ratio (**a**) and branched chain amino acid to tyrosine ratio (**b**)

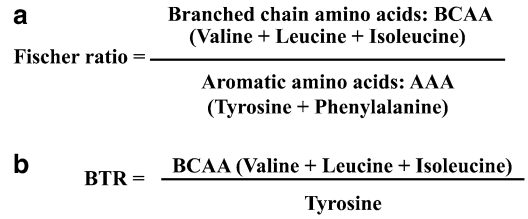
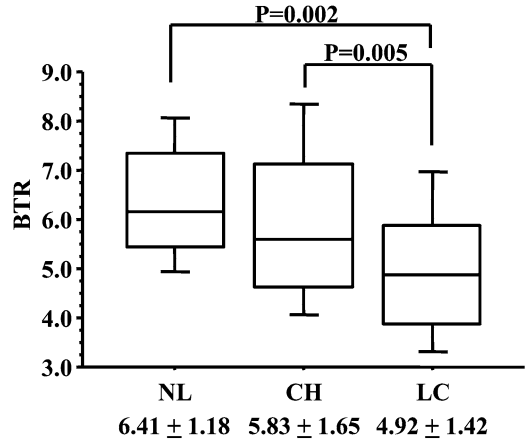


Fig. 5.2 Box and whisker plot of BTR according to the severity of liver disease. (NL, normal liver; CH, chronic hepatitis; LC, liver cirrhosis). The *central boxes* represent the values from the lower to upper quartiles (the 25th to 75th percentile). The line in the middle represents the median. The *horizontal line* extends from the minimum to the maximum value. Modified from Mizuguchi et al. [19]



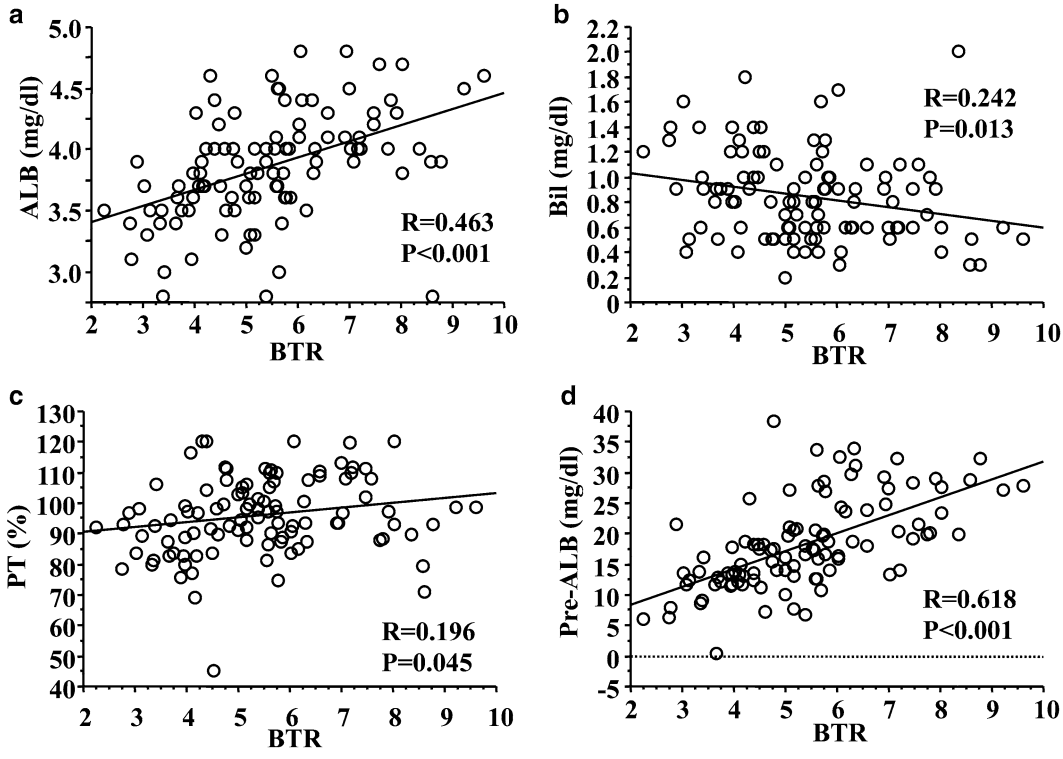


Fig. 5.3 Linear regression analyses of the relationships between the BTR and liver functional indicators such as ALB (**a**), bilirubin (**b**), PT (**c**), and pre-ALB (**d**). Modified from Mizuguchi et al. [19]

was correlated with albumin (ALB) ($R=0.463$; $P<0.001$) and pre-ALB levels ($R=0.618$; $P<0.001$) in HCC patients. However, the BTR exhibited very weak correlations with the bilirubin level ($R=0.242$; $P=0.013$) and PT ($R=0.196$; $P=0.045$) in these patients. Although the BTR indirectly reflects the severity of liver disease, it might also be useful as a liver function indicator. In the above-mentioned studies, the correlation between the BTR and other liver functional indicators varied depending on the severity of the patient's liver disease, and therefore, might have been affected by the selection bias introduced by the studies' inclusion criteria.

Relationships Between the BTR and Other Liver Functional Indicators

The clinical profiles of HCC patients who initially undergo hepatectomy can be divided into several clusters [20]. Cluster analysis is a type of multivariate analysis and is useful for elucidating the relationships among thousands of pieces of data, such as data obtained from microarray analyses of gene or protein expression in cancer tissue [21]. Cluster analysis makes it easier to understand the status of HCC patients, who display various levels of liver function and differing degrees of cancer progression, from a systematic point of view [20]. In a cluster analysis of the clinical profiles of HCC patients, the BTR was classified into the same cluster as liver functional indicators such as ALB, pre-ALB, and cholinesterase (Fig. 5.4). The serum levels of other proteins and the platelet count were also clustered into the same group as the BTR. These findings support the assertion that the BTR is correlated with the severity of liver disease.

Preoperative BTR and Postoperative Complications

Postoperative complications are inevitable in routine surgery [22]. Some complications are due to host-related factors such as an immunocompromised host, a poor nutritional condition, or the presence of a tumor [23, 24]. In addition, technical errors can also cause complications [22]. Surgeons try to avoid complications caused by technical factors by investigating what causes them.

We divided 105 consecutive HCC patients into high (>4.4) and low BTR (<4.4) groups (Table 5.1). The high BTR group consisted of 71 patients, and the low BTR group contained 34 patients [19]. Age, sex, etiology, pathology, ALB, bilirubin, hepatocyte growth factor (HGF), pre-ALB, alpha fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP) levels, PT, $ICGR_{15}$, BTR, platelet count tumor size, tumor number, vascular invasion, operation time, bleeding, operative procedure, and HCC stage were compared between the groups. The proportion of females ($P<0.001$), the frequency of HCV infection ($P=0.001$), and the frequency of liver cirrhosis were significantly higher ($P=0.005$) in the low BTR group than in the high BTR group. ALB (3.94 ± 0.44 vs. 3.69 ± 0.41; $P=0.001$), bilirubin (0.77 ± 0.35 vs. 0.98 ± 0.35; $P=0.001$), and pre-ALB (20.6 ± 7.2 vs. 13.6 ± 5.4; $P<0.001$) levels; as well as the $ICGR_{15}$ (11.4 ± 6.2 vs. 19.6 ± 8.39; $P<0.001$), platelet count (17.3 ± 11.8 vs. 11.9 ± 8.9; $P=0.0021$), and BTR (6.17 ± 1.24 vs. 3.88 ± 0.93; $P<0.001$) displayed significant differences between the groups. All of the complications encountered in our study are listed in Table 5.2. The incidence of complications after hepatectomy differed significantly between the groups (23.9 % vs. 52.9 %; $P=0.0008$). In addition, the frequency of grade IV and V complications was 23.5 % in the high BTR group, whereas it was 44.4 % in the low BTR group. Therefore, the BTR was not only able to predict the risk of complications but also predicted the severity of complications after initial hepatectomy for HCC.

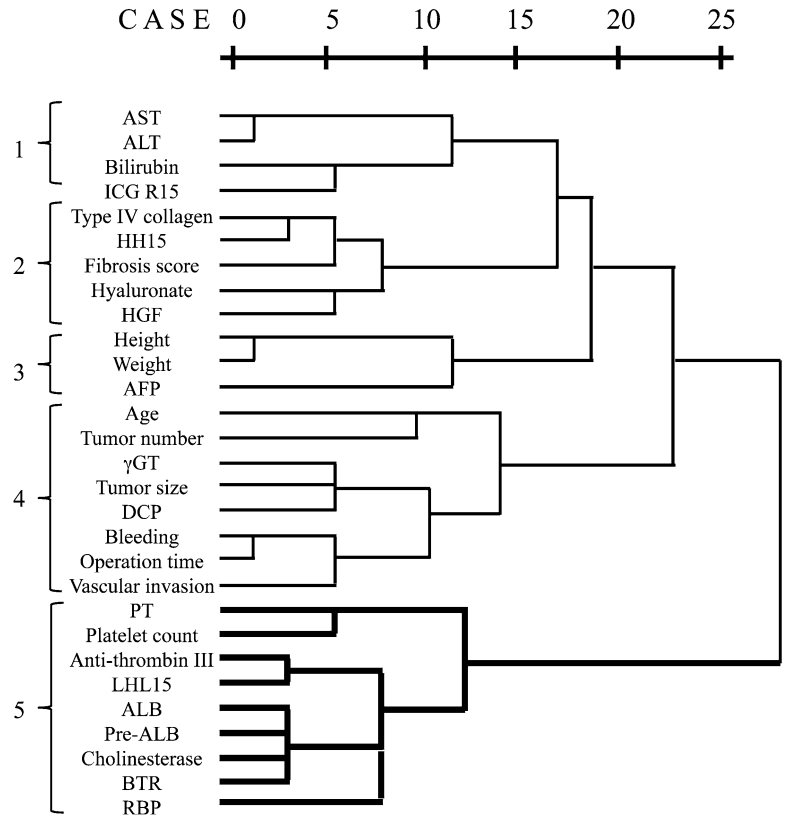


Fig. 5.4 Dendrogram of possible prognostic indicators, including tumor-related factors and operative variables, in 165 HCC patients. The indicators were divided into 5 clusters using Ward's criterion. AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched chain amino acid to tyrosine ratio; DCP, des-gamma-carboxy prothrombin; GGT, γ-glutamyl transpeptidase; HGF, hepatocyte growth factor; HH15, clearance index of ^{99}mtechnetium-galactosyl human serum albumin; $ICGR_{15}$, indocyanine green retention rate at 15 min; LHL15, hepatic uptake ratio of ^{99}mtechnetium-galactosyl human serum albumin; PT, prothrombin time; RBP, retinol-binding protein; *Dotted lines*, *tracks* indicating the relationships among ALB, HGF, and BTR. Modified from Nakamura et al. [20]

BTR as a Prognostic Factor in HCC Patients Who Undergo Hepatectomy

Posthepatectomy prognosis was compared between the high ($N=71$) and low BTR groups ($N=34$) [19]. The mean age of the study population was around 65 years, the mean tumor size was around 4 cm, and the mean number of tumors was 1.5. All of the study population had been selected for hepatectomy so their tumors were all resectable and localized in the liver. Therefore, some selection bias was inevitable in this type of study. The recurrence free-survival (RFS) period of the high BTR group was significantly longer than that of the low BTR group (Fig. 5.5a, 41.6±3.5 M vs. 19.8±2.8 M; $P<0.001$). In addition, the overall survival (OS) period of the high BTR group was significantly longer than that of the low BTR group (Fig. 5.5b, 53.1±2.9 M vs. 41.4±5.2 M; $P=0.021$). Thus, the BTR was able to predict both RFS and OS.

RFS differed depending on HCC stage (Table 5.3). Although RFS did not differ between the groups among the stage IV patients, it differed significantly between the high and low BTR groups in all other

Table 5.1 Clinical characteristics of the patients in the high BTR and low BTR groups of a consecutive series who initially underwent hepatectomy. Asteristics indicate significant differences (P < 0.05)

Factors	High BTR	Low BTR	*P*-value	Factors	High BTR	Low BTR	*P*-value
Age (Year)	64.3 ± 11.4	65.9 ± 9.1	0.496	Plt	17.3 ± 11.8	11.9 ± 8.9	0.021*
Sex (M:F)	61:10	16:18	<0.001*	HGF	0.34 ± 0.18	0.38 ± 0.14	0.293
Etiology B:C:alcohol	43:22:6	7:24:3	0.001*	T size	4.33 ± 3.62	4.22 ± 2.59	0.874
				T number	1.4 ± 0.9	1.5 ± 1.0	0.643
Pathology N:CH:LC	11:31:29	1:8:25	0.005*	VI Pos:Neg	19:52	12:22	0.369
ALB	3.94 + 0.44	3.69 ± 0.41	0.001*	AFP	2884 ± 14279	1721 ± 6226	0.656
Bilirubin	0.77 + 0.35	0.98 ± 0.35	0.001*	DCP	2994 ± 8375	3699 ± 16232	0.772
PT	97.6 + 12.4	92.6 + 13.1	0.059	Op time	350.5 + 175.2	357.6 + 272.7	0.874
Pre-ALB	20.6 ± 7.2	13.6 ± 5.4	<0.001*	Bleeding	726.4 + 850.9	833.1 + 1151.6	0.604
icgr15	11.4 + 6.2	19.6 ± 8.39	<0.001*	Op (Hr) (0:S:1:2:3)	29:18:14:8:2	17:12:2:2:1	0.230
BTR	6.17+1.24	3.88 ±0.93	<0.001*				
				Stage (1:2:3:4)	15:31:20:5	3:19:8:4	0.067

N normal liver, *CH* chronic hepatitis, *LC* liver cirrhosis, *ALB* albumin, *PT* prothrombin time, *ICG* indocyanine green retention rate at 15 min, *BTR* branched chain amino acid/tyrosine ratio, *Pit* platelet count, *HGF* hepatocyte growth factor, *T* tumor, *VI* vascular invasion, *Pos* positive, *Neg* negative, *AFP* alpha fetoprotein, *DCP* des-gamma-carboxy prothrombin, *Op* operation, *Hr* type of liver resection, *S* subsegmentectomy. Reproduced from Mizuguchi et al. [19] with permission

*(P < 0.05)

Table 5.2 Postoperative complications suffered by 105 HCC patients after initial hepatectomy

Complications	High BTR group ($n = 71$) (minor/major)	Low BTR group ($n = 34$) (minor/major)
Liver/Biliary		
Liver failure/insufficiency	–/1	–/3
Bile leakage	1/1	1/–
Hepatic arterial aneurysm	–/–	–/1
Pulmonary		
Pleural effusion (symptomatic)	1/1	–/1
Pneumonia	–/–	1/–
Genitourinary		
Renal insufficiency/failure	2/–	–/–
Gastrointestinal		
Ileus	1/–	–/–
Miscellaneous		
Wound infection/dehiscence	2/–	4/2
Ascites	5/1	4/1
Intra-ahdominal abscess	1/–	–/–
Total	13/4	10/8

Minor complications are graded as grade I, II, or III in the modified Clavien classification. Major complications are graded as grade IV or V in the modified Clavien classification. The χ^2 test followed by Fisher's exact test (post hoc 2 × 2) were used to compare the two groups ($P = 0.003$). Reproduced from Mizuguchi et al. [19] with permission

stages of the disease [stage I (56.1 ± 5.1 M vs. 4.0 ± 0 M; $P < 0.001$), II (44.3 + 4.9 M vs. 28.9 + 5.5 M; $P = 0.026$), and III (30.3 + 5.8 M vs. 11.2 + 3.3 M; $P = 0.049$)]. In this analysis, the significance of the difference became weaker as the stage increased. Therefore, the BTR might have been affected by tumor recurrence in the earlier stages of HCC. Another possible explanation is that oncological

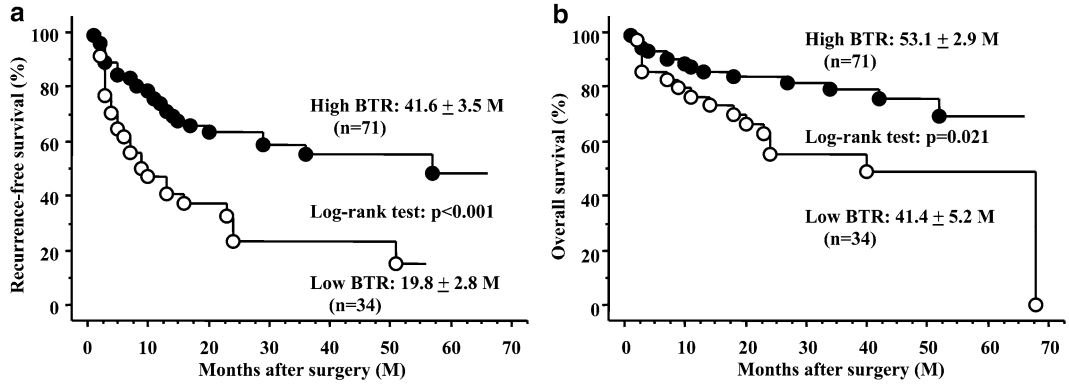


Fig. 5.5 Cumulative recurrence-free survival (**a**) and overall survival (**b**) curves for the HCC patients in the high and low BTR groups. *Closed circles*, high BTR group ($n=71$); *open circles*, low BTR group ($n=34$). Modified from Mizuguchi et al. [19]

Table 5.3 Mean recurrence-free survival period of high BTR and low BTR patients who initially underwent hepatectomy according to tumor stage

Stage	Total patients	High BTR	Low BTR	*P*-value
1	52.7 ± 5.7	56.1 ± 5.1	4.0 ± 0	<0.001
2	38.5 ± 3.9	44.3 ± 4.9	28.9 ± 5.5	0.026
3	25.1 ± 4.6	30.3 ± 5.8	11.2 ± 3.3	0.049
4	6.9 ± 2.2	5.2 ± 2.1	8.6 ± 4.2	0.618

Data: Mean ± standard error. Reproduced from Mizuguchi et al. [19] with permission

Table 5.4 Likelihood of disease-free survival according to Cox's proportional hazards model

Disease-free survival	Odds ratio	95 % confidence interval	*P*-value
Sex (Female)	0.37	0.14–0.95	0.039
BTR	0.57	0.37–0.86	0.008
Tumor size	1.24	1.11–1.38	<0.001
Tumor number	1.53	1.19–1.97	0.001

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prognostic factors other than the BTR were affected to a greater extent in the advanced stages of HCC than in the early stages. In fact, tumor size and number were found to be independent prognostic factors for RFS in HCC patients in addition to gender and the BTR (Table 5.4).

BTR-ALB Classification Highlights Amino Acid Imbalances and Can Be Used to Identify Unique Subgroups that Are at Risk of Early Recurrence

Albumin is the most abundant protein in the human body and is exclusively produced by the liver. Albumin production is an indicator of the relationship between cell functional status and cell proliferative status [25]. In normal cells, the balance between cell functioning and cell proliferation is maintained during physiological liver regeneration [26]. However, this balance can be disrupted by cancer development, which might be reflected by imbalances in the levels of albumin and the BTR.

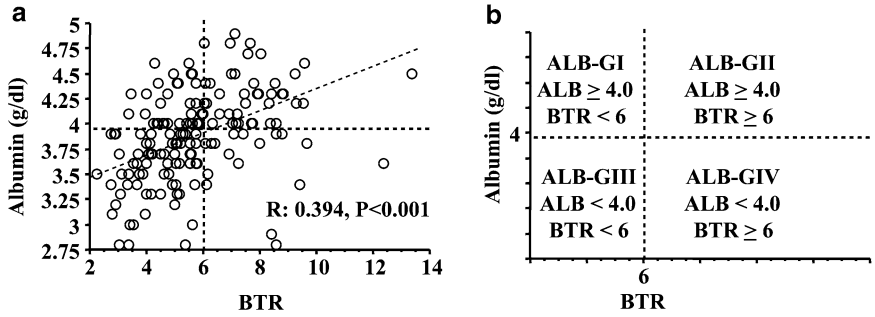

Fig. 5.6 Linear regression analysis of the relationship between ALB and BTR (**a**). The equation of the linear regression line is ALB = 3.357 + 0.092 X BTR ($R = 0.394$; $P < 0.001$). The ALB-BTR classification divides patients into four different subgroups depending on their ALB levels and BTR (**b**). Modified from Nakamura et al. [20]

Table 5.5 Clinical characteristics of the subgroups of the ALB-BTR classification

ALB-BTR	GI	GII	GUI	GIV
No. of patients	34	42	72	17
Etiology				
B	16	29	30	6
C	14	6	35	9
BC	2	0	1	0
NBNC	2	7	6	2
$P = 0.013$				
Stage				
I	6	7	8	3
II	12	19	33	8
III	11	12	25	4
IV	5	4	6	2
$P = 0.777$				
Background				
NL	2	12	5	3
CH	17	15	20	7
LC	15	15	47	7
$P = 0.003$				
Operation				
0	15	17	32	3
S	11	7	18	3
1	3	11	12	8
2	4	6	8	1
3	1	1	2	2
$P = 0.106$				

B hepatitis B alone, *C* hepatitis C alone, *BC* hepatitis B and C, *NBNC* non-B and non-C hepatitis, *NL* normal liver, *CH* chronic hepatitis, *LC* liver cirrhosis, *0* partial resection, *S* subsegmentectomy, *1* segmentectomy one, *2* segmentectomy two, *3* segmentectomy three. Reproduced from Nakamura et al. [20] with permission

The albumin-BTR classification (Fig. 5.6) divides HCC patients into four groups depending on their albumin levels and BTR [20]. In this system, high albumin levels were defined as albumin levels of 4.0 mg/dl or more, and low albumin levels were defined as albumin levels of less than 4.0 mg/dl. In addition, a high BTR was defined as a BTR of 6.0 or more, and a low BTR was defined as a BTR of less than 6.0. In our study, group I, the high albumin and low BTR group, consisted of 34 patients (Table 5.5); group II, the high albumin and high BTR group, consisted of 42 patients; group III, the

Table 5.6 Clinical characteristics of the subgroups of the ALB-BTR classification

ALB-BTR	ALB-GI	ALB-GII	ALB-GIII	ALB-GIV	ANOVA	I–II	III–IV
AST (IU/L)	45.2 ± 24.5	32.3 ± 12.6	62.1 ±49.9	37.5 ± 17.9	<0.001	0.004	0.049
ALT (IU/L)	45.2 ± 29.4	32.7 ± 18.5	50.8 ± 37.8	37.1 ±20.1	0.019	0.027	
Bil (mg/dl)	0.81 ± 0.36	0.81 ± 0.34	0.82 ± 0.39	0.54 ± 0.23	0.032		0.006
ICGR15 (%)	12.2 ± 6.7	9.7 ± 5.6	17.7 ± 9.1	8.04 ± 4.5	<0.001		<0.001
Col (ng/ml)	5.73 ± 1.62	4.95 ± 2.25	7.46 ± 3.14	5.21 ± 1.47	<0.001		0.011
HH15	0.59 ± 0.06	0.55 ± 0.06	0.66 ± 0.08	0.61 ± 0.07	<0.001	0.011	0.029
F score	2.7 ± 1.3	2.1 ± 1.7	3.1 ± 1.3	1.7 ± 1.4	0.005		0.002
HA (ng/ml)	139.3 ± 92.9	80.5 ± 74.5	246.8 ± 206.1	177.8 ± 144.9	<0.001	0.003	
HGF (ng/ml)	0.33 ± 0.11	0.33 ± 0.17	0.38 ± 0.15	0.32 ± 0.13			
Height	162.5 ± 8.9	163.5 ± 7.2	159.2 ± 8.9	162.9 ± 7.7	0.039		
Weight	62.1 ± 10.5	62.2 ± 10.6	61.8 ± 11.7	65.5 ± 9.4			
AFP (ng/ml)	4805 ± 20023	3134 ± 9814	1376 ± 4187	551 ± 977			
Age (Y)	62.9 ± 11.4	60.8 ± 11.8	68.2 ± 8.7	67.9 ± 7.9	0.001		
Tumor number	2.2 ± 2.8	1.7 ± 2.4	1.6 ± 1.1	2.5 ± 4.6			
GGT (IU/L)	107.1 ± 90.3	67.4 ± 76.5	106.4 ± 98.7	119.5 ± 204.4		0.043	
Tumor size	3.5 ± 3.2	3.9 ± 3.7	4.7 ± 3.3	5.5 ± 3.5			
DCP (mAU/ml)	1424 ± 6065	5377 ± 25688	4708 ± 13662	14362 ± 46798			
Bleeding (ml)	627.1 ± 720.1	507.4 ± 479.8	844.1 ± 1112.9	692.3 ± 599.6			
Op Time (min.)	372.3 ± 154.8	351.3 ± 138.6	346.1 ± 207.7	406.3 ± 164.7			
VI	0.5 ± 1.1	0.4 ± 0.8	0.5 ± 1.1	0.2 ± 0.4			
PT (%)	98.1 ± 11.9	97.4 ± 12.5	88.5 ± 13.1	90.3 ± 10.6	<0.001		
Plt ($X10^4$ ml)	13.6 ± 5.3	16.8±12.6	14.1 ± 9.5	16.8 ± 8.1			
ATIII (%)	91.2 ± 15.9	95.3 ± 12.1	77.5 ± 17.1	91.1 ± 15.8	<0.001		0.004
LHL15	0.93 ± 0.03	0.94 ± 0.02	0.89 ± 0.06	0.92 ± 0.03	<0.001	0.003	
ALB (g/dl)	4.18 ± 0.21	4.31 ± 0.31	3.55 ± 0.29	3.62 ± 0.33	<0.001		
Pre-ALB (mg/dl)	20.8 ± 6.1	24.1 ± 7.1	13.7 ± 4.9	20.8 ± 6.5	<0.001	0.037	<0.001
Cho1E (IU/L)	264.5 ± 77.1	288.3 ± 73.8	181.3 ± 48.1	227.3 ± 64.4	<0.001		0.002
BTR	4.97 ± 0.72	7.71 ± 1.37	4.39 ± 0.96	7.86 ± 1.65	<0.001	<0.001	<0.001
RBP (mg/dl)	3.14 ± 1.11	3.36 ± 1.26	2.01 ± 1.11	4.28 ± 3.08	<0.001		<0.001

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *Bil* bilirubin, *ICGR15* indocyanine green retention rate at 15 min, *Col* type IV collagen, *HH15* clearance index of ^{99m}technetium-galactosyl human serum albumin, *F* fibrosis, *HA* hyaluronic acid, *HGF* hepatocyte growth factor, *AFP* alpha fetoprotein, *GGT* γ-glutamyl transpeptidase, *DCP* des-gamma-carboxy prothrombin, *Op* operation, *VI* vascular invasion, *PT* prothrombin time, *Pit* platelet count, *ATIII* anti-thrombin III, *LHL15* hepatic uptake ratio of ^{99m}technetium-galactosyl human serum albumin, *ALB* albumin, *CholE* Cholinesterase, *BTR* branched chain amino acid to tyrosine ratio, *RBP* retinol-binding protein. Reproduced from Nakamura et al. [20] with permission

low albumin and low BTR group, consisted of 72 patients; and group IV, the low albumin and high BTR group, consisted of 17 patients.

HCV infection was often encountered in GIII and GIV, liver cirrhosis was common in GIII, and surgical complications were frequently seen in GIII. The main difference between GI and GII was in their BTR (Table 5.6); however, the alkaline phosphatase (AST) and alanine transaminase (ALT) levels of these two groups also differed significantly. Furthermore, the BTR, AST and bilirubin levels, and ICGR15 of GIII were significantly different from those of GIV.

The RFS and OS of each subgroup of the ALB-BTR classification are shown in Fig. 5.7. Both RFS and OS were longer in GII than in the other groups although the significance of the difference varied (Table 5.7). The mean RFS of GI was shorter than those of the other groups, although again the significance of the difference varied. Although the ALB levels of the patients who underwent hepatectomy for HCC were maintained, the patients with low BTR were found to be at high risk of recurrence. The low BTR of GI could have been caused by uncontrolled hepatitis, which presents with high AST and ALT levels.

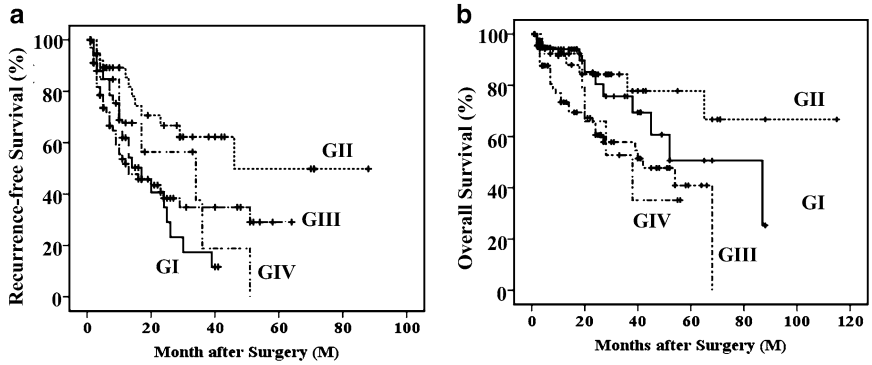

Fig. 5.7 Cumulative overall survival and recurrence-free survival curves of 165 consecutive HCC patients according to the ALB-BTR classification (**a** and **b**). The curves were produced using the Kaplan-Meier method, as described in the Methods section. ALB-BTR classification: *Solid line*, Group I (ALB-GI); *dotted line*, group II (ALB-GII); *single chain line*, group III (ALB-GIII); *double chain line*, group IV (ALB-GIV). Modified from Nakamura et al. [20]

Table 5.7 Mean overall survival period (MOS) and mean recurrence-free survival period(MRFS) according to the ALB-BTR classification

ALB-BTR classification	MOS (M)	ALB-GI (*P*-values)	ALB-GII (*P*-values)	ALB-GIII (*P*-values)	ALB-GIV (*P*-values)
ALB-GI	60.1 ± 5.6		0.328	0.063	0.308
ALB-GII	87.8 ± 7.0	0.328		0.006	0.088
ALB-GIII	40.4 ± 3.9	0.063	0.006		0.787
ALB-GIV	63.5 ± 4.1	0.308	0.088	0.787	
	MRFS (M)	ALB-GI	ALB-GII	ALB-GIII	ALB-GIV
ALB-GI	19.1 ± 2.4		0.001	0.738	0.316
ALB-GII	54.8 ± 7.6	0.001		0.008	0.075
ALB-GIII	27.8 ± 5.6	0.738	0.008		0.737
ALB-GIV	38.2 ± 3.2	0.316	0.075	0.737	

The ALB cut-off value was 4.0 g/dl, and the BTR cut-off value was 6.0. The HGF cut-off value was 0.35 ng/ml, and the BTR cut-off value was 6.0. The log-rank test was used for the statistical analyses

Putative Target of Adjuvant Nutritional Therapy for Preventing Early HCC Recurrence

BCAA administration has been tested in humans, but produced unsatisfactory clinical results [27]. In another study, BCAA administration altered the turnover of BCAA, but had no effect on hormone or protein metabolism [28]. On the other hand, perioperative nutritional support such as the enteral administration of BCAA reduced the frequency of surgical complications in HCC patients with cirrhosis [27, 29, 30], although it is still unknown whether BCAA administration improves the long-term prognosis of HCC patients [27]. A low BTR alone might not be sufficient for selecting patients that would benefit from treatments aimed at preventing early recurrence after hepatectomy. Rather, it was suggested that among patients with low BTR only those with high ALB levels are good candidates for such an approach [20]. To prove this hypothesis, further clinical studies with strict inclusion criteria, such as studies involving low BTR and high ALB patients, are needed.

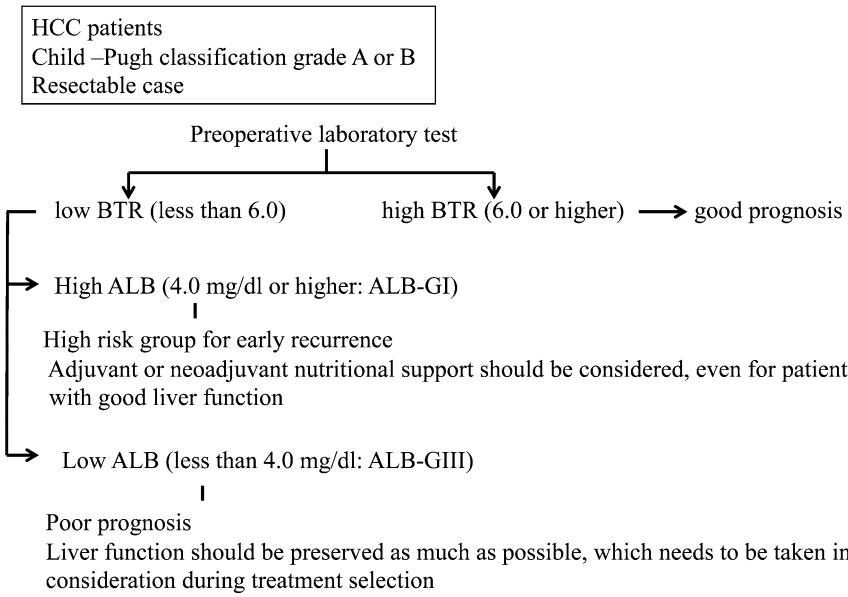

Fig. 5.8 Flow chart of the prognoses of HCC patients with good liver function who undergo hepatectomy. Modified from Nakamura et al. [20]

Strategy for the Surgical Management of HCC Patients Based on the ALB-BTR Classification (Fig. 5.8)

We have developed a strategy for the surgical management of HCC patients based on the ALB-BTR classification (Fig. 5.8). According to this method, patients with high BTR are expected to have a good prognosis, even those with low ALB values. Patients with high BTR and ALB levels are also predicted to have a generally good prognosis. On the other hand, patients with low BTR need to be carefully observed after hepatectomy. Among patients with low BTR, those with high ALB levels are considered to be at high risk of early recurrence after hepatectomy. Therefore, adjuvant or neoadjuvant nutritional support might be considered in this group. In addition, patients with low ALB levels and low BTR are expected to have a poor prognosis. In these patients, aggressive surgical management should be avoided, and the preservation of liver function should be actively considered during treatment selection.

Effects of Protein Metabolism in the Tumor Bearing State on the BTR

Tumors alter the systemic balance between metabolism and catabolism [31, 32]. Tumor stage progression increases systemic tyrosine levels without inducing phenylalanine degradation. It is likely that protein breakdown is responsible for the increased tyrosine concentrations observed in the advanced stages of tumor development [33]. On the other hand, tumors utilize BCAA to synthesize their own proteins [32]. As a result of this physiological mechanism, the BTR of HCC patients could decrease, and hence, act as a marker of tumor stage progression.

Metabolic Alteration of the BTR During the Inflammatory Response

Although amino acid metabolism in mild inflammation is not fully understood, amino acid alterations have been observed during sepsis [34]. The plasma levels of aromatic amino acids (phenylalanine and tyrosine) tended to be increased in septic patients, whereas the levels of branched chain amino acids (valine, leucine, and isoleucine) remained within the normal ranges. In addition, the surviving patients had decreased AAA levels and increased BCAA levels compared with the nonsurviving patients [35]. Therefore, the BTR could be used as a prognostic indicator not only for HCC patients but also for septic patients.

Conclusions

The BTR is correlated with not only the Fischer's ratio but also indicators of liver function. The preoperative BTR could be a useful predictor of surgical complications after hepatectomy in HCC patients who initially undergo hepatectomy, and it could also be useful for predicting the prognosis of these patients. Furthermore, the BTR-ALB classification could be used to identify patient groups that would benefit from BCAA supplementation as adjuvant nutritional support.

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Part II
Branched Chain Amino Acids: Status in Disease States

Chapter 6
Branched Chain Amino Acids in Heart Failure

Haipeng Sun and Yibin Wang

Key Points

- Branched chain Amino Acids are essential amino acids for protein synthesis, signaling, and metabolic activity.
- Its homeostasis is regulated largely by catabolic activities.
- Genetic defects in BCAA catabolic pathways lead to cardiomyopathies in human and animal models.
- Suppressed BCAA catabolic activities were observed in common forms of human heart diseases.
- In future studies, how BCAA catabolism is misregulated by pathological stressors in heart diseases, and how BCAA catabolic defects contribute to pathogenesis of heart failure, will need to be investigated.
- BCAA catabolism can be explored for potential diagnosis and therapeutic targets for heart failure.

Keywords Branched chain amino acid • Catabolism • Metabolic remodeling • Heart failure • Genetic disorder • Human disease

Abbreviations

BCAA Branched chain amino acids
BCKA Branched chain alpha keto-acids
mTOR Mammalian Target of Rapamycin
BCAT Branched chain amino-transferase

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BCKD Branched chain alpha keto acid dehydrogenase
TCA Tricarboxylic acid
BCKDK BCKD kinase
ROS Reactive oxygen species
DCM Dilated cardiomyopathy
HCM Hypertrophic cardiomyopathy

Introduction

BCAA Homeostasis

Essential amino acids are indispensable nutrient for cell growth, survival, and normal function. Three essential amino acids, leucine, isoleucine, and valine are collectively referred to as branched chain amino acids (BCAA) as they share a side-chain structure and common catabolic pathway (Fig. 6.1). BCAA provides a major component of building blocks for protein as well as nitrogen and carbon for biosynthesis of sterol, keto bodies, and glucose [1, 2]. In addition, a special function of BCAA, particularly leucine, is their signaling activity to cells [3–7]. BCAA promotes protein synthesis, cellular metabolism, and cell growth while repressing autophagy through regulation of mammalian Target of Rapamycin (mTOR) pathway [8]. Accordingly, BCAA-mediated intracellular signaling represents an important aspect of nutrient-cell interaction, and maintaining BCAA homeostasis is critical to normal physiology.

BCAAs are essential amino acids for animals and can only be acquired from external sources through food consumption. The homeostasis of free BCAA in serum is maintained at very stable levels achieved by balancing protein degradation and BCAA catabolic activities. BCAA catabolic activities are responsible to remove excess amino acids. The first step in BCAA catabolism is carried out by the Branched chain Amino-transferase (BCAT) which converts BCAA into branched chain alpha keto-acids (BCKA). BCKA are then decarboxylated by the Branched chain Alpha Keto Acid Dehydrogenase (BCKD) complex followed by multiple enzymatic steps, leading to the final catabolic products of acetyl-CoA and succinyl-CoA. They are eventually consumed through tricarboxylic acid (TCA) cycle during mitochondrial respiration. BCKD-mediated reaction is the rate-limiting and irreversible step of BCAA catabolism and its activity is the target of multiple regulatory mechanisms. Among them, phosphorylation on E1α subunit of BCKD inhibits its enzymatic activity. BCKD kinase (BCKDK) inhibits BCKD by phosphorylating BCKD E1α while BCKD phosphatase (also named PP2Cm) activates and dephosphorylates BCKD. This reversible BCKD phosphorylation contributes significantly to BCAA homeostatic maintenance [9].

BCAA Metabolism in Normal Heart

In mammals, BCAA catabolic activities have different tissue specific distributions among different species. In rat, the BCAA homeostasis is maintained by the cooperation between liver and extrahepatic organs [10]. The expression of BCAT, the gene catalyzes the deamination of BCAA, in liver is very low if there is any. The first step of BCAA catabolism catalyzed by BCAT occurs in nonhepatic tissues, such as brain, muscle, and kidney. The primary product, BCKA, is transported to and degraded in liver. In rat, more than 80 % of the total transaminase activity of BCAA occurs in skeletal muscle while more than 80 % of the BCKD activity expresses in liver [10]. On the other hand, in humans,

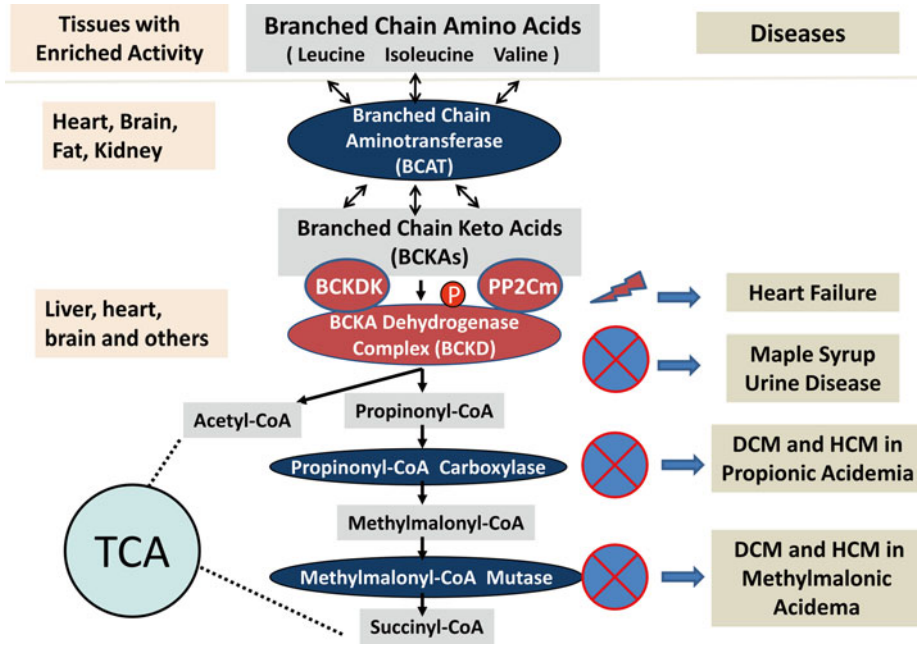

Fig. 6.1 Branched chain amino acids catabolic pathway and defects in heart diseases

BCAA catabolism demonstrates a different organ-specific pattern. It has been reported that skeletal muscle is the major organ that carries out both the BCAT and BCKD activities [10]. However, little is known about either the BCAA catabolic activity in heart or the role of cardiac BCAA catabolism in the whole-body BCAA homeostasis.

Heart muscle express all key BCAA catabolic genes, including BCAT, BCKDK, PP2Cm, and BCKD subunits. From a recent publication, Dr. David Chuang's lab demonstrated a ~50-fold increase of BCKD activity in mouse heart when BCKDK activity was inhibited. In the same study, the activity of BCKD in skeletal muscle increased ~11-folds upon BCKDK inhibition [11]. Therefore, it appears that the BCKD activity in cardiac muscle is heavily inhibited when animals are fed ad libitum. It has been suggested that BCKDK activity is high in skeletal muscle [12] and the BCKD activity is almost 100 % suppressed under normal conditions [10, 13]. In contrast, expression of PP2Cm (a BCKD activator) is highly enriched in myocardium while very low in skeletal muscle in adult mice [14, 15]. Therefore, highly coordinated regulation of BCAA catabolism is present in heart but the physiological role of BCAA catabolic activity in heart is unclear.

BCAA Function in Normal Heart

Although the BCAA's functions as protein building blocks, carbohydrate resource, and nutrient signal have been established at cellular level, little has been studied about the function of BCAA in heart. It is reasonable to anticipate that the primary utilization of BCAA in heart is for protein synthesis. On the other hand, BCAA is unlikely an important source energy relative to other two major contributors to fuels: glucose and fatty acid in normal heart. However, BCAA/BCKA is shown to inhibit pyruvate and fatty acid transport and utilization [16]. It is then possible that BCAA affects energy metabolism in heart through regulation of glucose and fatty acid metabolism. Another potentially important

function of BCAA, especially L-leucine, is the regulation of mTOR signaling in heart [17]. mTOR activity directly regulates protein synthesis [18, 19], insulin sensitivity [20–22], and autophagy [23], among many other vital cellular functions. Autophagy is essential for the turnover of organelles and macromolecules in the heart [24]. Therefore, BCAA may affect heart function through its signaling effects on metabolic activity, growth, and preservation. In addition, a recent report from D'Antona et al. showed that BCAA supplement promoted cardiac muscle survival in middle-aged mice [25]. The authors suggested that mTOR-mediated insulin signaling was involved in this beneficial effect. Nevertheless, besides these intriguing possibilities, the importance of BCAA homeostasis in normal physiology of heart has not been well understood.

BCAA Catabolic Defect Linked to Cardiac Disease

Although BCAA are essential for normal growth and function, excess amount of free BCAA metabolites can be pathological. Several human diseases, including maple syrup urine disease, methylmalonic acidemia, and propionic acidemia, are known genetic disorders resulting from mutations in BCAA catabolic enzymes (Fig. 6.1). Propionic acidemia and methylmalonic acidemia have been associated with dilated and hypertrophic cardiomyopathies [26–28], suggesting that BCAA catabolic defects can cause cardiac dysfunction. The fact that liver transplant corrected the clinical symptoms of cardiomyopathy in Propionic Aciduria patients further supports the hypothesis that accumulated BCAA metabolites are the culprit to trigger cardiac dysfunction in these patients [29].

With recent advancement in molecular genetics, the impact of BCAA catabolic defect on heart can be extensively explored in experimental animals. PP2Cm is one key regulator of BCKD via direct E1a dephosphorylation. Without functional PP2Cm, serum BCAA/BCKA accumulates in mice with genetic inactivation of PP2Cm [30]. PP2Cm deficient zebrafish displayed a significant loss of cardiac contractility [31]. PP2Cm deficient mice demonstrated accelerated heart failure following mechanical overload induced by transaortic constriction [32]. These results from research animal supports the conclusion that defects in BCAA homeostasis can have a significant adverse impact on cardiac function.

Cardiac Stress Alters BCAA Catabolism

In absence of genetic disorder, the concurrence of abnormal BCAA level and cardiac diseases indicates a relationship between them. A few nonbiased metabolomic studies found a higher BCAA level in blood from patients with cardiac diseases [33]. In addition, BCAA level is elevated in plasma and myocardium in rat with stress-induced heart failure [34, 35]. The association of cardiac disease and abnormal BCAA metabolism observed in these studies did not establish any causal relationship between these two phenomena. As the contribution of heart for the whole-body BCAA homeostasis appears small, it is less likely that damaged heart itself contributes to the increase of plasma BCAA level. BCAA metabolism in liver and/or other organs may be interrupted when cardiac function is compromised.

Locally in heart, the progression of cardiac failing is associated with a remodeling of metabolism [36, 37]. The myocardium shifts from a fatty-acids dominant bioenergetic state into more glycolytic state under stress. Bioenergenic defect develops and contributes to the disease progression [37]. Due to the energetic nature of these studies, most of current investigations are focused on fatty acids and carbohydrates. In contrast, amino acid metabolism is underexplored [38]. Recent studies found that,

in mice, PP2Cm is highly expressed in cardiac muscle cells. PP2Cm expression is significantly reduced at both mRNA and protein levels in hypertrophic and failing hearts [31]. Indeed, in failing heart, other BCAA catabolic genes, such as BCAT2 and BCKD subunits, were all downregulated significantly based on a profiling research [39]. These data suggests that suppressed BCAA catabolism represents another potentially important component of metabolic remodeling in diseased heart. Whether and how such defects contribute to the pathogenesis of heart failure remains to be further investigated.

Perspectives

The association between BCAA metabolic reprogramming and cardiac dysfunction is an underappreciated area for research. Recent studies showed that systemic defect of BCAA catabolism can contribute to heart failure. On the other side, when heart is under pathological stressor, both systemic and local BCAA catabolism in heart can be impaired. It is assured that more research in future will further validate the functional relevance of this connection and reveal the underlying mechanisms.

One question remains in this relationship is how stressed heart affects BCAA catabolism locally and/or systemically. Cardiac disease is not the only pathological process linked with defective BCAA metabolism. Recently, the abnormal serum BCAA level has been observed in patients with neurodegenerative, cardiovascular, and metabolic diseases [21, 33, 40, 41]. All of these studies made correlative observation, but the biological significance and underlying mechanisms have not been explored. It is possible that BCAA catabolic change is an overall response to stress in animals. If so, which organ mediates this response and how it responds are intriguing questions that remain to be addressed. Locally in heart, the BCAA catabolic enzymes are enriched yet with low basal activity under normal condition. Under stress, BCAA catabolic genes were downregulated in diseased heart. The molecular mechanism of this downregulation remains elusive. In addition, it remains unclear whether this suppression of gene expression and the resulting BCAA catabolic defect are part of global molecular and metabolic remodeling in stressed heart.

The second major question is how BCAA catabolic defect adversely impacts heart function. Defect in BCAA catabolism leads to accumulation of BCAA and likely the intermediate products such as BCKA. Firstly, it has been suggested that adverse impact of BCAA/BCKA accumulation on glutamine transport and induction of reactive oxygen species (ROS) contributes to neurological pathogenesis in Maple Syrup Urine Disease [42]. PP2Cm deficient mice shows significantly elevated reactive-oxygen species level [31] while BCKA treatment induced oxidative stress in cells [30]. It is plausible that oxidative stress induced by BCAA/BCKA accumulation contributes to cardiac dysfunction. Secondly, accumulated BCAA/BCKA can impact on energy metabolism in diseased heart. Heart consumes large amount of ATP generated mostly from lipid and glucose metabolism at a very high rate. Interference of energy homeostasis will impair cardiac contractility and performance pathological consequences. BCAA/BCKA can affect pyruvate and fatty acid utilization [16, 43]. Elevated BCAA/BCKA can potentially block bioenergenic production, leading to contractile dysfunction and accelerated heart failure under stress. Thirdly, mTOR plays a key role in regulating cellular processes such as insulin signaling, protein/lipid/glucose/nucleotide metabolism, and autophagy [44]. Autophagy plays a key role in cardiac pathology although it remains controversial about whether it is beneficial or detrimental [45–47]. It has been shown that mTOR plays an essential role in the hypertrophic response of the heart under stress to preserve cardiac function [18]. It is plausible that, when BCAA catabolism is impaired, elevation of BCAA concentration can lead to higher cardiac mTOR activity. As a result, fundamental physiological processes including protein turnover, lipid/glucose/nucleotide metabolism, and autophagy regulation will be interfered in heart. Fourthly,

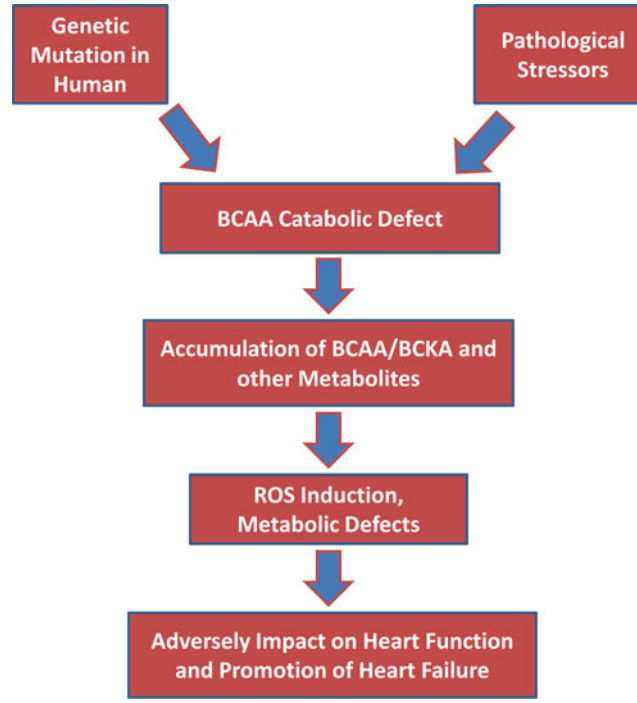

Fig. 6.2 Contribution of BCAA catabolic defects to pathogenesis of heart failure

BCAA and/or BCKAs metabolites can also have a direct impact on mitochondria function. Dilated cardiomyopathy is associated with defective mitochondrial respiratory chain in propionic aciduria or methylmalonic aciduria patients [48]. BCKA is reported to suppress respiration in brain mitochondria [49, 50]. However, the direct link between BCAA/BCKA impacted mitochondrial performance and contractile function in heart has not been established. In addition, how BCAA/BCKA affects mitochondrial function remains elusive. Nevertheless, the direct modification of mitochondrial function may impact cellular ROS and energy homeostasis, growth, and viability. In summary, the underlying mechanisms for the adverse effects of BCAA/BCKA accumulation in heart remain to be further investigated (Fig. 6.2).

Conclusions

The association of BCAA catabolic defect and cardiac diseases has been observed in both human and animal models, suggesting a potentially important contribution to the pathogenesis of the disease. However, the BCAA catabolic activity demonstrates different organ-specific pattern in rodents and human. Further investigation in basic science and clinical populations are needed to elucidate the complicated association between BCAA catabolism and heart dysfunction. The insights from these studies would shed new light in the pathogenesis of cardiac disease as well as other metabolic diseases, such as diabetics, obesity, and central nerves system abnormalities. Hopefully, the studies in both animals and human would provide clues to identify novel approaches of diagnosis and therapy for cardiomyopathy.

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Chapter 7
Mitochondrial tRNA Valine in Cardiomyopathies

M. Esther Gallardo, Teresa Galera, Rafael Garesse, and Belén Bornstein

Key Points

- Cardiomyopathies are diseases of the myocardium that can be complicated by heart failure, arrhythmias, and sudden death.
- Mitochondrial cardiomyopathies are a subgroup of cardiomyopathies produced by defects in the energetic metabolism.
- Cardiac involvement is reported in 20–25 % of patients with mitochondrial disorders.
- Mitochondrial cardiomyopathies can result from mutations in either nuclear or mitochondrial encoded genes.
- Mitochondrial $tRNA^{Leu}$, $tRNA^{Ile}$, and $tRNA^{Val}$ are mutational hotspots for mitochondrial cardiomyopathies.
- Most patients with mutations in the mitochondrial $tRNA^{Val}$ gene (*MT-TV*) present with a hypertrophic cardiomyopathy.
- The *MT-TV* gene should be included in the molecular diagnosis of patients when there is a high suspicion of mitochondrial cardiomyopathy.

Keywords Mitochondrial disease • Mitochondrial DNA • Cardiomyopathy • Mitochondrial cardiomyopathy • *MT-TV* • *MT-TL1* • *MT-TI*

Abbreviations

ARVC	Arrhythmogenic right ventricular cardiomyopathy
CM	Cardiomyopathy
CPEO	Chronic progressive external ophthalmoplegia
DCM	Dilated cardiomyopathy
HCM	Hypertrophic cardiomyopathy
MCM	Mitochondrial cardiomyopathy

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MD	Mitochondrial disease
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MiMyCa	Maternally transmitted mitochondrial cardiomyopathy and myopathy
MNGIE	Mitochondrial neurogastrointestinal encephalopathy
MT-TI	Mitochondrial tRNA isoleucine gene
MT-TK	Mitochondrial tRNA lysine gene
MT-TV	Mitochondrial tRNA valine gene
mtDNA	Mitochondrial DNA
OXPHOS	Oxidative phosphorylation
RC	Respiratory chain
RCM	Restrictive cardiomyopathy
tRNA	Transfer ribonucleic acid

Introduction

Cardiomyopathies (CM) are an important and heterogeneous group of diseases. They are defined as diseases of the myocardium with cardiac dysfunction that can be complicated by heart failure, arrhythmias, and sudden death [1]. They are classified into four main different entities: hypertrophic, dilated, restrictive, and arrhythmogenic right ventricular CM/ dysplasia (Fig. 7.1). Hypertrophic cardiomyopathy (HCM) is a clinically heterogeneous but quite common autosomal dominant genetic heart disease that probably is the most frequently occurring CM (estimated prevalence of about 1 of 500 [2–4]). HCM is defined by an unexplained left ventricular hypertrophy usually asymmetrical and that involves the interventricular septum. The clinical pattern is very heterogeneous. In fact, there are a lot of patients without symptoms during their whole life. In some cases HCM may lead to syncope or dysnea, sudden death, or congestive heart failure [1]. Dilated cardiomyopathy (DCM) is the most prevalent cause of congestive heart failure in young patients and besides an important cause of cardiac transplantation. It has an estimated prevalence of 36 of 100,000 in the US [5]. Dilated forms of CM are characterized by ventricular chamber enlargement and systolic dysfunction, thin wall thickness, and depressed left ventricular systolic function [6]. Restrictive cardiomyopathy (RCM) is a rare form of heart muscle disease and a cause of heart failure that is characterized by a pattern of ventricular filling in which increased stiffness of the myocardium causes ventricular pressure to rise with only small increases of volume [7]. Unlike HCM, DCM, and RCM, arrhythmogenic right ventricular cardiomyopathy (ARVC) is an uncommon form of inheritable heart muscle disease (estimated in 1:5,000) [4]. ARVC is defined histologically by the existence of progressive replacement of right ventricular myocardium with adipose and fibrous tissue often confined to a triangle of dysplasia comprising the right ventricular inflow, outflow, and apex [7].

Until now, more than 40 inborn errors in metabolism are reported to cause myocardial abnormalities. They can present with cardiac disease at any moment during life but frequently symptoms and signs of multiorgan system dysfunction appear in infancy or early childhood. Most genetic metabolic CM are inherited as autosomal recessive traits but some of them are X-linked. Another important group of diseases, called mitochondrial cardiomyopathies (MCM), a subgroup of CM produced by defects in the energetic metabolism, can result from mutations in either nuclear or mitochondrial encoded genes, showing the complexity of the inheritance patterns (e.g., maternal, autosomal recessive) [8]. This chapter will focus on MCM caused by mutations in the mitochondrial DNA (mtDNA) with an emphasis on the mitochondrial tRNA mutations and most specifically on mutations identified in the mitochondrial tRNA Valine gene (*MT-TV*).

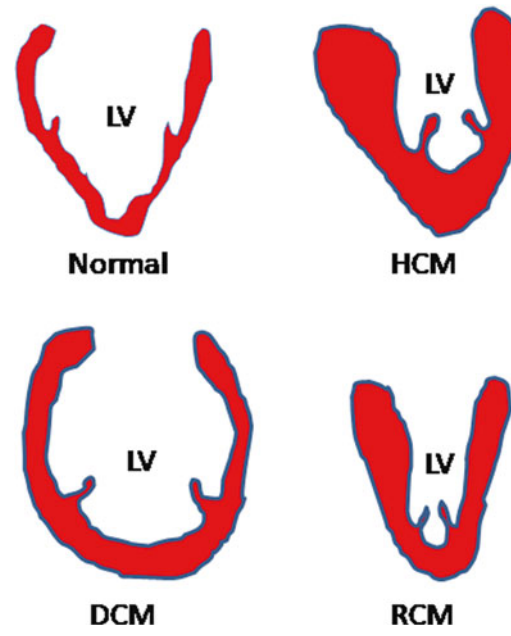

Fig. 7.1 Types of cardiomyopathies. The figure shows schematically different types of cardiomyopathies. *HCM* Hypertrophic cardiomyopathy, *DCM* Dilated cardiomyopathy, *RCM* Restrictive cardiomyopathy, *LV* Left ventricle (unpublished)

Mitochondrial Genetics and Cardiac Disease

Disorders of energy production are quite common among inborn errors of metabolism. Mitochondrial disorders (MD) are multisystemic diseases that may arise at any age, as a result of dysfunction of the respiratory chain (RC). Clinically, they usually involve multiple tissues although the most frequently and severely affected organs are those that place high demands on aerobic metabolism, such as brain, skeletal muscle, sensory organs, kidney, and cardiac muscle. Myocardium is one of the most energy-demanding tissues in the body because of continuous contractility needed for pumping the blood to the tissues. This energy is mostly supplied by the mitochondrion, the cellular organelle implicated in the generation of energy through the oxidative phosphorylation (OXPHOS). For that reason, it is not surprising that mitochondrial diseases can result in either HCM or DCM. CM usually does not appear as an isolated feature and it is usually a part of a multisystem involvement. As it has been mentioned above MCM can result from inherited or sporadic mutations in mitochondrial (mtDNA) or nuclear DNA. Recent studies have reported a minimum birth prevalence of approximately 1 of 8,000 for mitochondrial disorders caused by mutations in nuclear or mitochondrial genes although mtDNA mutations are more frequent [9, 10]. The human mtDNA is a 16,569 bp double-stranded circular molecule that encodes for 37 genes, 24 of which are involved in the translation mechanism (2 rRNAs and 22 tRNAs) (Fig. 7.2). The 13 remaining genes are responsible for the synthesis of respiratory chain subunits. Functional tRNAs are needed for the translation of these 13 proteins as it is shown by the fact that mutations in mitochondrial tRNA genes are normally associated with combined defects in the respiratory chain complexes [11–14]. However, there are also patients with a mutation in a mitochondrial tRNA gene that causes an isolated deficiency of a complex of the RC [14, 15]. Among the 900 genes that participate in the correct functioning of mitochondria only a few are located in the mtDNA, whereas the rest are encoded in the nucleus. That is the reason why about 50 % of adults and 80–90 % of children suspected to have a mitochondrial disease on the basis of biochemical and/or morphological data remain genetically undiagnosed [16].

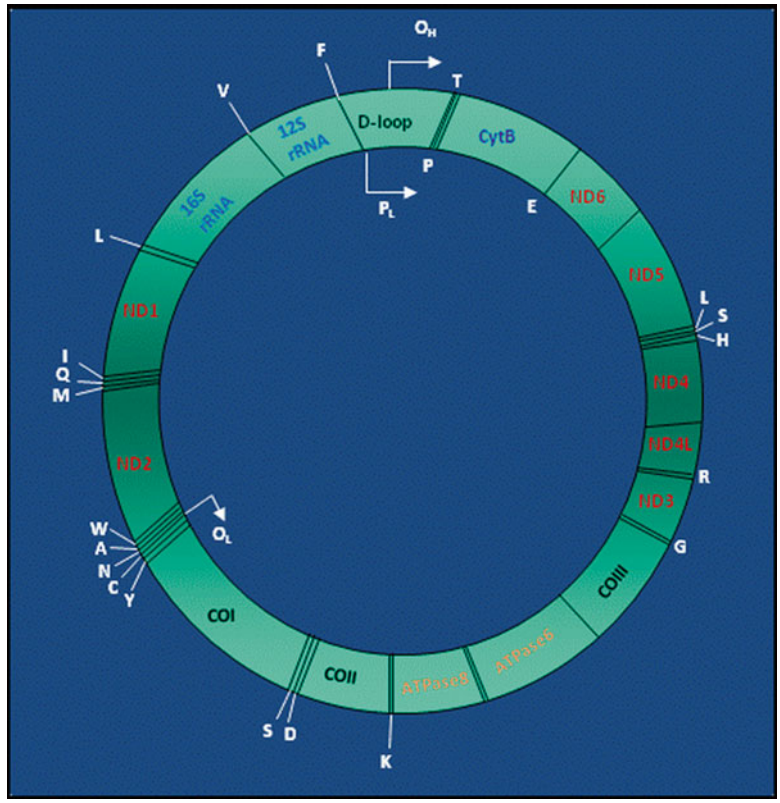

Fig. 7.2 Map of the human mitochondrial DNA. The human mtDNA is a circular double-stranded molecule with 16,559 base pairs of length. It encodes for 13 subunits of the oxidative phosphorylation system: The mitochondrially encoded NADH dehydrogenase subunits 1, 2, 3, 4, 4 L, 5, 6 (MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6), the mitochondrially encoded cytochrome c oxidase I, II, and III, (MT-CO1, MT-CO2, MT-CO3), the mitochondrially encoded cytochrome b (MT-CYB), the mitochondrially encoded ATP synthase 6 and 8 (MT-ATP6, MT-ATP8), 2 rRNAs: rRNA12S, and rRNA16S (MT-RNR1, MT-RNR2), and 22 tRNAs. The $tRNA^{Val}$ gene (MT-TV) located between the rRNA12S and rRNA16S is shown (unpublished)

MtDNA mutations obey to different genetic rules than those applied to Mendelian diseases. First of all, mtDNA is maternally inherited. Second, there are multiple copies of mtDNA in each cell. Homoplasmy is the situation in which all mtDNA copies are identical and on the contrary heteroplasmy is described when two or more sequence variants exist in a cell or individual. In fact, when the ratio of mutant to normal mtDNA exceeds a critical threshold, then the respiratory chain function will be impaired and the disease appears. The threshold at which symptoms manifest depends on the tissue involved. Although the presence of heteroplasmy has been a common pathogenic criterion for mitochondrial mutations [13], homoplasmic mutations are being increasingly recognized as causes of MD [13, 17]. Another aspect of the mitochondrial genetics is the mitotic segregation of the multiple copies of mtDNA that explains why the level of mutant mtDNA can change during life.

According to the limited number of reported clinical studies, cardiac involvement is published in 20–25 % of patients with mitochondrial disorders [18–20]. Cardiac manifestations include CM that variably affects the clinical outcome of patients. The course may be stable for many years but rapid deterioration may occur [8]. In spite of the improvement of our knowledge of the molecular mechanisms of these diseases the molecular diagnosis is beginning to have serious implications in the routine management of this type of patients.

tRNA Mutational Spectrum Associated with CM

Mitochondrial diseases (MDs) are a clinically heterogeneous group of disorders caused by dysfunctional mitochondria, the organelles that generate energy for the cell. These diseases can present at any age, and their clinical symptoms reflect a primary defect in tissues with high oxidative demand. Usually, changes in blood parameters such as lactate, pyruvate, carnitine, amino acids, and urine organic acids are described in these disorders as well as deficiencies in enzymes of the RC. Although these diseases can result from mutations in the nuclear genome most of them are caused by mutations in the mtDNA. In spite of the fact that only 10 % of the mtDNA molecule is composed by tRNA genes most of the reported mtDNA mutations associated with MD relies on these genes suggesting that these genes are prone to mutation [17]. This conclusion is also supported by a recent study, in which it is confirmed that mitochondrial tRNA genes are mutational hotspots with a frequency of deleterious mutations (excluding one of the most frequent mutations, the m.3243 A>G) that was almost six times more than that occurring in the protein-coding regions [21]. Until now mutations in all mitochondrial tRNAs have been described associated with several MD (see MITOMAP database: http://www.mitomap.org/). However, the mitochondrial tRNA Leu (*MT-TL1*), tRNA Lys (*MT-TK*), and tRNA Ile (*MT-TI*) appear to be more susceptible to mutation [22]. In fact, there have been described 35 mutations associated with MD in the *MT-TL1*, 22 in the *MT-TK*, and 20 in the *MT-TI*. By contrast, only three mutations in the mitochondrial tRNA Asp (*MT-TD*) and two in the mitochondrial tRNA Tyr (*MT-TY*) have been found.

The predominance of neurologic and neuromuscular manifestations in MD has generally masked the presence of other, but not less important, clinical phenotypes, such as cardiac complications. This may be one of the fact that has prevented the diagnosis of mitochondrial cardiomyopathies (MCMs) [14]. Nowadays, mitochondrial defects are being increasingly recognized to play an important role in the pathogenesis of hereditary cardiomyopathies [8]. Indeed, sporadic or inherited mutations in mitochondrial DNA (mtDNA), specifically in the mtDNA transfer ribonucleic acid (tRNA) genes, or in the nuclear genome, have been associated with hypertrophic and dilated cardiomyopathy [23, 24]. In the same way cardiac conduction abnormalities have also been associated with different mtDNA rearrangements [25].

Based on the prevalence data of mtDNA diseases and the frequency of cardiac involvement, at least 1 in 10–15,000 of the general population will be affected. In 1991, the first mitochondrial DNA (mtDNA) point mutation in the *MT-TL1* gene (m.3260A>G) associated with a maternally transmitted MCM and myopathy was identified and the acronym MiMyCa was described [26]. Since then, several mutations in the mtDNA, mostly in mitochondrial tRNA genes have been associated with different MCM phenotypes (HCM and DCM) [25]. These diseases are preferentially linked to mutations in the *MT-TL1*, and *MT-TI* genes [8, 27, 28]. The *MT-TL1* gene provides instructions for making a specific form of tRNA, present only in mitochondria, called $tRNA^{Leu(UUR)}$ and the mitochondrially encoded tRNA isoleucine also known as *MT-TI* is a tRNA which in humans is encoded by the mitochondrial *MT-TI* gene. During protein assembly, these molecules attach to the amino acids Leucine and Isoleucine (both branched chain amino acids) and insert it into the appropriate locations in the protein. In Table 7.1 are shown the mutations in the *MT-TI* and *MT-TL1* genes that have been associated with CM. A remarkable fact is that if we consider all the mitochondrial tRNAs the ones with more mutations associated to CM are the mitochondrial $tRNA^{Leu}$, $tRNA^{Ile}$, $tRNA^{Lys}$, and $tRNA^{Val}$ (the latter described in more detail in the next section).

Cardiomyopathies Due to Mitochondrial tRNA Val Mutations

The mitochondrially encoded tRNA Valine is a transfer RNA encoded by the mitochondrial *MT-TV* gene. *MT-TV* is a 69 nucleotide RNA (located in position 1602–1670 bp of the mtDNA molecule). The $tRNA^{Val}$ attaches to a particular branched chain amino acid, Valine, and inserts it into the suitable

Table 7.1 Mutations described in the mitochondrial tRNAs of branched chain amino acids

	Mutation	Inheritance	Mutant mtDNA percentage	Tissues	Disease	Functional study	Reference
tRNA $^{Leu(UUR)}$	m.3303C>T	Maternal	100 %	Muscle and blood	MimyCa	No	[40]
		nd	100 %	Muscle and heart	HCM, hepatomegaly and renal failure	No	[41]
	m.3243A>G	This mutation has been associated with different clinical phenotypes, several of them displaying cardiomyopathy: MELAS+HCM [42], [43]; Encephalomyopathy+HCM [44]); Diabetes+CM+ Deafness [45]					
	m.3260A>G	Maternal	>85 %	Muscle	MiMyCa	Yes (Transmitochondrial cybrids in [46]	[26]
tRNA ^{Ile}	m.4269A>G	nd	nd	Muscle, blood and heart	Encephalomyopathy and DCM	Yes (Transmitochondrial cybrids in [47]	[48]
	m.4277T>C	nd	100 %	Blood	Isolated HCM	Yes 1. tRNA^{ILE} steady-state levels decreased 2. Pathogenicity in transmitochondrial cybrids [49]	[50]
	m.4295A>G	Maternal	90,4 %	Heart	Isolated HCM	No	[51]
	m.4300A>G	Maternal	>95 %	Heart	Isolated HCM	No	[52]
		Maternal	100 %	Blood	Isolated HCM	Yes 1. tRNA^{ILE} steady-state levels decreased 2. Pathogenicity in transmitochondrial cybrids [29]	[50]
	m.4316A>G associated with m.3395A>G in *MT-ND1* gene	Maternal	97 %	Blood	HCM and hearing loss	No	[53]
	m.4317A>G	nd	nd	Heart	Fatal infantile CM	No	[54]
	m.4320C>T	nd	88 %	Muscle	Encephalocardiomyopathy	No	[55]

In the table are listed the most frequent mutations described in the mt-tRNA^{Ile} (*MT-TI* gene) and mt-tRNA$^{Leu\ (UUR)}$ (*MT-TL* gene) associated with cardiomyopathy
nd not determined, *CM* cardiomyopathy, *HCM* hypertrophic cardiomyopathy, *DCM* dilated cardiomyopathy, *MiMyCa* maternally transmitted mitochondrial cardiomyopathy and myopathy
Table unpublished

locations in many different proteins. The $tRNA^{Val}$ molecule is involved in the assembly of proteins that carry out OXPHOS.

Mitochondrial diseases caused by mutations in the *MT-TV* gene are not very frequent. In 1996, the first mutation in this gene (m.1642G>A) was described in a patient with a MELAS phenotype (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) [29]. Since then, a total of eleven mutations in the *MT-TV* gene associated with a wide range of phenotypes, including MELAS, Leigh syndrome, CPEO (Chronic progressive external ophthalmoplegia), MNGIE (mitochondrial neurogastrointestinal encephalopathy), or HCM have been identified (Table 7.2).

Five of these mutations (m.1606G>A, m.1607 T>C, m.1630A>G, m.1642G>A, m.1644G>T) were heteroplasmic in all patients studied and three were homoplasmic (m.1624C>T, m.1628C>T, m.1643A>G) (Table 7.2). The m.1659 T>C can also be considered homoplasmic, because in all tissues analysed (blood, muscle, buccal epithelia, and urinary epithelia) the heteroplasmy level was higher than 98 % [22]. The transition (m.1644G>A) was shown both in heteroplasmy (85 %) [30] and homoplasmy [14]. Interestingly, the patient with the m.1644G>A mutation in homoplasmy has a more severe clinical phenotype, including cardiac involvement which was absent in the patient with the mutation in heteroplasmy (Table 7.2). Finally, the heteroplasmy levels of the m.1658 T>C sequence change were not determined in the study, although the electropherogram shows heteroplasmy levels near to 100 % [31].

Only five mutations from the eleven published in the *MT-TV* gene have already been associated with cardiac conduction abnormalities and HCM within a neurological presentation (m.1624C>T, m.1628C>T; m.1644G>A; m.1644G>T and m.1659 T>C) ([11, 14, 22] (Table 7.2; Fig. 7.3). These data suggest that although the *MT-TV* gene is not a region prone to mutation compared with other mitochondrial tRNAs, such as *MT-TL*, *MT-TI*, and *MT-TK*, a relatively high percentage of mutations in this gene (45 %: 5/11) are associated with MCM. In consequence, the *MT-TV* gene should also be included in the molecular diagnosis of patients when there is a high suspicion of MCM [14]. Interestingly, all patients with *MT-TV* mutations associated with MCM presented with a hypertrophic subtype (Table 7.2).

Although the nucleotide changes described in the *MT-TV* gene associated with HCM were thought to be pathogenic following several molecular criteria only two (m.1624C>T; m.1644G>A) were probed to be pathogenic in functional assays (Table 7.2). The pathogenicity of the m.1624C>T nucleotide change was confirmed by means of transmitochondrial cybrids [32, 33], and a selective reduction of the steady-state mt-$tRNA^{Val}$ level in muscle from the patient harbouring the m.1644G>A transition, supports both the cardiac and the neurological involvement of this mutation [14]. Due to the lack of sample it could not be confirmed whether this mutation may lead to a conformational change or impaired aminoacylation capacity of the mt-$tRNA^{Val}$ [14].

A particular feature of mutations in mitochondrial tRNAs and in mtDNA in general is that a clear correlation between genotype-phenotype does not exit. That means that the same mutations are associated to different clinical phenotypes (clinical heterogeneity) and the same disease is associated with different mutations (genetic heterogeneity). As examples of the clinical heterogeneity, the homoplasmic mutation m.1624C>T in the *MT-TV* gene can generate from mild to severe phenotypes between the family members [13] or even mutations at the same nucleotide of the *MT-TV* gene (m.1644G>A and m.1644G>T) result in different phenotypes (Hypertrophic cardiomyopathy plus MELAS or Leigh Syndrome respectively) [11, 30, 34].

There are several explanations for the absence of genotype-phenotype correlation. Among them are the nuclear background, the heteroplasmy levels and its tissue distribution, the mtDNA haplotype, or the total mtDNA copy number [35]. The importance of the nuclear background in the clinical phenotype is highlighted in a recent study, in which the $tRNA^{Val}$ steady-state level in transmitochondrial cybrids carrying the m.1624C>T mutation in homoplasmy (10 % referred to controls) differs significantly of the level observed in muscle biopsy with the same mutation in homoplasmy (<1 % referred

Table 7.2 Mutations described in the *MT-TV* gene associated with different clinical phenotypes

Mutation	Inheritance	Level of heteroplasmy	Tissues	Disease	Cardiac problems	Sex, age	Functional study	Reference
m.1606G>A	Maternal	67 %	Muscle	Neurological disorder	No	Man, 48	No	[56]
	Sporadic	70 %	Muscle	Neurological disorder	No	Woman, 37	No	[35]
m.1607 T>C	Maternal	41,2 %	Blood and muscle	nd	nd	nd, 13	No	[21]
m.1624C>T	nd	100 %	Blood	nd	nd	Woman, 15 Man, 38	No	[21]
	Maternal	100 %	Muscle	Leigh syndrome, HCM	Yes, HCM	A family	Yes. Pathogenicity in transmitochondrial cybrids	[13, 32]
	nd	nd	nd	Suspected mito disease	nd	nd	No	[10]
m.1628 C>T	nd	100 %	Muscle	Pigmentary Retinitis, muscle atrophy and HCM	Yes, HCM	Man, 14	No	[14]
m.1630A>G	Maternal	90 % 70 % 80 %	Muscle Blood Myoblasts	MNGIE	No	Woman, 16	No	[12]
	Maternal	75 % 95 % 60 %	Blood Urine Fibroblast	MELAS	No	Woman, 15	Yes. Pathogenicity in transmitochondrial cybrids	[33]
m.1642G>A	Maternal	94 % 54 % 95 %	Muscle Blood Occipital cortex	MELAS	No	Man, 16	No	[29]
	Sporadic	60 % 80 %	Blood Muscle	MELAS	No	Boy, 10	No	[39]
m.1643A>G	Maternal	100 %	Muscle, Blood, Urine, oral mucosa	Encephalomyelopathy	No	Girl, 3	No	[50]

m.1644G>A	Sporadic	85 %	Muscle	MELAS	No	Woman, 37	No	[30]
	nd	100 %	Muscle	Neurological and gastrointestinal problems, HCM and myopathy	Yes, HCM	Man, 32	Yes. $tRNA^{Val}$ steady-state levels decreased	[14]
m.1644G>T	Maternal	65;80;87 %	Blood	Leigh syndrome	Yes. (1/3)[a] HCM	Woman, 43	No	[11]
		71;87;90 % respectively	Muscle			Man, 38 Girl, 3		
m.1658T>C	Maternal	nd	Blood	CPEO	No	Woman, 19	No	[31]
m.1659T>C	Maternal	98 %	Muscle and blood	Neurological disease	Yes HCM	Woman, 17	No	[22]
		99 %	Buccal and urinary epithelia					

In this table a revision of the mutations identified in the *MT-TV* gene associated with different clinical phenotypes is shown

[a]In this case, three patients in the same family were described to have mitochondrial disease. The girl with the higher heteroplasmy level manifestates the disease earlier and was the only who presented cardiomyopathy

nd not determined, *MELAS* mitochondrial Encephalomyopathy, Lactic acidosis, and stroke-like episodes, *CPEO* Chronic Progressive External Ophthalmoplegia, *MNGIE* Mitochondrial Neurogastrointestinal Encephalopathy disease

Table unpublished

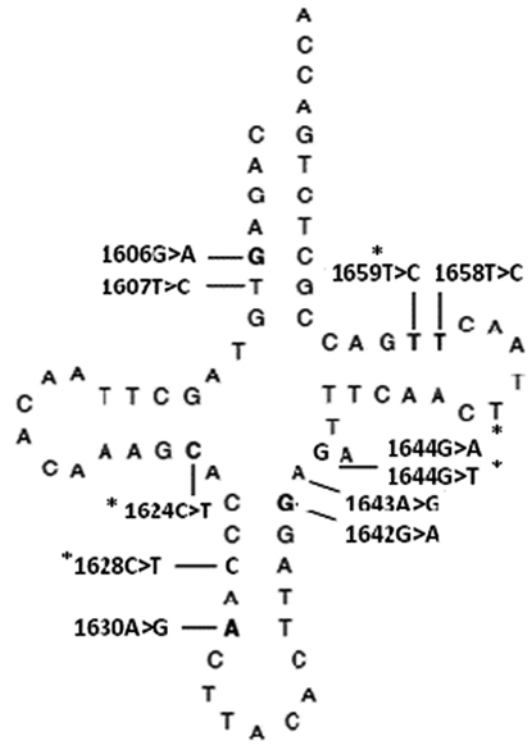

Fig. 7.3 Schematic representation of the mitochondrial tRNA^{Val} structure. A total of 11 mutations in the *MT-TV* gene associated with a wide range of phenotypes, including MELAS, Leigh syndrome, CPEO, MNGIE, or HCM have been represented. Mutations specifically associated with CM are highlighted with an *asterisk*. *HCM* Hypertrophic cardiomyopathy, *MELAS* Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, *CPEO* Chronic progressive external ophthalmoplegia, *MNGIE* Mitochondrial neurogastrointestinal encephalopathy disease (unpublished)

to controls) [32]. To date, the most pronounced decrease of the steaty-state mitochondrial tRNA^{Val} in cardiac and in skeletal muscles has been observed in the presence of this mutation associated with infantile and fatal Leigh syndrome together with severe cardiac failure in one of ten children affected [13]. Regarding the quantity of mutant mtDNA, although there are examples of maternally inherited mutations in which the severity of the disease correlates with the number of mutated copies [11], there are also examples in which the level of heteroplasmy do not correlate with the presence or severity of the disease. As an example the mutation m.1630A>G (not associated with a cardiac dysfunction; Table 7.2) has been reported to be in higher levels in a healthy mother (93 % in blood and 98 % in urine) than in her affected daughter (75 % in blood, 95 % in urine, and 60 % in fibroblast) [33].

Pathogenic mutations in mitochondrial tRNAs can alter the secondary structure or change one highly conserved base to another base, abolish the tertiary structure, and lead to dysfunction. Mutations in them affect biogenesis and function of mitochondrial tRNAs by several mechanisms, including transcription, maturation, posttranscriptional modification, structure, stability, aminoacylation, capability of binding to elongation factor thermo-unstable (EF-Tu), and Codon Reading [33, 36].

The pathogenic effect mediated by a structural change is in accordance with the work performed by [37]. In this study they demonstrated that mutations in different mitochondrial tRNAs but at the same position of the cloverleaf originate the same clinical phenotype. For example, mutations located at position 5 of the cloverleaf (m.1606G>A and m.7512 T>C in the *MT-TV* and *MT-TS* genes respectively) produce neurological symptoms, myopathy, and hearing loss, and mutations at the position 72 (m.3303C>T and m.8363G>A of the *MT-TL1* and *MT-TK* respectively) are associated with CM. On the contrary, mutations at the same position of the same gene have been reported to be associated with different phenotypes. For example, m.1644G>A and m.1644G>T mutations in the tRNA^{Val} cause different clinical phenotypes, MELAS, or HCM with neurogastrointestinal manifestations in the first case [14, 30] and adult Leigh syndrome in the second case [11, 34].

Regarding the *MT-TV* gene, its location between the 12S and the 16S ribosomal RNAs could explain by an indirect way how mutations in this gene could originate a pathogenic phenotype. Both rRNAs and the $tRNA^{Val}$ are transcribed as a polycistronic messenger with the posterior cleavage by the RNAse P-like endonuclease [38]. For that reason, it has been hypothesised that mutations in the $tRNA^{Val}$ could interrupt the normal cleavage of the polycistronic messenger and, this could be the reason of the disease. However, in a study performed by [39] with the m.1642G>A mutation, no abnormality in the splicing data was observed. This data suggest that this mechanism by itself cannot explain the observed phenotype.

Due to the fact that the *MT-TV* gene is being increasingly recognized as cause of MCM, an important effort in improving the knowledge about the molecular mechanisms that could explain the reason why the disease appears must be carried out.

Conclusions

Mitochondrial dysfunction frequently affects the heart and can produce both hypertrophic and dilated CM. CM are among the most severe presentations of OXPHOS with a relatively high morbidity and mortality. Due to the fact that genetics and biochemical aspects of the mitochondrion and mitochondrial disorders are beginning to be better understood, the pathophysiologic features of the mitochondrial heart disease are also becoming to be understood.

In spite of the improvement of our comprehension of the molecular mechanisms of these diseases the molecular diagnosis is beginning to have serious repercussions in the routine management of these patients. Indeed, an appropriate diagnosis of a mitochondrial cardiomyopathy could have not only important clinical implications but also could open new possibilities towards the development of potential future therapeutic strategies for these diseases which at this moment only have palliative therapeutic approaches.

The discovery that the *MT-TV* gene is another mutational hotspot for MCM will allow physicians to offer better genetic counselling. However more exhaustive sequencing strategies that involve the use of next generation sequencing will be needed to identify new molecular genetic causes in patients with MCM.

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Conflict of Interest The authors report no conflict of interest.

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Chapter 8
Branched Chain Amino Acids on Psychomotor Performance

Krystyna Nazar

Key Points

- Psychomotor skills play an essential role in everyday life, occupational work and sport activities.
- Psychomotor performance depends mainly on brain function including cognitive functions, attention, concentration, verbal and spatial working memory, decision making and information processing speed.
- Branched chain amino acids (BCAA), especially leucine, can easily cross endothelial blood–brain barrier (BBB) and directly influence cortical function providing amine groups for synthesis of glutamate—the excitatory neurotransmitter and precursor of the inhibitory neurotransmitter—γ-aminobutyric acid (GABA).
- Increase in the plasma BCAA concentration causes rapid inhibition of phenylalanine, thyrosine and tryptophan transport across the BBB and consequently rapid decrease in the synthesis of catecholamines (dopamine and noradrenaline) and serotonin.
- Ingestion of small doses of BCAA improves psychomotor performance at rest and during exercise, reduces perceived fatigue and enhances mood, it is, therefore, recommended for athletes and for some patients.
- Inhibition of dopamine synthesis by higher doses of BCAA exerts negative effect on some brain functions and may impair psychomotor performance.

Keywords Glutamate • γ-aminobutyric acid • Serotonin • Dopamine • Central fatigue • Blood–brain barrier • Aromatic amino acids

Abbreviations

BCAA	Branched chain amino acids
BBB	Blood–brain barrier
BCAT	Branched chain aminotransferase
fMRI	Functional magnetic resonance imaging
GABA	γ-Aminobutyric acid
MCR	Multiple choice reaction
NMDA	*N*-methyl-D-aspartate

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Introduction

Psychomotor skill is the human ability to respond with maximal speed and accuracy to external events or goals. It depends on several brain functions, including cognitive functions, attention, concentration, verbal and spatial working memory and information processing speed. The psychomotor performance is assessed by lots of neuropsychological tests, which are directed to evaluate either the single brain function or the global accuracy and time of the response. These tests, especially combined with functional magnetic resonance of the brain provide the useful tool for evaluation of the status of patient with neurological and psychiatric diseases and to investigate the effects of some agents, such as food components, on the brain functions in patients and healthy subjects.

Branched chain amino acids, especially leucine, can easily cross endothelial blood–brain barrier. They are used not only for protein synthesis and energy yielding processes but can also influence brain functions by modulating neurotransmission in some brain structures. Effects of BCAA administration on brain function have been extensively investigated in patients with some psychiatric, neurological and other diseases and in healthy subjects [1]. The doses used in these studies varied greatly from 5–10 to 60 g of BCAAs in acute treatment up to 250 mg kg^{-1} d^{-1} during chronic treatment. Exceptionally high doses of BCAA (500–800 mg kg^{-1} d^{-1}) were given to young adults with phenylketonuria. Most of these studies did not demonstrate any adverse effects of BCAA supplementation administered either orally or in intravenous infusion. The adverse effects of chronic BCCA administration such as faster decline in the respiratory function and increased mortality ratio were described in patients with amyotrophic lateral sclerosis [2, 3], however, these findings were not confirmed by more recent meta-analysis [4].

Apart from the hypothesized role of leucine per se or its metabolites as neurotransmitters [5] there are two main mechanisms of BCAAs action on brain function, namely: modulation of glutamatergic and gabaergic signal transmission and decreasing the aromatic amino acid uptake by the brain subsequently limiting serotonin and catecholamine synthesis (Fig. 8.1).

BCAA and Glutamatergic and Gabaergic Signal Transmission

Catabolism of BCAAs in the brain cells provide amine groups for synthesis of glutamate—the excitatory neurotransmitter and precursor of the inhibitory neurotransmitter—γ-aminobutyric acid (GABA). The system of branched chain aminotransferases (BCAT) in neuron cytoplasm and astrocyte mitochondria regulates amounts of the released glutamate for neurotransmission and GABA synthesis. The balance of these two fast acting transmitters plays a crucial role in the cortical functions determining psychomotor performance. This was evidenced by the study of Anticevic et al. [6] with healthy volunteeres submitted to spatial working memory test with infusion of ketamine—the antagonist of NMDA (*N*-methyl-D-aspartate) glutamate receptor. The results showed a significant reduction of accuracy for working memory following ketamine administration. Basing on the detailed analysis of each element of the task response accompanied with functional neuroimaging (fMRI) the authors concluded that the mechanism of cognitive performance impairment by ketamine is connected with reduced NMDA conductance on GABA interneurons, leading to cortical disinhibition.

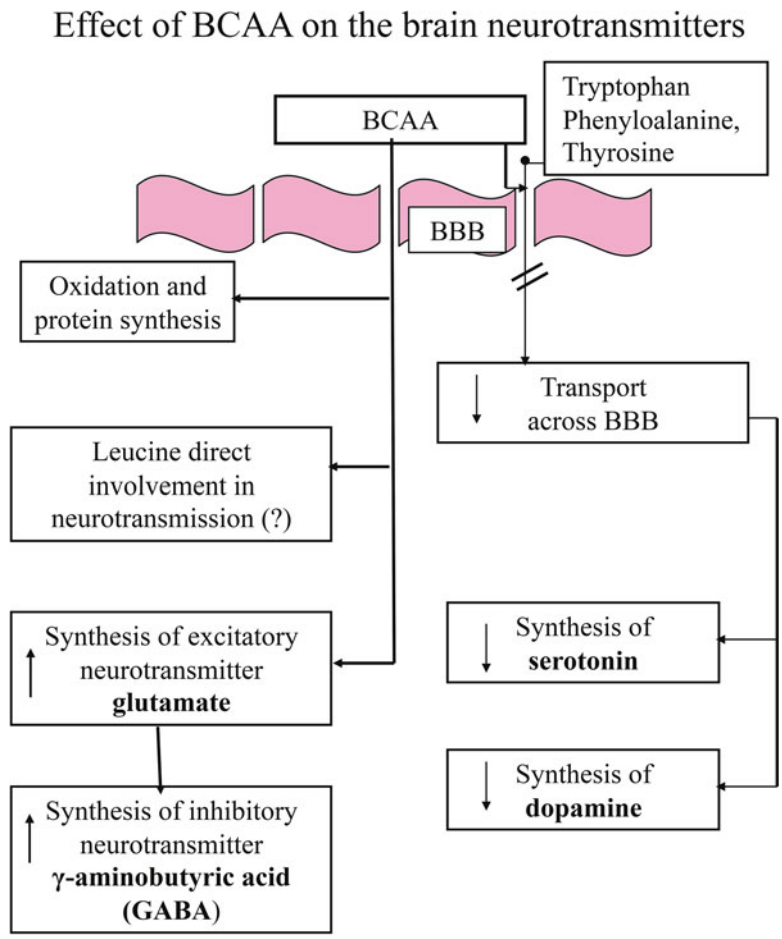

Fig. 8.1 Schematic presentation of the ways of BCAA acting on the brain neurochemistry. *BBB* denotes blood–brain barrier

BCAA and Serotoninergic and Dopaminergic Signal Transmission

Brain functions might be modified by BCAAs as a result of their influence on synthesis of the slow neuromodulatory neurotransmitters, such as dopamine and serotonin. Diminishment of the synthesis of dopamine and serotonin is caused by competitive inhibition of their precursors (phenylalanine, thyrosine and tryptophan) transport to the brain through the blood–brain barrier. Plasma BCAAs cross the blood–brain barrier through the receptor (transporter) in capillary endothelium shared by phenylalanine, thyrosine and tryptophan (Fig. 8.1). This receptor is competitive and under normal concentration of plasma amino acids almost fully saturated. Increase in plasma concentration of BCAAs causes, therefore, rapid inhibition of phenylalanine, thyrosine and tryptophan concentration in brain and consequently rapid decrease in the synthesis of catecholamines (dopamine and noradrenaline) and serotonin.

The studies on the effect of BCAA administration on brain function in healthy humans gained impetus after Newsholme et al. in 1987 [7] put forward the hypothesis on the role of brain serotonin as neurotransmitter responsible for fatigue during prolonged exercise. The assumption was based on the known effects of serotonin on arousal, lethargy, sleepiness and mood that may alter development

of central nervous system fatigue. According to the concept, synthesis of serotonin in the brain is accelerated during prolonged exercise because the ratio of BCAA to free tryptophan concentration in plasma is declined. This is because BCAA are taken from blood and oxidized in working muscles and tryptophan is liberated from its binding to albumin as a result of enhanced lipolysis in adipose tissue and competition of fatty acids for binding sites on albumin [8, 9]. Plasma concentration of the free tryptophan may increase also during a short-term intensive exercise, since a decline in blood pH diminishes tryptophan binding to albumin [9]. As it was mentioned above, BCAA and precursor of serotonin, free tryptophan compete for the same transporter in the blood–brain barrier. The synthesis of serotonin is catalyzed by tryptophan hydroxylase which is not saturated with substrate. Therefore, the changes of free tryptophan concentration in blood and its transport across the blood–brain barrier influence the rate of serotonin synthesis and its release in the brain.

The contribution of serotonin to the central nervous system fatigue was confirmed with pharmacological studies conducted with human subjects. It was demonstrated that drugs increasing brain activity of serotonin by inhibition of its re-uptake [10, 11] accelerate development of fatigue during running or cycling without changes in metabolism, cardiovascular or thermoregulatory adjustment to exercise.

Athletes use BCAA as dietary supplement to increase physical capacity and psychomotor performance. The beneficial effect of BCAAs on physical performance results mainly from influence on muscle metabolism and usually occurs after chronic supplementation. The ergogenic effect of acute BCAA administration before or during exercise is not convincingly documented [12–15]. More conclusive are the data concerning effect of BCAA ingestion on psychomotor performance [16]. The studies of Blomstrand et al. [17–19] and Hassmen et al. [20] demonstrated that the acute treatment with BCAA reduced perceived fatigue during prolonged exercise and improved psychomotor performance after exercise. In subjects who ingested 7.5 or 16 g of BCAA in flavored water solution, given in portions during the 30 km or marathon run, results of the color-word Stroop test (words, colors and color words) were improved after the run by 3–7 % in comparison with those obtained before exercise. Moreover, the subjects treated with BCAA did not show deterioration of the performance in the shape-rotation and figure-identification tasks. In the subjects who were given placebo there was no difference in the Stroop test results before and after exercise and there was impairment in the performance in the shape-rotation and figure-identification tests by 25 and 15 % after the run.

Mikulski et al. [21] investigated in a double blind manner the effect of a relatively small dose of BCAA (7 g) on psychomotor performance at rest and during exercise performed on bicycle ergometer with progressively increasing intensity until volitional exhaustion. Psychomotor skill was estimated on the basis of multiple choice reaction (MCR) time. The MCR task included 15 positive (red light or a sound) and 15 negative (green and yellow lights) stimuli applied in a randomized order. The subjects were asked to press and then to release, as quickly as possible, the button mounted on the right handlebar of the cycle ergometer in response to the red light, the button on the left handlebar in response to the sound and do not react to the negative stimuli. Using the same procedure it was previously showed that during graded exercise MCR time was shortening with increasing exercise intensity until 60–80 % of the maximal load is achieved and then it increased [22]. The exercise load associated with the best psychomotor performance (the shortest MCR time) was considered as the threshold of psychomotor fatigue. It appeared that 1 h after ingestion of a low carbohydrate pudding containing BCAA mixture, MCR time was shortened already before exercise in comparison with placebo trial performed with the same subjects (Fig. 8.2). Moreover, the threshold of psychomotor fatigue was shifted towards higher exercise loads (216 ± 10 vs. 253 ± 10 W) whilst there were no differences in the maximal power output and heart rate achieved by the subjects. The similar MCR test was also applied during treadmill running of changing velocity from 0 to 6.4 m s^{-1} simulating football match and lasting 90 min with 15 min resting break in the middle [23]. Ingestion of 7.0 g of BCAA one hour before exercise shortened MCR time at rest by approx. 10 % and the difference between BCAA and placebo trials was maintained throughout the whole exercise period (Fig. 8.3).

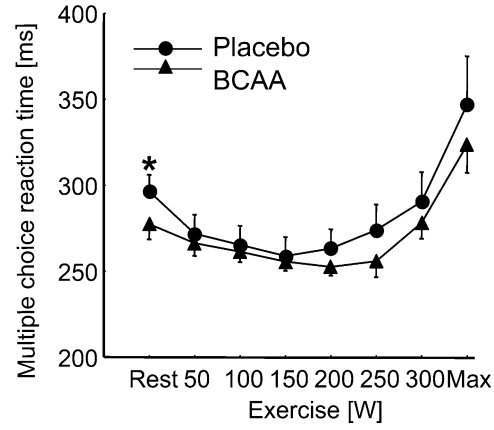

Fig. 8.2 Effect of BCAA on multiple choice reaction time measured before and during exercise with increasing intensity until volitional exhaustion. *Asterisk* denotes significant difference between BCAA and Placebo trial: Q2*$p<0.05$ (according to the data of Mikulski et al. [21])

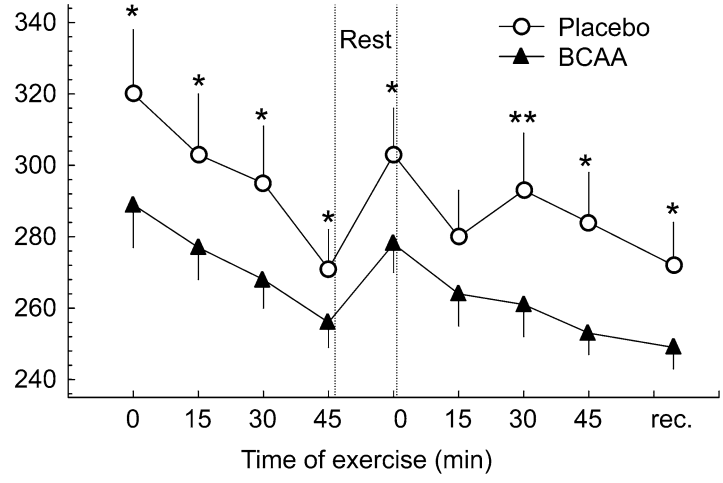

Fig. 8.3 Effect of BCAA on multiple choice reaction time measured before and during treadmill running with changing velocity simulating football match. *Asterisks* denote significant differences between BCAA and Placebo trial: *$p<0.05$, **$p<0.01$ (according to the data of Wisnik et al. [23])

It was hypothesized that lack of the effect of BCAA on physical working capacity may be related to the reduction of dopamine synthesis in the brain [24]. Another factor which may decrease effectiveness of BCAA action in the brain is an elevation of the plasma ammonia concentration. Ammonia can be produced in muscles by deamination of BCAA before their oxidation.

The studies on changes in brain function linked to reduction of dopamine synthesis are of special interest because increased activity of dopaminergic system has been considered as a pathogenetic factor in schizophrenia, mania, tardive dyskinesia and some other psychiatric or neurological diseases, while the decreased activity of this system occurs in Parkinson disease. Dopaminergic dysfunction in brain reward system was also implicated in addiction.

Gijsman et al. [25] demonstrated that a drink containing 10, 30 or 60 g of BCAA given to healthy subjects increased latency of the spatial recognition memory test and elevated plasma prolactin concentration which is indicative of lowered dopamine function. Both these changes were dose dependent. Scarna et al. [26] evaluated the effects of ingestion of a drink containing 60 g BCAA plus 2 g of

tryptophan on plasma levels of amino acids, prolactin and on cognitive tasks such as spatial recognition, pattern memory and decision making gambling tests in healthy subjects. The aim of tryptophan addition to BCAA was to prevent inhibitory effect on serotonin production. However, compared to placebo the ratio of BCAA to tryptophan did not change significantly. The new finding of this study was the adverse effect of BCAA with tryptophan on the decision making during gambling test.

To investigate the possible association of decreased dopaminergic activity with impaired emotion-based decision-making Sevy et al. [27] submitted healthy volunteers to the Iowa Gambling Test and battery of cognitive tasks evaluating perception, spatial working memory, visual attention working memory and verbal memory. The tests were performed after ingestion of high dose of BCCA mixture (60 g) or placebo. As a marker of dopaminergic activity plasma prolactin concentration was measured. Authors demonstrated that BCCA drink impaired emotion-based decision-making during gambling test and increased plasma prolactin without significant effect on cognitive tests applied in this study.

Conclusions

Analysis of studies concerning effects of BCAA in healthy humans shows that ingestion of low doses of BCAA may improve psychomotor performance and prevent loss of cognitive functions during intensive or long-term exercise. This indirectly supports the hypothesis of serotonin contribution to the central nervous system fatigue in spite of unchanged physical performance. BCAA supplementation may be, therefore, recommended to humans performing work requiring quick and accurate responses to the external stimuli, especially to athletes involved in team games. Application of higher doses of BCAA was shown to impair performance in some cognitive tests and exert an adverse effect on emotion-based decision-making during gambling tests. This was accompanied with the rise in plasma prolactin concentration which indicates the decreased dopamine function.

The studies concerning modulation of the brain signal transmission pathways by BCAA supplementation or dietary regimes may be important for better understanding of some psychiatric disorders, evaluation of treatment results and development of safe and effective therapeutical strategies.

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Chapter 9
The Branched Chain Amino Acids in the Context of Other Amino Acids in Traumatic Brain Injury

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Key Points

- Traumatic brain injury (TBI) is accompanied with phase-dependent changes in plasma amino acids with a different profile in the acute, subacute, and rehabilitation phase.
- During the acute phase, decreased plasma branched chain amino acid (BCAA) levels are associated with increased plasma aromatic amino acid (AAA) concentrations.
- High levels of phenylalanine are accompanied with decreased ICP and increased $SjvO_2$ while high levels of BCAA are associated with increased intracranial pressure (ICP) and decreased jugular venous oxygen saturation ($SjVO_2$).
- Nutrition aimed at correcting low plasma BCAA levels may not be appropriate in TBI patients while elevated AAA levels might be more beneficial.
- The exact pathophysiologic roles of BCAA and AAA within the pathogenesis of TBI has to investigated in more detail focusing on modulation of cerebral blood flow, neurotransmitter excitotoxicity, brain energy consumption, as well as calcium homeostasis.
- Further studies are required to elucidate the ideal composition of amino acid formulations if nutrition is to actively modulate brain function in the different posttraumatic phases.

Keywords Aromatic amino acids • Brain metabolism • Intracranial pressure • Jugular venous oxygen saturation • Nutrition • Secondary brain damage

Abbreviations

AA	Amino acids
AAA	Aromatic amino acids
BBB	Blood–brain barrier
BCAA	Branched chain amino acids
CaR	Calcium sensing receptor
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid

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EAA	Essential amino acids
ICP	Intracranial pressure
LAT1	Large neutral amino acid transporter 1
LNAA	Large neutral amino acids
$SjVO_2$	Jugular venous oxygen saturation
TBI	Traumatic brain injury

Traumatic Brain Injury (TBI)

The forces creating neurotrauma initiate a cascade of events starting with the primary mechanical impact leading to subsequent delayed nonmechanical damage. Within the cascades of secondary damage, impaired cerebral blood flow leads to inadequate oxygen supply followed by brain metabolic dysfunction which is exacerbated by excitotoxicity leading to cell death as well as inflammation [1]. Management of TBI focuses on prevention or reduction of secondary damage by preventing additional local and systemic insults. The state of the patient and the success of the treatment were initially monitored by controlling intracranial pressure (ICP) alone. However, contemporary knowledge shows that pathogenic alterations precede the increases in ICP after TBI [2]. Therefore, posttraumatic neuromonitoring should extend the classical measures of ICP and cerebral perfusion pressure (CPP) by including more downstream parameters as e.g., jugular venous oxygen saturation ($SjVO_2$) allowing to indirectly determine cerebral oxygen consumption [3]. Characteristic posttraumatic pathways leading to increased ICP and decreased $SjVO_2$, and also secondary brain edema formation, are schematically depicted in Fig. 9.1. In addition to preventing secondary brain damage the management of TBI includes measures to reduce intracranial hypertension, preserve adequate CPP, and optimize cerebral oxygenation [4]. This is achieved by combining several treatment strategies including amongst others sedation, controlled hypoventilation and hyperventilation, hemodynamic support, and temperature modulation [4]. Supportive nutritional therapy is one of the therapeutic interventions directly affecting the outcome after TBI as insufficient caloric supply during the first 5 posttraumatic days was associated with significantly increased mortality [5]. Today, provision of sufficient amounts of calories either by early enteral nutrition alone or by supplemental parenteral nutrition is strongly advocated by the official guidelines [6]. In recent years a continuous effort was made to determine the ideal amount of calories and to also improve the composition of the nutritional formulation [7]. However, amino acid supplementation in TBI patients is still performed based on recommendations for critically ill patients without considering potential variations and specific requirements in this specific disease. For example, in patients with cerebral injury administration of amino acid solutions bears the potential of providing precursors of neurotransmitters, which might lead to excitotoxicity and increased damage in the brain cells [8, 9] and therefore, have to be increased carefully in the blood. In view of possibly improving posttraumatic brain function by modulating certain cerebral processes, this review focuses on the amino acid requirements of TBI patients as well as the potential importance of different amino acids in the pathogenesis of secondary damage after TBI, mainly focusing on BCAA and AAA.

Changes in Plasma AA Levels After TBI

Stressful events are stereotypically accompanied by decreased plasma levels of BCAAs (isoleucine, leucine, and valine) and increased plasma levels of AAAs (phenylalanine, tyrosin, and tryptophan) [10]. As this derangement was associated with decreased survival in some diseases, e.g., sepsis [11], former treatment in trauma patients aimed at increasing plasma BCAA levels via

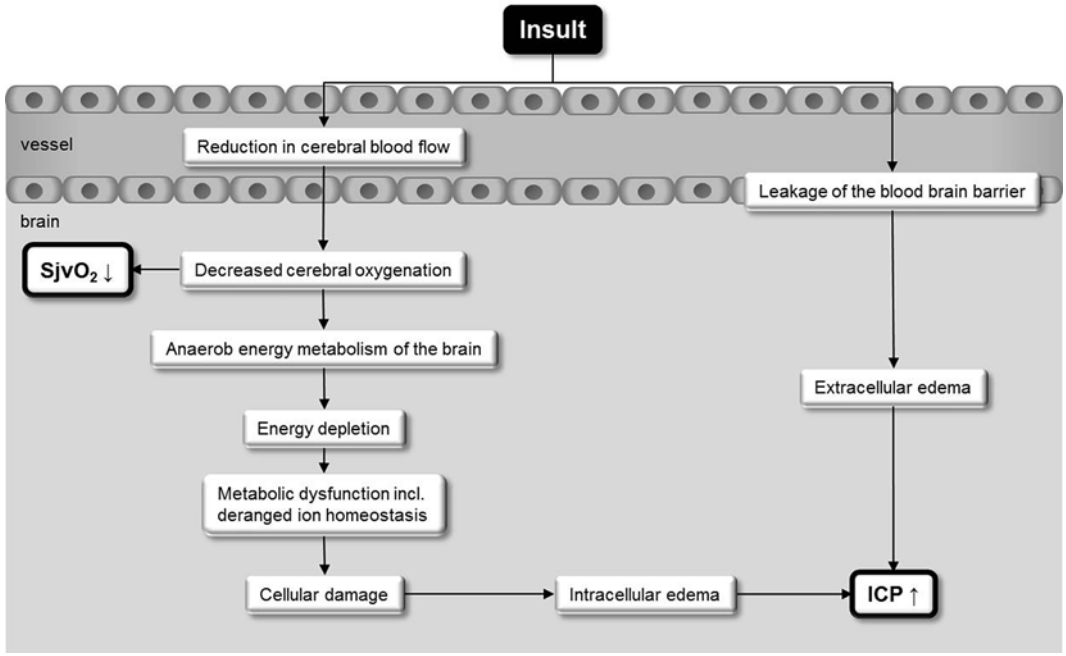

Fig. 9.1 Schematic overview of some major aspects of the secondary damage cascade occurring after TBI. Reduction in cerebral blood flow and leakage of the blood brain barrier lead to decreased jugular venous oxygen saturation and increased intracranial pressure

nutrition to yield a beneficial effect. However, clinical studies using BCAA-enriched parenteral or enteral nutrition in trauma patients yielded conflicting results (for review see de Bandt et al. [12]). Furthermore, the amino acid requirements between patients suffering from multiple trauma with and without TBI are different, reflected by the fact that TBI patients, for example, show statistically significant decreases in plasma glutamine levels during the acute phase compared to trauma patients without brain injury [13].

Plasma amino acid profile following TBI shows a complex pattern and course strongly influenced by the postinjury phase (Fig. 9.2), calling into question the applicability of the same amino acid formulations for trauma and brain trauma and even within the group of TBI patients for the acute and chronic phases. Up to 24 h after TBI, Suzuki and coworkers reported no significant changes in either the arterial and jugular venous plasma levels of BCAAs or any of the essential amino acids [14]. In another study, decreased arterial plasma valine was associated with increased plasma phenylalanine on the day of injury [15]. This discrepancy might be due to different analytical methods used as the changes were mild, but nonetheless statistically significant. In the following days postinjury, the arterial BCAA plasma levels were either within the normal range (leucine) or decreased with a nadir on day 2 (isoleucine) or day 3 (valine) [15]. As of day 4 all BCAA plasma levels returned to physiologic values. It is unclear if this observed normalization reflects endogenous correction or if this pattern is caused by the started nutritional therapy. While plasma phenylalanine was increased as of the day of injury and remained elevated until day 6, tyrosine was elevated on day 3 and 4 only [15]. To date, no study investigated the profile of plasma amino acid levels between day 6 postinjury and the rehabilitation phase, where venous plasma levels of all essential amino acids were shown to be decreased compared to controls [16, 17]. Interestingly, at 60 days after TBI plasma BCAA levels were still significantly decreased while AAA were normal [17]. However, taking a closer look at the single amino acids it becomes obvious that every essential single amino acid was significantly decreased to

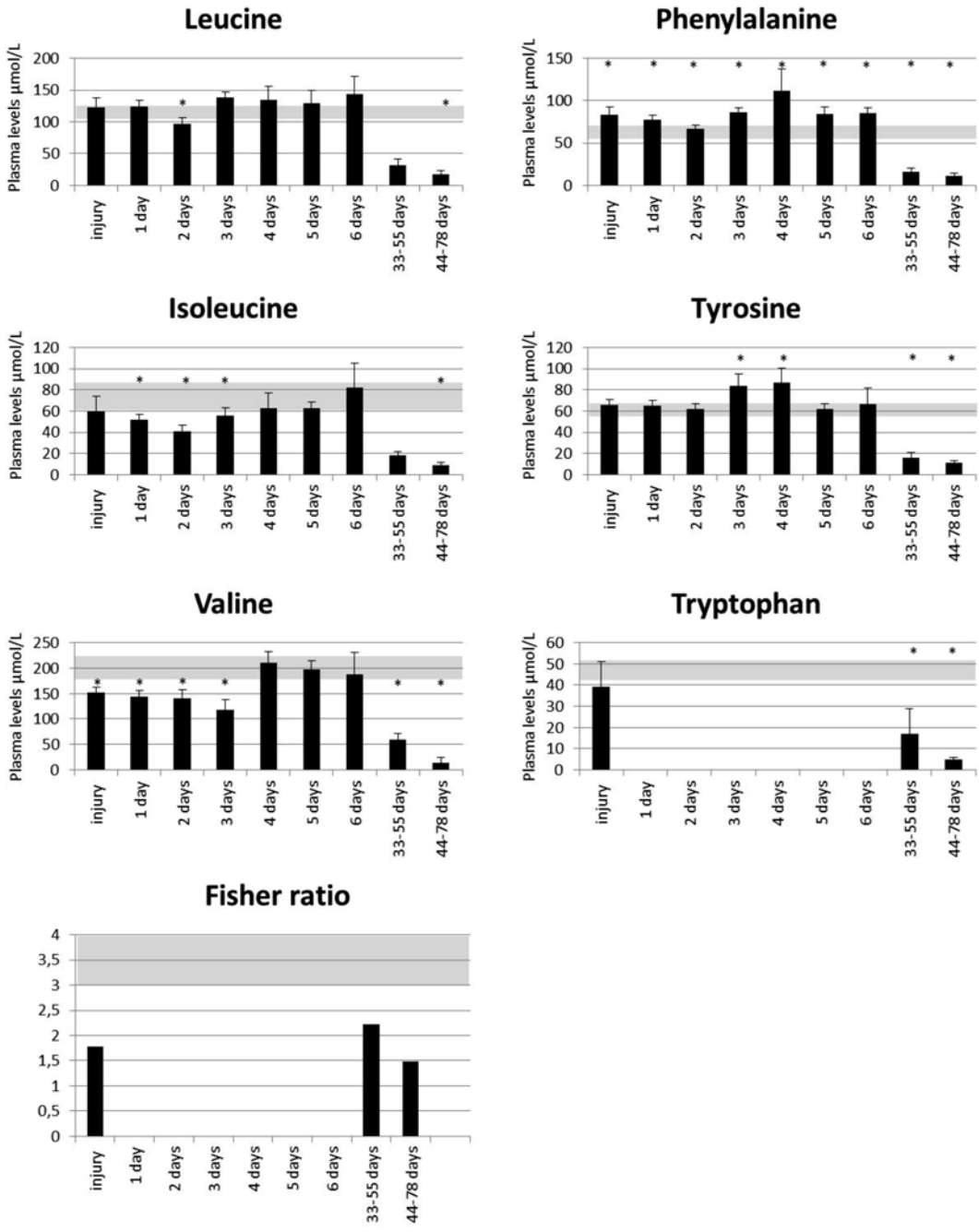

Fig. 9.2 Amino acid levels after TBI compiled from data published by Robertson et al. 1988, Aquilani et al. 2000, 2003(*$p < 0.05$ compared to control patients of the respective study, *blue horizontal bar* = reference range). Differentially regulated BCAAs (leucine, isoleucine, and valine) and AAAs (phenylalanine, tyrosine, and tryptophan) in the first week were followed by decreased levels after 1 or 2 months after injury

an extend of approximately 50 % with the exception of valine which was reduced to 18 %, accounting for the significant decrease in BCAA. Insufficient valine levels lead to disruption of the endoplasmic reticulum of neurons especially in the red nucleus region of the brain and impairs cellular protein synthesis [18]. This destructive effect of valine occurs in the presence of physiologic isoleucine and leucine levels, but not during a generalized BCAA deficiency. Therefore, in the rehabilitation phase after TBI special emphasis has to be laid on increasing plasma valine levels to avoid an isolated valine deficiency after restoring the levels of isoleucine and leucine. With more and more studies in favor of early nutritional support during the initial 48 h after trauma [19], amino acid supplementation was provided to increase the plasma availability of BCAA in TBI patients. However, the complex pattern of amino acid changes especially during the first week after TBI raises the question whether one single amino acid mixture is applicable to all phases after TBI.

Treatment of Changes in Plasma AA After TBI

To date, only few clinical studies investigated the responsiveness of posttraumatic plasma and brain amino acid levels to nutritional (enteral and parenteral) AA supplementation. Especially studies reporting changes in plasma levels of single AAs and not in groups of AAs are rare. For example, essential amino acids (EAAs) have been reported to be associated with a decreased infection rate in patients with different types of brain injury [20], but differences in the impact of single amino acids of these mixtures were not evaluated. Clinical trials investigating the changes of different AAs after TBI in association with quantified AA supplementations are listed in Table 9.1. These studies not only differed in the amount of administered AAs but also in the duration of treatment and the measurement of outcome. A "good" outcome, defined by an overall state not requiring continuous help was correlated with decreased loss of renal nitrogen in patients provided with an AA solution in addition to regular nutrition; however, a direct influence by the AA pattern was not observed [21]. Nutritional supplementation of AAs was not compared to a control group or to a different AA solution in this study, as performed by Ott and coworkers who compared two AA formulations during the acute phase following severe TBI [22]. Here, different AA mixtures resulted in different plasma levels and in different urea nitrogen levels [22] without, however, correlating with a better outcome or any other clinical parameters. Changes in plasma amino acid levels with and without treatment were also reported by other researchers [23, 24]. Unfortunately, the group of Petersen and coworkers used two different AA solutions without providing sufficient information on the actual administration, hindering an in-depth interpretation of the results. Of note, administration of EAAs 1 year after TBI leads to significantly different changes in the plasma levels of nonessential AAs compared to controls [25]. The plasma levels of the nonessential amino acids only increased in controls but not in the patients. The clinical relevance of this observation still has to be identified, especially as the study did not investigate the turnover rate of the AAs in the brain or other tissues. However, the study showed once more that at different time points following TBI single AAs react differently to the administration of AAs. Knowing that we are able to manipulate plasma AA levels at various posttraumatic time points, we have to also consider the impact and necessity of manipulating plasma amino acid levels. In this context, we need to address the questions if it really is the best solution to normalize plasma BCAA levels in every phase following TBI and if yes, which nutritional composition should be used?

In an initial attempt to address this question Vuille-Dit-Bille and coworkers could show that in TBI patients elevated plasma BCAA levels correlated with increased ICP and decreased $SjVO_2$, whereas high plasma phenylalanine levels were associated with decreased ICP and increased $SjVO_2$ [26]. Contrary to the currently existing rationale considered beneficial in metabolic/toxic encephalopathy [27], these results suggest that BCAA administration during the early posttraumatic phase might be harmful while AAA appear more beneficial in the treatment of TBI. These results should motivate us

Table 9.1 Clinical trials assessing the changes in plasma amino acids following traumatic brain injury (TBI)

		Amino acid content of the nutrition supplementation							Changes in plasma amino acids after nutrition					
		BCAA			AAA				BCAA			AAA		
Authors	Amino acid product	Leucine	Iso-leucine	Valine	Phenyl-alanine	Tryptophan	Tyrosine	Duration of treatment	Leucine	Iso-leucine	Valine	Phenyl-alanine	Trypto-phan	Tyrosine
Piek et al. [21]	Aminoplasmal® PO	107 mg/kg	61 mg/kg	81 mg/kg	53 mg/kg	25 mg/kg	–	6 days	±0 %	±0 %	+8 %	−1 %	−1 %	±0 %
Ott et al. [22]	Group 1: Travenol® 8.5 %; Group 2: Aminosyn® 8.5 %	+54 %	+53 %	+74 %	−28 %	−1 %	+29 %	16 days	+2 %	+21 %	+2 %	−30 %	−22 %	−56 %
Petersen et al. [24]	Vital®HN in patients with barbiturate coma; Jevity® in remaining patients	Jevity: 108 mg/kg Vital®HN: 98 mg/kg	Jevity: 54 mg/kg Vital®HN: 59 mg/kg	Jevity: 69 mg/kg Vital®HN: 68 mg/kg	Jevity: 90 mg/kg Vital®HN: 55 mg/kg	Jevity: 12 mg/kg Vital®HN: 15 mg/kg	Jevity: 90 mg/kg Vital®HN: 45 mg/kg	3 days	+6 %	+12 %	+5 %	±0 %	−33 %	±0 %
Ronne Engström et al. [23]	Glavamin®	3.9 g/patient	2.8 g/patient	3.6 g/patient	2.9 g/patient	0.9 g/patient	1.1 g/patient	single application	n/r	n/r	n/r	n/r	n/r	n/r
Borsheim et al. [25]	Drink (no further specification)	1.7 g/patient	0.8 g/patient	0.7 g/patient	0.5 g/patient	–	–	single application	±0 %	±0 %	±0 %	±0 %	±0 %	±0 %
Vuille-dit-Bille et al. [26]	Jevity® Plus	5880 mg/day versus 6860 mg/day	3000 mg/day versus 3500 mg/day	3720 mg/day versus 4340 mg/day	3240 mg/day versus 3780 mg/day	72 mg/day versus 84 mg/day	3240 mg/day versus 3780 mg/day	2 weeks	+22 %	+25 %	+35 %	−31 %	+5 %	+25 %

These trials investigated the impact of different amino acid products on changes in plasma amino acid levels in TBI patients. All formulations increased BCAA with an increase or decrease in AAA levels

to put into question the use of current AA formulations for the critically ill in TBI patients and to specifically address nutrition-dependent influences of brain metabolism and pathology, thereby preventing signs of potential worsening.

Effects of BCAA and AAA in TBI

Although several AA formulations are commercially available, their composition is mainly intended to correct the deranged plasma BCAA to AAA ratio by increasing the plasma BCAA levels without influencing the plasma AAA levels which elevated endogenously. However, with new data characterizing downstream cerebral alterations, new concepts and novel formulations need to be considered.

The composition of a supplementary AA formulation in TBI patients strongly depends on the effects of different plasma AA levels on

- brain AA levels.
- subsequent metabolism in face of pathophysiologic alterations.
- clinical signs/outcome.

Based on the study of Vuille-Dit-Bille and coworkers [26], the following section will delineate options for a modulatory role of BCAA and AAA focusing on pathways important in influencing ICP and $SjVO_2$.

Potential Modulatory Effects of BCAA and AA in TBI Pathophysiology

To evaluate the role of a specific AA in a certain pathophysiologic pathway it is essential to determine the tissue compartment of the brain where its action takes place. Conceivable compartments are the blood stream including vessel, the blood brain barrier, and the extracellular space between the brain cells or within the brain cells (neurons, astrocytes, or glial cells). Brain AA influx or efflux is calculated by the brain metabolic rate, defined as the arterial-jugular venous difference multiplied by the cerebral blood flow. Regarding BCAA, the decreased plasma levels during the first 2 days after injury correlated well with a reduced influx of BCAA, whereas higher plasma levels as of day 3 resulted in a higher influx of BCAA into the brain [15]. Therefore, it seemed reasonable to assume that the decreased availability in the blood stream consequently reduced the uptake of BCAA by the brain. In contrast, plasma levels and the cerebral metabolic rate of phenylalanine were not correlated in such a linear pattern. The arterial plasma phenylalanine levels were elevated on the day of injury, decreased on postinjury day 1, returned to the reference range on day 2, and were increased again on days 3–6 with a peak on day 4 [15]. The brain metabolic rate showed a net influx of phenylalanine on day 1, which decreases and turns to a net efflux on day 3 [15]. From day 4–6, a net influx of phenylalanine into the brain was reported. The differences in brain metabolism of BCAA and phenylalanine are schematically depicted according to Robertson et al. [15] in Fig. 9.3. Here, a similar amount of arterial plasma phenylalanine was associated with brain influx on day 1 and brain efflux on day 3, with the following possible causes and effects:

1. On day 1, high plasma phenylalanine levels increase phenylalanine influx to the brain due to unchanged or excessive transport related to increased expression of transporters or facilitated transport due to reduced competition mediated by decreased plasma BCAA levels (see below).
2. On day 3, transporters responsible for phenylalanine transport across the BBB are saturated and thus no phenylalanine reaches the brain tissue or transporters are functionally impaired.

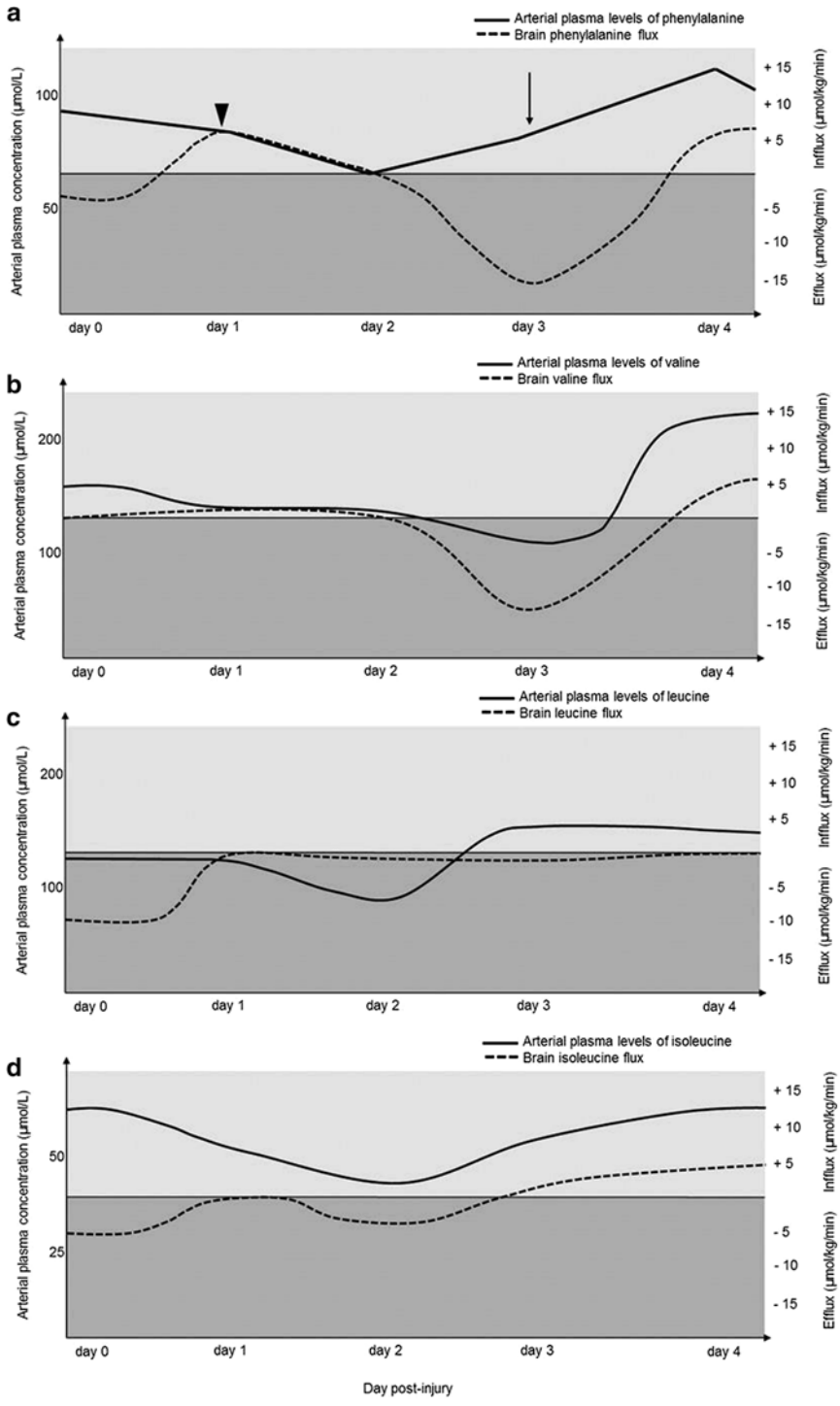

Fig. 9.3 Arterial plasma phenylalanine levels and brain flux in TBI patients up to 4 days postinjury compiled from data published by Robertson et al. 1988. Increased plasma phenylalanine levels resulted in brain influx or efflux. *arrowhead* = increased plasma level associated with brain influx; *arrow* = increased plasma level associated with brain efflux

The gradient observed between plasma and brain AA levels changes during nutritional treatment but the values do not necessarily show a parallel correlation. In this context, elevated plasma phenylalanine levels were associated with decreased brain content in several animal studies (for review see Huang et al. [28]). Therefore, if our interpretation is exclusively based on plasma BCAA and AAA levels in the context of the observed changes in ICP and $SjVO_2$ we can only hypothesize on the processes within the cellular compartment. In the following section we first discuss the likelihood of different amino acids to reach the brain tissue in patients with TBI in general and then point out several pathways on both sides of the BBB which could be influenced by isoleucine, leucine, valine, or phenylalanine.

Transport of AAs Across the BBB in Healthy Brain and in TBI

The entry of blood components into the brain starts with their passage of the luminal and the abluminal membrane of the BBB. In the healthy brain, tight junctions between the endothelial cells limit passive paracellular transport and AAs have to be transported actively into the brain (Fig. 9.4a). This is facilitated by different transporters with the most relevant ones depicted in Fig. 9.4b. Importantly, BCAA and AAA compete for transport via both the luminal and abluminal transporters, Large Amino acid Transporter 1 (LAT1) belonging to the L1 system [29]. The affinity of cerebral LAT1 is 15–50 μM in humans which is less than the amino acid concentrations in plasma [30]. At a first glance this notion seems to limit the transport capacities of large neutral amino acids (LNAA) to the brain, but upregulation of LAT1 protein in endothelial cells was observed in a rat study between 24 h and 5 days following spinal cord injury [31]. In skeletal muscle, LAT1 upregulation is associated with increased availability of EAAs due to their sustained transport into muscle tissue [32]. To date, there is no data available describing a potential downregulation of LAT1 due to negative supply of amino acids or the long-term effects of TBI on transporter expression. Current data suggest an upregulation and increased transport of LAT1 in the acute phase after TBI, whereas the selectivity of this transporter in terms of BCAA or AAA in TBI requires more investigations before any conclusion can be drawn on an increased transport of certain amino acids after TBI. LAT1 is not influenced by extracellular pH and is independent of sodium concentrations [30]; whereas astrocytes which also play a major role in the repair of the BBB after injury, were shown to modulate the transcription of LAT1 under in vitro conditions [33]. After TBI, the tight junctions initially remain intact as shown by ultrastructural analysis of human brain biopsies during the first 24 h [34] and thus blood components still have to be transported across the BBB. Upon lesion progression and especially during inflammation with morphological changes of the BBB including disrupted tight junctions, paracellular transport is increased [35], which has not yet been investigated for AAs in detail. An increased permeability of the BBB allowing leakage of molecules with a low molecular weight (0.3 kDa) from the blood into the brain has been reported up to 4 days postinjury [36]. As amino acids have an average mass of approximately 0.1 kDa it is likely to assume that despite the availability of special AA transporters in the endothelial cells of the BBB a certain amount of AAs might reach or leave the brain by passive paracellular transport during the first 4 days after TBI. As of day 5, the transport of AAs is likely to be restricted again to the active transporters. It remains unclear if these transporters still work properly or if these transport mechanisms are deranged as well. However, the changes in permeability of the BBB in week 1 that differ from those in week 2 after injury suggest a strong influence mediated by the administration of different amino acid compositions.

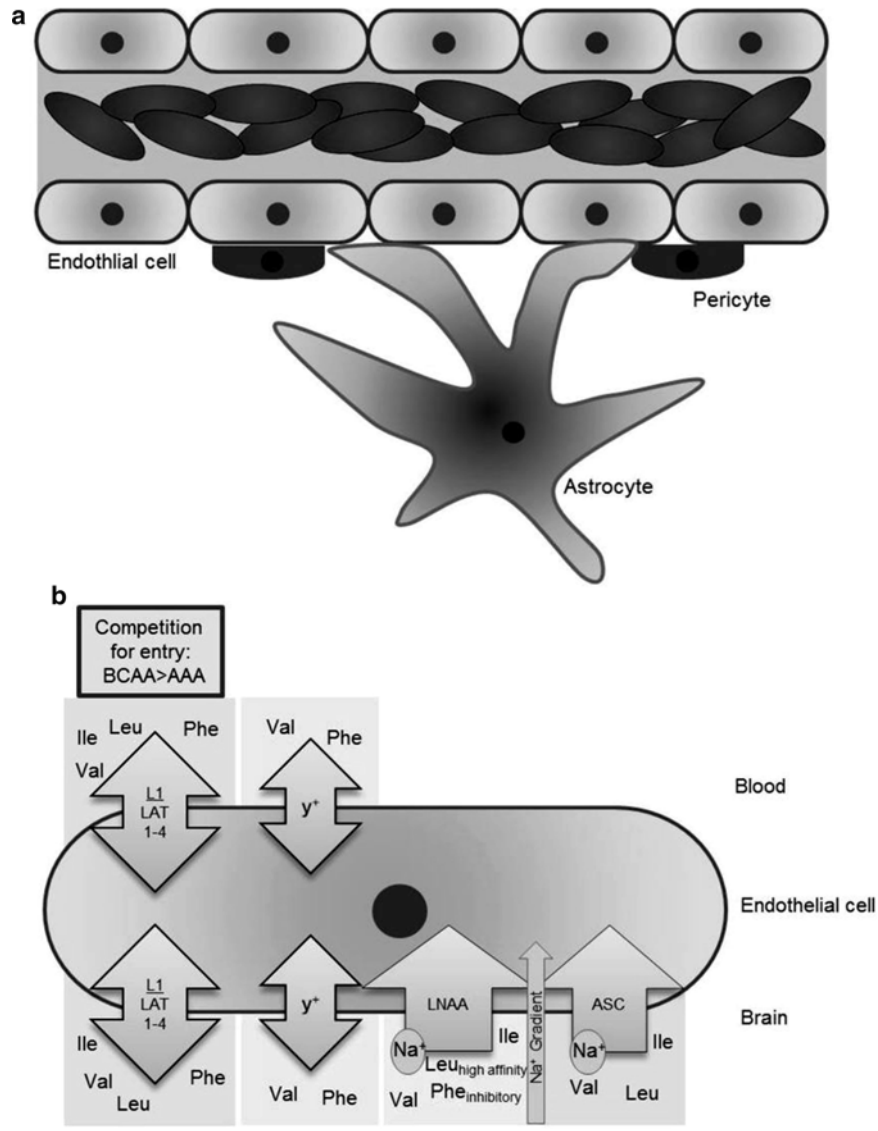

Fig. 9.4 (**a**) Cellular components of the blood brain barrier consisting of endothelial cells on the luminal side and astrocytes on the abluminal side. (**b**) Amino acid transporters at the blood brain barrier. Here, BCAAs and AAAs mainly compete to enter the brain via the L1 transporter located on the luminal side of the blood brain barrier. The export of amino acids from the brain is additionally supported by sodium dependent transporters. *LAT* L-type amino acid transporter, *LNAA* Large neutral amino acid transporter, *ASC* Alanine, serine, cysteine transporter

Possible Effects of BCAA and AAA on Brain Edema

Increased BBB permeability with its peak during the first days after injury leads to vasogenic edema [37]. This extracellular edema promotes secondary brain damage by compressing microcirculation which induces and aggravates cytotoxic edema formation [37]. In the first week after TBI, both extracellular and intracellular water accumulation exists in parallel induced by a plethora of different mechanisms (Fig. 9.5).

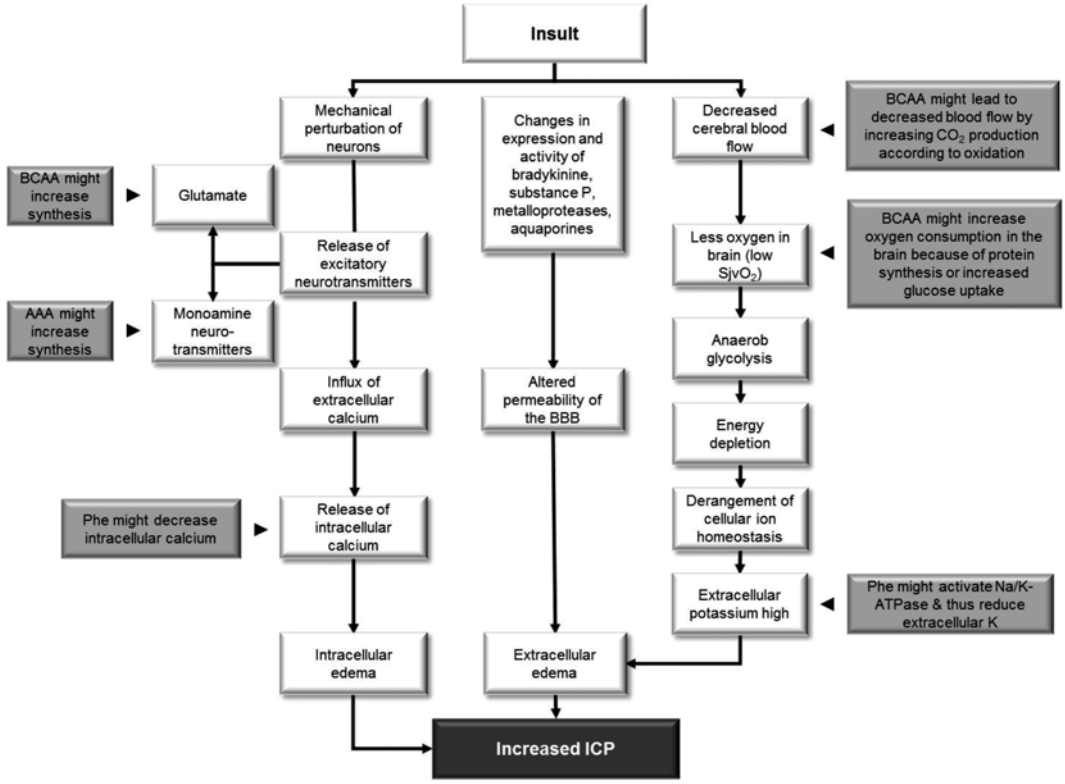

Fig. 9.5 Possible role of BCAAs and AAAs in the pathogenesis of secondary traumatic brain damage. BCAAs and AAAs can influence the pathways leading to increased intracranial pressure in different ways (*arrowheads*)

Vasogenic edema formation is mediated by vasoactive peptides like bradykinin or substance P as well as matrix metalloproteases which degrade the tight junction proteins of the BBB [37]. Aquaporins also play a major role in facilitating water influx and efflux [37]. Especially aquaporin-4 was suggested to be involved in altered amino acid and neurotransmitter metabolism due to increased glutamine, glutamate, and taurine levels in the brain of aquaporin-4 knockout mice [38, 39]. BCAA and AAA have not yet been investigated in aquaporin knockout mice.

Intracellular edema can only be attenuated when the cytotoxic agent is removed or the cytotoxic pathway is stopped. Some parts of the cytotoxic cascade are influenced by amino acids as e.g., glutamate-mediated excitotoxicity with disturbed calcium and potassium homeostasis, sustained intracellular water accumulation, and increased cell death [40]. BCAA provides a nitrogen shuttle for the synthesis of glutamate [41]. Thus increased levels of BCAA may potentially increase cerebral glutamate levels, possibly contributing to the elevated CSF glutamate levels [42]. In addition to glutamate, adrenergic transmitters are excitatory transmitters possibly inducing excessive neuronal activation. In this context, AAA are the precursors of norepinephrine, epinephrine, and dopamine which are increased in the CSF after TBI [9, 42]. Thus, both BCAA and AAA might increase the levels of excitotoxic neurotransmitters and therefore decreased levels of both types of AA in the brain after injury might have a rather protective effect and their concentrations have to be influenced carefully.

The release of neurotransmitters disturbs cellular calcium homeostasis. In this context, the calcium sensing receptor (CaR) which is overexpressed in mice after TBI [43] and facilitates increasing intracellular calcium is sensitive to AAA but not BCAA [44]. This, in turn, would not favor the use of AAA following TBI because high AAA levels might disturb the intracellular calcium homeostasis.

However, the AAA levels leading to intracellular calcium derangement due to CaR activation start in the micromolar range but show the half maximum effect not until a concentration of 2.5 mM [44]. Thus, the negative effect of AAA on the CaR in TBI is questionable because such high phenylalanine concentrations within the millimolar range rather occur in phenylketonuria. In TBI patients, plasma phenylalanine levels remain within the *micro*molar range despite being elevated [15]. In contrast, treatment of cultured neurons with phenylalanine resulted in an increased calcium efflux leading to decreased intracellular calcium levels, an effect depending on the modulation of the plasma membrane Ca^{2+}-ATPase [45]. Here, only 0.9 mM concentrations of phenylalanine were investigated. Further studies including minor concentrations are required to evaluate the effect of physiologic concentrations of phenylalanine on calcium homeostasis. Phenylalanine may thus have a beneficial effect in minor concentrations, although it is reported to be harmful in millimolar concentrations.

In addition to the derangement of the calcium homeostasis, potassium is also substantially altered in the brain of TBI patients. Potassium is increased in the extracellular space due to energy depletion and subsequent impairment of the Na^+/K^+-ATPase and failing inward potassium transport. Increased extracellular potassium can promote acidosis by activating K^+-H^+-antiporter which can induce brain swelling by secondary sustained vasodilation. At the same time, increased intracellular sodium accumulation due to failing transport against the existing concentration gradient can increase ICP due to sustained intracellular water accumulation. Phenylalanine can directly activate the Na^+/K^+-ATPase [46]. However, this requires energy; thus, without adequate energy supply increased levels of phenylalanine might rather exacerbate cerebral energy depletion than significantly ameliorating the deranged cellular ion homeostasis.

Possible Effects of BCAA and AAA on Cerebral Oxygen Consumption ($SjVO_2$)

The fact that lower $SjvO_2$ levels correlated with higher leucine and valine plasma levels [26] suggests either increased cerebral oxygen consumption or a decreased oxygen supply to the brain. Especially brain leucine oxidation is known to lead to increased CO_2 production [47]. This increase in CO_2 might result in increased cerebral vasodilation leading to brain swelling and elevated ICP as well as increased edema. Elevated ICP, in turn, could induce decreased oxygen supply by impairing cerebral perfusion [26]. On the other hand, in cortical prisms of the rat both isoleucine and leucine rather decreased CO_2 production and instead increased glucose uptake [48]. This sustained glucose uptake would provide a source of energy in brains of TBI patients even if the glycolysis occurs under anaerobic conditions. Nevertheless, oxidative glycolysis enabled by additional brain glucose uptake could explain an increased oxygen consumption of the brain and thus a decreased $SjVO_2$. Increased cerebral glucose uptake can also promote cellular acidosis and mitochondrial dysfunction due to glucose-induced production of free oxygen radical synthesis and subsequent edema formation [49]. Furthermore, BCAA might also increase cerebral protein synthesis [50], thereby augmenting oxygen consumption and thus lowering $SjVO_2$.

Clinical Signs and Outcome Correlated with Plasma Amino Acid Levels in TBI

Nutritional supplementation of amino acids in TBI patients using formulations with increased levels of BCAA and decreased levels of phenylalanine resulted in a positive nitrogen balance but not in differences in the neurological outcome [22]. The increase in the nitrogen balance in this study is assumed to refer to sufficient protein administration to the hypercatabolic TBI patient and is known

to be associated with decreased mortality [51]. Mortality was also decreased in another study comparing patients with cerebral hemorrhage when levels of BCAA were increased and levels of AAA were decreased in the parenteral nutrition [52]. While decreased mortality was not statistically significant the number of infections was significantly reduced in patients receiving more BCAA, possibly accounting for lower mortality. The same was observed in a study in brain injured patients including stroke, trauma, and anoxic coma patients when administration of an amino acid formulation with high BCAA and low AAA levels significantly reduced the infection rate [20]. Furthermore, BCAA supplementation was shown to enhance the cognitive recovery in TBI patients during the rehabilitation phase [53] even in a vegetative or minimally conscious state [54]. None of these studies reported a correlation between the plasma levels of certain amino acids and the clinical parameters, which would help to determine the right combination of amino acids for the treatment of TBI. The findings reported by Vuille-Dit-Bille and coworkers revealing differential effects depending on the increase in BCAA and AAA levels on ICP and $SjVO_2$ [26] prompt further in-depth research under clinical conditions to promote identification of the most beneficial nutritional composition and strategy. Thus, currently there are initial but conflicting and most notably insufficient clinical data to provide a recommendation for the right combination(s) of amino acids in TBI patients also considering the different post-traumatic phases.

Conclusions

Following TBI, deranged blood AA levels show a characteristic temporal profile with a specific pattern in the acute and chronic phase. While there is only limited data available assessing the impact of specific plasma AA levels on clinical parameters in TBI patients, there is growing evidence that current AA formulations for the encephalopathic critically ill aiming at restoring plasma BCAA levels might not be applicable for TBI patients. There are many possibilities for the BCAAs and AAAs to modulate pathways and to potentially influence the outcome of TBI. For this, further studies are required to elucidate their exact roles and provide a basis for the ideal phase-specific composition of amino acid solutions in TBI patients.

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Chapter 10
Branched Chain Amino Acids in Chronic Obstructive Pulmonary Disease

Tomoko Kutsuzawa and Munetaka Haida

Key Points

- Plasma concentrations of the branched chain amino acids leucine, isoleucine, and valine, are reduced or depleted in patients with chronic obstructive pulmonary disease.
- A new methodology, metabolomics, can identify perturbations in amino acid metabolism and can be used to stratify patients with chronic obstructive pulmonary disease according to their protein turnover and nutritional status.
- Glutamic acid in muscle tissue is reduced in patients with chronic obstructive pulmonary disease. The subgroup of patients with macroscopic emphysema demonstrated reduced branched chain amino acid, glutamine, and glutamic acid in muscle tissue.
- Enhanced amino acid release from muscle is seen in patients with chronic obstructive pulmonary disease during exercise.
- Plasma concentrations of BCAA are related to lactate accumulation in muscle during exercise in patients with chronic obstructive pulmonary disease.
- In pulmonary rehabilitation, nutritional support with essential amino acids containing high BCAA produced good results in patients with chronic obstructive pulmonary disease.

Keywords COPD • Muscle wasting • BCAA • Exercise • Lactate • Pulmonary rehabilitation

Abbreviations

COPD	Chronic obstructive pulmonary disease
BW	Body weight
BMI	Body mass index
FFM	Fat free mass
FEV_1	Forced expiratory volume in 1 s
EAA	Essential amino acids

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BCAA Branched chain amino acids
HRCT High-resolution computed tomography
NMR Nuclear magnetic resonance spectroscopy
TCA Tricarboxylic acid
MRS Magnetic resonance spectroscopy

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitation that is usually progressive. Previously, COPD was classified as emphysema and chronic bronchitis. COPD may be attributable to an enhanced chronic inflammatory response in the airways and lungs due to tobacco smoke and noxious particles or gases. Chronic inflammation leads to destruction of lung parenchyma and narrowing of the small airways. COPD patients have dyspnea, chronic cough, and sputum production. Airflow limitation is diagnosed using spirometry, and the finding of (forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC)) < 0.7 after treatment with a bronchodilator. Patients with COPD develop systemic features such as body weight (BW) loss and skeletal muscle dysfunction in addition to pulmonary impairment. Characteristic BW loss seen in COPD patients has a disproportionate decrease in fat free mass (FFM), and is known as pulmonary cachexia. About 25 % of patients with COPD develop cachexia [1].

Reduced FFM accompanies the decrease in muscle mass. However, muscle wasting can also occur as an isolated process in COPD patients of normal weight. Although patients with COPD reduce their physical activity due to exertional dyspnea, one can suppose that inactivity is not the only cause of muscle wasting. Multiple factors (for example, systemic inflammation, energy imbalance, hypoxia, or hormonal insufficiency) may also relate to muscle wasting (Fig. 10.1). Muscle wasting develops due to an imbalance of protein metabolism. Changes in protein metabolism may cause changes in amino acid profiles in plasma and muscles. The present review will cover several aspects of plasma and muscle levels of branched chain amino acids (BCAA) at rest and during exercise and with supplementation of BCAA or BCAA-rich protein in COPD patients.

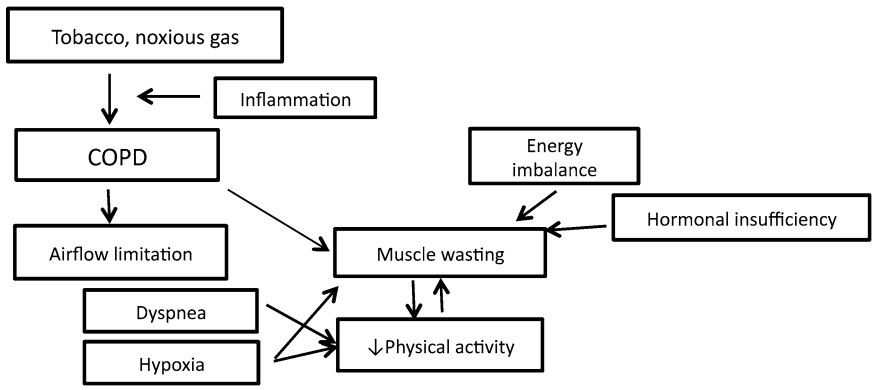

Fig. 10.1 Possible mechanism of muscle wasting in patients with chronic obstructive pulmonary disease (COPD). Tobacco smoke or noxious gas enhanced inflammatory response in the lung and airways. COPD patients have dyspnea and hypoxia due to airflow limitation, and this reduces their physical activity. Reduced FFM accompanies the decrease in muscle mass. Multiple factors (for example, reduced physical activity, systemic inflammation, energy imbalance, hypoxia, or hormonal insufficiency) may relate to muscle wasting (unpublished data)

Plasma BCAA in Patients with COPD (Table 10.1)

Since Morrison et al. [2] reported that the plasma concentration of amino acid profiles is altered in patients with COPD in 1988, several investigators have reported amino acid profiles in the plasma and skeletal muscles of patients with COPD [2–10]. Those findings are listed in Table 10.1.

Morrison et al. [2] found that the plasma concentrations of glutamine, glutamic acid, alanine, leucine, valine, and phenylalanine were decreased in COPD patients with severe airflow limitation. Most previous studies have shown that the plasma concentrations of BCAA (leucine, isoleucine, and valine) are reduced [4, 5, 7], but several studies [3, 8] have shown no significant decrease in plasma BCAA. It has been thought that the discrepancy of the changes in plasma BCAA concentration is caused by the difference in the disease in severity and/or body composition in COPD patients. COPD patients with low BMI and/or severe airflow limitation exhibit altered BCAA profiles, but those with a normal BMI and/or moderate airflow limitation do not. Yoneda et al. [6] demonstrated that decreased concentrations of BCAA in COPD are specifically related to weight loss and decreased muscle mass. In addition, a previous study [8] showed that the concentration of BCAAs correlated with BMI; thus, nutritional status relates to a low BCAA concentration. Engelen et al. [4] also demonstrated a positive correlation between FFM and plasma concentration of each BCAA. Engelen et al. [4] stratified COPD patients into macroscopic emphysema and minimal emphysema groups using high-resolution computed tomography (HRCT). The patients with macroscopic emphysema showed a severe decrease in forced expiratory volume in 1 s (FEV_1), BW loss, reduced leucine, and reduced total BCAA.

Recently, new methods using ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR) and/or mass spectrometry were developed and used to validate the measurement of low molecular weight metabolites in biological fluids or tissue extracts in a global/nontargeted manner (metabolomics studies). Ubhi et al. [9] and Rodrigues et al. [10] studied plasma metabolic profiles in COPD patients using metabolomics. Ubhi et al. [9] examined serum samples of COPD patients from the ECLIPSE study (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-Points). The NMR spectra of patients with severe airflow limitation were significantly different from controls by principal component analysis and partial least squares (PLS) discriminant analysis (DA) due to decreases in 3-hydroxyisobutyrate, isobutyrate, BCAAs, methionine, and trimethylamine. Metabolomics can be used to identify perturbations in amino acid metabolism that can be used to stratify patients with COPD according to their protein turnover and nutritional status. Rodrigues et al. [10] also reported difference in metabolic profiles between control subjects and COPD patients (Fig. 10.2). Plasma values of individual metabolites, such as valine, alanine, and isoleucine, were lower in COPD patients than in healthy subjects.

Decreases in plasma BCAA relate to decreases in FFM in COPD patients [4]. Systemic inflammation evoked by tobacco smoke is thought to be a possible mechanism of decrease in FFM. Pouw et al. [3] investigated the relationship between plasma amino acids and lipopolysaccharide binding protein (LBP) in COPD patients and reported that LBP was negatively correlated with the plasma concentrations of the amino acids alanine, glutamine, and asparagine.

Skeletal Muscle BCAA in Patients with COPD (Table 10.2)

Low BCAA concentrations are associated with a decrease in FFM and with muscle wasting. A few studies have investigated the amino acid profile in skeletal muscles of patients with COPD [3–5]. Target muscles were the tibialis anterior [3] or vastus lateralis [4, 5]. In muscle, several amino acids (glutamine, glutamic acid, alanine, phenylalanine, tyrosine, and BCAA) play prominent roles in overall protein metabolism, and are frequently measured in muscle biopsies.

Table 10.1 Plasma amino acid profile in patients with chronic obstructive pulmonary disease

Author	Year of publication	Subjects		Lung function		Nutritional state		Sum AA	Sum BCAA	Valine	Leucine	Isoleucine	Glutamine	Glutamic acid
		Healthy	COPD											
Morrison [2]	1988	11	8	FEV1	0.67±0.2 L	%IBW	76 %	NA	NA	↓	↓	→	↓	↓
Pouw [3]	1998	8	12	FEV1 %pred	32±2*	BMI	21.5±1.3*	↓	NA	→	→	→	↓	↓
						FFMI	15.9±0.6*							
Engelen [4]	2000	28	28	FEV1 %pred	41±15	BW loss	9/19	→	↓	→	↓	→	→	→
			14	FEV1 %pred Emphy−	49±16	BW loss	3/11	→	↓	→	↓	→	→	→
			14	FEV1 %pred Emphy+	33±10	BW loss	6/8	→	↓	→	↓	↓	→	→
Engelen [5]	2001	8	14	FEV1 %pred	37±12	FFM	52.9 kg (not depleted)	NA	NA	→	↓	→	→	NA
Yoneda [6]	2001	30	30	FEV1	0.85±0.25 L	%IBW	79.8±9.6	NA	→	↓	→	↓	↑	↑
				FEV1 %pred	48.1±6.4	%IBW	≥90	NA	→	→	→	→	↑	→
						%IBW	<90	NA	↓	↓	↓	↓	↑	↑
Rutten [7]	2006	7	9	FEV1 %pred	48±4*	BMI	20±1*	NA	↓	↓	↓	↓	NA	↓
			7	FEV1 %pred	53±6*	BMI	28±1*	NA	→	→	→	→	NA	→
Kutsuzawa [8]	2009	7	23	FEV1 %pred	50±4*	BMI	21.7±2.8	→	→	→	→	→	↑	→
Ubhi [9]	2012	66	31	FEV1 %pred	25.8±6.5	BMI	25.8±6.5	NA	NA	↓	↓	↓	↑	NA
						FFM	50.5±13.9							
Rodríguez [10]	2012	12	18	FEV1	1.34±0.39 L	BMI	24±4	NA	NA	↓	NA	↓	→	→
				FEV1 %pred	46±12									

Values are mean ± SD, *: mean ± SEM

AA amino acid, *BCAA* branched chain amino acid, *%pred* percent of predicted value, *FEV1* forced expiratory volume in 1 s, *Emphy* emphysema, *IBW* ideal body weight, *BMI* body mass index, *FFM* fat free mass, *BW* body weight, *FFMI* fat free mass index, *NA* not available

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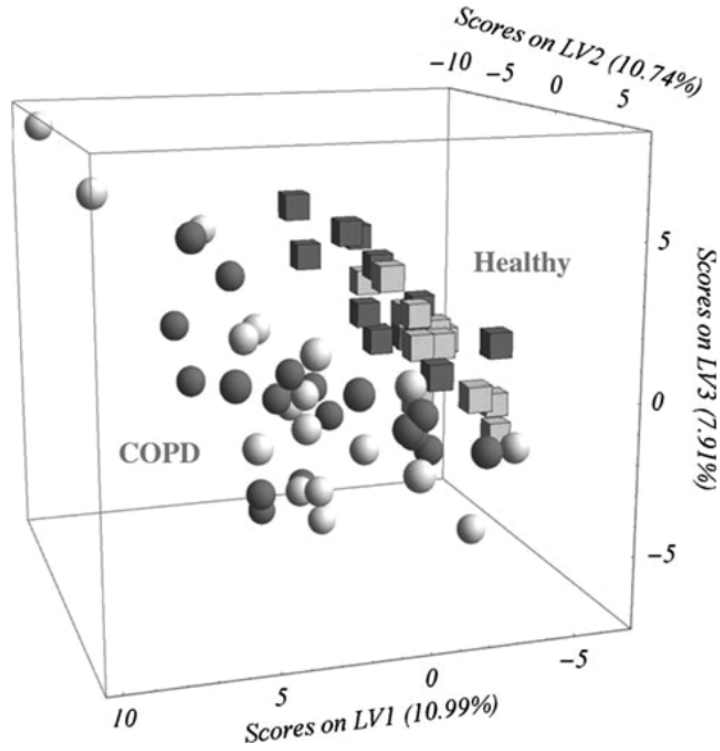

Fig. 10.2 Difference in metabolic profiles of COPD patients and healthy controls. Resting individual metabolic profiles in COPD patients (*spheres*) and healthy subjects (*cubes*), including pretraining (*black symbols*) and posttraining (*grey symbols*). The results are expressed by the three Latent Variables (LV1, 2, and 3) of the partial-least square discriminant analysis (PLS-DA) (from Ref. 10, with permission)

Pouw et al. [3] measured amino acid concentrations in plasma and tibialis anterior muscles of 12 COPD patients (BMI 21.5 ± 1.3 kg/m^2). The study demonstrated no significant difference in BCAA concentration between patients and healthy controls in plasma and muscle. Although plasma glutamine was found to be decreased in patients in the present study, an increase in glutamine and decrease in glutamic acid in muscle were demonstrated. Engelen et al. [4] also studied amino acid profiles in plasma and the vastus lateralis muscle. Twenty-eight COPD patients had low glutamic acid levels in the muscle in the present study, the same findings as Pouw's study, and no significant differences in plasma BCAA, glutamine, and alanine were found between patients and healthy controls. In addition, the subgroup of COPD patients with macroscopic emphysema demonstrated reduced BCAAs, glutamine, glutamic acid, alanine, phenylalanine, and tyrosine in the muscle. Patients with macroscopic emphysema were frequently found to have depleted FFM, suggesting that their total daily energy intake is insufficient for energy expenditure. In this situation, plasma amino acids from muscle breakdown are used in gluconeogenesis to supply glucose for energy; therefore, the muscle amino acid concentrations decrease. Engelen et al. [5] also investigated the amino acid profile of the vastus lateralis muscle in COPD patients (severe airflow limitation, FEV_1 % pred. 37 ± 12). Glutamic acid and valine in the muscle of COPD patients was reduced, the same as with previous studies.

Table 10.2 Muscle amino acid profile in patients with chronic obstructive pulmonary disease

Author	Year of publication	Subjects		Lung function		Nutritional state		Muscle	Sum AA	Sum BCAA	Valine	Leucine	Isoleucine	Glutamine	Glutamate
		Healthy	COPD												
Pouw [3]	1998	8	12	FEV1 %pred	32 ± 2*	BMI FFM	21.5 ± 1.3* 15.9 ± 0.6*	Anterior tibialis	→	NA	→	→	→	↑	↓
Engelen [4]	2000	28	28	FEV1 %pred	41 ± 15	BW loss	9/19	Vastus lateralis	→	→	→	→	→	→	↓
			14	FEV1 %pred Emphy−	49 ± 16	BW loss	3/11		→	→	→	→	→	↑	↓
			14	FEV1 %pred Emphy+	33 ± 10	BW loss	6/8		↓	↓	↓	↓	↓	↓	↓
Engelen [5]	2001	8	14	FEV1 %pred	37 ± 12	FFM	52.9 kg (not depleted)	Vastus lateralis	→	NA	↓	↓	NA	→	↓

Values are mean ± SD, *: mean ± SEM

AA amino acid, *BCAA* branched chain amino acid, *%pred* percent of predicted value, *FEV1* forced expiratory volume in 1 s, *IBW* ideal body weight, *FFM* fat free mass, *FFMI* fat free mass index, *NA* not available, *FEV1* forced expiratory volume in 1 s, *Emphy* emphysema, *BMI* body mass index, *FFM* fat free mass, *BW*, body weight, *FFMI* fat free mass index, *NA* not available

Unpublished

BCAA and Exercise

Previous studies [3–5] have demonstrated profound alterations in skeletal muscle amino acid status in patients with COPD at rest. The alterations of muscle amino acids may contribute to muscle dysfunction in patients with COPD. During exercise, six amino acids (glutamine, aspartate, asparagine, and the BCAAs) are metabolized in muscle tissue [5]. Muscle energy metabolism might be affected by BCAAs during exercise as energy sources. Energy expenditure greatly increases in skeletal muscle during exercise, and then BCAA oxidization maximally increases twofold to threefold [11]. In addition, BCAAs might contribute to energy metabolism during exercise as substrates that expand the pool of tricarboxylic acid (TCA) cycle intermediates [11] (Fig. 10.3).

Glutamic acid, another amino acid, is known to play an important role in metabolism of amino acids in muscle. Glutamic acid is generated from BCAA and α-ketoglutarate via reversible transamination in the presence of branched chain aminotransferase (BCAT). During the first minutes of exercise, glutamic acid generates TCA cycle intermediates via the alanine aminotransferase reaction (pyruvate + glutamic acid → alanine + α-ketoglutarate) in healthy human muscle [12] (Fig. 10.3).

Changes in Amino Acid Profiles Preexercise and Postexercise

Engelen et al. [5] examined the effect of submaximal, constant work rate exercise (Vo_2 max 20 % of predicted for 20 min) on amino acid profiles in plasma and muscle in 14 COPD patients and 8 healthy subjects. Plasma amino acid profiles of COPD patients at rest showed decreases in leucine in plasma, and decreases in glutamic acid, valine, and luecine in muscle, as is shown in Tables 10.1 and 10.2. After exercise, COPD patients with severe airflow limitation (FEV_1 37 ± 12 % predicted) showed an increase in alanine and glutamine in plasma, and a decrease in total amino acids, glutamine, and glutamic acid in muscle. In contrast, healthy subjects did not show significant change in the amino acid profile in muscle. These findings suggest enhanced amino acid release from muscle in patients with COPD during exercise.

Rodríguez [10] studied the plasma metabolic profile at rest and during a constant work rate exercise at 70 % of pretraining peak work rate in COPD patients, whose BMI were 24 ± 4 kg/m^2, and healthy subjects using ^{1}H-NMR. The patients with COPD had significantly lower plasma levels of valine, alanine, and isoleucine compared to controls at rest before training. Healthy and COPD patients presented with significant changes in metabolic profiles in response to exercise. In healthy controls, plasma levels of glutamine, alanine, valine, and isoleucine were significantly reduced during and after exercise. In COPD patients, the effects of exercise were similar to those seen in healthy controls, except for alanine. The increase in plasma alanine after exercise is consistent with the findings of Engelen et al. [5].

Rodríguez also investigated the metabolic profile after 8 weeks of training [10]. With endurance training, healthy controls had increased percent changes of each metabolite, including valine, isoleucine, and glutamic acid, but patients with COPD did not. This finding suggests that training effects on plasma amino acid profiles are altered in COPD patients compared to healthy subjects.

BCAA and Lactate

As previously mentioned, BCAA can relate to lactate production. Several studies have demonstrated that reduced oxidative capacity in skeletal muscles related to an accelerated lactate response to exercise in patients with COPD [13]. The relationship between plasma BCAA levels and muscle pH

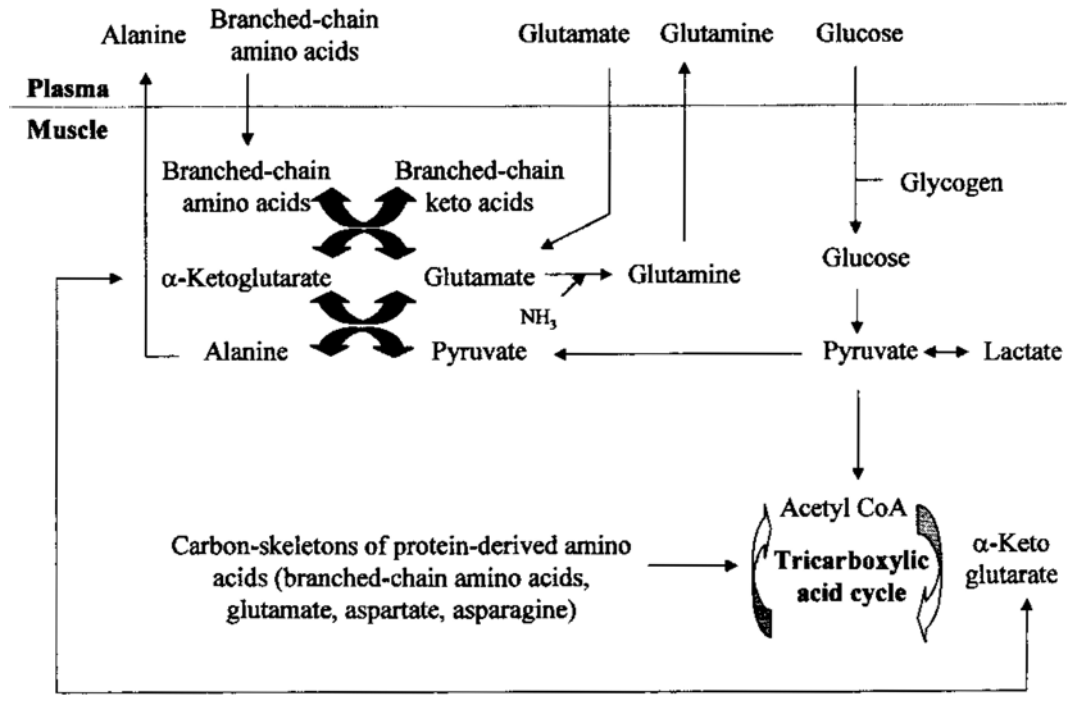

Fig. 10.3 Schematic overview of normal muscle amino acid metabolism during short-term exercise. During the first minutes of exercise, glutamic acid and BCAA have an important role (from Ref. 5, with permission)

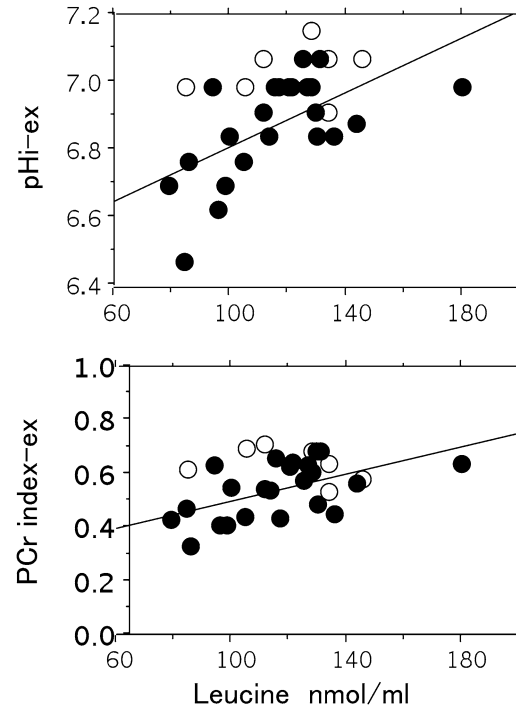

Fig. 10.4 Relationship between leucine level before exercise and muscle pH and phosphocreatine. Muscle pH and phosphocreatine at completion of exercise correlated with plasma leucine level in COPD patients. (*open circle*) healthy subjects, (*filled circle*) COPD patients, *pHi-ex* muscle pH at completion of exercise, *PCr index-ex* phosphocreatine/(phosphocreatine + inorganic phosphate) at completion of exercise (from Ref. 8, with permission)

during exercise in COPD patients was investigated using ^{31}P magnetic resonance spectroscopy (MRS) [8]. ^{31}P-MRS can dynamically and noninvasively measure intracellular muscle pH (pHi) and high-energy phosphate compounds in skeletal muscle. A decrease in pHi during exercise suggests lactic acid accumulation in exercising muscle. It was found that plasma concentrations of leucine, isoleucine, and valine correlated with pHi and phosphocreatine at the completion of exercise [8]. These findings suggest that the plasma concentrations of BCAAs contribute to alterations in muscle energy metabolism during exercise in COPD patients (Fig. 10.4).

Supplementation of BCAA

Effects of Supplementation of BCAA on Protein Turnover

In patients with COPD, loss of body weight or of FFM affects prognosis, and additional dietary protein is an attractive approach to gain BMI and FFM. Researchers at Maastricht University have investigated the effects of sip feeding of milk protein (casein or whey, both of which contain large amounts of BCAA), on protein turnover in patients with COPD using stable isotope methodology [14]. Their studies have shown that sip feeding of milk protein was able to increase whole-body protein synthesis in normal-weight COPD patients. In addition, they found that anabolic response was enhanced in patients with COPD during sip feeding of BCAA-low soy protein adding BCAA, compared with soy protein [15]. Thus, sip feeding of BCAA-rich protein can play a role in gaining muscle mass.

Engelen et al. [14] examined the influence of casein or whey protein on protein turnover during exercise in COPD patients using the stable isotopic method. COPD patients performed constant cycle exercise (50 % of peak work rate) for 20 min. They demonstrated that net protein synthesis was higher during casein feeding in COPD due to decrease in protein breakdown.

Effects of Supplementation of BCAA on Lactate During Exercise

BCAA supplementation can be linked to lactate metabolism during exhaustive exercise. Several studies have examined the effects of BCAA ingestion on lactate metabolism during long-term, exhaustive exercise in healthy subjects [16]. MacLean et al. [16] reported that lactate release and arterial lactate values were lower in a group given BCAAs than in a control group. Only a few studies have examined the effects of amino acid supplementation on exercise in COPD patients. The effect of BCAA ingestion on muscle pH in patients with COPD was investigated using ^{31}P-MRS [17]. COPD patients performed repeated bouts of short-term handgrip and ingested BCAA (8.0 g) 1 h before the second bout. Changes in muscle pH were smaller in the second bout than in the first bout, suggesting that BCAA can help to prevent metabolic acidosis. Thus, BCAA ingestion can improve exercise capacity in COPD patients.

Pulmonary Rehabilitation and BCAA Supplementation

In pulmonary rehabilitation, nutritional support with BCAA, essential amino acids, and BCAA-rich protein has been applied in COPD patients [18–21]. The effects of BCAA and/or protein supplementation on endurance training in COPD are still controversial. In addition, there have only been a few randomized control trials of nutritional support of BCAA and/or BCAA-rich food in COPD patients.

Menier et al. [18] examined the effect of BCAA supplementation (375 mg/7 kg BW/daily) on oxygen consumption symptom limitation (Vo_2 SL) before and after retraining in COPD patients. Vo_2 SL after a 5-week retraining significantly increased both in patient groups with and without BCAA supplementation, but there was no difference between those groups. Kubo et al. [19] investigated the effect of BCAA-rich food during pulmonary rehabilitation in eight COPD patients. Four patients consumed 200 mL of BCAA-rich food containing 8 g protein per day, and other patients received nutritional instruction. There was no significant difference in 6-min walking distance between the groups with and without supplementation. However, COPD patients without supplementation showed a decrease in albumin after rehabilitation, but the group with supplementation did not, suggesting that protein imbalance developed in the group without supplementation.

A pilot study on supplementation of essential amino acids (EAA) in COPD patients was performed by Baldi et al. [20]. The patients were randomly assigned to pulmonary rehabilitation with or without EAA ingestion containing high and balanced BCAAs. After a 12-week rehabilitation, the EAA group showed more BW and FFM gain compared to the control group. The findings suggest that EAA supplementation to pulmonary rehabilitation has the potential to stabilize or recover body weight in depleted COPD patients.

Dal Negro et al. [21] randomized patients with severe COPD to receive essential amino acids (EAA, 8 g/day) or a placebo for 12 weeks. Patients in both groups could not participate in the pulmonary rehabilitation program. After 12 weeks, EAA patients significantly increased FFM, muscle strength, daily steps, and serum albumin compared to placebo patients. The results suggest that supplementation of specific amino acids is able to stimulate protein synthesis and to restore muscle mass which can aid in improving physical activity.

Therefore, further study is needed to determine optimal protein intake (kinds of protein, amino acid content, and dosage) in order to gain muscle mass or to improve exercise limitation.

Conclusions

Plasma and muscle concentrations of amino acids are altered in patients with COPD, especially in depleted patients. Reduced levels of BCAA affect muscle energy metabolism during exercise. Nutritional support for pulmonary rehabilitation is needed in COPD patients. Further study is needed to determine prescription nutritional support.

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Part III
Branched Chain Amino Acids and Liver Diseases

Chapter 11
Identification of Branched Chain Amino Acids; Underlying Molecular Pathways Using Transcriptomic Analysis: Application to Cirrhosis

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Key Points

- Oral administration of BCAA is vital in improving prognosis in patients with chronic liver disease.
- BCAA can improve albumin synthesis, hepatic encephalopathy, insulin resistance, and suppress hepatocarcinoma, leading to higher event-free survival rate and better quality of life.
- The effects of BCAA on global gene expression in liver and skeletal muscle and the molecular mechanisms underlying the improvement in cirrhotic rats were addressed using DNA microarray analysis.
- Genes whose expression in BCAA supplemented group was reversed by more than twofold in liver cirrhosis group in the microarray analysis were selected to elucidate the mechanism behind the improvements in nutritional and metabolic disorders.
- The decrease in the expression of fatty acid translocase (*FAT*)/*Cd36*, glutamine synthetase, and pyruvate dehydrogenase kinase isoenzyme 4 which promotes lower uptake of fatty acids, lower ammonia incorporation, and higher uptake of glucose, may provide as an alternative energy source to BCAA, resulting in slower catabolism of BCAA and skeletal muscle protein, maintaining BCAA blood levels.

Keywords Branched chain amino acid (BCAA) • Liver cirrhosis • Transcriptomic analysis • Nutritional status • Insulin resistance • Hypoalbuminemia • Hepatic encephalopathy • Hepatocarcinoma

Abbreviations

AAA	Aromatic amino acids
ALT	Alanine aminotransferase
Arg	Arginase

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BCAA	Branched chain amino acids
BCKDK	Branched chain α-keto acid dehydrogenase kinase
CCl_4	Carbon tetrachloride
Cdo	Cysteine dioxygenase
Col α1(I)	Type I collagen alpha 1
Col α1(III)	Type III collagen alpha-1
Col α1(V)	Type V collagen alpha 1
CPS	Carbamoyl phosphate synthetase
ECM	Extracellular matrix
FAT/Cd36	Fatty acid translocase
Glud	Glutamate dehydrogenase
Gs	Glutamine synthetase
HCC	Hepatocellular carcinoma
HE	Hepatic encephalopathy
Hmgcr	3-Hydroxy-3-methylglutaryl coenzyme A reductase
HSC	Hepatic stellate cells
IFN	Interferon
mTOR	Mammalian target of rapamycin
Otc	Ornithine transcarbamylase
PDH	Pyruvate dehydrogenase
Pdk4	Pyruvate dehydrogenase kinase isoenzyme 4
PPARα	Peroxisome proliferator-activated receptor alpha
RPA	RNase protection assay
Slc2a4	Solute carrier family 2 (facilitated glucose transporter), member 4
Sqle	Squalene epoxidase
Sqs	Squalene synthetase
Tgf-β1	Transforming growth factor β1

Introduction

In patients with liver cirrhosis, blood levels of branched chain amino acids (BCAA; valine, leucine, and isoleucine) are decreased, and blood levels of aromatic amino acids (AAA; tyrosine, phenylalanine, free tryptophan) are increased. The decrease in blood BCAA levels results from BCAA being used as an energy source and as a compensation for hyperammonemia. BCAA oxidation, which is controlled by by-products of leucine transamination, can provide a key energy source for skeletal muscle [1–3].

It is evident that oral administration of BCAA in liver cirrhosis has beneficial effects, including the suppression of oxidative stress and inflammation [4], and also delays the development of serious complications, such as hypoalbuminemia in animals and humans [5, 6]. These effects are attributable to the roles of BCAA as regulators of protein synthesis and degradation and as key precursors for glutamine and alanine synthesis. BCAA have also been shown to prevent the occurrence of hepatocellular carcinoma (HCC) in liver cirrhosis, especially in overweight or obese patients [7].

In this chapter, we review the current therapeutical benefits of BCAA supplementation. In addition, our recent work that established the regulatory gene pathways underlying the improvements in an animal model of liver cirrhosis is described.

Abnormal Amino Acid Metabolism in Patients with Liver Cirrhosis

In patients with liver cirrhosis, levels of BCAA are decreased in blood, whereas levels of AAA and methionine are increased [8]. In particular, BCAA levels decrease in advanced fibrosis (F4 stage) and tyrosine levels increase with the progression of hepatic fibrosis [9].

The decreases in BCAA levels can be attributed to a few causes. One of which is that BCAA has a significantly higher combustion rate in patients with liver cirrhosis as compared to healthy subjects and is therefore utilized more readily than glucose and fatty acids as an energy substrate [10]. Patients with liver cirrhosis also show a positive correlation between clearance rate of BCAA and ammonia levels in blood [11]. BCAA is metabolized in skeletal muscle to compensate for the normal processing of ammonia by the urea cycle in livers. As a result, BCAA levels in blood are decreased in patients with liver cirrhosis.

Nutrition Support and Improved Prognosis Using BCAA Supplementation in Patients with Liver Cirrhosis

Late evening snacks supplemented with BCAA are effective in the improvement of nitrogen balance for the fasting state upon awakening in liver cirrhosis patients as glycogen stores are markedly depleted in the atrophic liver [12]. The change of nonprotein respiratory quotient for 3 months was significantly higher than when the control snack of the same number of calories was administered [13]. BCAA, especially leucine [14, 15], can induce the phosphorylation of the downstream effectors of mammalian target of rapamycin (mTOR) which is a major effector of cell growth and proliferation with a regulatory effect on protein synthesis [16, 17]. In addition, leucine also promotes hepatic albumin synthesis [18] and has the effect of increasing albumin levels in blood in patients with liver cirrhosis [1, 2]. The improved nutritional status of patients with liver cirrhosis given BCAA may be due to the additive effects of BCAA functioning both as an energy substrate and as a promoter of albumin synthesis.

Reduction of Hepatic Injury and Suppression of Onset of Liver Cirrhosis by BCAA

Another reported function of leucine is that it stimulates hepatocyte growth by promoting the secretion of hepatocyte growth factor, which regulates cell growth, cell motility, and morphogenesis, and also increases DNA synthesis in the liver, thereby reducing hepatic injury [19, 20]. On the other hand, valine has been documented to regulate the maturation and function of monocyte-derived dendritic cells, which are professional antigen-presenting cells that are vital in the stimulation of the innate and adaptive immune reactions [21]. The onset of liver cirrhosis is related with oxidative stress and immune response; however, the mechanism by which BCAA reduces oxidative stress and the suppression of dendritic cells in patients with liver cirrhosis is unknown.

Amelioration of Hepatic Encephalopathy by BCAA

Hepatic encephalopathy (HE) refers to disturbance of consciousness as a result of liver disease. The causes of cirrhosis-related encephalopathy are as below: (1) hyperammonemia due to reduced ammonia processing; and (2) abnormal metabolism of monoamine neurotransmitters in the brain due to increased AAA arising from defective liver metabolism.

BCAA-enriched amino acid infusion solution is effective in the treatment of HE. BCAA is absorbed by skeletal muscle and the brain, especially so in the brain where BCAA increases the rate of ammonia metabolism and this transfer of BCAA suppresses AAA transfer. These effects are hypothesized to improve HE. However, the wake-up effect of BCAA infusion therapy is influenced by the severity of liver disease [22]. Ammonia levels are reduced during the metabolism of BCAA as it is used as a source of glutamine production. In the instance if the liver is severely damaged, there is a risk of deterioration or prolongation of coma as the nonfunctioning urea cycle is unable to process ammonia.

Improvement of Insulin Resistance Through BCAA Administration in Patients with Liver Cirrhosis

Leucine also has been reported to play an important role in glucose metabolism in skeletal muscle [23, 24]. Isoleucine also exerts a hypoglycemic effect as it stimulates glucose uptake in the skeletal muscle and decreases glucose production in the liver [25]. Oral administration of BCAA-enriched nutrients suppresses the elevation of postprandial blood glucose in patients with liver cirrhosis [26]. With regards to the effect of BCAA on insulin resistance, HbA1c level in patients with nonalcoholic steatohepatitis-related cirrhosis is decreased after BCAA administration [27]. However so, BCAA does not improve insulin resistance in chronic hepatitis C patients [28].

Suppression of Liver Cancer and Prevention of Recurrence by BCAA

Liver cirrhosis patients with a body mass index larger than 25 have a significantly reduced risk of liver cancer when given BCAA [7]. Obesity is a characteristic associated with insulin resistance [29], and insulin resistance is a risk factor for liver cancer [30]. Recent evidence has suggested that HCC can be suppressed by BCAA as it inhibits insulin-induced angiogenesis [31].

In liver cancer patients, the administration of BCAA-enriched oral nutrients or BCAA granules has been found to have several beneficial effects, lowering the incidence of ascites and peripheral edema [32], the Child-Pugh score [33] after transarterial chemoembolization, the Child-Pugh score from the early stage after radiofrequency ablation [34], and the deterioration in liver function and liver fibrosis 1 year after liver tumor resection [35]. In view of these findings, patients should ideally continue to consume BCAA from the period before the onset of liver cancer throughout cancer treatment and thereafter to prevent liver cancer and its recurrence.

Benefits of long-term administration of BCAA include reduced aggravation of liver failure, increased event-free survival rate, improved quality of life [36], and reduced liver cancer incidence [37]. The prevention of liver cancer and suppression of liver fibrosis by BCAA appears to be related to insulin resistance [14], however, there is a need for further elucidation of the mechanisms, including its relation to oxidative stress. These outcomes indicate that BCAA does not simply improve nutrition in liver cirrhosis patients but also contributes to the suppression of HCC.

New Clinical Applications for BCAA

Recent studies on hepatitis-C patients have shown that patients given BCAA with zinc had higher continuation of interferon (IFN) therapy [38], and that valine had an effect on viral load reduction and eradication during IFN therapy [39]. BCAA thus may be a potential adjuvant for IFN therapy.

In addition, application of BCAA as a treatment from the hepatitis stage could be significant in preventing the further progression of fibrosis. Further research on these topics and on elucidating the precise molecular mechanisms underlying the action of nutritional intervention by BCAA supplementation on the pathophysiological states of liver cirrhosis is anticipated.

Identification of Molecular Pathways Mediating the Effects of Supplementation of BCAA on CCl_4-Induced Cirrhotic Rats

With the recent advancements in genomic research, DNA microarray technology has been employed to monitor altered gene expression and to characterize different disease states for diagnoses involved in liver injury [40], fibrogenesis, liver cirrhosis, and hepatocarcinogenesis [41–43]. Using DNA microarray analysis (GeneChip Rat Genome U34_A Array, Affymetrix, Santa Clara, CA, USA), we investigated the effects of BCAA supplementation on hepatic and skeletal muscle gene expression profiles and their relationships in carbon tetrachloride (CCl_4)-induced chronic cirrhosis in 3-week-old male Wistar rats [44].

There were three groups of rat where Group 1 ($n=6$) was given a basal diet with vehicle only. Groups 2 and 3 ($n=10$, each) were administered 50 % 1 ml/kg of bodyweight CCl_4 by hypodermic injection twice a week and fed the same basal diet. Hepatic injury in groups 2 and 3 was confirmed by serum hepatotoxicity (alanine aminotransferase (ALT) and albumin) at week 19. According to the Child-Pugh classification, rats administered with CCl_4 repeatedly for 19 weeks were confirmed to have decompensated cirrhosis in which liver functions were impaired, leading to abnormal metabolic processes of glucose, protein–amino acids, lipids, vitamins, and minerals. Therefore, following this 19-week period, the diets of group 1 (normal control group, NC) and group 2 (liver cirrhosis-casein group, LC) were changed to a 20 % crude protein (casein derived) diet (Fischer ratio: 2.8), whereas 20 % of the crude protein of group 3 (AL) consisted of equal compositions of casein and BCAA nutritional supplement (Aminoleban EN powder mix; Fischer ratio: 38, Otsuka Pharmaceutical, Tokyo, Japan) diet (Fischer ratio: 6.8).

Effects of BCAA on General Characteristics

BCAA tended to suppress the decrease in body weight observed in LC, although no significant difference was observed. However, dietary intervention with BCAA significantly attenuated the elevation of ALT activity to 73.4 ± 25.1 from 111.7 ± 55.2 in LC.

Elevated levels of plasma AAA or a decreased Fischer ratio is correlated with the development of hepatic encephalopathy through enhanced brain AAA uptake and disturbed neurotransmission [9]. In order to determine the state of abnormalities in amino acid and protein metabolism, amino acid concentrations in plasma, liver, skeletal muscle, and brain were measured. Plasma, muscle, and brain BCAA concentrations of AL were significantly higher than those of LC, while AAA in brain was significantly lower. Similar tendencies were observed in other tissues thereby reflecting an improved protein nutrition status (Fig. 11.1). In particular, we found that the increase in the Fischer ratio in AL can be attributed to the increase in BCAA, as well as by the decrease in AAA due to an improved ability to metabolize AAA in liver.

Decreased hepatic protein synthesis in liver cirrhosis also contributes to hypoalbuminemia, which is another important indicator in the severity of liver cirrhosis. The synthesis and secretion of albumin in primary hepatocytes have been reported to be increased by BCAA through the activation of transcription factor mTOR when the Fischer ratio is maintained at an appropriate concentration [45, 46]. It was observed in our study that BCAA slightly improved (2.7 ± 0.4 in AL to 2.6 ± 0.7 in LC) the

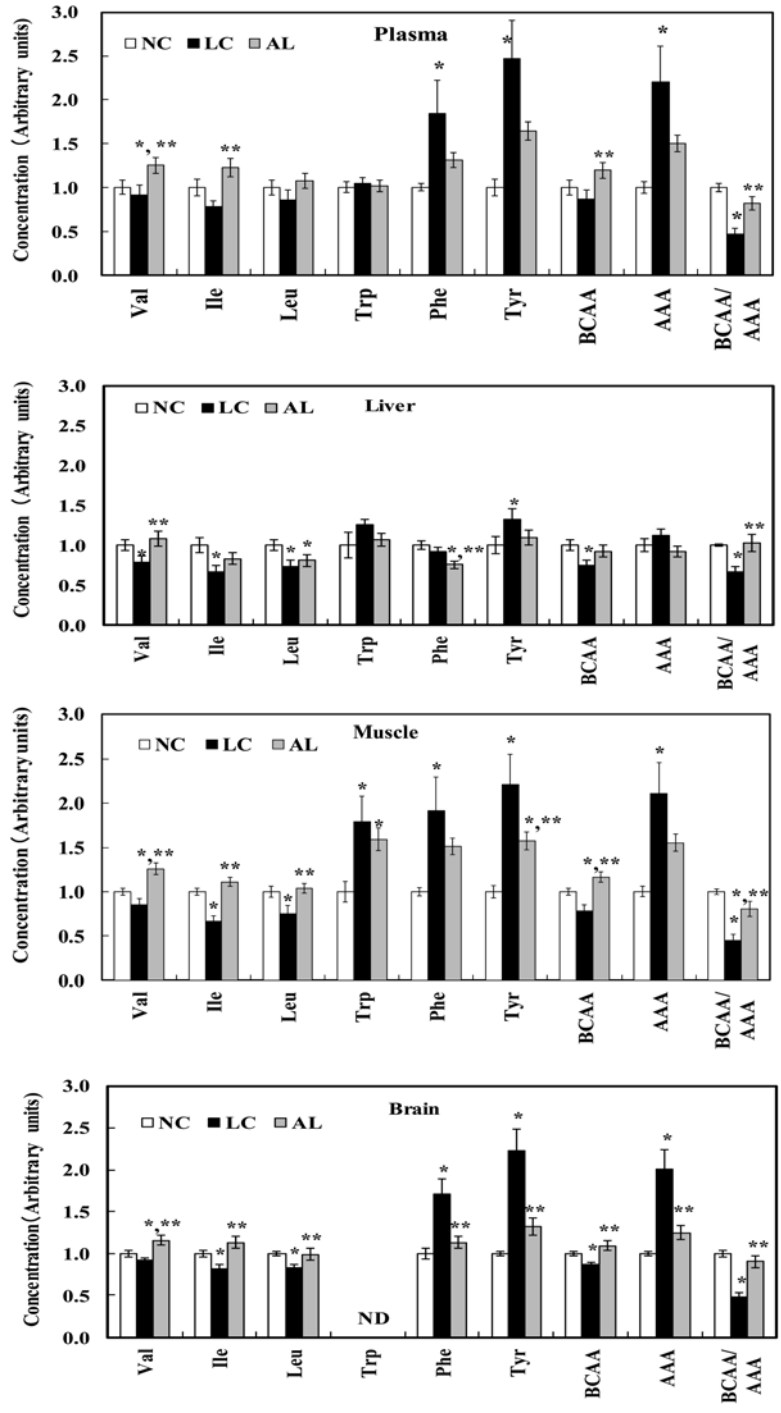

Fig. 11.1 Effects of CCl_4 administration and BCAA supplementation on concentrations of BCAA, AAA, and Fischer ratio in plasma, liver, muscle, and brain [44]. *BCAA* branched chain amino acids, *AAA* aromatic amino acids, *Fischer ratio* the molar ratio of BCAA to AAA, *ND* not detected. Data are values relative to the concentration of amino acid in NC, which was assumed as 1. Values are the means ± SE ($n = 6$ in NC, $n = 10$ in LC, and AL). *NC* normal control, *LC* liver cirrhosis-casein, *AL* amino acid plus liver cirrhosis-casein. $^{*}P < 0.05$ vs. NC, $^{**}P < 0.05$ vs. LC. We have hereby received permission to reproduce this figure from John Wiley and Sons (License No: 3125250988663)

decreased serum albumin concentrations in LC. The reason for this phenomenon remains unclear, although it is speculated that BCAA might first ameliorate the amino acid imbalance and then later improve the reduced serum albumin concentrations (hypoalbuminemia).

Effects of BCAA Supplementation on Hepatic and Skeletal Muscle Gene Expression Profiles

In order to investigate the molecular mechanisms fundamental to the ameliorative effects of BCAA supplementation, genes whose expression in AL was reversed compared to the expression in LC were selected. Genes showing an expression change of twofold or greater between LC and NC were chosen, and their expression levels were further compared against to those of AL. In liver, it was observed that the up-regulation by LC of 28 genes was attenuated in AL or even completely reversed. Conversely, down-regulation shown in LC was attenuated in AL for 9 genes (Table 11.1). In skeletal muscle, 20 genes showed differential changes (all downregulated, none upregulated) between AL and LC (Table 11.2). Expression levels of these differentially changed genes were validated by RNase protection assay (RPA) (Fig. 11.2).

Table 11.1 Genes affected by LC and AL in rat liver [44]

Accession no.	Description	Fold change	
		LC > NC	AL < LC
AF065161	Cytokine-inducible SH2-containing protein	13.9	2.0
M69246	Collagen-binding protein (gp46)	6.5	1.4
M29249	3-Hydroxy-3-methylglutaryl coenzyme A reductase	5.3	1.6
M28671	Rearranged IgG-2b	5.3	1.9
M91595	Insulin-like growth factor binding protein-2	4.0	1.5
M96630	Sec61 homologue	3.7	1.6
S81353	Sulfated glycoprotein-1	3.7	1.2
X77934	Amyloid precursor-like protein 2	3.7	1.4
M22360	Plasma proteinase inhibitor alpha-1-inhibitor III	3.5	2.6
M74494	Sodium/potassium ATPase alpha-1 subunit truncated isoform	3.5	1.4
U50842	Ubiquitin ligase (Nedd4) protein	3.2	1.2
L22654	Antiacetylcholine receptor antibody gene	3.2	1.4
U82612	Fibronectin (fn-1)	3.0	1.9
X12535	Ras-related protein p23	3.0	1.3
M22993	Alpha-1 inhibitor III (alpha-1-I3)	2.8	2.6
S56937	3-Methylcholanthrene-inducible UDP-glucuronosyltransferase	2.8	1.4
S80431	Delta 4-3-ketosteroid 5 beta-reductase = 37 kDa protein	2.8	1.7
M63991	Thyroxine-binding globulin (TBG)	2.8	1.4
X92097	Transmembrane protein rnp21.4	2.8	1.4
U39875	EF-hand Ca2+ -binding protein p22	2.6	1.3
L18889	Calnexin	2.5	1.4
X90710	Alcohol dehydrogenase protein	2.5	1.7
M91234	VL30 element	2.3	2.5
U72741	36 kDa beta-galactoside binding lectin	2.3	1.4
J02679	NAD(P)H-menadione oxidoreductase	2.1	1.5
Z75029	Heat-shock protein 70	2.1	2.0

(continued)

Table 11.1 (continued)

Accession no.	Description	Fold change	
D14564	L-Gulono-gamma-lactone oxidase	2.0	1.6
M95591	Hepatic squalene synthetase	2.0	1.3
		LC<NC	AL>LC
L37333	Glucose-6-phosphatase (G6Pase)	7.0	1.3
AA866302	4-Hydroxyphenylpyruvic acid dioxygenase	6.1	1.9
M81183	Insulin-like growth factor I gene	2.8	1.3
X78855	Cation transporter	3.0	1.2
J03959	Uricase	3.0	2.1
AA945054	Cytochrome b-5	3.0	2.0
H33255	Thymine glycol DNA glycosylase	2.0	2.3
AA799980	Protein phosphatase 1B, magnesium dependent, beta isoform	2.0	1.3
M36410	Sepiapterin reductase	2.0	1.4

NC normal control, *LC* liver cirrhosis-casein, *AL* amino acid plus liver cirrhosis-casein
Results are expressed as fold changes to LC in gene expression
LC>NC: Gene expression in LC was twofold or greater than that in NC
AL<LC: Gene expression in AL was twofold or less than that in LC
LC<NC: Gene expression in LC was twofold or less than that in NC
AL>LC: Gene expression in AL was twofold or greater than that in LC
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Table 11.2 Genes affected by LC and AL in rat muscle [44]

Accession no.	Description	Fold change	
Glycolysis and gluconeogenesis		LC>NC	AL<LC
M92919	Phosphorylase kinase alpha-SUBUNIT	4.6	1.9
D10655	Dihydrolipoamide acetyltransferase	4.0	1.5
M98826	Phosphorylase kinase gene	3.7	1.6
S79213	Cytosolic regulatory subunit of type 1 protein phosphatase	3.7	1.7
Z12158	Pyruvate dehydrogenase E1 alpha form 1 subunit	3.5	1.6
D00092	Dihydrolipoamide acetyltransferase	3.2	1.7
L01793	Glycogenin	3.2	1.5
X16043	Phosphatase 2A catalytic subunit isotype alpha	2.8	1.9
AF034577	Pyruvate dehydrogenase kinase isoenzyme 4	2.6	2.5
AJ005046	Muscle fructose-1,6-bisphosphatase	2.0	2.5
Lipid and fatty acid metabolism			
AF072411	Fatty acid translocase/Cd36	8.6	1.5
AI237731	Lipoprotein lipase	2.5	1.5
U83880	Glycerol-3-phosphate dehydrate dehydrogenase	2.1	1.9
Stress response			
AA848563	Heat-shock 70 kDa protein 1B	6.1	3.7
S75280	70 kDa heat-shock protein precurso	4.3	1.6
U95727	DnaJ homolog 2	4.0	1.7
AI176546	Heat-shock protein 1, alpha	3.5	1.6
AI172247	Xanthine dehydrogenase	2.8	1.5
Z27118	Heat-shock protein 70	2.6	2.8
Y17295	Thiol-specific antioxidant protein	2.1	1.5
M34253	Interferon regulatory factor 1 (IRF-1)	2.5	2.1
Amino acid metabolism			
AA852004	Glutamine synthetase 1	4.3	2.6
AA942751	Tyrosine 3-monooxygenase	4.0	1.7

(continued)

Table 11.2 (continued)

Accession no.	Description	Fold change	
AA900516	Peptidyl arginine deaminase, type II	3.0	1.7
S45812	Monoamine oxidase A	2.8	1.5
S61973	NMDA receptor glutamate-binding subunit	2.5	1.7
Transcription regulator			
AF022081	Small nuclear RING finger protein	2.1	1.6
Signal transduction			
M64301	Extracellular signal-related kinase (ERK3)	10.6	1.9
S81584	Transforming growth factor, beta receptor 1	4.6	3.5
D30041	RAC protein kinase beta	2.6	1.7
X13905	ras-related rab1B protein	2.1	11.3
Structure elements			
S82383	Tropomyosin isoform 6	3.0	1.7
J02780	Tropomyosin (TM-4)	2.6	1.7
Unclassified			
M58040	Transferrin receptor	36.8	2.3
X77934	Amyloid precursor-like protein 2	27.9	1.7
Z46614	Caveolin	21.1	2.1
D14568	Calcineurin B	17.1	4.0
L22558	Adenylyl cyclase-activated serotonin receptor	11.3	8.6
L13619	Insulin-induced growth-response protein (CL-6)	12.1	2.0
M15191	Beta-tachykinin	6.5	5.3
AF000899	p58/p45	5.7	4.9
D14425	Calcineurin B	4.6	1.6
AF052596	SNAP-23	4.3	1.5
D10655	Dihydrolipoamide acetyltransferase	4.0	1.5
AA942751	Tyrosine 3-monooxygenase	4.0	1.7
U31816	Calcium channel alpha-1S subunit (ROB1)	3.0	2.8
M93401	Methylmalonate semialdehyde dehydrogenase gene	2.8	1.7
M74494	ATPase, Na+/K+ transporting, alpha 1 polypeptide	2.6	2.5
M26686	Carboxyl methyltransferase	2.5	1.9
X76988	Gal beta 1,3 galNAc alpha 2,3-sialyltransferase	2.5	2.0
AA850734	Vascular endothelial growth factor	2.1	1.7
L20913	Vascular endothelial growth factor form 3	2.3	2.1
U57042	Adenosine kinase mRNA	2.1	1.9
U52663	Peptidylglycine alpha-amidating monooxygenase	2.1	1.5
U68562	Chaperonin 60	2.1	1.5
AA874813	Mitofusin 2	2.0	1.7
AI237654	Up-regulated by 1,25-dihydroxyvitamin D-3	2.0	2.3

NC normal control, *LC* liver cirrhosis-casein, *AL* amino acid plus liver cirrhosis-casein
Results were expressed as fold changes to LC in gene expression
LC > NC: Gene expression in LC was twofold or greater than that in NC
AL < LC: Gene expression in AL was twofold or less than that in LC
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Hepatic fibrosis, a common condition preceding liver cirrhosis, is well known to develop in hepatic stellate cells (HSC), which can be activated in the case of toxic insult such as CCl_4 exposure, alcohol abuse, viral hepatitis, or a biliary or autoimmune disease [47, 48]. The proliferation of activated HSC results in the secretion of a large amount of extracellular matrix (ECM) proteins, including collagens and other matrix components such as proteoglycans, fibronectins, and hyaluronic acid [49]. Accumulation of ECM due to imbalances in the synthesis and degradation of these proteins leads to a

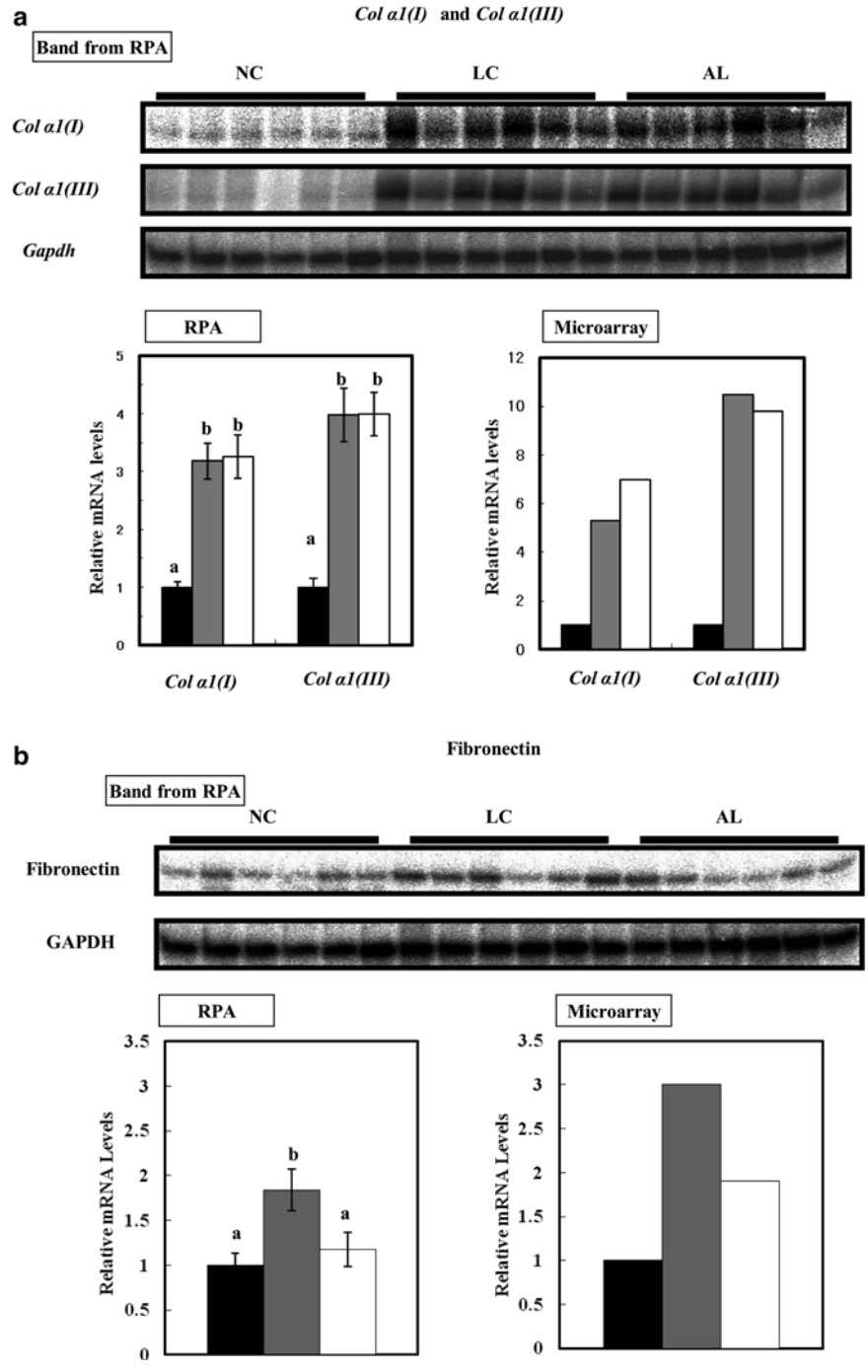

Fig. 11.2 Validation of the expression of differentially changed genes of DNA microarray analysis by RNase protection assay (RPA) [44]. Results are expressed as means ± SE of the fold changes normalized to GAPDH mRNA expression. *Different letters* indicate significant differences ($P<0.05$) among NC, LC, and AL. *NC* normal control (*filled square*), *LC* liver cirrhosis-casein (*filled gray square*), *AL* amino acid plus liver cirrhosis-casein (*open square*). (**a**) *Col α1(I)* type I collagen alpha 1, *Col α1(III)* type III collagen alpha 1. (**b**) *Fibronectin*. (**c**) *Hmgcr* HMG-CoA reductase, *Sqs* squalene synthetase, *Sqle* squalene epoxidase. (**d**) *Arg* arginase, *Otc* ornithine transcarbamylase. (**e**) *Cps* carbamoyl phosphate synthetase, *Glud* glutamate dehydrogenase. (**f**) *Slc2a4* solute carrier family 2 (facilitated glucose transporter), member 4. We have hereby received permission to reproduce this figure from John Wiley and Sons (License No: 3125250988663)

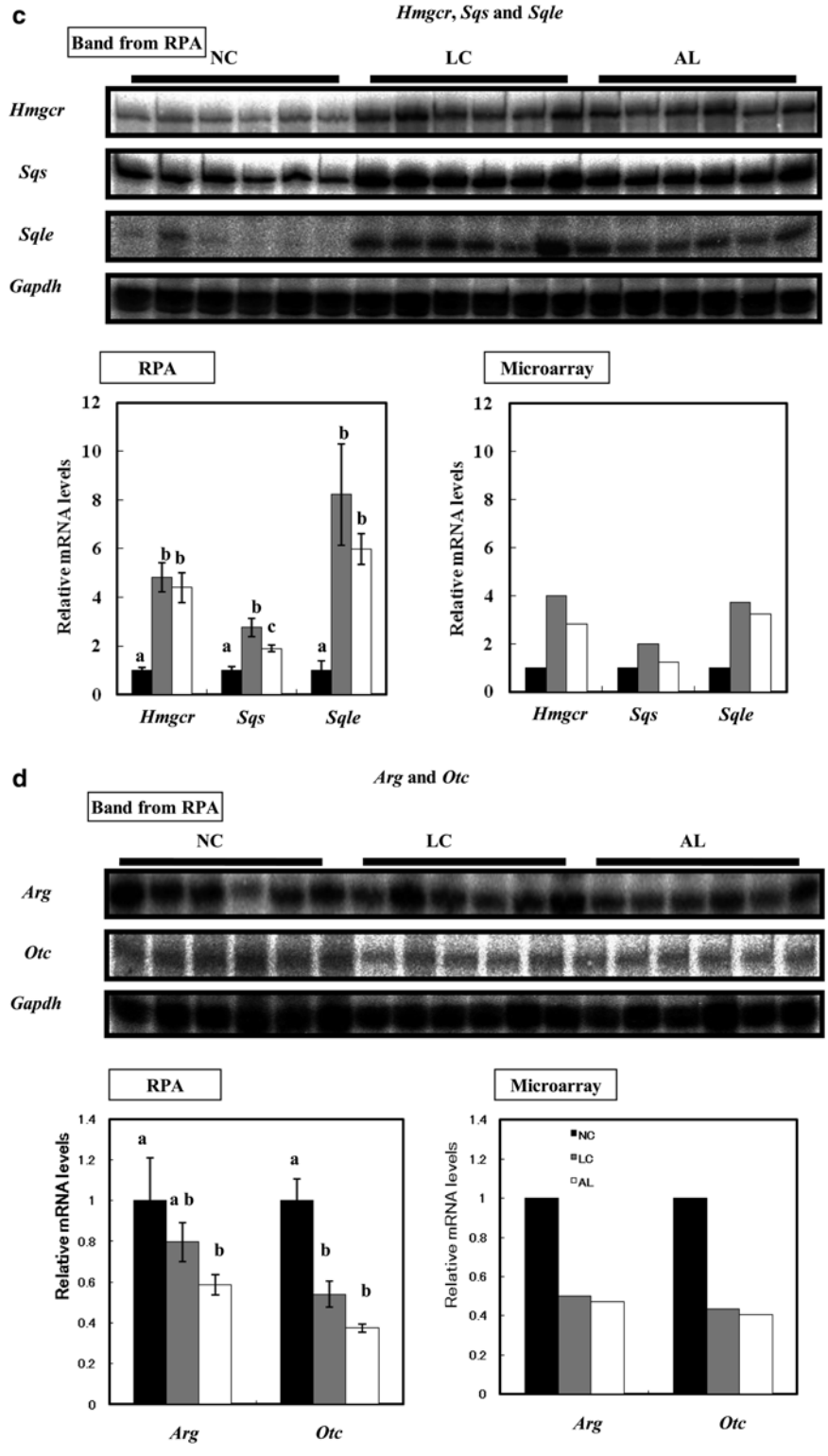

Fig. 11.2 (continued)

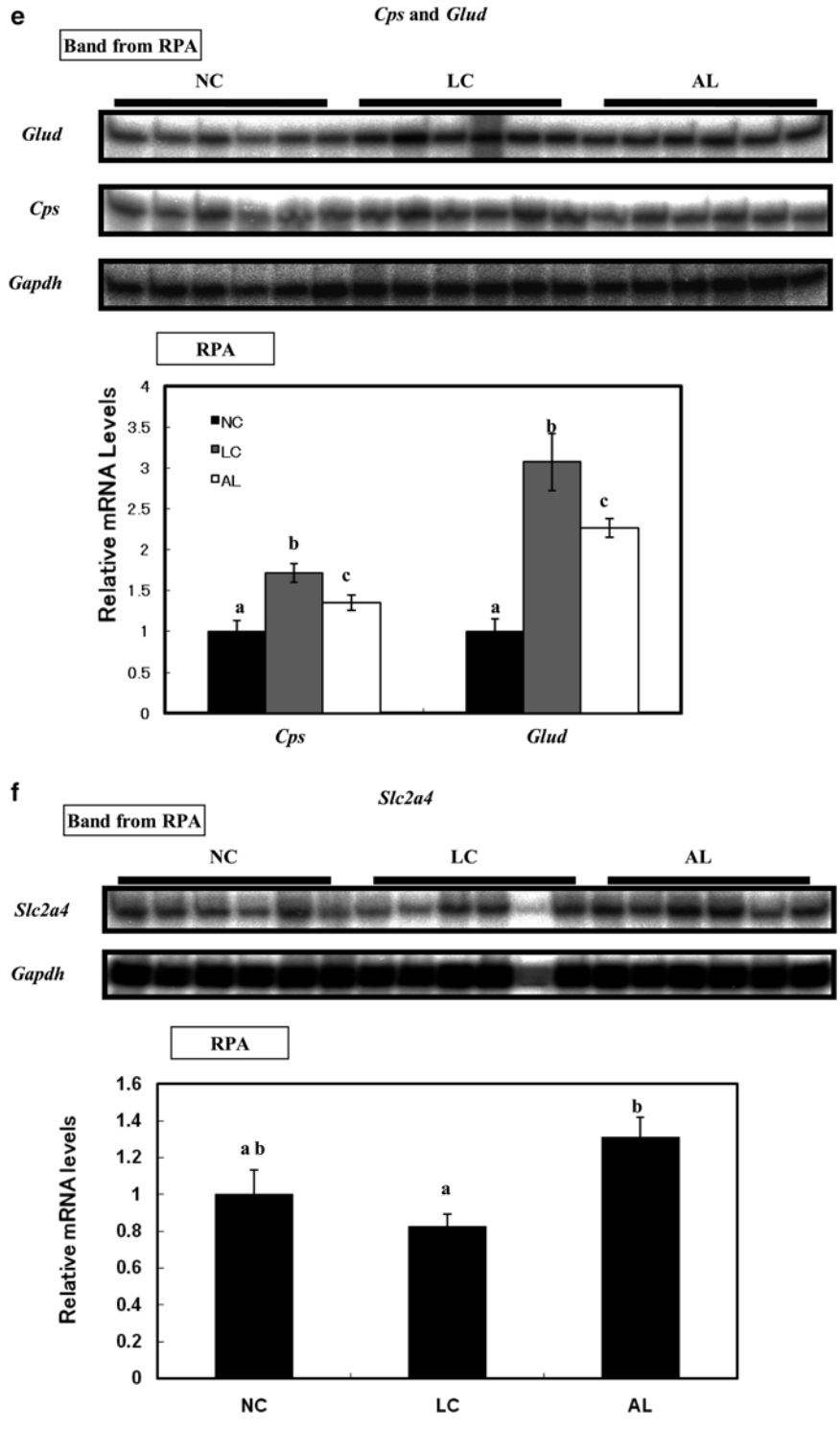

Fig. 11.2 (continued)

distortion of the hepatic architecture, resulting in liver fibrosis proceeding to cirrhosis [50]. It can be postulated that the process of hepatic fibrogenesis is regulated by the differential expression of a number of critical genes. In the present study, genes involved in some key pathways of fibrogenesis were screened by transcriptomic analysis. Transforming growth factor β1 (TGF-β1) is known to be a crucial cytokine/growth factor mediator responsible for HSC activation. The increased expressions of *Tgf-β1* (4.0-fold) and *transforming growth factor β3* (10.6-fold) were observed in LC, which could possibly activate HSC to increase the production and accumulation of ECM, and thereby up-regulate the expression of *type I collagen alpha 1* (*Col α1(I)*, 32.0-fold), *type III collagen alpha-1* (*Col α1(III)*, 8.0-fold), *type V collagen alpha 1* (*Col α1(V)*, 2.3-fold), *fibronectin* (3.0-fold), and *laminin gamma 1* (2.0-fold). Fibronectins are secreted by fibroblasts as an ECM glycoprotein, which is induced by TGF-β1. The expression of *fibronectin* was decreased (1.9-fold) in AL. This indicated that BCAA can prevent CCl_4-induced liver cirrhosis by the prevention of progressive fibrosis as well as the attenuation of the activity of profibrotic cytokine TGF-β1.

In LC, there was an increase in the expression of *3-hydroxy-3-methylglutaryl coenzyme A reductase* (*Hmgcr*, 5.3-fold), *hepatic squalene synthetase* (*Sqs*, 2.0-fold), and *squalene epoxidase* (*Sqle*, 3.7-fold) which are enzymes involved in the biosynthesis of cholesterol. HMGCR is the primary rate-limiting enzyme, SQS is downstream from HMGCR and modulates the first committed step, and SQLE acts distally to SQS. These up-regulations in LC were reversed in AL—*Hmgcr* (1.6-fold), *Sqs* (1.3-fold), and *Sqle* (1.6-fold), which suggests that BCAA can reduce cholesterol synthesis, which would be particularly effective in cirrhosis patients who are overweight or obese.

Due to excessive levels of ammonia entering the brain thereby causing injury to the mitochondria electron transport chain, ammonia is strongly implicated in the pathogenesis of hepatic encephalopathy [51] as mentioned earlier. Ammonia, derived mostly from bacterial degradation in the gut, is carried to the liver through the portal vein and then is processed in the urea cycle and glutamine synthesis pathway. In patients with mild cirrhosis, the enzymatic activity that processes harmful ammonia is decreased by more than half, resulting in hyperammonemia due to this impaired hepatic capacity [52]. In our study, the gene expression of *transcarbamylase* (*Otc*), which is a regulator of ammonia incorporation into the urea cycle, and of *arginase* (*Arg*), which is the final enzyme of the cycle, was observed to be down-regulated by half in LC and AL in both DNA microarray and RPA. On the other hand, the gene expression of two vital enzymes involved in ammonia disposal was reversed in AL compared with LC. One of them is *carbamoyl phosphate synthetase* (CPS), which mediates the incorporation of first waste nitrogen into the cycle, and the other is *glutamate dehydrogenase* (GLUD), which converts glutamate to α-ketoglutarate. From these alterations we hypothesize that there is a reduced demand for ammonia disposal by these enzymes in BCAA-supplemented rats.

Similarly to the liver, the skeletal muscle is also an vital organ for the metabolism of amino acids, ammonia, and glucose [10]. BCAA is metabolized mainly in muscle by a pathway starting from branched chain aminotransferase. The pathogenesis of decreased plasma BCAA concentrations in liver cirrhosis has yet to be elucidated, however, several metabolic abnormalities have been proposed to be the cause, including hyperinsulinemia, hyperglucagonemia, catecholamines, hyperammonemia, and starvation. The decline in BCAA concentrations is possibly linked to the process by which excess ammonia is disposed. Skeletal muscle detoxifies blood ammonia by using it in the synthesis of glutamine from glutamate. The change in ammonia metabolism in liver cirrhosis is responsible for the depletion of BCAA as well as the elevation of glutamine in blood and muscle [53]. The key process of ammonia incorporation is regulated through the actions of *glutamine synthetase* (*Gs*). From our study, BCAA supplementation ameliorated the increase in glutamine concentration in plasma in AL (0.97 ± 0.050 arbitrary unit) from that in LC (1.18 ± 0.39) to that of NC (1.00 ± 0.057). There was also an increase in the expression of *Gs* (2.6-fold). This indicates the decrease in the amount of ammonia to be processed in AL, leading in turn to a decline in BCAA consumption. This suggests that BCAA plays a principal role in protein metabolism regulation in order to preserve nitrogen balance by decreasing

muscle protein catabolism and amino acid efflux. It also suggests that the skeletal muscle is dependent on the exogenous supply of BCAA to satisfy its metabolic requirements in liver cirrhosis.

In lieu of the loss of glycogen storage and the poor gluconeogenic capacity due to the atrophy of liver parenchymal cells in cirrhosis, the efficiency of carbohydrate as a physiological energy substrate is drastically decreased, and thus resulting in an acceleration in energy utilization from lipids and proteins [54, 55]. The expression of *fatty acid translocase* (*FAT*/*Cd36*), involved in the transport of long-chain fatty acids for oxidation and subsequent energy production, as well as lipoprotein lipase, which functions dually as triglyceride hydrolase and as the ligand factor for receptor-mediated lipoprotein uptake, was up-regulated (8.6-fold) in response to liver cirrhosis. These effects were reversed (1.5-fold) in AL, indicating that under BCAA supplementation, low utilization of fatty acid in cirrhotic skeletal muscle occurs. On the other hand, some studies have also demonstrated that L-leucine improves blood glucose concentration by the induction of *solute carrier family 2 (facilitated glucose transporter), member 4* (SLC2A4) translocation to the cell membrane and facilitating non-insulin-mediated glucose uptake in skeletal muscle [56]. In the current study, skeletal muscle expression of *Slc2a4* was essentially higher (1.6-fold) in AL, which may serve as another mechanism underlying the promotion of glucose uptake with BCAA supplementation in skeletal muscle.

Pyruvate dehydrogenase (PDH) is an enzyme linking the glycolysis metabolic pathway to the citric acid cycle. One of the upstream regulators of PDH is pyruvate dehydrogenase kinase isoenzyme 4 (PDK4), which is responsible for the phosphorylation and inactivation of PDH. In addition, PDK4 has been reported to be regulated by peroxisome proliferator-activated receptor alpha (PPARα), which is also an activator of branched chain α-keto acid dehydrogenase kinase (BCKDK), a rate-limiting enzyme in an irreversible step in the pathways for BCAA catabolism [57]. The increase in *Pdk4* (2.6-fold) expression in LC was attenuated (2.5-fold) in response to BCAA supplementation, implying that BCAA plays a crucial role for glucose availability in skeletal muscle and additionally for the control of amino acid hypermetabolism.

According to these findings, the improvement of protein-energy malnutrition of BCAA on cirrhosis in skeletal muscle can be summarized as shown in Fig. 11.3. In the case of cirrhosis, due to impaired glucose synthesis there is a decline in glucose availability in skeletal muscle. Consequently, the uptake of fatty acids through *FAT*/*Cd36* is activated for use as an energy source. The increase in the expression of *Pdk4* may cause the suppression of glucose metabolism and the enhancement of amino acid metabolism, which is likely to lead to the use of amino acids as energy sources. Likewise, the up-regulation of *Gs* may implicate an increase in the consumption of BCAA for ammonia processing. In this way, protein catabolism in skeletal muscle is enhanced, leading to a greater demand for BCAA, thus diminishing BCAA concentrations in blood. Conversely, with BCAA supplementation, glucose uptake was promoted by increased expression of *Slc2a4*. At the same time, the down-regulation of *FAT*/*Cd36*, *Gs*, and *Pdk4* results in the lower uptake of fatty acids, lower ammonia incorporation, and higher uptake of glucose, and this serves as an energy source without using endogenous BCAA. Therefore, the catabolism of BCAA and skeletal muscle protein was suppressed, maintaining BCAA concentrations in blood.

Conclusions

In this chapter, we have highlighted the effectiveness of BCAA and its potential as a nutraceutical in the prevention of liver cirrhosis and possibly HCC. Our study clarified for the first time, using global gene expression analysis, the molecular mechanisms by which hepatic fibrosis is reversed and how BCAA supplementation improves the nutritional status in liver cirrhosis. This analysis can provide new insights into how BCAA are involved in the regulatory gene networks in cirrhosis.

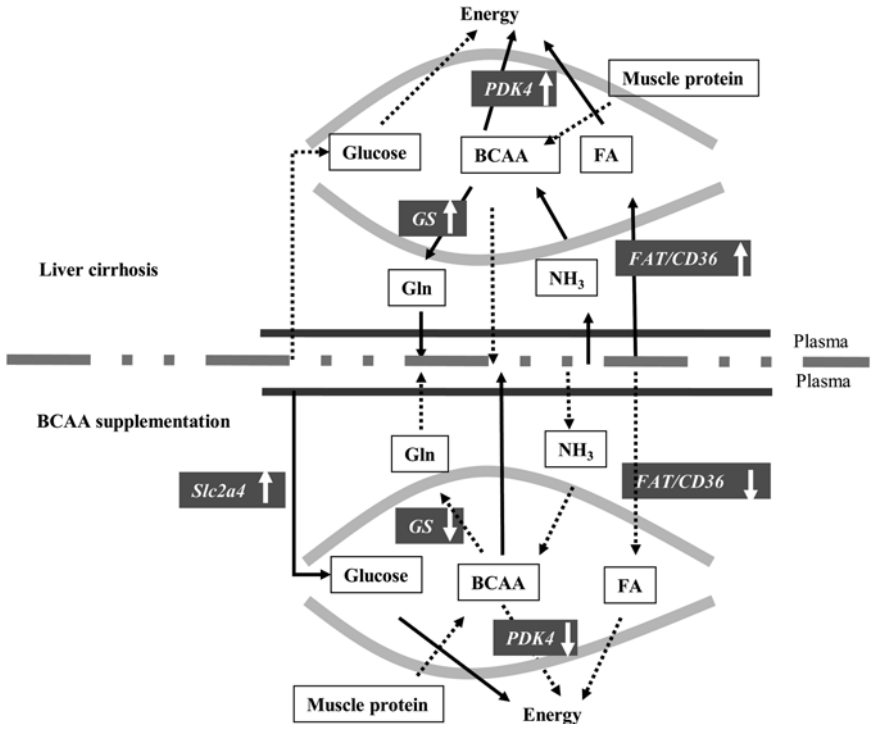

Fig. 11.3 Schematic representation of the metabolic changes by the occurrence of liver cirrhosis and BCAA supplementation [44]. *BCAA* branched chain amino acids, *FA* fatty acids, *Gln* glutamine, *Pdk4* pyruvate dehydrogenase kinase isoenzyme 4, *Gs* glutamine synthetase, *Fat* fatty acid translocase, *Slc2a4* solute carrier family 2 (facilitated glucose transporter), member 4. We have hereby received permission to reproduce this figure from John Wiley and Sons (License No: 3125250988663)

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Chapter 12
Branched Chain Amino Acid Supplementation and Plasma Albumin

Masashi Kuwahata and Yasuhiro Kido

Key Points

- Plasma albumin level is one of the most common parameters used in evaluating nutritional status.
- Protein content and quality in a meal influence the regulation of albumin synthesis.
- Amino acids regulate albumin gene expression through intracellular concentration of pyridoxal 5′-phosphate (PLP).
- Food intake activates mammalian target of rapamycin (mTOR) signaling cascade and stimulates albumin synthesis.
- Leucine promotes albumin synthesis via the inhibition of polypyrimidine tract-binding protein (PTB), which represses translation of albumin mRNA.
- Effect of branched chain amino acids (BCAA) on the function of neonatal Fc receptor (FcRn), which is involved in the regulation of the half-life of albumin, requires clarification.

Keywords Plasma albumin • Branched chain amino acids • Hepatocyte nuclear factor 1 • Pyridoxal 5′-phosphate • Mammalian target of rapamycin • Polypyrimidine tract-binding protein • Albumin metabolism

Abbreviations

BCAA	Branched chain amino acids
COP	Colloid osmotic pressure
HMA	Human mercaptoalbumin
HNA	Human nonmercaptoalbumin
HNF1	Hepatocyte nuclear factor 1
C/EBP	CCAAT/enhancer-binding protein
PLP	Pyridoxal 5′-phosphate
PMP	Pyridoxamine 5′-phosphate
AAA	Aromatic amino acids

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mTOR	Mammalian target of rapamycin
S6K1	Ribosomal protein S6 kinase-1
eIF	Eukaryotic initiation factor
4E-BP1	eIF 4E-binding protein-1
TOP	Terminal oligopyrimidine tract
PTB	Polypyrimidine tract-binding protein
FcRn	Neonatal Fc receptor

Introduction

Albumin is the major protein produced in the liver and normally accounts for over 50 % of total plasma protein content. Its plasma level is widely measured in routine clinical examinations in order to evaluate nutritional status, liver function, or pathophysiological condition. Furthermore, it is known that the plasma albumin level is inversely related to morbidity and mortality [1–3]. There are many factors regulating plasma albumin levels under various conditions. One of the most important factors regulating levels involves ingestion of a meal, especially the supply of protein. Chronic protein deficiency under conditions of adequate nonprotein caloric intake leads to marked hypoalbuminemia. This may result from the net loss of albumin from both intravascular and extravascular pools, causing kwashiorkor.

Branched chain amino acids (BCAA) are a group of essential amino acids comprising valine, leucine, and isoleucine. In Japan, pharmacological supplementation of BCAA is widely used to improve hypoalbuminemia in patients with decompensated liver cirrhosis [1, 4]. Presently, it is known that BCAA have a role not only as protein constituents, but also as signal molecules for some physiological functions.

In the literature on albumin metabolism, a great deal of information exists concerning the regulation of albumin synthesis. This chapter focuses on the effects of the supply of amino acids, including BCAA, on the regulation of albumin synthesis.

Characterization of Plasma Albumin

Albumin is the most abundant protein in plasma, being present at concentrations of approximately 4.0 g/dL. The plasma albumin level is the net result of three dynamic processes: synthesis, distribution, and degradation. Thus, at any given moment, these processes must be in balance in order to maintain a stable plasma albumin level. Albumin has many biological functions [5, 6], such as maintenance of colloid osmotic pressure (COP), transport of endogenous and exogenous compounds, and antioxidation. The level of plasma albumin is one of the most common parameters used in evaluating nutritional status.

Human albumin is a single-chain polypeptide composed of 585 amino acid residues [7], and its heterogeneity is a result of posttranslational modifications such as oxidation and glycation. Albumin contains 35 cysteine residues. Thus, albumin forms 17 disulfide bonds and has one free thiol group in the cysteine residue at the 34th position from the N-terminal (Fig. 12.1). Plasma albumin is divided into two types depending on its redox state: reduced form (human mercaptoalbumin: HMA) and oxidized form (human nonmercaptoalbumin: HNA) [8]. HMA has a free thiol form in the cysteine residue. On the other hand, the cysteine residue in HNA forms a disulfide with small thiol compounds or is oxidized with a sulfinic or sulfonic acid. The percentage of HMA within total albumin is about 60 % in plasma from healthy subjects and decreases in pathophysiological conditions [8, 9].

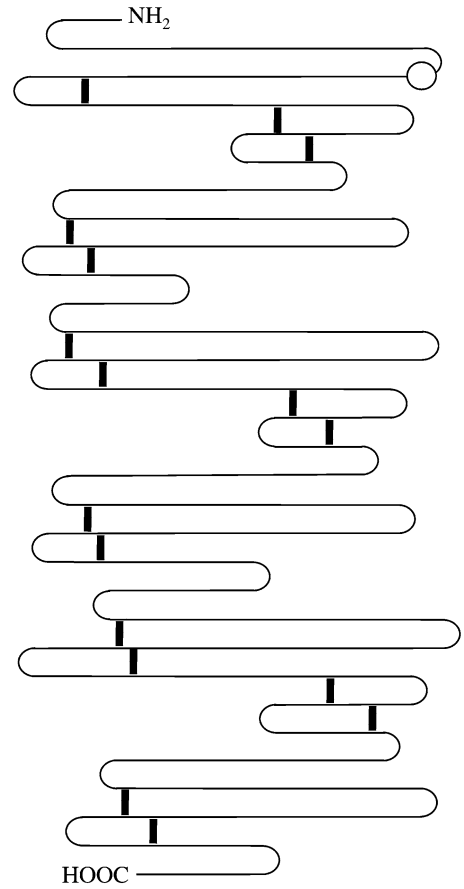

Fig. 12.1 Disulfide bonding pattern of human albumin. The *bold lines* indicate the disulfide bridges. The cysteine residue at the 34th position is shown by the *open circle*

An in vitro study [6] showed that the redox status of albumin affects its functional properties, including protease susceptibility, ligand-binding affinity, and antioxidant activity. Furthermore, a clinical study [9] indicated that treatment with a low oxidized albumin preparation was an independent predictor for reduction of body weight in cirrhotic patients with edema. This therefore suggests that oxidized albumin is associated with water retention in patients with chronic liver diseases.

Albumin has an unusually long half-life. Studies of radioiodinated albumin catabolism in healthy young males indicated that the apparent biological half-life of the radiolabeled albumin, as determined from the plasma radioactivity disappearance curve, ranged from 12.7 to 18.2 days [10]. Binding of glucose to albumin typically occurs in vivo and is known to involve nonenzymatic glycation. Glycation-induced modifications have an important impact on albumin functional properties, mainly related to conformational alterations [11]. The glycation of albumin affects its ligand-binding affinity and antioxidant activity. On the other hand, the glycated albumin level may reflect the status of blood glucose more rapidly than hemoglobin. Recent studies [12] suggest that the glycated albumin can serve as one of the clinical indices to predict blood glucose variability.

Albumin is synthesized in the liver. Of the many factors influencing albumin synthesis, the most important seems to be nutritional status. A previous study [13] showed that albumin fractional synthesis rate, which represents the percentage of the intravascular albumin pool synthesized per day, increased during the enteral feeding of amino acids and glucose in healthy subjects. However, the synthesis of another hepatic protein, fibrinogen, was unaffected by such enteral feeding [13].

Protein content and quality in a meal influence the regulation of albumin synthesis. Specifically, contents of essential amino acids, including BCAA, may be an important factor in the regulation of albumin synthesis rate [14, 15].

When the plasma albumin level is less than 3.5 g/dL, it is called hypoalbuminemia. Hypoalbuminemia has been shown to be associated with increased mortality and morbidity rates in patients with chronic disease [1], surgical patients [2], and elderly persons [3]. Albumin exists in both intravascular and extravascular compartments. Extravascular albumin comprises 55–64 % of the body's total albumin mass in healthy adults [10]. In malnutrition where the plasma albumin level is likely to fall, the extravascular albumin can be mobilized to compensate for the intravascular losses. Furthermore, the long half-life of albumin allows changes in the plasma concentration only after long periods of malnutrition. The level of plasma albumin is a poor indicator for short-term nutritional assessment. Hypoalbuminemia indicates that a malnourished state has been present for a long period of time.

Transcriptional Regulation of Albumin Gene Expression by COP and Amino Acid Nutrition Status

Albumin synthesis is regulated predominantly at the transcriptional level of albumin gene expression in the liver. Cis-acting promoter elements in albumin gene are highly conserved among species. The albumin promoter is composed of a TATA motif and six upstream binding sites for nuclear proteins [16]. Among these six binding sites, hepatocyte nuclear factor 1 (HNF1) and CCAAT/enhancer-binding protein (C/EBP) binding sites strongly activate the transcription of the albumin gene in liver-specific manner. Furthermore, it has been reported that luciferase reporter constructs driven by the human albumin gene promoter are synergistically activated by HNF1 and C/EBPα in cultured cells [17]. Both HNF1 and C/EBPα are particularly important factors for the transcription of the albumin gene.

Hyperalbuminemia is rarely observed, except in cases of acute dehydration. Serum COP is believed to control the hepatic synthesis and/or secretion of plasma proteins, including albumin. A previous study [5] indicated that intravenous infusion of albumin in rats decreased transcriptional activity of the albumin gene in the liver. The molecular basis for the regulation of albumin gene transcription by COP was investigated in cultured cell experiments [16]. When hepatoma cells were exposed to 5 % (w/v) albumin, binding of nuclear extracts from the cells to HNF1 binding site was decreased and transcriptional activity from the albumin promoter containing an HNF1 binding site was reduced. Furthermore, the HNF1 mRNA level was decreased in cells exposed to 5 % albumin. These results indicate that the decreased albumin gene transcriptional activity, resulting from increased COP, was due to decreased expression of HNF1 (Fig. 12.2). Feedback regulation of albumin gene transcription by serum colloids, including albumin, may serve as a specific homeostatic mechanism to maintain the steady-state level of plasma albumin.

Notwithstanding homeostatic regulation, one of the most important factors influencing plasma albumin levels is the supply of amino acids [13–15]. In our previous study [18], rats were nourished by infusion of total parenteral nutrition solutions containing 0 % or 3.3 % amino acids for a week. The level of albumin mRNA in the liver of amino acid-infused rats was found to be about fivefold higher than that in the liver of amino acid-depleted rats. The binding activity of nuclear extracts to HNF1 binding site was also determined. The binding activity of extracts prepared from the liver of amino acid-infused rats was greater than that of amino acid-depleted rats. We previously reported [19] that vitamin B_6 modulates albumin gene expression through a novel mechanism involving inactivation of tissue-specific transcription factors by direct interaction with pyridoxal 5′-phosphate (PLP). The PLP concentration in the liver of the amino acid-infused rats was significantly decreased, to almost a half that of amino acid-depleted rats, while pyridoxamine 5′-phosphate (PMP) concentration was increased

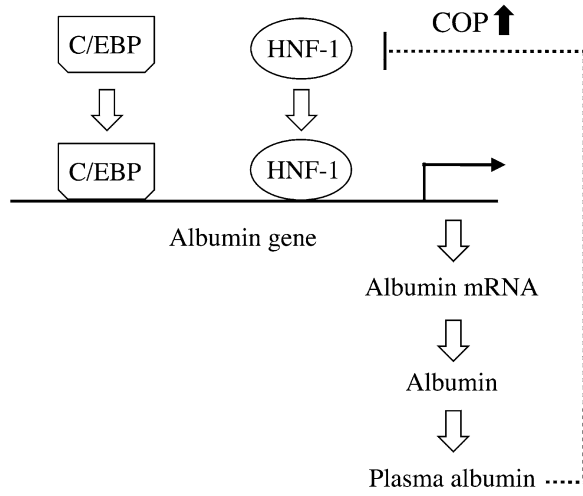

Fig. 12.2 Feedback regulation of albumin gene transcription. Increased COP caused by serum colloids, including albumin, decreases the level of transcription factor HNF1 in liver. The decreased level of HNF1 reduces the transcriptional activity of albumin gene

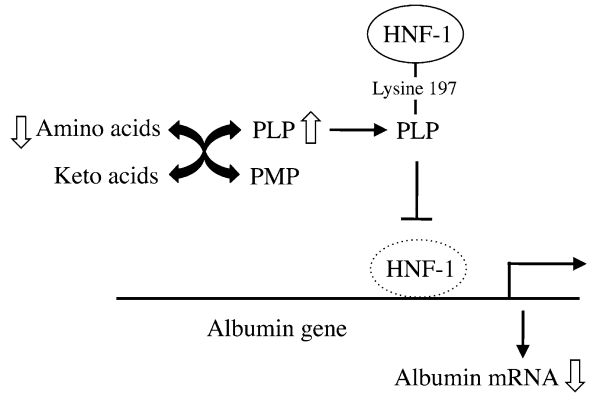

Fig. 12.3 Proposed regulatory mechanism of albumin gene expression by amino acids. Deficiency of amino acid supply may cause increase in liver PLP concentration. DNA-binding activity of HNF1 is inhibited by binding of PLP to the lysine residue at the 197th position in HNF1. As a result, the transcriptional activity of the albumin gene is decreased

proportionally [18]. Amino acids, transported into hepatic cells, will undergo transamination reactions catalyzed by aminotransferases. Since PLP is a coenzyme of all aminotransferases, and enzyme-bound PLP is converted to PMP during transamination, continuous influx of amino acids may decrease PLP and elevate PMP concentrations in the steady state. We have proposed that PLP interacts with tissue-specific transcription factors by forming a Schiff base between its aldehyde group and primary amino groups, most commonly the epsilon-amino group of lysine residues, resulting in a decreased DNA-binding activity of the transcription factors. In order to elucidate the molecular mechanism whereby DNA-binding activity of HNF1 is inhibited by PLP, we examined the site of attachment of PLP in vitro. Determination of the amino acid sequence of PLP-containing peptide revealed that PLP was bound to lysine 197 of the HNF1 molecule [20]. Inasmuch as lysine 197 lies in the homeodomain of HNF-1, PLP binding to this lysine residue would render HNF1 molecule less accessible to the HNF1 binding site to albumin gene (Fig. 12.3). These observations suggest that enhancement of albumin gene expression in the liver by an increased supply of amino acids can be explained by elevated binding of HNF1 to the DNA-binding site, which in turn is caused by a decrease in the intracellular level of PLP by the increased amino acid supply. It is not clear whether this regulation is limited to certain amino acid types, such as BCAA.

It has been reported that BCAA influence albumin gene expression [21]. In the study, the effect of the molar ratio of BCAA to aromatic amino acids (AAA) on albumin gene expression was examined using a hepatoblastoma-derived cell line, HepG2, cultured in serum-free medium. The results indicated that albumin gene expression was stimulated according to the increase in BCAA/AAA ratios from 0.1 to 10, but at 30 its expression was inhibited. The possibility exists that the molar ratio of BCAA to AAA is associated with albumin gene expression, although the addition of serum to culture medium may mask the function of amino acids.

Translational Regulation of Albumin mRNA by Food Intake and BCAA

Dietary intake is the most important factor for nutrition management. When compared with the fasted state, albumin synthesis significantly increases in response to feeding in healthy subjects [13, 15]. It has been reported that 97–98 % of albumin mRNA sequences in normal rat liver are present in membrane-bound polysomes [22]. When rats are fasted for 24–30 h, albumin mRNA sequences are released from membrane-bound polysomes to enter the free cytosol fraction [22, 23], and refeeding a mixture of amino acids restores albumin mRNA to membrane-bound polysomes [23]. Translational regulation thus plays an important role in albumin synthesis in response to food intake.

Food intake-induced stimulation of protein synthesis is in large part due to modulation of signaling through mammalian target of rapamycin (mTOR) complex [24]. The mTOR complex is activated by hormones, such as insulin, and BCAA, especially leucine, in the liver. Moreover, mTOR is a nutrient-sensing kinase. Two substrates of mTOR include the ribosomal protein S6 kinase-1 (S6K1) and the translational repressor eukaryotic initiation factor (eIF) 4E-binding protein-1 (4E-BP1) [24]. Phosphorylation of 4E-BP1 results in its dissociation from eIF4E, thereby permitting eIF4E to interact with eIF4G to form the active eIF4F complex, which mediates the binding of mRNA to the 40S ribosomal subunit. Phosphorylation and the resulting activation of S6K1 enhance the phosphorylation of ribosomal protein S6, which is implicated in mediating translational control of mRNAs containing a 5′-terminal oligopyrimidine tract (TOP) sequence. The mRNAs encoding most ribosomal proteins, as well as several elongation factors, contain a 5′-TOP sequence. Thus, the activation of mTOR signaling following food intake stimulates translation initiation (Fig. 12.4). A previous study [25] showed that 78 different mRNAs had increased polysome association in rat liver after feeding. However, 36 of the 78 mRNAs lack a 5′-TOP sequence. Albumin mRNA incorporation into polysome was also observed to increase in the experiment. Because the albumin mRNA lacks a 5′-TOP sequence, the results suggest that its translation may be regulated through a unique mechanism.

Our previous study [26] demonstrated that polypyrimidine tract-binding protein (PTB), which is a sequence-specific RNA-binding protein, in extracts prepared from rat liver interacts with the coding region of albumin mRNA. Furthermore, immunodepletion of PTB from rabbit reticulocyte lysate caused an increase in albumin mRNA translation in the lysate. The PTB binding site in human albumin mRNA is located within the 20-base coding region from nucleotides 54 to 73 [27]. The exact sequence requirements for binding of PTB are not identified, but sequence analysis showed that the sequence of the region from nucleotides 62 to 73 of albumin mRNA is similar to that of a known PTB consensus sequence. We speculate that the albumin mRNA-PTB complex is localized in the light polysomal fraction containing the preinitiation complex of translation machinery, in which PTB inhibits formation of the mature initiation complex. We also speculate that the cytoplasmic fraction contains the albumin mRNA-PTB complex as well. The distribution of albumin mRNA may be affected by binding of PTB. The effects of food intake on regulation of albumin synthesis through binding of PTB to albumin mRNA were investigated [28]. Levels of albumin mRNA-PTB complex were increased in liver extracts from fasted rats. No significant differences in PTB levels in liver homogenates were found between fed and fasted rats. However, PTB levels in the cytoplasmic

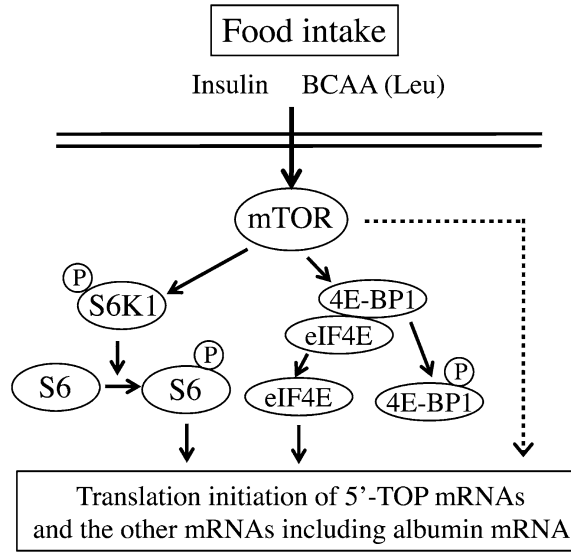

Fig. 12.4 Model of mTOR-dependent regulation of translation initiation in response to food intake. Food intake increases mTOR activity and further facilitates the phosphorylation of S6K1 and 4E-BP1. *Dashed lines* indicate an interaction with an unknown molecule or complex that regulates translation initiation

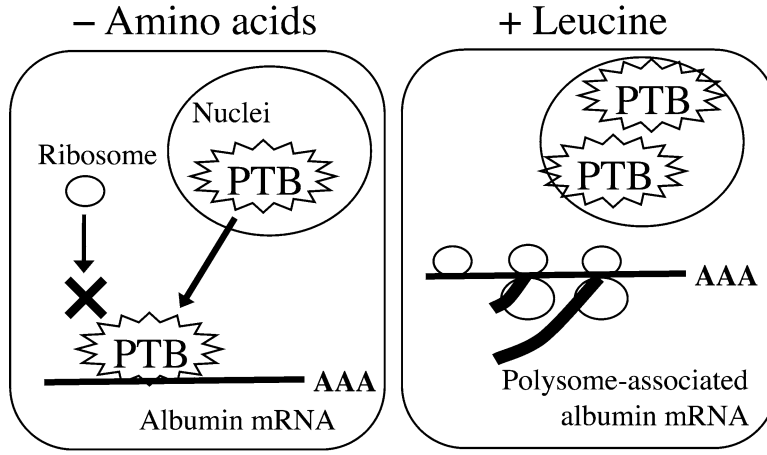

Fig. 12.5 Proposed regulatory mechanisms of albumin mRNA translation via the nucleocytoplasmic transport of PTB by leucine. Binding of PTB to albumin mRNA attenuates its translation. Addition of leucine decreases the levels of albumin mRNA-PTB complex and stimulates the nuclear import of PTB. As a result, translation efficiency of albumin mRNA is increased

fraction were higher in fasted rats than in fed rats. In refed rats, the PTB level in the cytoplasmic fraction returned to a level comparable to that in fed rats, but was inhibited by treatment with rapamycin, a mTOR inhibitor. Localization of PTB is regulated by food intake through mTOR signaling, and alterations in the level of albumin mRNA-PTB complex play a role in mediating the effects of food intake on albumin synthesis in the liver. In a study using human hepatoma HepG2 cells [27], nuclear export of PTB was observed in cells cultured in amino acids-free medium, and the level of albumin mRNA-PTB complex was greater than in cells cultured in a medium containing all 20 amino acids. The addition of leucine, but not valine or isoleucine, to amino acids-free medium increased albumin secretion and stimulated the nuclear import of PTB (Fig. 12.5). Thus, leucine regulates the localization of PTB

and promotes albumin synthesis by inhibiting the formation of albumin mRNA-PTB complex in hepatoma cells. Leucine-induced changes in the abundance of albumin mRNA-PTB complex in liver may regulate the translation efficiency of albumin mRNA.

Regulation of Albumin Turnover

As compared with albumin synthesis, there is little research on albumin distribution and degradation. However, it is now understood that the albumin degradation process is controlled by a receptor-mediated mechanism. The major histocompatibility complex-related neonatal Fc receptor (FcRn) for IgG binds not only IgG but also albumin in a pH-dependent manner [29]. FcRn is widely expressed in several organs and tissues. Circulating IgG and albumin are pinocytosed nonspecifically and are transported to acidic endosomes where they bind FcRn. The binding of albumin and IgG to FcRn is observed at acidic pH but not at neutral pH. FcRn-ligand complexes then return to the cell surface membrane, where exposure to the neutral pH of the bloodstream triggers the release of the ligands [30]. The albumin and IgG that do not bind to FcRn progress to lysosomes for proteolytic degradation (Fig. 12.6). The half-life of albumin is shortened in FcRn-deficient mice, and the plasma albumin concentration of FcRn-deficient mice is less than half that of wild type mice [29]. These results indicate that FcRn protects albumin from degradation. Furthermore, it seems that the activity of albumin synthesis is upregulated in liver of FcRn-deficient mice, probably in response to a lower osmotic pressure caused by a lower plasma albumin concentration in the absence of FcRn. Thus, FcRn recycling is fundamentally important for albumin homeostasis. Whether FcRn plays a role in the distribution of albumin to the vascular and the extravascular spaces has not yet been determined.

In cirrhotic patients, both albumin synthesis and degradation rates of albumin are decreased, and the biological half-life of albumin is prolonged [1]. As a result, the percentage of HNA within total albumin increases with the progression of liver cirrhosis [9]. Pharmacological supplementation with BCAA could improve the prolonged half-life of albumin in cirrhotic patients [1]. Furthermore, a

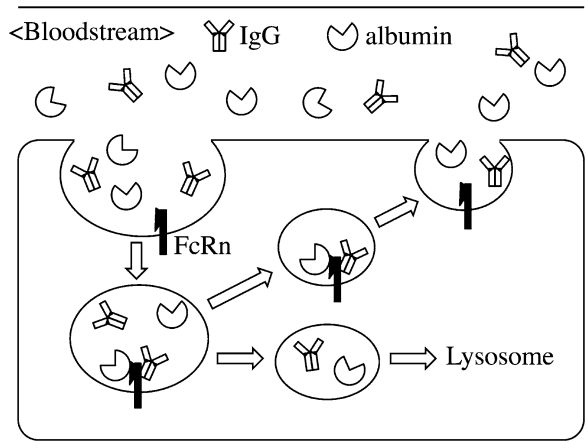

Fig. 12.6 Model of FcRn-mediated albumin recycling. Albumin and IgG in the bloodstream are continuously taken up by pinocytosis. The low pH in the vesicles allows binding of albumin and IgG to FcRn. FcRn-ligand complexes are exported to the cell surface, where exposure to the higher physiological pH of the bloodstream triggers release of albumin and IgG into the circulation. When the concentrations of albumin and IgG are high, FcRn is saturated and unbound ligands undergo lysosomal degradation

previous study [4] showed that 8-week BCAA supplementation did not change the plasma total albumin level significantly in cirrhotic patients. However, the percentage of HMA within total albumin significantly increased in the study. An animal study [31] showed that continuous supplementation with BCAA in rats with chronic liver disease improved the oxidized/reduced ratio of plasma albumin and activated mTOR signaling in the liver. Thus, supplementation with BCAA may improve abnormal albumin metabolism through the activation of albumin synthesis in rats with chronic liver disease. In chronic liver disease, it is supposed that the activity of FcRn-mediated recycling of albumin may increase following decreases in albumin synthesis activity. However, whether BCAA supplementation modulates the function and expression of FcRn remains to be clarified.

Conclusions

Plasma albumin has many physiological and biochemical properties. The level of plasma albumin is used as a major index in health and disease. There are many factors that influence albumin metabolism, including synthesis, distribution, and degradation. It seems that BCAA are among the most important nutrients for stimulating albumin synthesis. At present, clarification of the regulatory mechanisms of the half-life of albumin is advanced. Thus, future studies should evaluate the effects of BCAA on the homeostatic regulation of plasma albumin.

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Chapter 13
Late Evening Snack, Branched Chain Amino Acids, and Cirrhosis

Chizu Koreeda

Key Points

- Patients with liver cirrhosis (LC) have decreased gluconeogenic capacity, which leads to decreased availability of sugar and increased availability of fat especially during the fasting period from after the evening meal until morning.
- Furthermore, due to gluconeogenesis requirements for amino acids being met by the breakdown of muscle protein, skeletal muscle is decreased and the nitrogen balance becomes negative.
- Thus, patients with LC present with protein energy malnutrition (PEM).
- Branched chain amino acid (BCAA) therapy for LC is effective for ameliorating this condition by correcting PEM and improving both quality of life (QOL) and prognosis.
- A late evening snack (LES) using Aminoleban EN for LC has beneficial effects on hepatic parenchymal cells in addition to nutritional effects.
- A LES using BCAA is both safe and useful, as nutritional and dietary therapy, comprising one part of the four-meal-a-day dietary intervention.

Keywords Protein energy malnutrition (PEM) • Nonalcoholic fatty liver disease (NAFLD) • Late evening snack (LES) • Liver diseases • Liver cirrhosis (LC) • Branched chain amino acid (BCAA) • Quality of life (QOL) • Overnight starvation

Abbreviations

PEM	Protein energy malnutrition
BCAA	Branched chain amino acid
HBV	Hepatitis B viruses
HCV	Hepatitis C viruses
NAFLD	Nonalcoholic fatty liver disease
TG	Triglyceride

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LES	Late evening snack
LC	Liver cirrhosis
npRQ	Nonprotein respiratory quotient
AAAs	Aromatic amino acids
MPS	Muscle protein synthesis
MPB	Muscle protein breakdown
ASPEN	American Society for Parenteral and Enteral Nutrition
QOL	Quality of life
AUC	Area under the curve
GSA	Galactosyl serum albumin
99mTc-GSA	Technetium-99*m* diethylene triamine pentaacetic acid galactosyl serum albumin
n.s.	Not significant
SD	Standard

Introduction

Cirrhosis of the liver is caused by factors such as hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, and autoimmunity.

In recent years, cirrhosis related to nonalcoholic fatty liver disease (NAFLD) has also gained recognition. NAFLD results from excessive dietary intake and is associated with accumulation of triglyceride (TG) in hepatocytes, obesity, and insulin resistance.

NAFLD can manifest as a range of conditions, from simple steatosis with a good prognosis to nonalcoholic steatohepatitis, a chronic progressive disease exhibiting hepatic dysfunction as well as infiltration of inflammatory cells and fibrosis.

Nutritional intake in patients with NAFLD constitutes an energy overload due to high carbohydrate intake with a high-fat diet or as an excessive lipid intake. Therefore, nutritional and dietary interventions are the basic treatment for NAFLD.

In patients with chronic hepatitis C, a viral hepatitis, nutritional support enhances the efficacy of antiviral treatment, and an iron-restricted diet has been proven to effectively reduce the damaging effects of the viral infection on the liver.

In summary, various nutritional issues govern various complications as well as the prognoses of patients in the terminal stage of chronic hepatitis and cirrhosis. Nutritional and dietary interventions are therefore important for preventing the complications of these liver diseases.

Various aspects of the efficacy of treatment with BCAA formulations as nutritional and dietary interventions in liver cirrhosis (LC) have been investigated, and the dosages and dosing regimens have been examined to assess effectiveness.

We investigated the usefulness of consuming a late evening snack (LES) as a nutritional and dietary intervention for LC.

Energy Metabolism in Patients with LC

Approximately 80 % of patients with LC present with an abnormality of energy alone, protein alone, or both, i.e., protein energy malnutrition (PEM).

Energy production in healthy people consists of approximately 50 % from carbohydrate, 30 % from fat, and 20 % from nitrogen compounds (protein). Since patients with cirrhosis primarily have decreased glycogen storage in the liver and impaired gluconeogenesis due to hepatic fibrosis, the

availability of carbohydrate as an energy substrate is decreased while the availability of fat is increased during the fasting period from after the evening meal until morning. In addition, due to the requirements for gluconeogenesis from amino acids being met by the breakdown of muscle protein, skeletal muscle is decreased and the nitrogen balance becomes negative.

The nonprotein respiratory quotient (npRQ) in patients with LC after an overnight fast is sufficiently decreased so as to be equivalent to that in healthy people after a fast of approximately 2–3 days, indicating that patients with LC are in a state of starvation during the night [1].

Decreased npRQ in patients with LC correlates with the severity of cirrhosis and prognosis, and the prognosis is poorest in patients with npRQ below 0.85 [2].

In chronic hepatitis C and C-virus related cirrhosis, hepatitis C virus (HCV) itself suppresses signal transmission of insulin, leading to insulin resistance, which results in worsening of energy metabolism and PEM. Long-term persistence of this condition is likely to lead to further worsening of the disease state of liver LC.

Liver Diseases (Chronic Hepatitis, Cirrhosis) and BCAA

The liver plays a central role in regulating metabolism, and various metabolic abnormalities are often found in patients with chronic liver diseases [3, 4]. Branched chain amino acids (BCAAs) have branched aliphatic side chains and consist of valine, leucine, and isoleucine.

BCAA is not only an ingredient of protein but also a source of glutamate and eliminates the toxicity of ammonia in skeletal muscles via glutamine synthesis.

A clinical study conducted by Plauth et al. [5] proved that intravenous administration of BCAA improves hepatic encephalopathy due to hyperammonemia. A decrease in the ratio of BCAAs to aromatic amino acids (AAAs) in serum is a characteristic of LC and is attributed to several factors including decreased nutrient uptake into skeletal muscles, increased metabolism, and ammonia detoxification [6]. A low serum BCAA/AAA ratio leads to decreased biosynthesis and albumin secretion in hepatocytes [7] and is linked to the prognosis of patients with chronic hepatic diseases [8].

Usefulness of LES as a Nutritional Therapy

Loss of skeletal muscle mass, or sarcopenia, is the most frequent complication in those with LC, occurring in 60 % of the patient population [9, 10]. Skeletal muscle mass is maintained by the balance between muscle protein synthesis (MPS) after meals and muscle protein breakdown (MPB) in the fasting state. LC is characterized by the starvation state facilitated by early conversion of glucose to lipid after absorption from the gastrointestinal tract [11, 12].

Following an overnight fast, lipids account for 75 % of total calories utilized in patients with cirrhosis and reflects the rates of increase in ketogenesis and gluconeogenesis, whereas in healthy subjects this metabolic profile only occurs after 2–3 days of starvation, i.e., after fasting [13]. Amino acids are further consumed as a source of energy with an increasing rate of gluconeogenesis during fasting [14].

This accelerates the loss of muscle protein and decreases MPS, resulting in sarcopenia. However, an increased caloric intake alone is not sufficient to reduce sarcopenia. In summary, patients with LC are in a state of anabolic resistance. One potential strategy for reducing sarcopenia in liver LC with suppressed MPB and stimulating protein synthesis is to prolong the fed state.

The longest postabsorption phase occurs between the evening meal and breakfast of the next day. This period may therefore offer the best opportunity to reverse anabolic resistance.

LES shortens the duration of this prolonged physiological fasting and thereby potentially preserves skeletal muscle mass by reducing recruitment of amino acids from skeletal muscle (reduced MPB) and increasing the rate of skeletal MPS.

LES is a simple, safe, and inexpensive intervention; however, it may exacerbate gastroesophageal reflux, existing sleep disorders, and abnormal glucose tolerance [15–17].

The beneficial effect of caloric supplementation before sleep in patients with LC was first described in the report by Swart et al. [18].

They investigated the effect of a late evening meal on nitrogen balance in nine patients with LC. The patients ingested the same amounts of calories and the same amounts of protein per day, in three meals a day during the first 1 week, followed by either four meals a day consisting of their usual meals plus a late evening meal or six meals a day. The results showed nitrogen balance to be improved in patients who received an additional late evening meal, and confirmed that this meal reserves 1 g of nitrogen per day, demonstrating that the late evening meal improved nitrogen metabolism efficiency. Continuations of such dietary therapies for long periods reportedly improve the nutritional condition of cirrhotic patients and increase their lean body mass. Furthermore, Miwa et al. [19] advocated the four-meal-a-day diet, i.e., three meals plus LES before sleep to shorten the fasting period until the next morning, as a treatment for the overnight starvation state.

The guidelines of the American Society for Parenteral and Enteral Nutrition (ASPEN) also recommend divided meals including LES [20].

Actual Implementation of LES

A patient consumes a LES of approximately 200 kcal before sleep without changing the total daily energy intake of approximately 30 kcal/kg/day or slightly more. Instructions given by a dietitian are important for preventing weight increase due to excessive energy intake during this treatment. Requirements for LES include: (1) having approximately 200 kcal; (2) containing appropriate proportions of carbohydrate (sugar), lipid, and protein; (3) containing BCAA; and (4) being easily digested. A light meal of rice balls or a piece of bread may be acceptable, but supplementation with BCAA has been shown to be desirable since BCAAs are insufficient in patients with cirrhosis [21]. Therefore, oral nutrients for liver failure patients meeting these requirements (Aminoleban EN, 210 kcal/pack (Fig. 13.1); Hepan ED, 310 kcal/pack) are ideal as LES based on the advantages of being easily consumed, having excellent digestion and absorption profiles, and no need for cooking.

It is also important to understand LES as part of the patient's lifestyle. Since meals are eaten at different times of the day by different patients, dietary conditions including the contents of meals should be documented for the entire day using a clock to determine the duration of fasting from after the evening meal until breakfast. On that basis, the timing of meals should be adjusted and the timing of LES consumption determined. When administration of one pack is difficult, the patient may consume half a pack before sleep and the remainder may be eaten upon awakening as divided doses or when waking during the night to go to the toilet.

Effects of LES

Yamauthi et al. [22] examined whether administration of an oral nutritional formula for liver failure (Aminoleban EN) (Fig. 13.1) before sleep improves the nutritional condition in patients with LC. The ratio of urine creatinine, an indicator of muscle protein amount, to total urinary excretion of

Total energy	210kcal
Protein	13.5g
Amino acids (Fisher's ratio= 38)	
L-valine	1.60g
L-leucine	2.04g
L-isoleucine	1.92g
L-threonine	0.13g
L-tryptophan	0.07g
L-arginine hydrochloride	0.30g
L-histidine hydrochloride	0.19g
L-lysine hydrochloride	0.24g
Fat (rice oil)	3.50g
Carbohydrates (dextrin)	31.05g
Minerals [a]	
Vitamins [b]	
Deionized water	180ml
PH	5.5-7.0

[a] Minerals include trace amounts of magnesium sulphate, calcium glycerophosphate, potassium iodide, potassium chloride, sodium dihydrogenphosphate dihydrate, sodium ferrous citrate, cupric sulphate, zinc sulphate and manganese sulphate. [b] Vitamins include retinol palmitate, ergocalciferol, bisbentiamine, riboflavin, pyridoxine HCl, cyanocobal-amin, folic acid, sodium l-ascorbate, tocopherol acetate, phytonadione, calcium pantothenate, nicotinamide and biotin.

Fig. 13.1 Composition of the Aminoleban EN®, per 50 g/200 mL (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan)

3-methylhistidine is considered to be a good indicator of the muscle protein metabolism rate. Patients in the before-sleep administration group clearly had lower levels of 3-methylhistidine and lower 3-methylhistidine-to-creatinine ratios than those in the postprandial administration group.

In the report by Sako et al. [23], eight patients with LC were given Aminoleban EN (Fig. 13.1) as a LES between 22:00 h and 23:00 h: four patients were prescribed Aminoleban EN as a LES from the beginning, two switched from a daytime snack to LES, and the remaining two received one additional pack as a LES. Calorie and protein intakes were not controlled since all subjects were outpatients. Administration of LES for 3 months resulted in a dramatic reduction in muscle cramps. These results showed LES to be effective for improving the quality of life (QOL) of patients with liver cirrhosis.

The study conducted by Sakaida et al. [24] showed that 1-week administration of Aminoleban EN (Fig. 13.1) as a LES to inpatients increased the burn-up fraction of sugar, decreased the burn-up fraction of lipids, and improved the npRQ.

At the same time, improvement of postprandial hyperglycemia was confirmed based on the reduction in the area under the curve (AUC) obtained by connecting the blood glucose levels by a line [25].

Suzuki et al. [26] studied blood glucose levels in 47 patients with chronic viral liver failure using a three-meal-a-day diet (the day before starting the LES regimen) and a four-meal-a-day diet including LES. Blood glucose levels were reduced 2 h before and after the evening meal as well as 2 h after breakfast, before lunch, and before evening meals, especially in patients with cirrhosis, with significant decreases in the mean and the highest blood glucose levels. This demonstrated that consuming a LES improves blood glucose levels in patients with chronic liver failure of viral origin, especially those with cirrhosis.

In addition, the results revealed that patients with a higher creatinine height index used as an indicator of skeletal muscle mass achieve greater improvement of npRQ, demonstrating the importance of preventing muscle atrophy in patients with LC.

This raises the possibility that BCAA replacement therapy increases the serum BCAA level and the tyrosine molar ratio in patients with maintained muscle mass while BCAA is used as energy in patients whose muscle mass is not maintained [27].

LC is associated with increased ammonia production, and BCAA is consumed in muscles to process ammonia, leading to amino acid imbalance. Anabolism requires a protein intake of approximately 1.09/kg/day in a healthy person compared to approximately 1.39/kg/day in patients with cirrhosis. Administration of BCAA granules to patients with cirrhosis but without PEM before sleep reportedly increases the albumin level even in those who show no albumin increase after administration following a regular meal [28].

Nakaya et al. [21] investigated whether intake of a BCAA-enriched nutrient mixture as LES improves the nutritional state and QOL as compared to intake of ordinary food as LES, after long-term (3 months) LES therapy, in patients with HCV-related cirrhosis. Significant improvements in the serum albumin level and npRQ were observed with the BCAA mixture intake. No marked improvement of QOL was observed in either group. In contrast, LES reduced general malaise, a characteristic symptom of LC, in both groups. Fatigability was ameliorated in the BCAA mixture group but was unchanged in the ordinary food group.

Koreeda et al. [29] in a longer investigation administered BCAA-enriched LES (Aminoleban EN) (Fig. 13.1) to patients with LC for 6 months to assess hepatic reserve and nutrient metabolism using technetium-99*m* diethylene triamine pentaacetic acid galactosyl serum albumin (99mTc-GSA). This study included 17 patients with LC who were admitted and followed up at the Third Department of Internal Medicine, Kansai Medical University Hospital, between March 2000 and July 2006. All investigations were conducted in conformity with Ethical Principles for Medical Research Involving Human Subjects as described in the Declaration of Helsinki. Signed informed consent for participation in the study was obtained from all patients. The inclusion criteria consisted of patients diagnosed with LC with no BCAA-enriched agent intake before the study. The diagnosis of LC was based on blood biochemical tests and findings of diagnostic imaging including a computed tomography scan, echography, and magnetic resonance imaging, as well as a liver biopsy.

Patients received a low-protein diet for LC with calories set at 1,500 kcal/day (40 g protein/day), taken at 07:00 h, 12:00 h, and 18:00 h. A BCAA-enriched agent (Aminoleban EN; Otsuka Pharmaceutical, Tokyo, Japan) (Fig. 13.1) was administered as a LES of 50 g (protein: 13.5 g; BCAA: 6 g; calories: 210 kcal) twice a day (at 22:00 h before bedtime and at 10:00 h or 15:00 h). Thus, a total of 1,920 kcal was administered per day (protein: 67 g; lipid: 37 g). Furthermore, nutritional guidance was provided to all patients by a national registered dietician prior to initiation of this study.

The patients' compliance with the LES administration was assessed at a medical interview. Six months after initiation of the LES administration, the following parameters were measured in patients who maintained >85 % compliance: serum albumin, tyrosine, molar ratio of BCAA to tyrosine (BTR), and ammonia levels. An analysis was performed to determine whether significant differences were present between baseline levels and those measured at the 6-month follow-up. A significant improvement was observed in the "mild" group in both the mean albumin levels (3.42–3.75 g/dL) (Fig. 13.2) and mean tyrosine levels (106.1–96.1 mmol/L) (Fig. 13.3). On the other hand, there was no significant improvement in the mean BTR (4.55–4.95) (Fig. 13.4).

Furthermore, the R_{max} values in the 17 patients did not significantly improve following LES administration ([before: 0.23 ± 0.10] vs. [after: 0.25 ± 0.12 mg/min]). In both the "severe" and "mild" groups, there was no significant change in R_{max} values between baseline and follow-up at 6 months (Fig. 13.5). In the <70 group, the R_{max} values increased from 0.18 to 0.22 mg/min ($P < 0.05$), serum albumin increased from 3.1 to 3.5 g/dL ($P < 0.05$), and tyrosine decreased from 125 to 113 mmol/L ($P < 0.05$). In the ≥70 group, no significant changes in nutrient values were observed (Fig. 13.6).

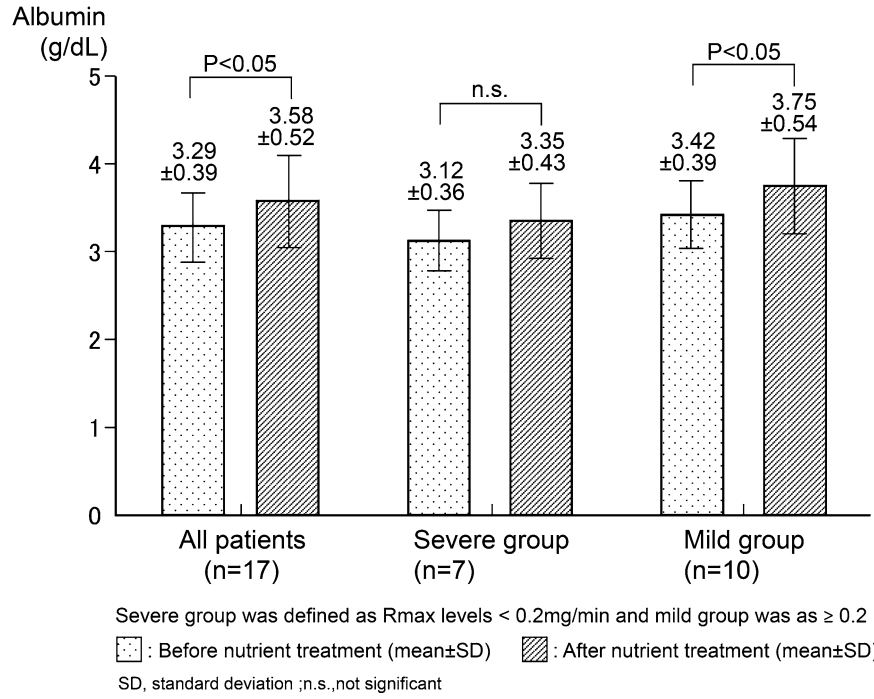

Fig. 13.2 Effect of nutrient treatment for hepatic function (Albumin)

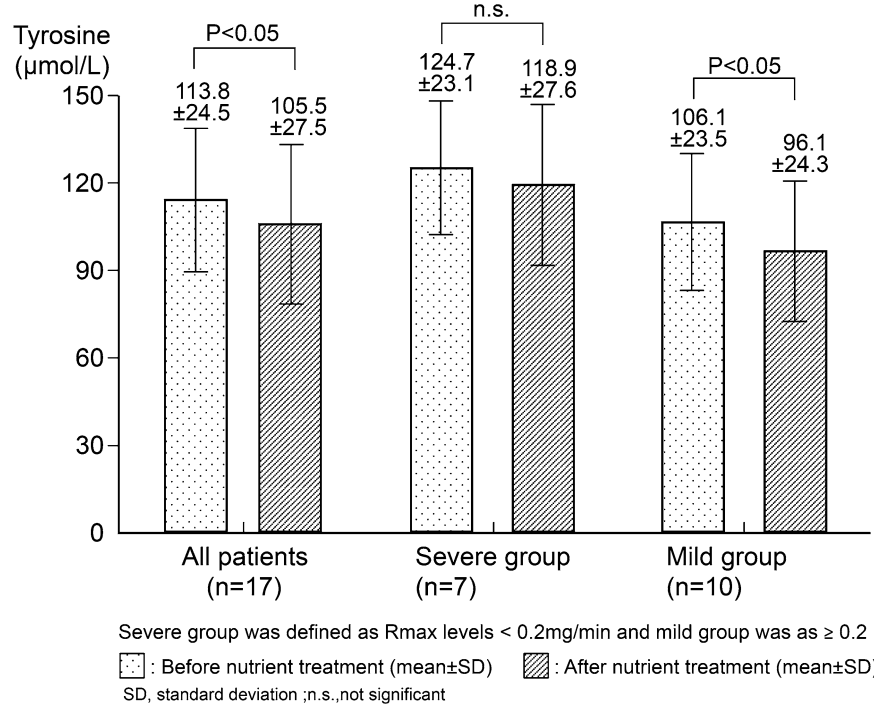

Fig. 13.3 Effect of nutrient treatment for hepatic function (Tyrosine)

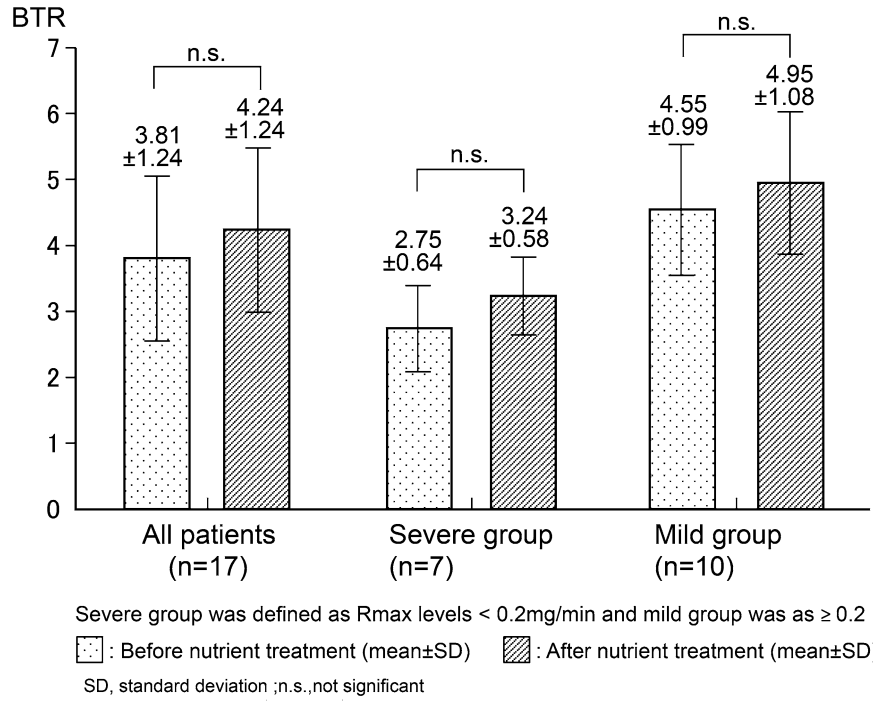

Fig. 13.4 Effect of nutrient treatment for hepatic function (BTR)

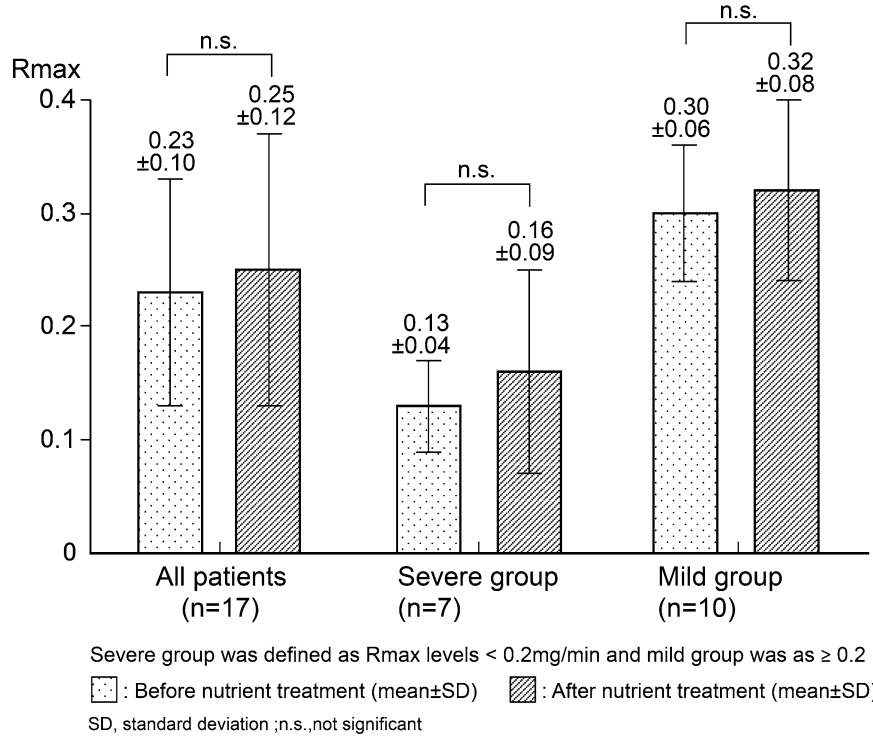

Fig. 13.5 Evaluation of R_{max} before and after nutrient administration

	Rmax (mg/min)		Serum albumin (g/dL)		Tyrosine (μmol/L)		BTR	
	Before	After	Before	After	Before	After	Before	After
Age (years)								
<70 (n=7)	0.18±0.08	0.22±0.11*	3.1±0.4	3.5±0.5*	125.2±22.8	113.2±25.7*	3.1±1.0	3.7±0.8
≥70 (n=10)	0.27±0.10	0.28±0.12	3.5±0.3	3.6±0.6	103.7±22.4	98.7±28.6	4.4±1.4	4.7±1.4
Body mass index								
<25 (n=12)	0.21±0.10	0.25±0.12*	3.1±0.4	3.5±0.6*	113.5±28.8	111.6±30.5	3.7±1.2	4.1±1.0
≥25 (n=5)	0.26±0.11	0.26±0.12	3.3±0.3	3.7±0.1*	114.6±11.4	91.1±9.3*	4.0±1.5	4.7±1.8*

*$P < 0.05$.
BTR, molar ratio of branched-chain amino acids to tyrosine; Rmax, maximal removal rate; SD, standard deviation.

Fig. 13.6 Result of R_{max}, serum albumin, tyrosine, and BTR compared with before and after nutrient treatment (mean ± SD)

Finally, in the group with a BMI < 25 kg/m^2, the R_{max} values increased from 0.21 to 0.25 mg/min ($P < 0.05$) and serum albumin increased from 3.1 to 3.5 g/dL ($P < 0.05$). On the other hand, in the group with a BMI ≥25 kg/m^2, serum albumin levels increased from 3.3 to 3.7 g/dL ($P < 0.05$), tyrosine decreased from 114 to 91 mmol/L ($P < 0.05$), and BTR increased from 4.0 to 4.7 (Fig. 13.6) [29].

The results suggested that consuming a LES in the form of Aminoleban EN may allow overnight starvation to be avoided in patients with LC and has beneficial effects on hepatic parenchymal cells in addition to nutritional effects. The authors also suggested that nutritional intervention in the early stage of LC is beneficial, and that it is important for patients with malnutrition to ingest BCAA-enriched LES long term to boost hepatic parenchymal cell mass. It is also important to determine the R_{max} value serving as the cut-off between mild and severe conditions.

Morihara et al. [30] assessed the effect of BCAA treatment as LES on the maintenance of liver function in patients with cirrhosis who had undergone radiofrequency ablation for hepatocellular carcinoma using Child-Pugh scores. The results demonstrated that BCAA given as a LES improved both serum albumin and total bilirubin levels, thereby improving the Child-Pugh score significantly.

A recent search by Cynthia et al. [31] using PubMed, EMBASE, Google scholar, and OVID databases focused on the effects of LES in patients with liver cirrhosis not related to hepatocellular carcinoma.

Published reports were evaluated to examine the effects of LES on regulation of substrate utilization (short-term studies) and nutritional outcomes (long-term studies).

LES decreased lipid oxidation and improved nitrogen balance, irrespective of the composition or type of formulation used. Daytime isocaloric isonitrogenous snacks did not have the metabolic or clinical benefit of LES. Furthermore, LES decreased skeletal muscle proteolysis. No studies have examined its effect on muscle protein synthesis. There was inconsistent translation into an increase in lean body or skeletal muscle mass. Improved QOL was noted but a decrease in mortality or the need for transplantation has not been reported. The optimal composition of LES has not been defined, but, based on mechanistic considerations, BCAA as supplemental LES holds the most promise.

LES, in fact, holds the most promise as an intervention for reversing the anabolic resistance and sarcopenia of liver cirrhosis with improved QOL in patients with LC. Long-term benefits and improved survival await critical evaluation.

The treatment guidelines for LC edited by The Japanese Society of Gastroenterology recommend implementation of BCAA treatment and energy administration before sleep, as LES, in patients with LC with recommendation grades of A and B, respectively, positioning both therapies as valid nutritional interventions for improving energy metabolism in LC [31].

Conclusions

While conclusive evidence that consuming a LES improves clinical outcomes is lacking, this intervention, especially BCAA-enriched LES, is as yet the only treatment strategy that is simple, inexpensive, and relatively free of side effects with the potential to reverse PEM in patients with LC.

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Chapter 14
Branched Chain Amino Acids and Organ Transplantation

Toshimi Kaido

Key Points

- Pretransplant nutritional condition is closely related to posttransplantation outcome.
- In particular, protein-energy malnutrition is common in patients with end-stage liver disease requiring liver transplantation (LT), and is closely associated with posttransplant risk of morbidity and mortality.
- Accurate nutritional assessment and adequate perioperative nutritional treatment are essential for improving outcomes after LT.
- The overall survival rate in patients with low skeletal muscle mass was found to be significantly lower than in patients with normal/high skeletal muscle mass.
- Perioperative nutritional therapy including branched chain amino acids is useful for patients with sarcopenia, whose prognosis is poor without nutritional therapy.

Keywords Liver transplantation • Nutritional assessment • Nutritional therapy • Branched chain amino acids • Sarcopenia • Bioelectrical impedance analysis • Body cell mass • Skeletal muscle mass

Abbreviations

LT	Liver transplantation
BCAAs	Branched chain amino acids
BIA	Bioelectrical impedance analysis
BCM	Body cell mass
LDLT	Living donor liver transplantation
BTR	BCAA-to-tyrosine ratio
MELD	Model for end-stage liver disease
ESPEN	EUROPEAN Society for Parenteral and Enteral Nutrition
DDLT	Deceased donor liver transplantation
POD	Postoperative day

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Introduction

Pretransplant nutritional condition is closely related to posttransplantation outcome. In particular, protein-energy malnutrition is common in patients with end-stage liver disease requiring liver transplantation (LT), and is closely associated with posttransplant risk of morbidity and mortality [1–5]. Therefore, to improve a patient's outcome after LT, it is necessary to have accurate nutritional assessment and adequate perioperative nutritional treatment. In this chapter, we review perioperative nutritional assessment and treatment for patients undergoing LT and describe the usefulness of branched chain amino acids (BCAAs) in improving nutritional status and optimizing outcomes after LT.

Pretransplant Nutritional Assessment Using Bioelectrical Impedance Analysis

In patients anticipating general surgery, nutritional assessment is performed using subjective global assessment, anthropometry including body mass index and muscle arm circumference, and biological markers such as prealbumin (transthyretin) and albumin. However, in patients undergoing LT, these parameters are not available due to decompensated cirrhosis. Edema and ascites can lead to the overestimation of anthropometric parameters, and underlying liver dysfunction affects biological markers. In 2008, we introduced body composition analysis into nutritional assessment using bioelectrical impedance analysis (BIA) in patients undergoing LT. BIA measures the body's resistance to flow (impedance) of alternating electrical current at a designated frequency between points of contact on the body. As the water in body tissue is conductive, the measurement of body impedance can indirectly provide information on the body's tissue content including total body water, fat-free mass, and skeletal muscle mass. The prevalence of using BIA to estimate body composition is increasing because BIA is easy to perform, noninvasive, and quick; exhibits high interobserver reproducibility; and has been highly correlated with hydrostatic weighing, dual energy X-ray absorptiometry, and deuterium isotope dilution techniques in specific populations. However, the accuracy of BIA declines in patients with massive ascites and edema [6–8]. Regarding reproducibility, Erceg et al. examined the repeatability of measurement results by the BIA method in adults and children [9]. The intraclass correlation coefficients for men, women, and children were greater than 0.99, and the coefficients of variation for the BIA system for men, women, and children were small, which suggested strong repeatability of BIA results.

Body mass can be divided overall into two compartments: fat mass and fat-free or lean body mass. The multicompartment body composition model partitions lean body mass into skeleton and integument, skeletal muscle and visceral organs, and total body water; these are further partitioned into intracellular and extracellular water [10]. The concept of body cell mass (BCM), proposed by Moore et al. [11] in 1963, reflects the cellular components of the body involved in biochemical processes and energy metabolism. BCM is defined as the sum of intracellular water and fat-free mass, including skeletal muscle and viscera, but excluding bone mineral mass [11]. BCM is altered by nutritional status, physical activity level, and disease states; therefore, BCM can serve as a biomarker of these processes [12]. BCM comprises the metabolically active and protein-rich compartments in the body and is known to be depleted in patients with protein-energy malnutrition [13]. Consequently, BCM is regarded as a useful parameter for assessing malnutrition in patients with cirrhosis [14]. In living donor LT (LDLT), Kaido et al. showed that preoperative low BCM was an independent risk factor for posttransplant sepsis and death due to infection [15]. This strongly suggests the importance of the effect of nutritional status, including skeletal muscle mass, on posttransplant outcomes.

Significance of Sarcopenia in Liver Transplantation

Sarcopenia, defined as a low level of muscle mass, is associated with an increased risk for age-related decline in muscular strength and functional ability [16, 17]. It has recently been demonstrated that sarcopenia is an independent predictor of lower disease free and overall survival in various kinds of diseases [18–21]. Sarcopenic obesity has been shown to reduce survival in patients with solid tumors of the respiratory and gastrointestinal tracts [18]. Moreover, sarcopenia has been found to be an adverse prognostic factor in patients with colorectal liver metastasis and pancreatic cancer [19–21]. In patients with liver cirrhosis, malnutrition is caused by the decreased protein synthesis and disturbed energy metabolism that result from liver dysfunction. In cirrhotic patients, protein malnutrition can cause a decrease in skeletal muscle mass. Hayashi et al. reported that the skeletal muscle index, measured as % arm muscle circumference and arm muscle area, in patients with liver cirrhosis was significantly lower than in healthy subjects [22]. However, the impact of pretransplant sarcopenia on outcomes after LT remains poorly understood. Englesbe et al. recently reported that central sarcopenia strongly correlated with postliver transplant mortality, using the size of the psoas muscle as measured by computed tomography scan [23]. However, the total psoas area is merely part of skeletal muscle mass and might not correctly reflect whole body skeletal muscle mass. In contrast, BIA can easily and automatically measure whole body skeletal muscle mass. Most recently, Kaido et al. used BIA to examine the skeletal muscle mass in 124 patients undergoing LDLT [24]. These authors reported that the median ratio of preoperative skeletal muscle mass was 92 % (range 67–130 %) of the standard mass. The preoperative skeletal muscle mass was significantly correlated with the BCAA-to-tyrosine ratio (BTR) ($r=-0.254$, $p=0.005$) (Fig. 14.1) and BCM ($r=0.636$, $p<0.001$). However, there were no significant correlations between the preoperative skeletal muscle mass and age, sex, total lymphocyte count, zinc, prealbumin, Child-Pugh classification, or Model for End-stage Liver Disease (MELD) score [24]. Regarding the reason for the negative correlation between skeletal muscle mass and the BTR, Kaido et al. speculated that BCAAs in patients with cirrhosis are mainly metabolized in skeletal muscle. Therefore, the more skeletal muscle mass, the more BCAA consumption, which leads to a decrease in the BTR. Moreover, the overall survival rate in patients with low skeletal muscle mass was found to be significantly lower than in patients with normal/high skeletal muscle mass ($p<0.001$) (Fig. 14.2) [24]. In a multivariate analysis, low skeletal muscle mass was found to be an independent risk factor for death after transplantation [24]. Sarcopenia would therefore be an independent parameter that reflects mainly liver dysfunction in conventional scores, including the Child-Pugh and MELD scores.

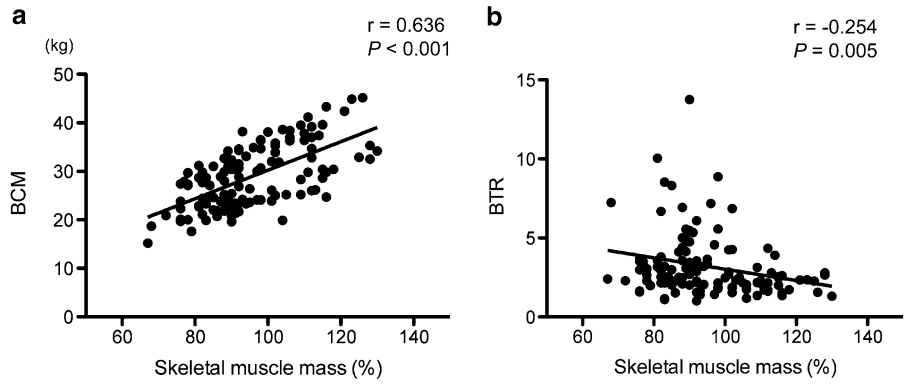

Fig. 14.1 Correlation between the skeletal muscle mass value and body cell mass (**a**) and branched chain amino acids to tyrosine ratio (**b**). The preoperative skeletal muscle mass was significantly correlated with the branched chain amino acids-to-tyrosine ratio ($r=-0.254$, $p=0.005$) and body cell mass ($r=0.636$, $p<0.001$)

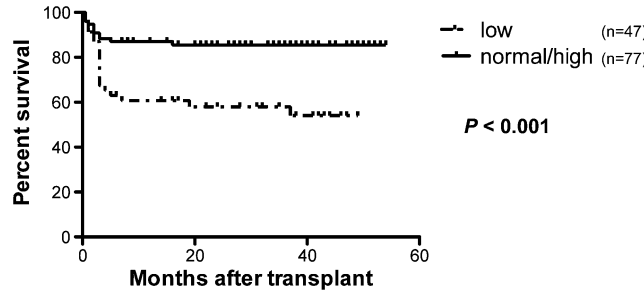

Fig. 14.2 Overall survival rates according to skeletal muscle mass value. The overall survival rate was significantly lower in patients with low skeletal muscle mass than in patients with normal/high skeletal muscle mass ($p < 0.001$)

Significance of Peritransplant Administration of BCAAs

The beneficial effects of BCAAs in patients with liver cirrhosis have been extensively demonstrated [25–33]. For example, administration of BCAAs has been shown to improve nutritional status, hepatic encephalopathy, glucose tolerance, liver generation, and quality of life. However, the usefulness of BCAAs has not been specifically investigated in patients with end-stage liver disease who are on the liver transplant waiting list. Long-term nutritional supplementation with oral BCAA was found to be beneficial in slowing the progression of hepatic failure and prolonging event-free survival in liver cirrhosis in two randomized controlled studies [28, 33]. These findings suggest the usefulness of BCAAs for candidates for LT. Recently, in an open-label, randomized, controlled parallel group study, Kawamura et al. investigated the long-term role of BCAAs in 56 patients with Child class A cirrhosis without major complications [32]. In this study, the oral BCAA supplementation group had less mean annual change in their MELD and Child-Pugh scores than the control group. The incidence of overall major cirrhotic complications, including hepatocellular carcinoma and esophagogastric varices, was lower in the BCAA group than in the control group. The authors concluded that early interventional oral BCAAs might help maintain the health of patients during the liver transplant waiting period by preserving hepatic reserve in cirrhosis. However, patients categorized as Child class A are not usually listed as LT candidates.

Recently, some investigators have reported the beneficial effect of pretransplant BCAA supplementation. Kaido et al. retrospectively analyzed pre- and perioperative predictors, including nutritional factors such as BCAA supplementation for the prevention of posttransplant infectious complications, in 100 consecutive adult patients who underwent adult LDLT [15]. Multivariate analysis showed that low preoperative BCM and the absence of preoperative oral BCAA-enriched nutrient supplementation were of independent prognostic significance for posttransplant sepsis (Table 14.1). Shirabe at al. retrospectively analyzed the effects of preoperative oral supplementation with BCAAs on postoperative bacteremia after LDLT for chronic liver failure in 236 patients who underwent adult LDLT [34]. Multivariate analysis revealed that lack of BCAA supplementation was an independent risk factor for postoperative bacteremia. These findings strongly suggest that pretransplant BCAA supplementation has a beneficial effect on reducing posttransplant infection, even in patients with Child class B or C cirrhosis.

As for perioperative administration of BCAA-enriched nutrients, Yoshida et al. performed a randomized pilot study in 25 adult LDLT recipients to evaluate its usefulness for treating postoperative metabolic abnormalities [35]. In the control group ($n = 13$), an ordinary diet was adjusted to 30–35 kcal and 1.2–1.3 g of protein per kilogram of ideal body weight per day according to the European Society for Parenteral and Enteral Nutrition (ESPEN) guideline [36] (Fig. 14.3). In the BCAA group ($n = 12$), 2 packages of BCAA-enriched nutrients (420 kcal and 27 g protein) were administered from 7 days until 1 day before LDLT. This regimen was continued from 3 days until 4 weeks after LDLT. Total energy intake as well as protein intake was adjusted to be the same in both groups. Postoperatively, the BTR and retinol binding protein significantly improved in the BCAA group compared with the

Table 14.1 Multivariate analysis of factors affecting posttransplant sepsis

Variable	Odds ratio	95 % CI	*p*
Preoperative low BCM	4.633	1.493–17.701	0.032
Absence of preoperative BCAA enriched nutrient mixture	3.201	1.202–7.849	0.020

Preoperative low body cell mass (BCM) and the absence of preoperative oral branched chain amino acids (BCAA)-enriched nutrient supplementation were of independent prognostic significance for posttransplant sepsis

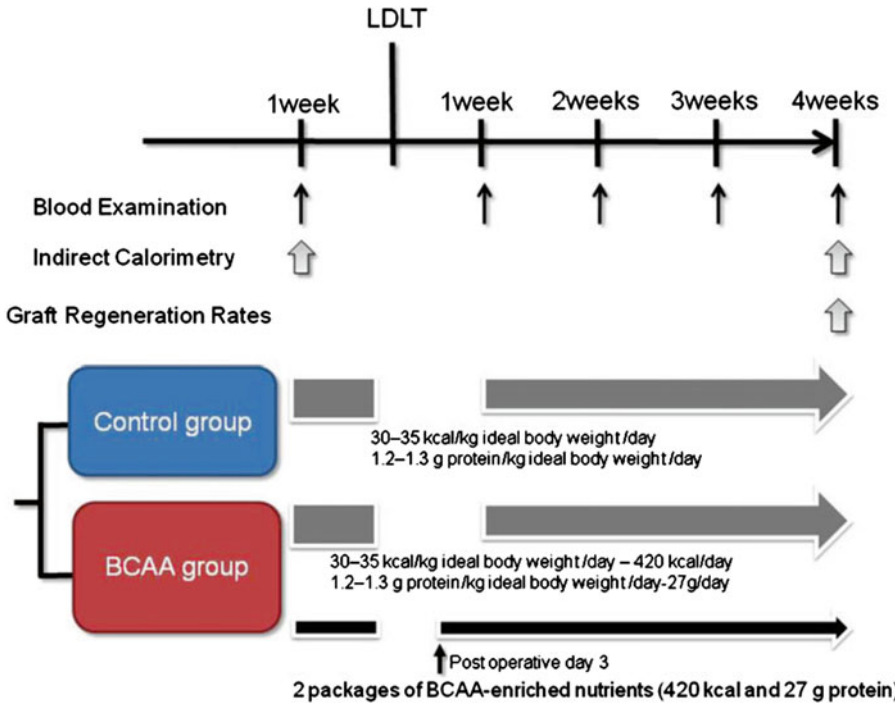

Fig. 14.3 Schematic presentation of the intervention schedule. Reference [35] with permission from Springer

control group (Fig. 14.4). The nonprotein respiratory quotient significantly increased from 1 week before LDLT to 4 weeks after LDLT in the BCAA group (0.77 ± 0.05 to 0.84 ± 0.06, $p = 0.022$), but not in the control group (0.78 ± 0.04 to 0.81 ± 0.05, $p = 0.318$). Moreover, the mean oxidative rate of carbohydrates at 4 weeks after LDLT compared with that at 1 week before LDLT was significantly increased in the BCAA group ($p = 0.016$) but not in the control group ($p = 0.223$) (Fig. 14.5). As for infectious complications, there were no significant differences in the occurrence of bacterial cytomegalovirus and fungal infections between the two groups. While a large multicenter randomized controlled trial is still necessary, this pilot study showed that BCAA-enriched enteral nutrients could improve the nutritional and metabolic disorders associated with end-stage liver disease in the early posttransplant period.

Tietge et al. examined plasma amino acid levels in patients with end-stage cirrhosis and in patients with stable graft function in the long-term course after LT [37]. The investigators observed that only aromatic amino acid levels returned to normal with normalization of liver function, whereas BCAA levels improved but remained significantly lower than in healthy controls, indicating persistent changes in muscular amino acid metabolism after LT. These findings suggest that not only pretransplant but also posttransplant BCAA supplementation would be useful to improve the nutritional and metabolic disorders of patients during the long term after LT.

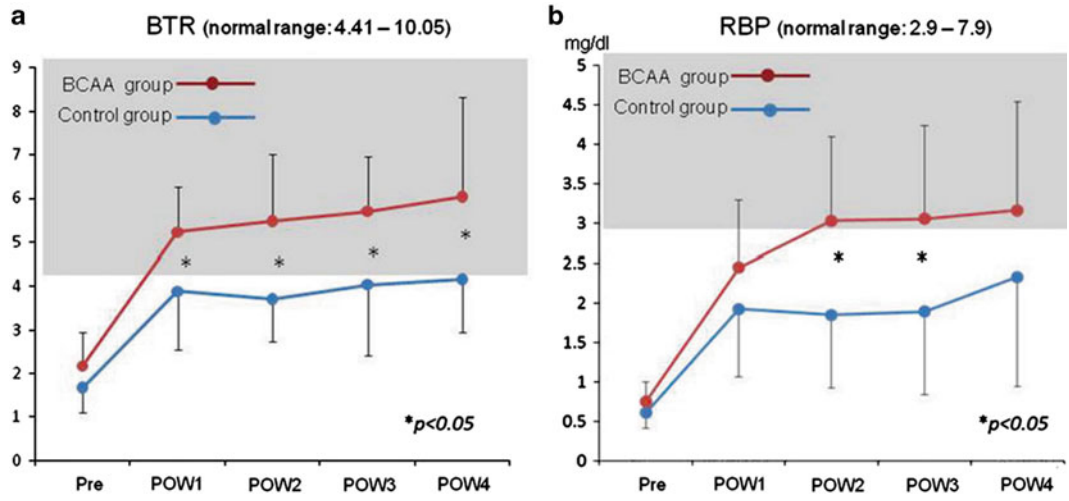

Fig. 14.4 Branched chain amino acids to tyrosine ratio (BTR) (**a**) and retinol binding protein (RBP) (**b**) measured in the perioperative periods of living donor liver transplantation. Postoperatively, the BTR and RBP significantly improved in the branched chain amino acids (BCAA) group compared with the control group. Reference [35] with permission from Springer

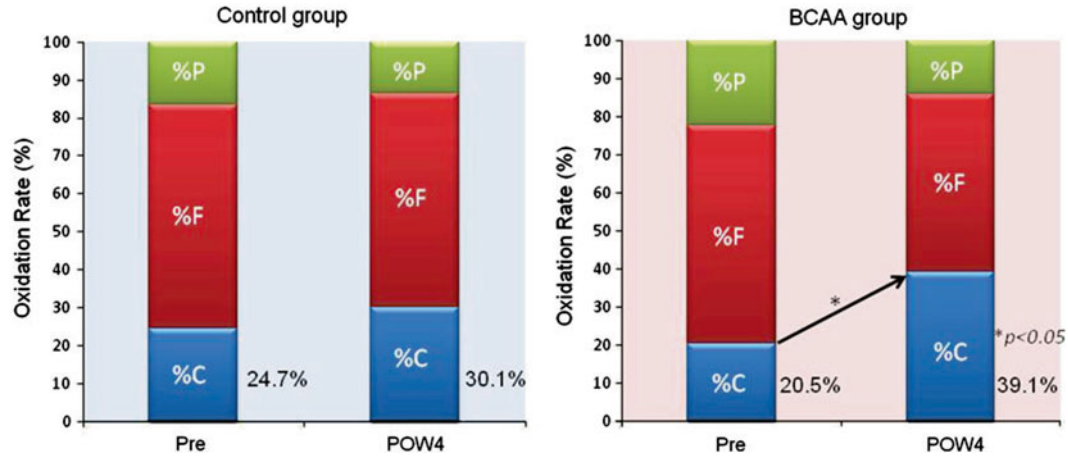

Fig. 14.5 Analysis using indirect calorimeter for oxidation rates of carbohydrate, fat, and protein. The mean oxidative rate of carbohydrates at 4 weeks after living donor liver transplantation (LDLT) compared with that at 1 week before LDLT was significantly increased in the branched chain amino acids (BCAA) group ($p = 0.016$) (**a**) but not in the control group ($p = 0.223$) (**b**). Reference [35] with permission from Springer

Perioperative Nutritional Therapy with BCAA in Liver Transplantation

A planned preoperative nutritional intervention can be performed for most patients anticipating LDLT, since the transplantation date for LDLT is known in advance, unlike in deceased donor LT (DDLT). In cases of LDLT, it would be desirable to start preoperative nutritional therapy, as well as rehabilitation, at the time of referral of a potential recipient, approximately a few months before LT, to more effectively increase skeletal muscle mass and BCM.

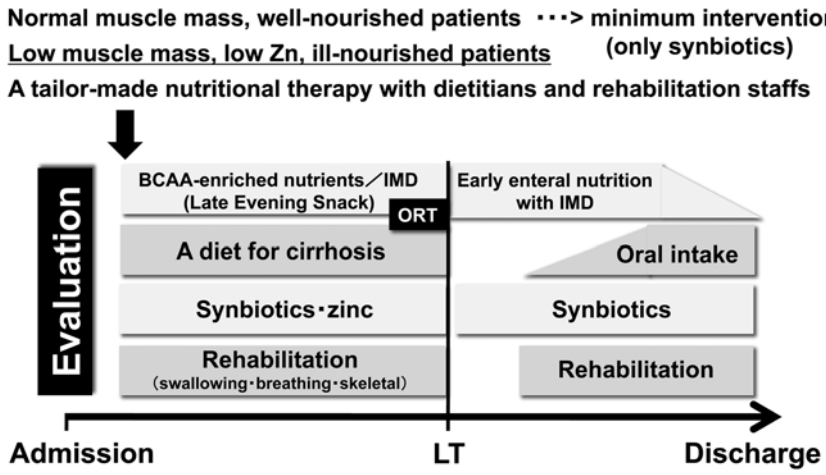

Fig. 14.6 Our current tailor-made perioperative nutritional therapy in liver transplantation. For well-nourished patients, we perform minimum nutritional intervention with synbiotics only. In contrast, we start nutritional intervention for ill-nourished or low muscle mass patients with dietitians and rehabilitation staffs according to their nutritional status at admission

We have implemented tailor-made perioperative nutritional therapy in LT. For well-nourished patients, we perform minimum nutritional intervention with synbiotics only. In contrast, we start nutritional intervention for ill-nourished patients according to their nutritional status at admission (Fig. 14.6). In our department, preoperative nutritional therapy is administered for approximately 2 weeks before LDLT after BIA assessment on admission. Typically, the therapy consists of the following three components: a nutrient mixture enriched with BCAAs (Aminoleban EN®; Otsuka Pharmaceutical Co., Tokyo, Japan) or BCAA nutrients (Livact®; Ajinomoto Pharma Co., Tokyo, Japan) as a late evening snack; synbiotics using a supplementation product enriched with glutamine, dietary fiber, and oligosaccharide (GFO®; Otsuka Pharmaceutical Factory, Tokushima, Japan) three times daily and a lactic fermented beverage containing 5×10^8/mL of *Lactobacillus casei* Shirota strain (Yakult 400®; Yakult Honsha Co., Tokyo, Japan) once a day; and 1.0 g/day of Polaprezinc (Promac D®; Zeria Pharmaceutical Co., Tokyo, Japan) for patients with low serum zinc level. To maintain a total caloric intake of 35–40 kcal/kg and a protein intake of 1.2–1.5 g/kg including BCAA nutrients according to the guidelines of the ESPEN, dietitians adjust the type and amount of food for each patient [36].

The ESPEN guidelines for enteral nutrition after surgery, including organ transplantation, recommend starting enteral feeding within 24 h of surgery [38]. The reason is because several studies have shown that early enteral nutrition lowers the rate of postoperative complications due to infections, as well as the duration of hospitalization [7]. These guidelines indicate early tube feeding for patients who cannot tolerate early oral nutrition or who are obviously malnourished at the time of surgery. Since most patients who undergo LT have suffered poor nutrition, liver recipients are appropriate recipients of early enteral nutrition.

We start early postoperative enteral nutrition within the first 24 h after surgery through a tube jejunostomy for enteral nutrition placed in the proximal jejunum using a 9-French enteral tube at the time of surgery. The starting total daily caloric intake until postoperative day (POD) 3 of 10–15 kcal/kg is gradually increased to reach 25–35 kcal/kg. As an enteral nutrient, we prefer a new immune-modulating diet (IMD) enriched with hydrolyzed whey peptide (MEIN®; Meiji Dairies Co., Tokyo, Japan), a protein complex derived from milk, based on the findings in our previous report [39]. Kaido et al. reported that early enteral nutrition with the new IMD enriched with hydrolyzed whey peptide

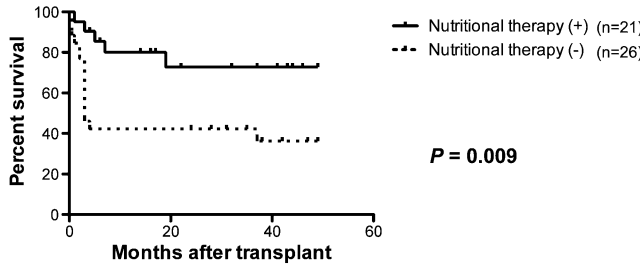

Fig. 14.7 Overall survival rates according to perioperative nutritional therapy in patients with preoperative low skeletal muscle mass. Perioperative nutritional therapy has been shown to significantly increase overall survival in patients with low skeletal muscle mass ($p=0.009$)

could prevent posttransplant bacteremia and posttransplant hyperglycemia [39]. The initial infusion rate is 20 mL/h. If well tolerated, the enteral infusion rate is increased to 40 mL/h by POD 5. In patients with severe edema of the small intestine or severe diarrhea, we usually decrease the speed of IMD to 20 mL/h (=20 kcal/h) or use an oral rehydration solution. After confirmation of improvement in the edema or diarrhea, we resume our nutritional therapy regimen. Plank et al. reported that pre- and postoperative IMD in patients undergoing DDLT might help hasten posttransplant recovery and reduce postoperative complications due to infections [40].

Only after swallowing ability can be confirmed is oral nutrition started, usually around POD 5. Dietitians calculate the daily amounts of protein and carbohydrate required for each recipient and adjust the speed of the enteral nutrition according to the oral intake. Enteral feeding is stopped when the patients can tolerate adequate oral intake containing solid food. For synbiotics, all patients receive both the above supplementation products three times daily and a lactic fermented beverage once a day via the feeding tube or orally until discharge.

In this chapter, we have described how the overall survival rate after LT in patients with low skeletal muscle mass has been observed to be significantly lower than in patients with normal/high skeletal muscle mass ($p<0.001$) (Fig. 14.2). However, perioperative nutritional therapy has been shown to significantly increase overall survival in patients with low skeletal muscle mass ($p=0.009$) (Fig. 14.7) [24]. Perioperative nutritional therapy therefore plays an important role in the treatment of end-stage liver disease requiring LT.

Conclusions

Accurate nutritional assessment and adequate perioperative nutritional treatment are essential for improving outcomes after LT. In particular, perioperative BCAA supplementation helps to prevent posttransplant bacteremia and improve metabolic disorders. Moreover, perioperative nutritional therapy including BCAA is useful for patients with sarcopenia, whose prognosis is poor without nutritional therapy.

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Chapter 15
Basic Aspects in Prevention of Posttransplant Bacteremia by Branched Chain Amino Acids

Ken Shirabe, Toru Ikegami, Tomoharu Yoshizumi, and Yoshihiko Maehara

Key Points

- Posttransplant bacteremia is one of the most serious complications after liver transplantation.
- Unfortunately, there are few therapies for preventing posttransplant bacteremia currently available.
- For prevention of bacteremia, nutritional support is a promising strategy.
- After orthotopic liver transplantation (OLT), serum levels of branched chain amino acids (BCAAs) generally decrease and severely decreased levels of BCAAs after OLT have been reported to be a risk factor for posttransplant bacteremia.
- Pretransplant and/or posttransplant BCAA supplementation may be promising nutritional strategy in patients undergoing OLT and living donor liver transplantation (LDLT).
- In this chapter, we review previous studies on the beneficial effects of BCAA supplementation in OLT and LDLT for preventing bacteremia and its mechanism of action, and provide a perspective on avenues for future research.

Keywords Branched chain amino acids • Orthotopic liver transplantation • Living donor liver transplantation • Bacteremia • Oral supplementation • Enteral nutrition

Abbreviations

BCAA	Branched chain amino acid
OLT	Orthotopic liver transplantation
LDLT	Living donor liver transplantation
mTOR	Mammalian target of rapamycin

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Introduction

Despite recent advances in perioperative management and surgical techniques, postoperative mortality and morbidity associated with bacteremia after orthotropic and living donor liver transplantation (OLT and LDLT) are still prevalent. Bacteremia is the most serious complication, and the most frequent cause of in-hospital death after OLT and LDLT [1, 2].

Risk factors for bacteremia after liver transplantation have been widely reported. For example, Singh et al. [1] reported that diabetes mellitus and serum albumin levels were independent predictors of bacteremia in patients undergoing orthotropic liver transplantation. Meanwhile, Iida et al. [2] reported that Child-Pugh class C, massive pleural effusion or ascites before the operation, ABO incompatibility, older donor age, and postoperative cytomegalovirus infections were independent risk factors in LDLT patients. Alexopoulos et al. [3] reported that the incidence of posttransplant bacteremia reached 42 % in patients achieving a model for end-stage liver disease score of ≥40. These data suggest that, in liver transplant recipients, deteriorated liver function before transplantation is related to postoperative bacteremia.

Nutritional support therapy after OLT and LDLT has been examined over the last 30 years (Table 15.1). The goal of nutritional support in most previous studies was prevention of posttransplant sepsis. After OLT, administration of 1.5–2.0 g of protein/kg body weight was reported to be necessary to achieve a positive nitrogen balance [4]. Reilly et al. [5] designed a randomized prospective study in which they randomly assigned 28 hypoalbuminemic cirrhotic patients to receive, immediately after liver transplantation, one of three regimens: group 1, no nutritional support ($n=10$); group 2, total parenteral nutrition (TPN) (35 kcal/kg/day) with standard amino acids (1.5 g/kg/day) ($n=8$); or group 3, isocaloric isonitrogenous TPN with added branched chain amino acids (BCAAs; $n=10$). TPN with either standard or BCAA-enriched amino acids is tolerated well immediately after successful liver transplant. A positive nitrogen balance was achieved, and the large protein loads did not worsen encephalopathy. Nutritional

Table 15.1 Beneficial effects of perioperative nutritional support except BCAA in reducing infections in patients undergoing liver transplantation

Authors	Year	Reference	Study design	Patients number	Nutrition therapy (infection rates)
Rayes et al.	2002	[8]	Prospective	95 (32 with selective bowel decontamination, 31 with Lactobacillus, and 32 with placebo)	Postoperative early enteral nutrition with Lactobacillus (13 %) and control (48 %)
Rayes et al.	2005	[9]	Prospective	66 (33 with lactic acid bacteria and fiber and 33 with fibers only)	Postoperative early enteral nutrition with lactic acid bacteria and fibers (3 %) and only fibers (48 %)
Kaido et al.	2010	[7]	Prospective	30 (10 with postoperative early immuno-modulating nutrition and 20 with conventional enteral diet)	Postoperative early enteral nutrition with immuno-modulating diet (10 %) and control (50 %)
Eguchi et al.	2011	[10]	Prospective	50 (25 with peripoerative Bifidobacterium breve, Lactobacillus casei, 25 without synbiotics)	Peioperative synbiotics (4 %) No symbiotics (24 %)
Ikegami et al.	2012	[6]	Retrospective	192 (135 with enteral nutrition within 48 h 57 without enteral nutrition)	Early enteral nutrition (5.9 %) No early enteral nutrition (24 %)

support may improve respiratory muscle function, allowing earlier weaning from ventilatory support. A shortened ICU stay justifies the expense of TPN. Nevertheless, the number of patients in each group was too small to be evaluated, and while the authors showed the necessity of amino acids, they did not clearly show the beneficial effects of BCAAs over conventional amino acids.

Our recent study [6] showed that the incidence of early graft loss was eightfold higher in recipients with massive intraoperative blood loss without early enteral nutrition, and that the main cause of early graft loss was bacteremia. An immune-modulating diet supplemented with arginine, glutamine, and omega-3 fatty acids was reported to have beneficial effects of enteral nutrition preventing bacteremia after LDLT [7]. Several previous prospective, randomized studies [8–10] have shown that perioperative synbiotic treatment prevents infectious complications in patients after elective OLT and LDLT. These studies confirmed the importance of nutritional support, especially enteral nutrition, for the prevention of bacteremia after liver transplantation. Nevertheless, the best nutritional substrate for preventing bacteremia remains unclear. Herein, we focus on the effects of BCAA supplementation in the light of prevention of posttransplant bacteremia.

Serum Levels of BCAA After Liver Transplant

Tietge et al. [11] investigated hepatic amino acid metabolism in patients with liver cirrhosis and over the long term after OLT. After OLT, BCAA levels remained subnormal, although higher than in cirrhosis. BCAA levels decreased with increasing catecholamine and insulin levels. These authors concluded that, despite normal liver function, BCAA levels remain subnormal after OLT. This decreased BCAA level after OLT was accompanied by normalization of aromatic amino acid levels, and this change in amino acid profile was observed in adults and children who had undergone OLT. The decreased serum BCAA levels might be explained by an increased requirement for BCAAs after OLT. Mager et al. [12] showed that the total BCAA requirement increased after OLT by measuring the oxidation of L-[1-^{13}C] phenylalanine to $^{13}CO_2$ after a primed, continuous infusion of the tracer. Furthermore, Luzi et al. [13] demonstrated that whole-body proteolysis, protein synthesis, and leucine oxidation were reduced in comparison to previously obtained values in a normal population. The rate of phenylalanine appearance across the forearm was equally suppressed in healthy adults and those who had undergone OLT, under condition of a euglycemic/insulin clamp. Conversely, whole-body leucine flux was not suppressed by insulin in adults after OLT, suggesting that the site of aberrant BCAA metabolism is insulin-sensitive tissues (liver or muscle). This may be partly due to insulin resistance and suppression of insulin secretion mediated by immunosuppressive therapy prescribed in the post-OLT period.

Previous studies have shown that decreased serum BCAA levels are related to posttransplant bacteremia. Munoz et al. [14] showed that a logistic regression model constructed using the BCAA/aromatic amino acid ratio predicted the occurrence of sepsis after OLT 77 % of the time. Figueiredo et al. [4] demonstrated that lower BCAA levels after liver transplantation were strongly associated with an increased likelihood of postoperative infections. Interestingly, Roth et al. [15] also showed decreased levels of BCAAs in orthotropic liver transplant patients suffering from sepsis. Taken together, these data suggested that BCAA supplementation after both OLT and LDLT may be useful for preventing bacteremia.

Effects of BCAA Supplementation for Prevention of Bacteremia

The effects of BCAA supplementation in patients who have undergone liver transplantation have been evaluated in only a few studies (Table 15.2). We previously found in Child-Pugh class C patients, no BCAA supplementation was a risk factor for bacteremia [16]. Kaido et al. demonstrated that low

Table 15.2 Beneficial effects of perioperative BCAA supplementation in reducing infections in patients undergoing liver transplantation

Authors	Year	Reference	Study design (pre/post-translant)	Patients number	Nutrition therapy (infection rates)
Reilly et al.	1990	[5]	Prospective (post-transplant supplementation)	28 (10 with no nutritinal support, 8 with TPN with standard amino acid, 10 with TPN added BCAA)	Positive nitrogen balabce, short en length of ICU stay, compared with no nutrition support. No difference between TPN with and without BCAA
Shirabe et al.	2011	[16]	Retrospective (pre-transplant)	236 (129 with BCAA supplementation and 107 with control)	BCAA supplementation (6.7 %) and control (22.0 %)
Kaido et al.	2012	[17]	Retrospective (pre-transplant)	100 (37 with BCAA supplementation and 63 with control)	BCAA supplementation (26 %) and control (52 %)
Yoshida et al.	2012	[19]	Prospective (post-transplant)	24 (12 with BCAA supplementation and 12 with control)	BCAA supplementation (25 %) and control (33 %)

preoperative body cell mass and the absence of preoperative BCAA supplementation and hydrolyzed whey peptide, which is easily absorbed as BCAA, were significant risk factors for posttransplant bacteremia [17, 18].

Yoshida et al. [19] designed a randomized prospective pilot study for evaluating the effect of post-transplant BCAA supplementation in patients who underwent LDLT. Although there were only 12 patients treated with BCAAs and 12 without BCAAs, synthesis of short-turnover protein, amino acid profile, and nonprotein respiratory quotient were significantly improved in the patients with BCAA supplementation. Although there was no significant difference in the incidence of sepsis between the two groups, a large-scale multicenter trial is necessary to determine the effect of BCAAs on prevention of bacteremia.

Mechanism Underlying the Beneficial Effects of BCAAs

Preoperative oral BCAA supplementation may reduce the incidence of posttransplant bacteremia in LDLT patients. The mechanism underlying this effect remains unclear. One possible mechanism is the improvement of nutritional status. Mattick et al. [20] showed that the changes following trauma and sepsis, termed systemic inflammatory response syndrome, elicit major changes in carbohydrate, protein, and energy metabolism. When these events persist for too long they result in a severe depletion of lean body mass, multiple organ dysfunction, and eventually death. Recently, various effects of BCAAs on liver, skeletal muscle, and the immune system have been examined (Table 15.3). The beneficial effects of BCAAs in critically ill patients can be understood more easily by considering the close relationship between muscle and liver, known as muscle-liver cross-talk. BCAAs are metabolized in two major steps that occur differentially in muscle and liver. In muscle, BCAAs are reversibly transaminated to the corresponding α-keto acids. For the complete degradation of BCAAs, the α-keto acids must travel to the liver to undergo oxidation. The liver, in contrast to muscle, does not significantly express branched chain aminotransferase. Thus, BCAA degradation is under the joint control of both liver and muscle. Recent evidence [21–23] suggests that in liver, BCAAs may perform signaling functions, more specifically activation of the mTOR (mammalian target of rapamycin) signaling

Table 15.3 The mechanism of BCAA

Organs	Competent cells	Activated pathway	Effects
Liver	Hepatocyte	mTOR pathway	Protein synthesis ↑ Glucose uptake ↑ Glycogen storage ↑
Muscle	Skeletal muscle cell	mTOR pathway AKT pathway	Protein degradation ↓ Glucose uptake ↑ Glycogen storage ↑
Immune system	Myeloid dentric cell		Proliferation ↑ IL-12 production ↑
	Lymphocyte		Proliferation ↑ Natural killer activity ↑
	Macrophage Neutrophil		Phagocytic function ↑

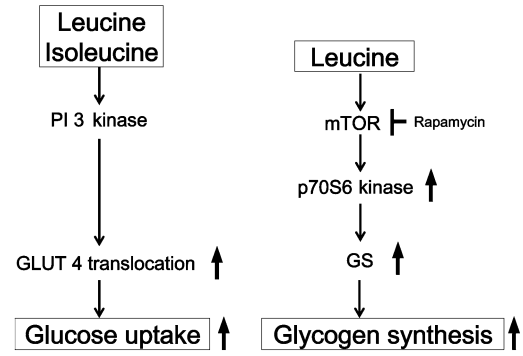

Fig. 15.1 Leucine and isoleucine regulate glucose uptake and glycogen synthesis via the PI3 kinase (phosphatidylinositol 3-kinase) and mTOR (mammalian target of rapamycin) pathways

pathway, influencing a wide variety of metabolic and synthetic functions, including protein translation, insulin signaling, and oxidative stress following severe injury and infection. In an in vitro study, BCAAs, especially leucine, activated the mTOR signaling pathway and inhibited protein degradation, resulting in the promotion of protein synthesis [21, 22]. Furthermore, in a cirrhotic rat model, leucine activated glycogen synthase via mTOR signaling and improved glucose metabolism [23]. Thus, BCAAs activate both PI3 kinase and mTOR pathways, and glucose uptake and glycogen synthesis are upregulated (Fig. 15.1). As mentioned previously, in a prospective study by Yoshida et al. [19], synthesis of short-turnover protein, amino acid profile, and nonprotein respiratory quotient were significantly improved after LDLT.

This metabolic improvement, such as the improvements in protein synthesis and glucose metabolism, may occur in immunocompetent cells. There have been several reports suggesting that BCAA supplementation can restore or regulate naturally acquired immunity. In previous cohort studies [24, 25], the BCAA supplementation groups showed elevated absolute lymphocyte counts. Bassit et al. [26] reported that BCAA supplementation restored the ability of peripheral blood mononuclear cells to proliferate in response to mitogens after long-distance intensive exercise. In a review article, Calder et al. [27] emphasized the necessity of BCAAs for lymphocytes to be able to synthesize protein, RNA, and DNA, and to divide in response to stimulation. Previous studies have shown that BCAAs increase the absolute lymphocyte number count [26, 28], and this may partly explain the reduced incidence of bacteremia in the BCAA group.

Nakamura et al. [29, 30] reported that, in patients with chronic liver disease, the phagocytic functions of neutrophils and the natural killer activity of lymphocytes obtained from patients with liver

cirrhosis were restored by oral supplementation with BCAAs. Even in patients with advanced cirrhosis, Kakazu et al. [31, 32] reported that an elevation of the BCAA level improved the function of myeloid dendritic cells, and that this was beneficial to immune function. Our previous retrospective study [16] showed that, particularly in Child-Pugh class C patients who underwent LDLT, the lymphocyte ratio tended to be higher in the BCAA group than in the non-BCAA group. The beneficial effects of BCAA supplementation on posttransplant bacteremia may reflect the restoration of immune function.

Perspective on BCAA Therapy for the Prevention of Posttransplant Bacteremia with Special Reference to Skeletal Muscle Loss

Sarcopenia is primarily used to describe skeletal muscle loss that occurs during aging [33]. It can occur in patients with a variety of chronic illnesses, such as cancer, cardiovascular disease, bone fractures, and chronic liver disease. Among patients with liver cirrhosis, >40 % were reported to have accompanying sarcopenia [34] (Table 15.3). Although there are many causes of sarcopenia in cirrhotic patients, one of the most important causes is thought to be malnutrition (Table 15.4). Malnutrition has been reported in 60–80 % of patients with cirrhosis. However, identification of malnutrition is extremely difficult in such patients [35, 36]. Measurements of albumin and prealbumin levels do not necessarily reflect nutritional status, because hepatocellular protein synthesis is usually impaired in these patients. The assessment and interpretation of body weight is also difficult because of the presence of ascites, pleural effusion, and peripheral edema. The nonprotein respiratory quotient (npRQ) is a unitless number estimated from carbon dioxide production, and is used to evaluate the nutritional status of liver cirrhotic patients [36], although its measurement is sometimes difficult to perform in the clinical setting.

Thus, sarcopenia evaluated by computed tomography may be the most objective measurement of malnutrition of cirrhotic patients. Recently, evaluation of muscle loss in patients with liver cirrhosis was reported to be an important and a novel predictor of survival. Montano-Loza et al. [34] showed that sarcopenia was not correlated with the degree of liver dysfunction evaluated by a conventional scoring system. Thus, the extent of sarcopenia can be an independent marker of survival in patients with end-stage liver failure. Recently, Englesbe et al. [37] and Kaido et al. [38] reported that central sarcopenia strongly correlates with mortality after OLT. Sarcopenia in candidates for LT may be due to an extended catabolic phase and muscle breakdown in those with liver failure.

Table 15.4 The causes of sarcopenia in the patients with liver cirrhosis

Classification	Effects of liver cirrhosis on muscle
Exercise	Disuse atrophy
Malnutrition	Caloric disturbance
	Protein disturbance
	Both
Metabolic change	Insulin resistance ↑
	Resting energy expenditure ↑
	Hyperammonemia
Humoral factors	BCAA ↓
	IGF 1 ↓
	Myostatin ↑

A number of complex metabolic alterations occur in liver disease that are unique to cirrhosis and affect skeletal muscle growth and atrophy responses. These include dysregulation of fatty acid oxidation and ketogenesis, gluconeogenesis from amino acids, glycogenolysis, and the use of BCAAs in skeletal muscle as a source of energy [21–23]. BCAAs also modulate signal transduction pathways as a messenger in skeletal muscle, preventing muscle atrophy. BCAAs, particularly leucine, have anabolic effects on protein metabolism by increasing the rate of protein synthesis and decreasing the rate of protein degradation in resting human muscle. These effects are likely to be mediated through changes in the signaling pathways controlling protein and glycogen synthesis. Therefore, BCAA deficiency can cause basic energy impairment in liver, skeletal muscle, and immune cells. On the other hand, as previously mentioned, decreased levels of BCAA were found in patients with septic OLT. Therefore, both sarcopenia and a high incidence of bacteremia may be due to low serum levels of BCAAs in posttransplant patients.

A more direct explanation of the relationship between sarcopenia and bacteremia could be provided by focusing on the profiles of other amino acids, such as glutamine. In plasma, the concentrations of threonine, glutamine, valine, cysteine, methionine, leucine, lysine, tryptophane, arginine, BCAAs, and the essential amino acids are correlated with age. These results indicate that there is an age dependency of the amino acid pattern in skeletal muscle and plasma. Stuerenburg et al. [39] showed that in myositis, which is a change seen in aged skeletal muscles, glutamate levels were significantly reduced. This study provides evidence that an alteration in glutamine levels is correlated with aging and might reflect increased proteolysis in aged and diseased human skeletal muscle. In chronic liver disease, skeletal muscle is able to seize ammonium during hyperammonemia, releasing glutamine [40]. In a recent experimental study, Jia et al. [41] administered carbon tetrachloride to rats repeatedly for 19 weeks as a decompensated cirrhosis model, and thereafter gave them a BCAA-enriched diet or normal diet for 5 weeks. Downregulation of fatty acid translocase/Cd36, glutamine synthetase, and pyruvate dehydrogenase kinase isoenzyme 4 is believed to promote lower uptake of fatty acids, lower ammonia incorporation, and higher uptake of glucose, and thus, to provide an energy source without using BCAAs. This study showed that, in patients with liver cirrhosis, serum BCAAs may be exhausted as a result of detoxication of ammonia in skeletal muscle. Glutamine would be produced in this process, and then disposed of via the liver.

Glutamine is the most abundant nonessential free amino acid in the healthy human body [40]. It is synthesized de novo, predominantly in skeletal muscle. Low glutamine levels, which have been detected in patients during critical illness, have been associated with poor outcomes [42]. Loss of muscle mass, with no evident upregulation of glutamine synthesis, has been inferred to contribute to insufficient glutamine production [43]. Thus, apparent glutamine deficiency, brought about by the increased glutamine requirements of immune cells, enterocytes, and hepatocytes, is thought to occur, and has led to the idea that glutamine is a "conditionally essential" amino acid in critically ill patients. It is widely accepted that immune system responses such as T-cell proliferation, B cell differentiation, macrophage phagocytosis, antigen presentation, cytokine production, and enterocyte barrier function target intestinal bacteria.

Under a stress condition, such as postliver transplantation and liver function deterioration, glutamine exhaustion may easily occur without BCAA supplementation. In these patients with glutamine exhaustion, bacteremia may occur owing to bacterial translocation from gut and/or impaired immunity (Fig. 15.2). Thus, especially in sarcopenic patients who have undergone liver transplantation, and who are supposed to have impaired glutamine production activity in their skeletal muscle, BCAA supplementation may be beneficial for maintaining glutamine metabolism.

By analyzing body composition, especially in the light of sarcopenia, custom-made nutritional support such as BCAA supplementation should be examined, and may be realized in the very near future.

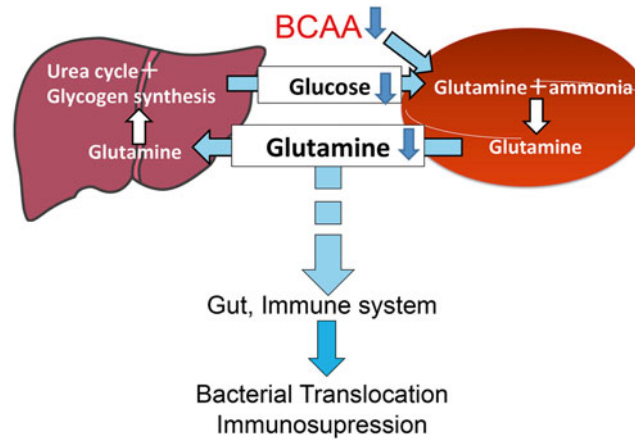

Fig. 15.2 Hypothesis for the development of posttransplant bacteremia in light of muscle-liver cross-talk. Decreased BCAA levels can cause glutamine deficiency and this may be a cause of enterocyte and immune system dysfunction. Alternative explanations of posttransplant bacteremia are possible, especially in patients with sarcopenia

Conclusions

Recently, the various effects of BCAAs on liver, skeletal muscle, and host immunity have been examined and demonstrated in patients with end-stage liver disease [44]. BCAA supplementation preliver and post-liver transplantation may provide essential nutritional support for preventing posttransplant bacteremia. This nutritional support may improve protein synthesis and naturally acquired immunity. Furthermore, in patients with sarcopenia, which is a commonly observed condition in candidates for liver transplantation, BCAA supplementation may improve the outcome after liver transplantation. Further large-scale clinical trials are necessary to test this hypothesis.

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Chapter 16
Branched Chain Amino Acids and Postoperative Quality of Life

Takehiro Okabayashi, Akihito Kozuki, Tatsuaki Sumiyoshi, and Yasuo Shima

Key Points

- Patients with chronic liver disease often reach a state of protein-energy malnutrition, which may influence patient outcomes following surgery and subsequent quality-of-life (QOL).
- Recent assessments of QOL integrate a biochemical health model with a social science model that is based on the patient's subjective perception of functioning and well-being across a range of physical, mental and social aspects of life.
- Since most liver neoplasms occur in patients with chronic liver disease, hepatic resection could potentially reduce QOL in these patients by further compromising liver function.
- Advances in surgical technology and perioperative management have led to hepatic surgical procedures, including liver resection and radiofrequency ablation, being the mainstay of curative treatment for not only hepatocellular carcinoma (HCC), but also metastatic liver tumours.
- Hepatic surgery is still associated with postoperative morbidities due to the inevitable deterioration of liver function following a reduction in functioning liver mass.
- In general, it is recommended that nutrition is individualized according to a patient's nutritional status and monitored to ensure well being and nutritional adequacy.
- Based on the clinical assessment, dieticians should therefore educate patients and carers about sodium and fluid restriction, and appropriate food choices.
- Branched chain amino acid (BCAA) nutritional supplementation improves postoperative QOL over the long term after hepatic resection by restoring and maintaining nutritional status and whole-body kinetics.
- BCAA may also inhibit carcinogenesis in heavier patients with cirrhosis and play a key role in liver regeneration.
- Individualized intervention is thus recommended based on patient's nutritional status.

Keywords Branched chain amino acids • Quality-of-life • Surgery • Liver • Liver cirrhosis • Chronic liver diseases

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Abbreviations

QOL	Quality-of-life
HCC	Hepatocellular carcinoma
BCAA	Branched chain amino acid
PEM	Protein-energy malnutrition
LES	Late-evening snack
GLUT	Glucose transporters
BM	Bone marrow

Introduction

In recent years there has been an increasing coincidence between a patient's point of view and the assessment of their health status. Traditional medical outcomes, which are important endpoints for clinicians, need to be integrated with patients' survival rate after adequate management for various diseases. However, it would be of note that it has been considered patients' opinions on health status, reflecting how they really feel, and how much their disease affects their way of living to be more important, because the treatment consensus over almost all disorders has been established to some extent.

Liver is the central organ for nutrient production and metabolism [1]. Patients with chronic liver disease often become severely malnourished, which can seriously damage their capacity for liver regeneration [2] and increase the risk of hepatocellular carcinoma (HCC). In particular, a state of protein-energy malnutrition (PEM) [3] affects patient outcomes following management as shown by quality-of-life (QOL) estimations [4]. Recent assessments of QOL integrate a biochemical health model with a social science model that is based on the patient's subjective perception of their physical, mental and social functioning and well-being [5].

Recent advances in surgical technology and perioperative management have made hepatic surgical procedures, such as liver resection and radiofrequency ablation, the mainstay of curative treatment for both primary and metastatic liver tumours [6]. However, since most liver neoplasms occur in patients with chronic liver disease, hepatic resection could potentially reduce QOL in these patients by further compromising liver function. Hence, it is important to consider QOL among the more traditional treatment outcomes of operative mortality and long-term survival rates. Some recent studies implicated an important role for nutritional support using branched chain amino acids (BCAA) in the surgical management and postoperative QOL of patients undergoing hepatic resection for liver neoplasms [7, 8]. In this article, we revisit the basic concept of BCAA administration and review how BCAA supplementation affects long-term self-estimated QOL and health in these patients.

Quality-of-Life in Patients with Chronic Liver Diseases

Evaluation of QOL

A QOL assessment concept was developed in the mid-1990s by integrating biochemical and social science models of health assessment [9, 10]. According to the modern concepts of health-related QOL, the principle criteria guiding a patient's acceptance of treatment are often subjective. In patients with hepatobiliary disease, this could include the patients' feelings about their potential QOL following suggested surgical, medical or palliative interventions. Indeed, such perceptions could be more relevant to acceptance of treatment than predicted length of life, because patients are frequently more concerned about quality and disability than about longevity [11]. This is especially true with chronic

Table 16.1 SF-36 and NHP score

SF-36	Physical functioning (PF)	PF scale measures the extent to which physical activities are limited for reasons of health
	Role physical (RP)	RP scale measures how physical health impacts work and daily activities
	Bodily pain (BP)	BP scale measures limitations due to pain
	General health (GH)	GH scale measures how a subject sees personal health and the potential for decline
	Vitality (VT)	VT scale measures how tired/full of energy subject feels
	Social functioning (SF)	SF scale measures how much physical or emotional problems interfere with normal social activities
	Role emotional (RE)	RE scale measures the impact of emotional problems on work and daily activities
	Mental health (MH)	MH scale measures the general state of feeling (e.g. depressed, happy, peaceful)
	Physical Component (Summary) Score (PCS): PCS summarizes the physical health component of SF-36: PF, RP, BP and GH	
	Mental Component (Summary) Score (MCS): MCS summarizes the mental health component of SF-36: VT, SF, RE and MH	
NHP	The Nottingham Health Profile is a multi-dimensional, 45-item questionnaire designed to measure subjective health status. Part 1 comprises six dimensions of health: physical mobility, pain, sleep, energy, social isolation and emotional reactions. Part 2 consists of seven aspects of daily life (i.e. paid employment, jobs around the house, social life, personal relationships, sex life, hobbies and interests and holidays)	

SF-36 short form-36 questionnaires, *NHP* the Nottingham health profile questionnaires

diseases, where survival is not at risk for a long time, and the goal of interventions is to maintain symptom-free and community-living patients.

This conceptualisation is based on the World Health Organization definition of health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity". This definition is so broad that it includes elements that are beyond the traditional domain of medicine and healthcare systems. Opportunity, education, spiritual attitudes, social security, working satisfaction, social relationships and goods availability are elements of QOL that are independent of medicine. Various questionnaires, usually self-administered, have been developed to assess QOL. From the 1990s on, there has been a growing emphasis on assessing QOL in patients with cancer, and this assessment may be as important as the evaluation of long-term survival. The most widely used generic instruments to assess QOL for patients with cancer are the European Organization for Research and Treatment of Cancer (EORTC) quality-of-life questionnaire (QLQ)—the EORTC QLQ-C30—and the Functional Assessment of Cancer Therapy—General (FACT-G) [12, 13]. More recently, a study about health-related QOL of chronic liver disease patients with and without hepatocellular carcinoma (HCC) reported that impaired QOL is not associated with the presence of cancer itself, but is dependent on the level of liver function, indicating the importance of preserving liver function [14]. In recent years, the Italian versions of the Medical Outcome Study Short Form-36 (SF-36) and the Nottingham Health Profile (NHP) questionnaires, two generic instruments assessing patients' well-being, have been validated and used to compare the impacts of chronic diseases in a general population and to determine health policies and resource allocation (Table 16.1) [15].

Poor Health-Related Quality-of-Life of Patients with Cirrhosis

Globally, cirrhosis/chronic liver disease of varying aetiologies is responsible for major mortality and morbidity. Liver cirrhosis is one of the commonest causes of hospitalisation, and a number of studies over the past decade have convincingly demonstrated that health-related QOL is significantly impaired in patients with cirrhosis compared to the general population (Fig. 16.1) [16].

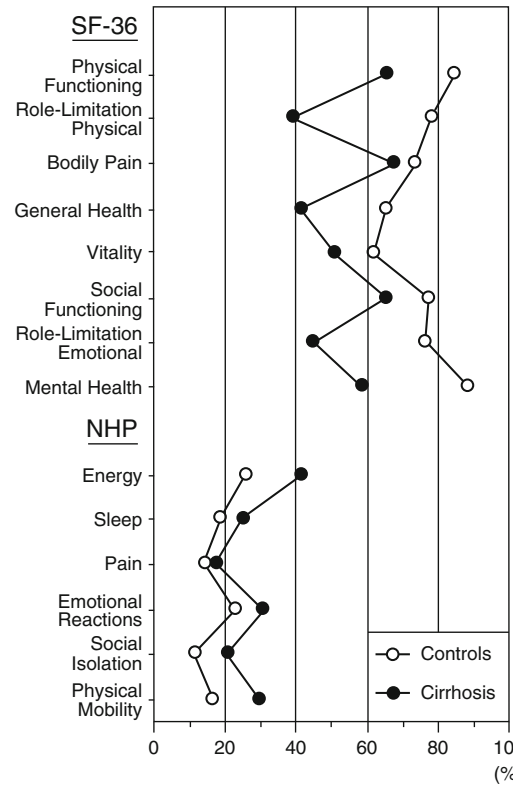

Fig. 16.1 Evaluation of QOL in cirrhosis according to both SF-36 and NHP score [16]. All domains of the SF-36 scored significantly lower in cirrhosis in comparison to normative population [23]

Hepatic Encephalopathy

Hepatic encephalopathy is the occurrence of confusion, an altered level of consciousness and coma as a result of liver failure. In the advanced stages it is called hepatic coma or coma hepaticum. The mildest form of hepatic encephalopathy presents as forgetfulness, mild confusion and irritability, and thus is difficult to diagnose clinically, but it may be demonstrated on neuropsychological testing. The progression of hepatic encephalopathy is characterised by an inverted sleep-wake pattern (sleeping by day, being awake at night), followed by lethargy and personality changes, then by worsened confusion, and finally, a progression to coma [17]. Hepatic encephalopathy thus has a significant impact on the patient's health-related QOL. In addition, minimal hepatic encephalopathy is a part of the spectrum of overt hepatic encephalopathy, with a characteristic cognitive profile that cannot be diagnosed clinically [18]. On follow-up, patients with minimal hepatic encephalopathy are more likely to develop overt hepatic encephalopathy, as compared with cirrhotics without minimal hepatic encephalopathy [19]. Hepatic encephalopathy is associated with poor prognosis and is an independent predictor of survival [20].

Hyperammonaemia

Hyperammonaemia is a condition characterised by raised serum ammonia levels. Mild and transient hyperammonaemia can often be asymptomatic and is usually triggered by protein loads and catabolic states. In symptomatic cases, the clinical features may be variable and episodic. Many cases present with acute mental status changes characterised by confusion, personality changes, irritability, ataxia,

visual disturbance, lethargy and somnolence may also report nausea, vomiting and hyperventilation. More severe cases can lead to encephalopathy characterised by stupor and coma. The pathogenesis of hyperammonaemic encephalopathy remains unclear. Changes in mental status have been attributed to high levels of ammonia and the presence of other organic acids, with raised brain ammonia concentrations sometimes present even when the serum ammonia level is normal [21]. Hyperammonaemia can also cause encephalopathy via the inhibition of glutamate uptake by astrocytes [22]. The resulting astroglial processes surround the brain microvessels of the blood–brain barrier and swell in the presence of advanced hepatic encephalopathy. However, despite these significant astroglial changes, the barrier function remains intact, suggesting that cytotoxic rather than vasogenic mechanisms predominate in the pathogenesis of hepatic encephalopathy [23]. As with delirium, elderly patients may present with several concomitant predisposing factors for developing hyperammonaemia and encephalopathy.

Hepatitis Viral Infection and Health-Related QOL

Chronic viral hepatitis infection has been associated with a significant reduction in health-related QOL that is not related to the severity of liver disease. Possible pathophysiological mechanisms affecting QOL in such cases of HCV infection include alterations in mood (increased anxiety and depression) and cognition, together with changes in both the midbrain serotoninergic and striatal dopaminergic systems, irrespective of viraemia or state-ofliver function [24]. The existence of brain alterations directly caused by HCV is evidenced by reported deficits in attention, executive function and verbal learning, by electroencephalogram recordings slowing in the absence of liver cirrhosis and/or substance abuse disorder, and atypical changes on magnetic resonance spectroscopy in HCV-infected patients. It remains controversial whether QOL in patients with HBV-associated cirrhosis is reduced compared to that in patients with HCV-associated or HBV/HDV-associated cirrhosis. Another study showed less impairment of QOL in patients suffering from HBV-associated cirrhosis compared to other causes of cirrhosis such as HCV or cholestasis [25]. However, overall the number of chronic hepatitis B patients reporting reduced QOL compared to patients with hepatitis C was small. Hence, more studies are required to confirm the poor QOL in patients with hepatitis B viral infection.

Serum Sodium and Ascites

A previous study demonstrated the importance of serum sodium concentration for health-related QOL in cirrhosis, whereby patients with hyponatraemia reported a marked impairment in health-related QOL compared to patients with normal serum sodium concentration [26]. Hyponatraemia is common in patients with cirrhosis and ascites and its frequency increases with disease progression [27]. Hyponatraemia with concomitant hypo-osmolality is associated with an adaptive response of the central nervous system aimed at preventing the passage of fluid from the extracellular to the intracellular space and the development of cerebral edema. In patients with marked hyponatraemia, a low-grade cerebral edema exists despite this adaptive brain response and increased ammonia levels also play a role in low-grade cerebral edema [28]. The value of serum sodium concentration in the prediction of health-related QOL persisted after adjustment for possible confounding factors related to the severity of liver failure. Furthermore, serum sodium level was an independent predictive factor in both physical and mental summary scores of health-related QOL, and in six of the eight domains of the SF-36: role physical, physical functioning, general health, social functioning, vitality and mental health [26].

QOL in Patients Undergoing Liver Resection

Branched Chain Amino Acids (BCAA) Improved Both PEM and QOL

Protein-energy malnutrition (PEM) is a common finding in chronic liver disease and affects about 50 % of patients with liver cirrhosis [4]. Since malnutrition adversely affects clinical outcomes, guidelines of the European, American and Japanese Societies for Parenteral and Enteral Nutrition advocated nutritional support for cirrhotic patients [29, 30], recommending the following consensus nutrition standard: 35–40 kcal/kg/day in energy and 1.2–1.5 kcal/kg/day in proteins [29]. However, such standards are not always pertinent and should be altered depending on conditions such as race, intensity of daily activity, PEM, glucose intolerance, protein intolerance and obesity. Flexible handling of the ESPEN guideline is therefore necessary, and calorimetry might be the best way to assess the nutritional status of patients with liver cirrhosis. Possible treatments for PEM include BCAA supplementation and a late-evening snack (LES). BCAA effectively corrects protein malnutrition by increasing plasma albumin, and prolongs event-free survival in patients with advanced cirrhosis [4]. Since energy deficiency is related to worse survival rates, it is also important to address the early-morning energy starvation that is typical in cirrhosis and equivalent to a 3-day starvation period in healthy individuals. To this end, the ESPEN and ASPEN guidelines recommend a LES or divided meals to reduce the starvation period between dinner and breakfast. BCAA as a LES is an ideal supplement in patients with cirrhosis because it provides more energy and protein than the ordinary enteral formula or BCAA granules, significantly reduces fatigue, and improves energy metabolism, protein levels and nitrogen balance [31]. Furthermore, a Hong Kong study also showed that long-term dietary supplementation with BCAA twice daily significantly reduced complications such as ascites and peripheral edema, and improved survival in patients undergoing chemoembolization for HCC [32].

QOL After Curative Hepatectomy

Postoperative QOL

Since most liver neoplasms occur in patients with chronic liver disease, hepatic resection could potentially reduce QOL in these patients by further compromising liver function. QOL assessment has proven to be a valuable parameter for such patients and surgeons and may be helpful in determining the optimal treatment. As an outcome parameter, QOL is considered as important as disease-free and overall survival. In various benign and malignant liver diseases, surgical management is a common procedure with intent-to-cure treatment, as a result of recent advances in surgical technology and perioperative management. Although major and minor liver resections are both safe procedures, little is known about postoperative QOL in these patients [33]. Recent studies indicated that QOL returns to baseline within 3–6 months after liver resection for malignancies in most cases (Fig. 16.2) [34].

BCAA-Enriched Nutritional Support in Surgical Patients (Table 16.2)

In a prospective randomized clinical trial, the San-in Group of Liver Surgery [13] studied the effects of long-term oral administration of BCAA after curative resection of HCC. Between 2 and 3 weeks after surgery, 75 patients were randomized to receive oral BCAAs (Aminoleban EN) at 100 g/day for 1 year, and another 75 patients were assigned to a control group. Flapping tremor was less common, body weight was increased and performance status was better in the BCAA-treated group than in

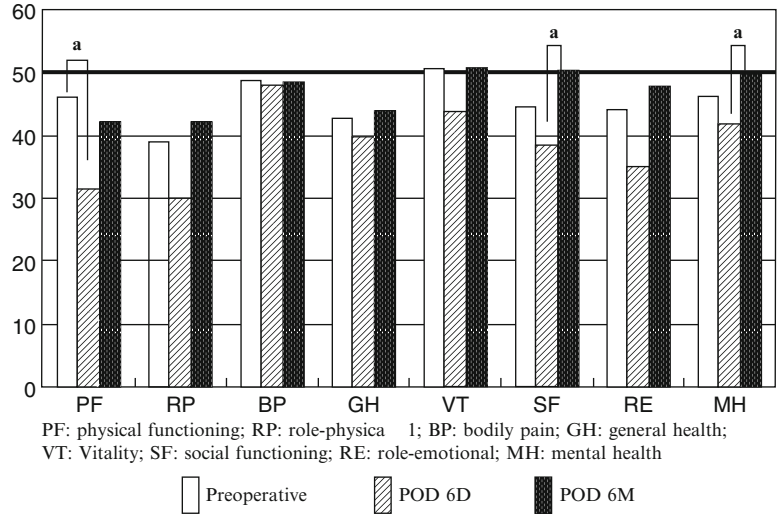

Fig. 16.2 Postoperative QOL after liver surgery ($^{a}P<0.05$) [34]. The scores of all eight parameters also decreased immediately after the operation (at POD 6D). However, they recovered at POD 6M to levels comparable to those before the operation [34]

Table 16.2 Effect of BCAA-enriched nutritional support in surgical patients with liver cancer

Author [reference]	Study design	Administration	Benefits of BCAA
San-in group [13]	Prospective 150 patients	Aminoleban EN	• Improved clinical features (body weight) • Improved laboratory data (red blood cell, serum albumin level and Fischer molar ratios)
Meng et al. [8]	Prospective 44 patients	Aminoleban EN	• Shorter postoperative hospital stay • Higher haemoglobin level, higher sodium level, higher albumin level and lower bilirubin during the postoperative course
Togo et al. [36]	Retrospective 43 patients	LIVACT	• Rapid improvement in protein metabolism and inhibition of progression to liver cirrhosis
Okabayashi et al. [34]	Retrospective 36 patients	Aminoleban EN	• Shortened hospitalisation after surgery • Restoration of peripheral lymphocyte count and serum total cholesterol level at 3 months after the operation
Okabayashi et al. [37]	Retrospective 112 patients	Aminoleban EN	• Reduced morbidity associated with postoperative complications • Shortened duration of hospitalisation
Ishikawa [38]	Prospective 24 patients	Aminoleban EN	• Higher serum erythropoietin levels after liver surgery
Okabayashi et al. [35]	Prospective 96 patients	Aminoleban EN	• Significant improvement in QOL after hepatectomy • Restored and maintained nutritional status
Ichikawa et al. [39]	Prospective 56 patients	LIVACT	• Reduced early recurrence after hepatic resection in patients with HCC

controls throughout the 1-year period (Fig. 16.3a). BCAA treatment also significantly increased red blood cell and serum albumin levels in patients with Child grade B and C disease. Substantially similar effects were observed in patients treated with major hepatic resection. The San-in Group summarized that long-term oral nutritional support with BCAAs after resection of HCC is beneficial in

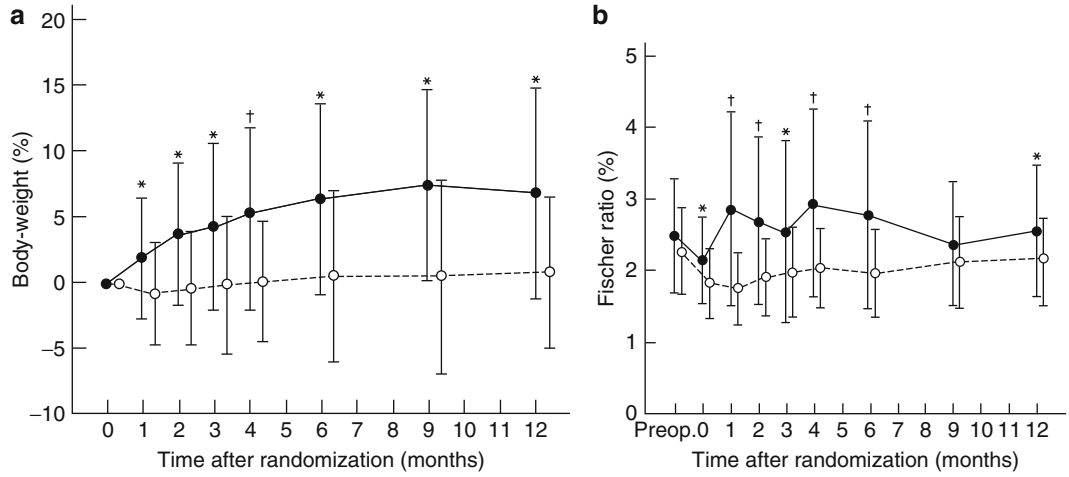

Fig. 16.3 The effect of BCAA [13]. Figure 16.3a, Percentage changes in body-weight in patients treated with BCAA (*black circle*) and controls (*white circle*). Values are mean (standard deviation). *$P<0.01$, †$P<0.001$ versus control group. Figure 16.3b, Changes in Fischer's molar ratio in patients with BCAA (*black circle*) and controls (*white circle*). Values are mean (standard deviation). *$P<0.05$, †$P<0.001$ versus control group [13]

improving clinical features and laboratory data without increasing the rate of tumor recurrence, particularly in patients with advanced cirrhosis or after major hepatic resection (Fig. 16.3b).

In a prospective study, Meng et al. [8] evaluated the effect of BCAA treatment in patients undergoing liver resection for HCC. A prospective randomized controlled clinical trial was conducted involving 44 patients. The BCAA group (21 patients) received Aminoleban EN in addition to a normal diet for 12 weeks and the control group (23 patients) received an isonitrogenous and isocaloric diet only. The BCAA group had a shorter hospital stay, and showed a significantly higher haemoglobin level, higher sodium level, higher albumin level and lower bilirubin level during the postoperative course (Fig. 16.4). The authors concluded that Aminoleban EN is safe to administer and does not have significant adverse effects, while contributing to a shorter hospital stay and quicker improvement of liver function in the early postoperative period (Fig. 16.4).

In a retrospective study involving 43 elective hepatectomized patients, Togo et al. [36] evaluated the usefulness of granular BCAA after hepatectomy for liver cancer complicated with liver cirrhosis. In the BCAA group (21 patients), postoperative ascites and edema tended to improve earlier than in the control group (22 patients), and nutritional status based on serum albumin and total protein levels recovered immediately after liver surgery in the BCAA group. Furthermore, the BCAA group showed a more rapid improvement in hyaluronic acid and type IV collagen 7S levels compared to controls.

In a large retrospective study involving 112 elective hepatectomized patients, Okabayashi et al. [37] evaluated the effects of BCAA-enriched nutrient support for patients undergoing liver resection for HCC. These patients were divided into two groups: 40 patients received perioperative supplementation of a BCAA-enriched nutrient mixture (BCAA group) and 72 patients had no supplement (control group). Laboratory data, postoperative complications, duration of hospitalisation and survival were compared between groups. The overall incidence of postoperative complications was lower in the BCAA group (17.5 %) than in the control group (44.4 %) ($P=0.01$). Among the postoperative complications, surgical site infection and bile leakage were observed in 5 % of patients in the BCAA group and in 15.3 % and 12.5 % of patients in the control group, respectively. Ascites appeared after the surgery in 7.5 % of patients in the BCAA group and in 16.7 % of control patients, while the duration of hospitalisation was significantly shorter in the BCAA group than in the control group ($P<0.05$).

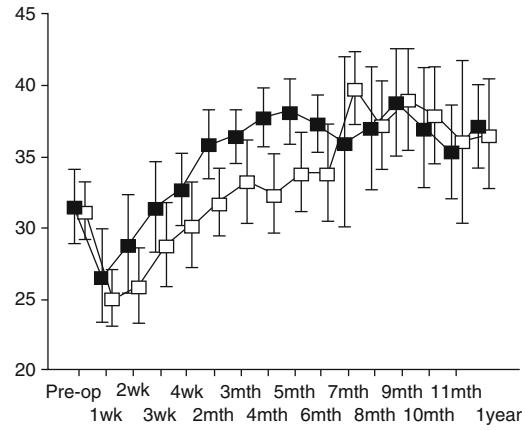

Fig. 16.4 The effect of BCAA on postoperative albumin levels [14]. Mean albumin level (g/dL; 95 % confidence interval). (*Black square*), BCAA group; (*white square*), control group. Albumin level was significantly different in favour of the BCAA group in the postoperative period [14]

The authors suggested that their perioperative BCAA supplementation protocol is clinically beneficial in reducing the morbidity associated with postoperative complications and in shortening the duration of hospitalisation of patients with chronic liver disease who undergo liver resection for HCC.

In a prospective study involving 24 elective hepatectomized patients, including some with a non-hepatitis liver, Ishikawa et al. [38] studied the benefits of perioperative oral nutrition (ON) with BCAA. The patients (20 with malignant liver tumors and 4 with benign liver tumors) were randomly assigned to receive perioperative ON with BCAA (11 patients, BCAA group) or a usual diet (13 patients, control group). The BCAA group received a BCAA supplement twice daily plus a usual diet for 14 days before operation and on days 1–7 after operation. Two of the eleven patients in the BCAA group developed postoperative complications, as compared with 3 of the 13 patients in the control group (18.2 % vs. 23.1 %, $P=0.7686$). Among patients with non-hepatitis, serum erythropoietin (EPO) levels on POD 3, 5 and 7 were significantly higher in the BCAA group than in the control group ($P=0.0174$, $P=0.0141$ and $P=0.0328$, respectively). The short-term ON support with BCAA was thus associated with higher serum EPO levels in patients with non-hepatitis who underwent curative hepatic resection, and higher EPO levels might be beneficial in protecting liver cells from ischemic injury and preventing intraoperative haemorrhage associated with lower perioperative levels of alanine aminotransferase and aspartate aminotransferase in serum.

In a prospective randomized clinical study, Okabayashi et al. [35] assessed the impact of oral supplementation with BCAA-enriched nutrients on postoperative QOL in patients undergoing liver resection. To our knowledge, this was the first prospective clinical study evaluating an association between perioperative supplementation of BCAA and postoperative QOL. Patients were randomly assigned to receive BCAA supplementation (BCAA group, $n=48$) or a conventional diet (control group, $n=48$). Postoperative QOL and short-term outcomes were regularly and continuously evaluated in all patients using a short-form 36 (SF-36) health questionnaire and by measuring various clinical parameters. This study demonstrated a significant improvement in QOL after hepatectomy for liver neoplasm in the BCAA group based on the same patients' preoperative SF-36 scores (Fig. 16.5). Perioperative BCAA supplementation preserved liver function and general patient health in the short term compared to a normal diet. The authors on this study concluded that BCAA supplementation improves postoperative QOL after hepatic resection over the long term by restoring and maintaining nutritional status and whole-body kinetics.

In a prospective randomized clinical study, Ichikawa et al. [39] studied the effect of oral supplementation with BCAA on the development of liver tumorigenesis after hepatic resection in HCC patients. Fifty-six patients were randomly assigned to receive either BCAA supplementation orally for 2 weeks before and 6 months after hepatic resection (BCAA group, $n=26$) or a conventional diet

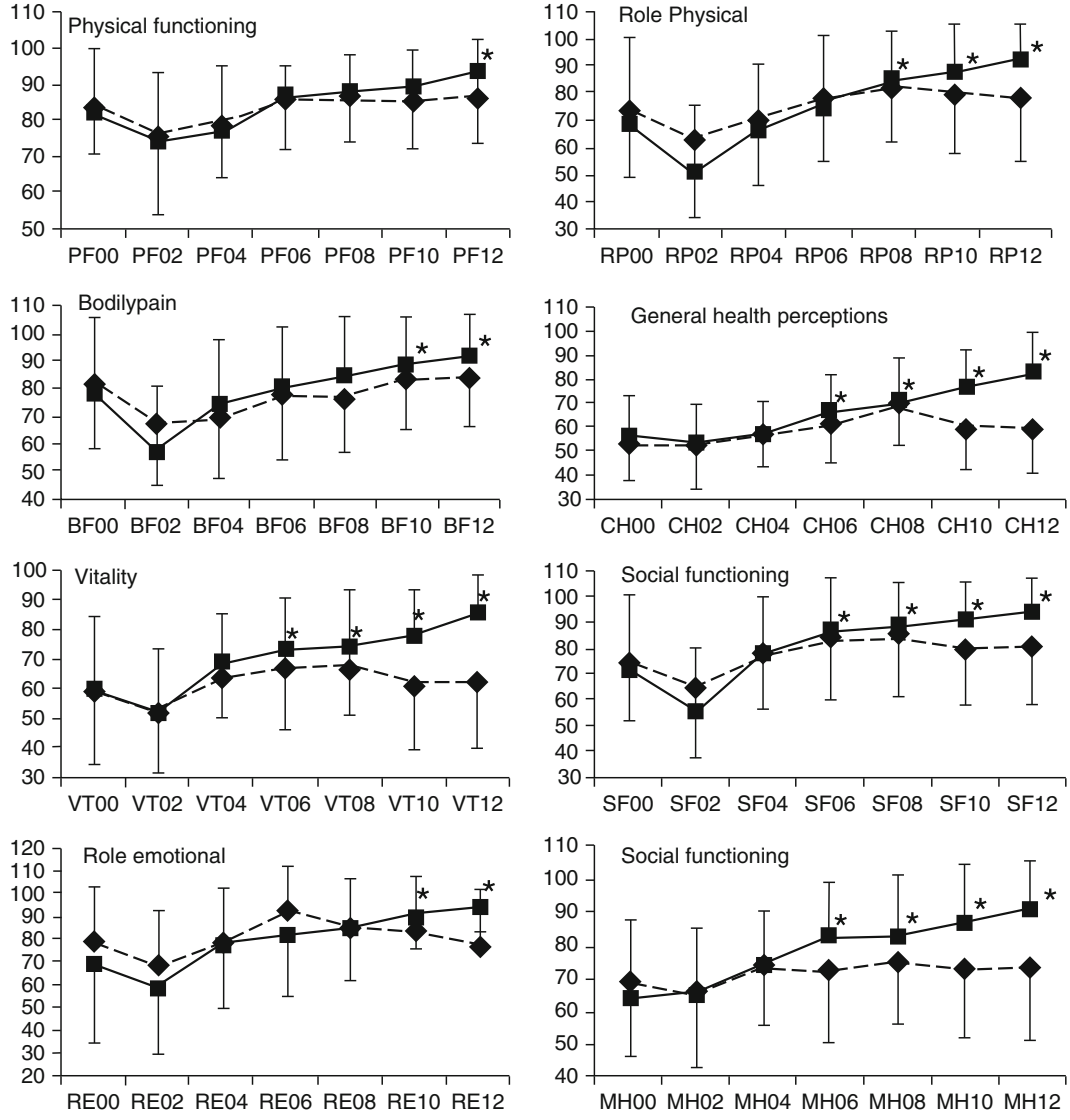

Fig. 16.5 Trends of Norm-based score by SF-36 in the group administered BCAA and the control group [35]. Trends of Norm-based score by SF-36 in the group administered BCAA (*solid line*) and the control group (*broken line*) (*$P<0.05$, compared to preoperative score) [35]. All scores decreased immediately after the operation. However, general health measures including perceptions of health and well-being, vitality, social functioning, and mental health improved by 6 months postoperative to at least preoperative levels in the AEN group [35]

(control group, $n=30$). Postoperative tumor recurrence was continuously evaluated in all patients by measuring various clinical parameters. Recurrence rate at 30 months after surgery was significantly better in the BCAA group than in controls. Interestingly, tumour markers, including AFP and PIVKA-II, significantly decreased at 36 months after liver resection in the BCAA group in comparison to the control group. These findings therefore indicated that oral supplementation of BCAA also reduces the incidence of early recurrence after hepatic resection in patients with HCC, and this treatment regimen offers potential benefits for clinical use in such patients, even in cases with a well-preserved preoperative liver function.

BCAA Improved Perioperative Insulin Resistance

HCV infection causes insulin resistance [40], which is a known risk factor for HCC and reduced long-term survival. Insulin resistance is therefore a potential therapeutic target in patients with HCV infection. BCAA may also play an important role in improving insulin resistance, and in experimental studies using rodents, BCAA induced glucose uptake in skeletal muscle, adipocytes and hepatocytes. Furthermore, in a rat model of liver cirrhosis induced by CCl_4, leucine and isoleucine promoted glucose uptake in skeletal muscle [41]. This effect might occur due to up-regulation of the glucose transporters 4 and 1 (GLUT4 and GLUT1) and/or the rapamycin-dependent activation of glucose synthase in skeletal muscle. Interestingly, a recent human study [42] found that oral supplementation of BCAA for 4 and 6 weeks reduced HOMA-IR in two cases with HCV-related liver disease.

Glucose metabolism is generally adversely affected in patients following major surgery, with hyperglycemia a possible result of postoperative insulin resistance due to reduced glucose uptake by skeletal muscle, adipose tissue and liver [43]. Patients may also develop hyperglycemia due a combination of surgical stress and postoperative insulin resistance. Indeed, insulin resistance after major surgery is well documented and its development is related to the magnitude of surgery [44]. Insulin infusion support to maintain normal glucose levels thus reduces morbidity and mortality rates in critically ill patients, and preoperative management of whole-body insulin resistance is required. Interestingly, preoperative oral administration of carbohydrate reduces postoperative insulin resistance in patients with colorectal resection [43]. Furthermore, short-term infusion of amino acids following colorectal surgery can reduce insulin resistance since endogenous glucose production and glucose clearance is decreased [45]. However, it is uncertain whether preoperative dietary supplementation with carbohydrates and BCAA improves postoperative insulin resistance.

To address this question, Okabayashi et al. [40] conducted a randomized clinical trial in which 26 patients undergoing a hepatectomy for the treatment of a hepatic neoplasm either received a supplement of carbohydrate and BCAA prior to surgery or had no supplement. The postoperative blood glucose level and the total insulin requirement for normoglycemic control during the 16 h following hepatic resection were determined using a closed-loop glycemic control system. Postoperative insulin requirements for normoglycemic control in the group with preoperative nutritional support was significantly lower than that in the control group ($P = 0.039$), indicating that preoperative oral administration of carbohydrate and BCAA is clinically beneficial and reduces postoperative insulin resistance in patients undergoing hepatic resection.

Future: Improving Postoperative QOL According to Liver Regeneration Following the Administration of BCAA

Liver failure is a potentially life-threatening condition for which organ transplantation is the only definitive therapy. However, the current shortage of available livers for transplant results in the death of many patients while awaiting transplantation. Thus, it is imperative that new approaches for repairing the liver are developed, so that the need for transplanting a partial or complete human liver to cure the patient can be eliminated. Presently, cell-based therapies represent one of the most promising alternative solutions to entire or partial liver transplantation. Unfortunately, human livers would still be required as a source of cells and the isolation of human hepatocytes remains difficult and inefficient. Furthermore, differentiated hepatocytes cannot yet be effectively expanded in culture, greatly limiting the cell numbers obtained from each liver. Numerous studies have therefore concentrated on culturing and differentiating stem cells from different sources that can be readily isolated using non-invasive procedures, to give rise to hepatocytes both in vitro and in vivo. An added advantage of a stem-cell approach is that many of these cell populations can be expanded significantly in vitro,

making it possible to generate large numbers of cells for transplantation from a fairly small initial number. Since some of these stem cell populations are present within the adult, and could thus be isolated from the patient to be treated, the production of personalized, immunologically matched hepatocytes is possible [46].

Liver regeneration is unique among organ systems [47]. In the adult liver, there are two major populations of cells that have been thought to explain liver regeneration and/or repair. The first consists of unipotential cells, specifically hepatocytes and bile duct epithelial cells that regenerate during normal tissue turnover. The second population consists of bipotential cells, intrahepatic liver stem cells and/or oval cells that can differentiate into hepatocytes and bile duct epithelial cells. Recent reports suggest that bone marrow (BM) stem cells may harbor unexpected developmental plasticity [48], although it remains unclear precisely how and to what extent BM cells contribute to liver regeneration, and whether it is by cell fusion, transdifferentiation or both. Previous studies reported that under certain conditions BM cells are recruited into the liver and become not only Kupffer cells, endothelial cells, oval cells, stromal cells and cholangiocytes, but also functioning hepatocytes [48]. Further examination is required to address the possible association between BCAA and liver regeneration, especially the mechanisms by which BM-derived extra-hepatic stem cell develop after liver resection with perioperative BCAA supplementation.

Liver cirrhosis negatively impacts the patient's nutritional status, with derangements in energy expenditure and in protein, carbohydrate and fat metabolism. Common complications of cirrhosis such as ascites, hepatic encephalopathy and esophageal varices require appropriate nutritional intervention. However, for patients with hepatic encephalopathy, the evidence is controversial; while some studies indicate that protein restriction is beneficial, others found that this strategy does not have apparent benefits in acute encephalopathy. For patients unable to tolerate animal proteins, other protein sources should be considered, such as proteins from vegetables or BCAA-enriched formulations [46]. In general, it is recommended that nutrition is individualized according to a patient's nutritional status and monitored to ensure well being and nutritional adequacy. Following the assessment, dieticians should educate patients and carers about sodium and fluid restriction and appropriate food choices. Nutrient-dense meals, snacks and oral supplements are recommended.

Conclusions

Supplementation of BCAA has been shown to improve the nutritional status and QOL in patients with cirrhosis, preventing complications and prolonging survival, including in those patients undergoing chemoembolization and liver resection for HCC. BCAA may also inhibit carcinogenesis in heavier patients with cirrhosis and play a key role in liver regeneration. Individualized intervention is recommended based on each patient's nutritional status.

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Part IV
Branched Chain Amino Acid Supplementation Studies in Certain Patient Populations

Chapter 17
Leucine-Protein Functional Adaptation in the Clinical Setting

Leonidas G. Karagounis, Magne Hugues, and David S. Rowlands

Key Points

- Skeletal muscle homeostasis is imperative for maintaining metabolic health and functional capacity of the individual.
- Loss of muscle mass is associated with impaired whole-body metabolic outcomes and function of skeletal muscle, leading to increased incidence of disease and social dependency.
- Metabolic disease and reduced physical capacity may be attenuated by dietary and exercise interventions aimed at promoting skeletal muscle anabolism
- Leucine, BCAA, or leucine-rich protein increase skeletal muscle protein synthesis rates.
- Aging-related disablement, disuse atrophy, cancer cachexia, and weight management may benefit from dietary leucine or leucine-rich protein supplementation.
- In elderly, 35 g whey protein may act as an optimal stimulant to muscle protein accretion, but as little as 6.7 g of EAA (2.8 g leucine) stimulated protein synthesis.
- Chronic leucine supplementation had no impact on skeletal muscle protein accretion and reduced plasma valine, suggesting a balance of amino acids or whole protein are required to achieve chronic gains in muscle mass.
- During immobility, some promising results obtained with BCAA/EAA supplementations highlight the potential benefit of leucine alone, but requires further investigation.
- Leucine supplementation under cancer cachexia condition appears efficient in maintaining both muscle anabolism and muscle mass, but these positive effects could be improved when leucine is combined with other nutrients or physical activity.
- There is some evidence for beneficial effects of leucine-rich proteins for mass retention with weight loss, but clinical studies are lacking to establish a clear benefit of free leucine supplementation in obese patients under caloric restriction.

Keywords Aging • Sarcopenia • mTOR • Cachexia • Weight loss • Obesity • Leucine threshold • Muscle protein synthesis

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Abbreviations

BCAA	Branched chain amino acids
EAA	Essential amino acids
mTOR	Mammalian target of rapamycin
PKB	Protein kinase B
MPS	Muscle protein synthesis
MPB	Muscle protein breakdown
HMB	β-Hydroxy-methylbutyrate
SPPB	Short Physical Performance Battery score

Introduction

Skeletal muscle is the most abundant tissue in the human body and its homeostatic regulation is imperative for maintaining metabolic health and functional capacity throughout life. This homeostatic profile is due to its remarkable plasticity and ability to adapt to numerous and varied environmental stimuli, such as, contractile activity and nutrient availability [1–4]. The adaptive ability of skeletal muscle is regulated, in part, by the rate of synthesis and breakdown, and hence turnover of skeletal muscle proteins (Fig. 17.1); turnover, therefore, determines the quality and quantity (protein balance) of muscle protein and intern the functional phenotype of the tissue. Any change in the rate of protein synthesis or degradation may result in net increases or decreases in skeletal muscle protein mass. On one hand, failure to maintain the appropriate skeletal muscle mass equilibrium can cause a dramatic loss of skeletal muscle mass and tissue function (contractile performance, strength) as in the case of disuse or disease-induced sarcopenia can lead to improved metabolic health and tissue function. On the other hand, under conditions of adequate nutrition and physical activity, muscle hypertrophy is observed. The aim of this chapter is to highlight the role leucine, a BCAA in maintaining muscle mass in several disease states as associated with sarcopenia, and in clinical weight management.

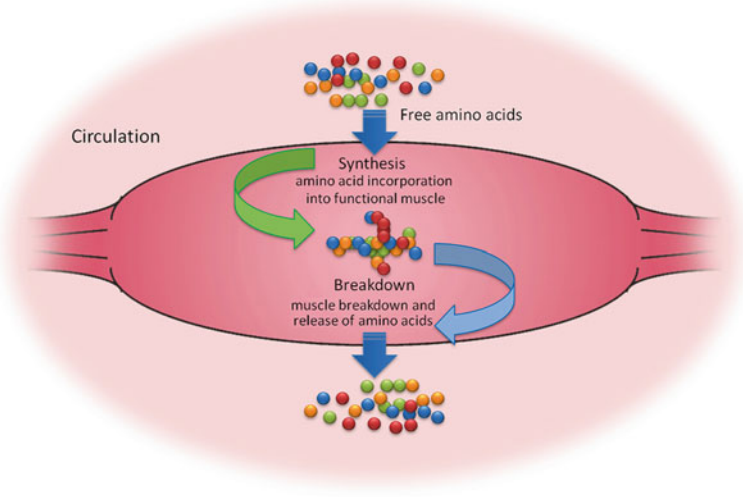

Fig. 17.1 Schematic showing the constant turnover of skeletal muscle. Free amino acids are taken up by the muscle and incorporated into protein while at the same time protein is broken down into free amino acids and released into the circulation

The Role of Amino Acids on Skeletal Muscle Protein Balance

As early as 1975, Buse and colleagues identified leucine as a possible regulator of skeletal muscle protein turnover [5] followed by a study in 1981 demonstrating the effect of feeding BCAAs on muscle protein synthesis in fasted rats [6] and humans [7]. A further subsequent torrent of research has confirmed BCAAs as potent stimulators of anabolic response in skeletal muscle. Work in vitro and in vivo has determined leucine as the most potent of the BCAAs [8, 9]. Elevated extracellular leucine following feeding elicits its effect on skeletal muscle by stimulating protein synthesis and inhibiting breakdown in the postprandial state [10–12]. However, the underlying molecular mechanisms, by which such changes in skeletal muscle protein turnover are regulated, are far from clear. Current knowledge has identified the insulin sensitive phosphoinositide pinase-3 (PI3K)/protein kinase B (PKB) cascade and downstream targets, mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinase (p70S6k) as being implicated in skeletal muscle protein synthesis by promoting protein translation and increasing rates of protein synthesis [13]. In contrast, a principal regulator of muscle proteolysis is the ATP-dependent ubiquitin-proteasome system (UPS). This system involves the ubiquitination of proteins by the ubiquitinating ligases muscle atrophy F-box (MAFbx/Atrogin 1) [14] and the muscle RING finger (MuRF1) [15] for degradation via the 26S proteasome complex. However, recent reports have suggested a possible mechanism interlinking the activation of the PKB signaling pathway and its regulation of the ubiquitin-proteasome dependent protein degradation [16].

The stimulatory effect of leucine on muscle protein synthesis is primarily mediated by the modulation of intracellular kinases such as mTOR and p70S6K kinase [17, 18] and are partially responsible for the translational process of proteins. Leucine is also insulinotropic in vivo, demonstrating the ability of leucine to modulate glucose homeostasis [19]. The underlying mechanisms by which leucine promotes and/or enhances insulin secretion from the pancreatic β-cell have not yet been fully elucidated. It is currently proposed that leucine stimulates insulin release in the pancreas via its mitochondrial oxidative decarboxylation as well as by activating glutamate dehydrogenase in the β-cells [20, 21]. Finally, leucine can act synergistically with exercise to increase the efficiency of muscle protein metabolism following exercise [22].

Leucine Supplementation and Sarcopenia

During the normal ageing process there is the propensity for a reduction in the metabolic and force generating capacity of skeletal muscle through multiple mechanisms. The *disablement pathway* [23] has classified these changes at a cellular level to include the attenuation of mitochondrial activity [24], reduced rate of muscle protein synthesis [25], impaired neuromuscular activation, and increased rates of muscle protein breakdown and inflammation [26], together leading to reduced skeletal muscle functionality, and ultimately to societal dependency (Fig. 17.2).

These observed multifactorial age-related attenuations in muscle mass and physical function have previously been defined at previous consensus meetings and encompassed under the term *sarcopenia* [27, 28]. The incidence of sarcopenia may be further amplified by the age-associated decline in physical activity and reduction in the amount and quality of nutrient ingestion commonly observed in elderly individuals [29, 30]. Such metabolic and physiological alterations to the muscle milieu contribute to the declines in physical function and mobility in the elderly, which in turn may lead to increased fall incidence and the inability to function independently in their society [28].

A principle cellular second messenger signaling system which is known to link both nutrient and mechanical stimuli to homeostasis of skeletal muscle mass is the mTOR pathway [31, 32]. However, the sensitivity and activation of this pathway as well as the rate of muscle protein synthesis in

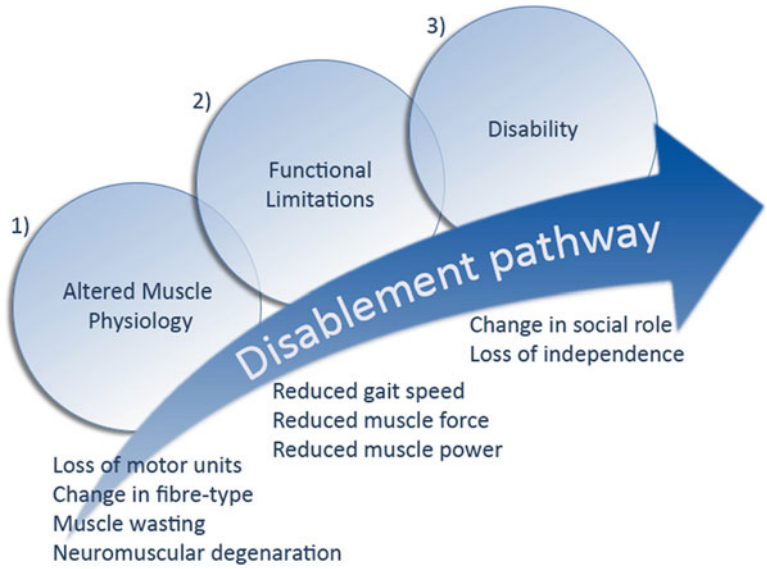

Fig. 17.2 Schematic representation of the disablement process leading to increased disability. Altered muscle physiology results in functional limitations leading to disability

response to BCAAs are attenuated in the elderly compared to young adults [25] (suggesting an age-related anabolic resistance). Age-related anabolic resistance may be reversed, or at least partly attenuated, by nutrition and exercise interventions. To this end, various nutritional interventions for reversing the anabolic resistance have been proposed and tested with different degrees of success including increasing leucine availability in isolation or in combination with protein. More specifically, it was recently shown that whey protein ingestion is able to stimulate skeletal muscle protein accretion with 35 g of whey protein acting as an optimal stimulant of this process in elderly men [33]. Data from animal studies suggest that leucine-rich ingested protein induced increased blood leucine concentrations (~×2 above fasting baseline) are able to restore the postprandial stimulation of both muscle protein synthesis and inhibition of protein degradation in elderly humans [34, 35]. For instance Katsanos et al. [35] showed that the ingestion of 6.7 g of EAA containing 41 % leucine resulted in increased muscle protein synthesis anabolism in elderly subjects (mean age 66 years ± 2.2) and this response was similar to that observed in young adults (28.8 ± 2.6). These observations were supported by Rieu et al. [36] who evaluated the impact of meals enriched with leucine on muscle protein synthesis in the elderly (males, mean age: 69.5 ± 0.8 years). Subjects received semiliquid meals administered over a 5-h period, with 50 mL provided every 20 min. The leucine-enriched diet was supplemented with 0.052 g leucine per kilogram body weight (providing an additional ~3.6 g leucine compared with 2.2 g protein-bound leucine). At the end of the feeding period, the muscle protein fractional synthetic rate was 56 % higher in the leucine-supplemented group (Fig. 17.3). Increased protein synthesis in response to the leucine-supplemented diet was attributed to the 130 % increase in plasma leucine concentration, as only plasma free leucine concentrations differed between groups. Taken together, these data and those obtained in animals support the notion that a *leucine threshold* exists and must be overcome after protein ingestion to stimulate muscle protein synthesis above basal (rest) value [36–38].

As mentioned previously, age-related anabolic resistance may also be applicable to the effect of leucine in stimulating muscle protein synthesis [35–38], suggesting a higher leucine stimulus is required to achieve activation of the leucine threshold associated processes. Based on the positive effects obtained in acute feeding studies, it was assumed that the beneficial effect of leucine or

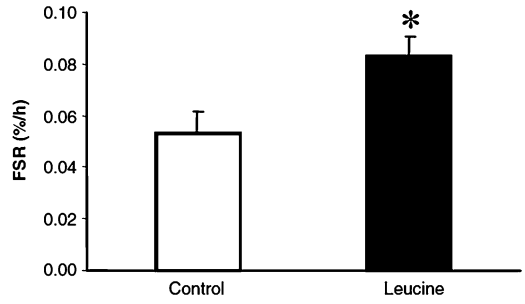

Fig. 17.3 Fractional synthesis rates (FSR) of myofibrillar muscle proteins in elderly volunteers fed a control diet or a leucine supplemented diet as a repeated bolus between 0 and 300 min. Values are means ± S.E.M. *Significantly different from control group at $P < 0.05$. Figure from [36] with permission from the American Physiological Society

leucine-rich protein supplementation on muscle protein synthesis could result in a significant increase in protein synthesis, muscle mass gain, and muscle function in elderly subjects. In healthy elderly women (mean age: 67 ± 1 years) who received 15 g of essential amino acids (EAAs) per day (providing 4.0 g leucine per day) over a 12-week period there was a 4 % increase of lean mass [39], but only a few studies have addressed the potential impact of chronic leucine supplementation in the elderly [40–42]. For example, healthy elderly men (mean age: 71 ± 4 years) who received a 7.5 g free leucine supplementation daily provided with each main meal (3 × 2.5 g at breakfast, lunch, and dinner) had no clear increase in total or lean body mass, nor muscle strength compared to a placebo group. It is noteworthy that a 18–25 % decline in basal plasma valine concentration was recorded in the leucine-supplemented group, suggesting an amino acid imbalance. The strongest explanation for the apparent discrepancy between acute and chronic effect of dietary leucine supplementation on muscle protein metabolism was proposed by Dardevet et al. [43] suggesting that the addition of free leucine over a normal protein diet might create a desynchronization between the leucine anabolic signal and the rise in all amino acids. The more rapid free leucine absorption compared to other amino acids may partly lower muscle protein accretion as it would only be stimulated on a very short period of time during the postprandial period. This is to say that the rate of leucine appearance and other amino acids in the circulation should be simultaneous in order to work synergistically in stimulating muscle protein accretion. Therefore rapidly absorbed leucine rich whole proteins (e.g., whey proteins) or leucine added to whole protein may be most beneficial to obtain synchronization of both the leucine signal and amino acid bioavailability.

Leucine Supplementation and Immobility

Decreased physical activity/immobility is commonly found in elderly individuals. Furthermore, muscle recovery in the elderly following immobilization or extended periods of inactivity is attenuated exacerbating the sarcopenic phenotype [44]. Further inactivity during recovery will lead to reduced motor control and reduced confidence, which may further increase the prevalence of falls, lengthen recuperation periods, and dependency. In addition, prolonged periods of immobility and skeletal muscle atrophy are often observed in patients submitted to bed rest due to surgery, sepsis, stroke, bone fracture, spinal cord injury, or traumatic injury. It is important therefore, to identify potential bioactive ingredients that may be beneficial in preventing the immobilisation-dependent reduction of skeletal muscle protein mass. Given the widely reported anabolic effect of leucine on skeletal muscle, much research has been carried out investigating the potential benefit of leucine supplementation alone or in combination with other EAA/BCAA supplementation as means of maintaining skeletal muscle mass

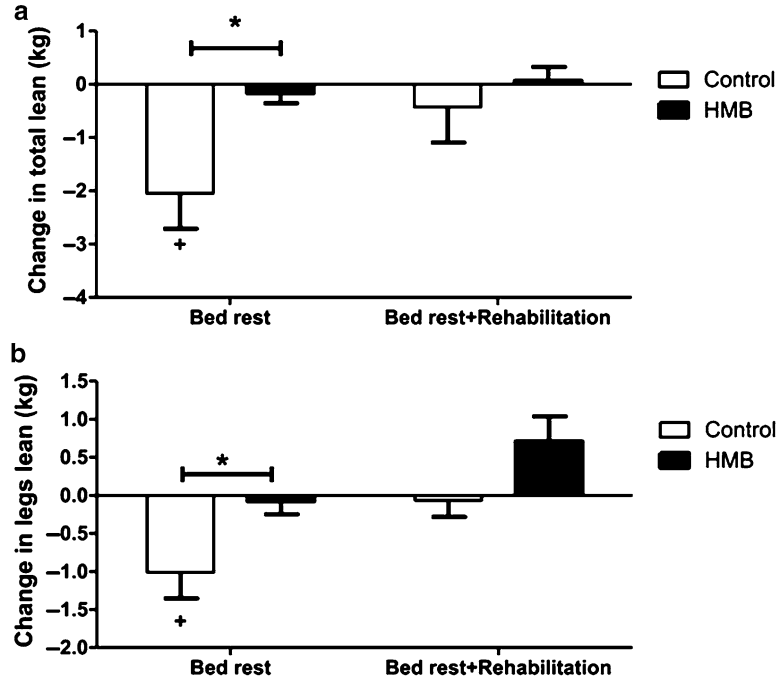

Fig. 17.4 Body composition (DEXA) changes over bed rest. *Top panel* (**a**): change in total lean mass over 10-day bed rest (bed rest) and change from baseline to end of exercise rehabilitation (bed rest + rehab). Values are presented as mean ± SEM for Control ($n = 8$) and HMB ($n = 10$, excluding potential outlier). (+) Difference from prebed rest value ($p = 0.02$, paired t-test); (*) difference between treatment groups ($p = 0.02$, ANOVA). *Bottom panel* (**b**): change in leg lean mass over bed rest and rehabilitation. (+) Difference from prebed rest value ($p = 0.02$, paired t-test); (*) difference between treatment groups ($p = 0.02$, ANOVA); trend toward increase from baseline to end of rehabilitation for HMB group ($p = 0.06$, paired t-test) and nonsignificant for Control group. Figure from [47], with permission from Elsevier Limited

during immobility. For example, Kobayashi et al. in 2006 tested BCAA infusion and EAA infusion in immobilized rats (246 mg kg^{-1} h^{-1} for 105 min and 600 mg kg^{-1} h^{-1} for 105 min, respectively). They observed no effect of BCAA infusion on muscle protein metabolism, whereas EAA infusion suppressed muscle protein breakdown [45]. In contrast, positive results of BCAA supplementation in attenuating muscle loss have been reported during short-term bed rest [46].

Leucine is also able to regulate muscle mass in an indirect manner via one of its metabolites known as β-hydroxy-β-methylbutyrate (HMB). Specifically, HMB has received much attention with regards to increasing muscle protein synthesis as well as attenuating immobility-induced muscle loss. In a recent study dietary HMB was investigated in terms of preventing immobility-induced muscle loss during 10 days of bed rest in elderly (67 years old) males and females. More specifically, it was recently reported that ingesting HMB for 5 days prior to 10 days of bed rest prevented muscle atrophy (as measured by DEXA) compared to a placebo (Fig. 17.4) [47]. Following the 10-day bed-rest period each subject underwent 8 weeks of rehabilitation with or without HMB. The rehabilitation consisted of exercise (circuit and resistance exercise which was carried out for 1 h duration 3 days per week (24 total sessions)). At the end of the exercise rehabilitation period, there was no significant difference from baseline in the change value for total lean mass from within each group. This is to say that HMB and rehabilitation did not result in any benefit in lean mass compared to rehabilitation alone. With regards to strength (change in isokinetic strength) and functionality measures (as measured by the Short Physical Performance Battery score (SPPB)), HMB did not result in any benefit compared to

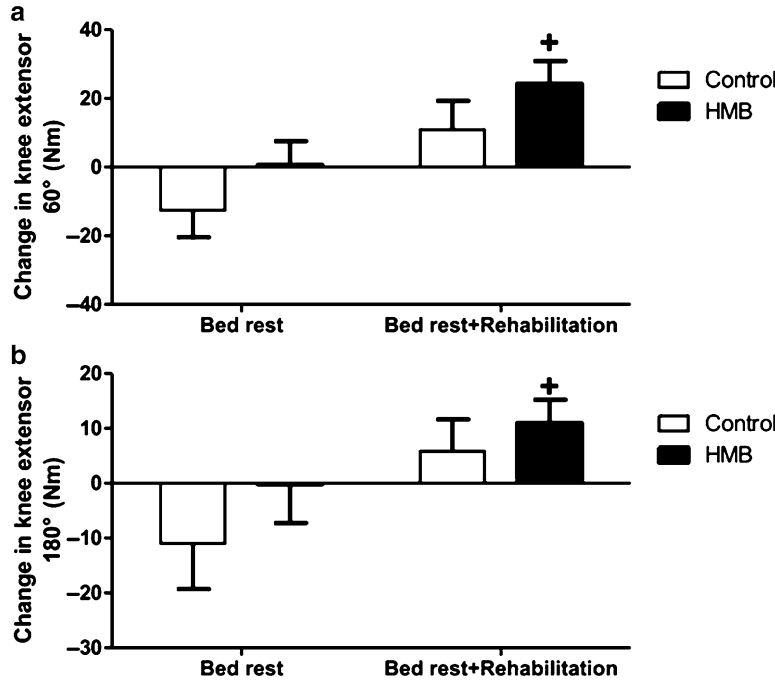

Fig. 17.5 *Top panel* (**a**): change in isokinetic knee extensor (60°) strength over 10-day bed rest and 8-week exercise rehabilitation. Values are presented as mean ± SEM. (+) Difference from prebed rest value ($P = 0.004$, paired t-test). *Lower panel* (**b**): change in isokinetic knee extensor (180°) strength over 10-day bed rest and 8-week exercise rehabilitation. (+) Difference from prebed rest value ($P = 0.03$, paired t-test). Figure from [47], with permission from Elsevier Limited

placebo (Fig. 17.5) [47]. This highlights the importance of measuring functional outcomes as opposed to muscle mass as an indicator of improved mobility. Of course preventing the amount of muscle loss may be important in preventing immobility-induced insulin resistance but further research is warranted in this area.

During immobility, the benefit of free leucine supplementation alone in the limitation of muscle disuse atrophy has not been thoroughly explored in humans. Some promising results obtained with BCAA/EAA supplementations highlight the potential benefit of leucine alone, but requires further investigation. During ageing, the main challenge is to induce the muscle mass recovery, and chronic free leucine supplementation is not sufficient to induce the recovery, with high-leucine content whole proteins or free-leucine enriched whole proteins proposed as more beneficial.

Leucine Supplementation and Cancer Cachexia

Given the anabolic effect of leucine in healthy individuals and the promising data showing improved muscle mass in sarcopenic individuals, leucine supplementation is a major candidate for the restoration of muscle protein anabolism and the overall attenuation of muscle mass loss observed in cancer cachexia. Numerous studies have investigated the role of BCAA supplementation in various models of cancer cachexia, demonstrating their capability to maintain protein synthesis and inhibit protein breakdown [48]. Leucine alone has been reported to elicit benefits in rodent models of cachexia. For

instance, a 3 % free leucine supplementation had a protective effect on muscle mass and myosin content loss [49] possibly via stimulation of protein synthesis and reduction of protein breakdown [50]. New nutritional strategies have been investigated whereby combining various nutrients and bioactive ingredients may help in the management of not only the observed loss of muscle mass, but also the reduced insulin resistance and increased systemic inflammation observed during cachexia. Given the beneficial effect of leucine on muscle protein synthesis in healthy individuals it was recently demonstrated that muscle protein synthesis may be stimulated in cachectic cancer patients by providing them with a specially formulated food, rich in leucine and proteins (24.2 g casein + 11.9 g whey + 4.16 g free leucine) [51].

Leucine supplementation under cancer cachexia condition appears to be efficient to maintain both muscle anabolism and muscle mass. However, these positive effects could be improved when leucine is combined with others nutrients or physical activity.

Leucine Supplementation and Weight Management

Obesity is a major public health issue often leading to insulin resistance and increased risk of metabolic syndrome. In humans, weight loss by means of calorie restriction leads to both reduced fat mass also reduced lean tissue mass, including skeletal muscle. Loss of lean mass associated with low caloric intake is further accentuated in obese elderly individuals (sarcopenic obesity) and may have further deleterious effects because of added stress due to excess body fat on the skeletal system, as expressed for example by a higher incidence of degenerative bone diseases in obesity. The anabolic stimuli of leucine or high-protein leucine containing diets may therefore be beneficial in weight loss strategies in obese individuals via attenuation of muscle mass losses during energy restriction [19, 52, 53]. In addition to these data, recent studies highlighted the ability of leucine to decrease food intake through mTOR activation in the hypothalamus [54, 55] and to decrease and stimulate the leptin secretion [56]. Finally, leucine exerts a thermogenic effect [57, 58] and increased weight and adipose tissue loss during energy restriction in rats [59].

Long-term BCAA or leucine supplementation, however, has not been evaluated in humans. We could find only two studies with some evidence for a role of leucine supplementation in humans for weight management. In the first study, Zemel et al. [60] tested a blend of vitamin B6 and leucine (750 mg three times/day, total daily dose: 2.25 g leucine) in young obese subjects. After 28 days of supplementation, fat oxidation was increased in the supplemented group, and insulin sensitivity was significantly improved. In the second study, Frestedt et al. [61] provided obese patients with a low-caloric diet (reduction in 500 calories per day) for 12 weeks supplemented with whey protein (i.e., leucine rich, at 20 g per day). The supplement increased the loss of body fat and the retention of lean muscle when compared with the placebo group. However, it was not possible to attribute the beneficial effect of such a supplement to unique leucine excess.

There is some evidence for beneficial effects of leucine-rich proteins for mass retention with weight loss, but there is a lack of clinical studies to establish a clear benefit of free leucine supplementation in obese patients under caloric restriction. However, such strategies should be further evaluated.

Conclusions

Leucine has long been identified as playing a major role in stimulating skeletal muscle protein accretion largely via stimulation of increased protein synthesis through the mTOR signaling pathway. However, under various catabolic conditions leading to muscle atrophy (sarcopenia, cachexia,

immobility), the leucine-stimulatory effect was not sufficient to limit the muscle atrophy. The current challenge is to overcome the anabolic resistance present in such situations. New nutritional approaches should include combinations of nutrients and bioactives with leucine and/or physical activity in order to promote anabolism.

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Chapter 18
Branched Chain Amino Acids Supplementation and Glycemic Control

Toshinari Takamura, Yumie Takeshita, and Shuichi Kaneko

Key Points

- Currently, BCAAs supplementation is clinically used and covered by insurance only for patients with chronic liver diseases.
- It remains unclear whether BCAAs improve insulin resistance in humans.
- Circulating levels of BCAAs are associated with insulin resistance both in animals and humans.
- BCAAs supplementation therapy, together with their metabolites and their target mTOR-related signaling pathway, may modulate insulin signaling and glucose metabolism depending on their dosage, site of action, duration, and synergies with background lipid profiles.
- BCAAs supplementation may improve energy metabolism and glucose tolerance in liver cirrhosis patients with more skeletal muscle volume.
- BCAAs-enriched supplement may be useful in ameliorating insulin resistance in male patients or in severely insulin-resistant patients with chronic liver disease.
- BCAAs increase muscle mitochondrial function in the young but not in the elderly.
- BCAAs therapy may exert a beneficial effect on HbA1c values in patients with marked insulin resistance in the skeletal muscle.

Keywords BCAA • Chronic liver disease • Glucose tolerance • Insulin resistance • mTOR • Type 2 diabetes

Abbreviations

AUC	Areas under curve
BCAA	Branched chain amino acids
BCATm	Mitochondrial form of BCAA transaminase

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BCKDH	Branched chain ketoacid dehydrogenase complex
BMI	Body mass index
BTR	Branched chain amino acid/tyrosine ratio
CHI	Creatinine height index
FPG	Fasting plasma glucose
HCC	Hepatocellular carcinoma
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HF	High-fat
H-IR	Hepatic insulin resistance index
HOMA-IR	Homeostasis model assessment of insulin resistance
IRI	Immunoreactive insulin
MAPR	Maximal mitochondrial ATP production rate
MCR	Metabolic clearance rate of glucose
mTOR	Mechanistic target of rapamycin
mTORC1	mTOR complex 1
npRQ	Nonprotein respiratory quotient
SC	Standard chow
OGTT	Oral glucose tolerance test

Introduction

Branched chain amino acids (BCAAs) that include valine, leucine, and isoleucine are essential amino acids and are not synthesized de novo in organisms. Currently, in Japan, BCAAs supplementation is clinically used and covered by insurance only for patients with chronic liver diseases. BCAA supplementation improves disorders of albumin metabolism, quality of life, subjective symptoms, and prognosis in patients with chronic hepatitis [1]. Insulin resistance increases in chronic liver disease and is a risk factor for the progression of liver pathology, the development of hepatocellular carcinoma (HCC), and a decrease in long-term survival [2, 3]. Therefore, insulin resistance is an important therapeutic target in patients at any stage of chronic liver disease. It remains unclear whether BCAAs improve insulin resistance in humans; both beneficial and deleterious effects of BCAAs are reported based on experimental and clinical observations. In the current review, we focus on the current issues on BCAA and glucose homeostasis from both experimental and clinical points of view.

Circumstantial Evidence of Circulating Levels of BCAAs and Insulin Resistance

It is well known that circulating levels of BCAAs are associated with insulin resistance both in animals and humans. Felig et al. found that plasma levels of valine, leucine, isoleucine, tyrosine, and phenylalanine are increased and correlated with serum insulin in obese subjects compared with age-matched and sex-matched controls [4]. BCAA and related metabolites such as C3 and C5 acylcarnitines are associated with cardiometabolic abnormalities independently of body mass index [5]. In addition, BCAA levels may predict future diabetes. In the Framingham Offspring Study, BCAAs, tyrosine and phenylalanine profiled in baseline specimens predict future diabetes development independently of age, body mass index, and fasting glucose [6]. These observations were confirmed in a comprehensive metabolomics profiling of obese versus lean humans [7]. A principal component analysis revealed that the

component most strongly associated with insulin resistance assessed by HOMA-R was not lipid-related, but rather comprised of the BCAAs and their metabolites C3 and C5 acylcarnitines.

Decreased catabolism of BCAA mediated by the mitochondrial form of BCAA transaminase (BCATm) and the branched chain ketoacid dehydrogenase complex (BCKDH) in the adipose tissue [8, 9], rather than increased consumption of BCAA [6], may contribute to increases in BCAA levels in insulin-resistant states. Indeed, adipose tissue expression of the genes involved in BCAA catabolism such as BCATm and BCKDH was positively associated with increase in insulin sensitivity after 3-month treatment with thiazolidinediones in humans [9]. In addition, plasma levels of BCAAs are reduced by surgical weight loss intervention by Roux-en-Y gastric bypass in human subjects with increased expression of genes for BCATm and BCKDH in the adipose tissue [8].

BCAA-Induced Insulin Resistance and Its Possible Underlying Mechanisms

Previous experimental studies have reported conflicting effects of BCAAs on glucose metabolism in vivo. BCAAs promote glucose uptake in skeletal muscle in a rat model of liver cirrhosis [10] and in sucrose-lipid-fed rats [11]. In adipocytes from diabetic db/db mice, leucine permits insulin to stimulate Akt when PI 3-kinase is inhibited [12]. Leucine reduces hepatic glucose production and insulinemia in insulin-resistant obese Zucker fa/fa rats [11]. In skeletal muscle isolated from nondiabetic rats [13], leucin promotes glucose uptake under insulin-free conditions by enhancing translocation of GLUT4 to the plasma membrane via PI3K and protein kinase C pathways independently of mechanistic target of rapamycin (mTOR), a serine/threonine protein kinase that senses and integrates a variety of environmental cues to regulate organismal growth and homeostasis [14]. However, in general, BCAAs/amino acids activate mTOR and thereby induce phosphorylation of 4E-BP1, stimulate the enzymatic activity of p70 S6K, and reverse hypoalbuminemia in the cirrhotic liver [15]. The mTOR signaling is also activated in obese/over-nutrient state and plays crucial roles in regulating metabolism in various organs responsible for energy homeostasis [14]. mTOR complex 1 (mTORC1) activation promotes adipogenesis by activating PPAR-γ in adipose tissue, enhances protein synthesis, mitochondrial biogenesis, and oxidative metabolism in muscles, promotes hepatic insulin resistance, gluconeogenesis, and lipogenesis by activating SREBP1, and promotes β cell growth and proliferation [14]. Paradoxically to the effect of mTORC1 in maintaining muscle mass, mTORC1 negatively regulates insulin-mediated PI3K activity by degrading insulin receptor substrates in skeletal muscle cells during long-term insulin treatment [16]. Because chronic activation of mTORC1 in the tissues of obese mice and humans appears to play a role in the development of insulin resistance and type 2 diabetes, the potential of mTOR inhibition with rapamycin to improve metabolic parameters has been tested in a variety of animal models. Unexpectedly, treatment of rodents with rapamycin leads to a deterioration of the metabolic profile. Rapamycin reduces adipose tissue size and β-cell mass and function, promotes hepatic gluconeogenesis, and thereby causes insulin resistance, hyperlipidemia, and glucose intolerance [14]. mTOR signal crosstalks with ERRalpha that is involved in the TCA cycle and lipid biosynthesis. Rapamycin treatment exacerbates hepatic lipid accumulation via inhibition of ERRalpha [17]. Indeed, we have experienced a case of type 2 diabetes whose glycemic control and fatty liver were exacerbated after administration of an anticancer drug everolimus that works as an inhibitor of mTOR (manuscript in preparation). On the other hand, rapamycin extends lifespan in mice despite metabolic impairment. Fang et al. recently found that detrimental metabolic effects of rapamycin treatment are only observed during the early stages of treatment and are reversed or diminished in mice treated for longer period (20 weeks), with better metabolic profiles, increased oxygen consumption and ketogenesis, and markedly enhanced insulin sensitivity [18]. These findings suggest that rapamycin treatment may exert both deleterious and beneficial metabolic effects depending on the treatment period. Therefore, the relation between mTORC1 activity and insulin sensitivity and

metabolic profile is suggested to follow a U-shaped curve, where too little or too much mTORC1 activity has a negative impact on systemic metabolism [14].

Given BCAAs as mTOR activators, BCAAs may also play a dual role in systemic glucose metabolism depending on their dosage, site of action, and duration. Indeed, interventions that alter circulating levels of BCAAs have exhibited quite paradoxical phenotypes in mice. Mice deleted with *BCATm* gene, which encodes the enzyme catalyzing the first step in peripheral BCAA catabolism, exhibited elevated plasma BCAAs and decreased adiposity and body weight, despite eating more food. *BCATm−/−* mice also exhibited increased energy expenditure, improvements in glucose and insulin tolerance, and protection from diet-induced obesity. These observations suggest that elevated BCAAs and/or loss of BCAA catabolism in peripheral tissues play an important role in regulating insulin sensitivity and energy expenditure. In this regard, elevation in circulating levels of BCAAs in insulin resistant animals and humans may be a compensation to keep energy homeostasis.

On the other hand, Newgard et al. fed Wistar rats on high-fat (HF), HF with supplemented BCAA (HF/BCAA), or standard chow (SC) diets [7]. Rats fed the HF/BCAA diet consumed less food than the HF group. Despite having reduced food intake and a low rate of weight gain equivalent to the SC group, HF/BCAA rats revealed impaired insulin signaling in liver and muscle equally as HF rats. Insulin resistance induced by HF/BCAA feeding was accompanied by chronic phosphorylation of mTOR, JNK, and Ser307 of IRS1 and by accumulation of multiple acylcarnitines in muscle, which were reversed by the mTOR inhibitor, rapamycin [7]. These findings suggest an interaction between excess fat and BCAA in development of insulin resistance that can occur independently of body weight. Pair-feeding of HF diet to match the HF/BCAA animals or BCAA addition to SC diet did not cause insulin resistance [7], suggesting that in the context of a dietary pattern such as high-fat diet, BCAA contributes to development of obesity-associated insulin resistance.

Clinical Interventions of BCAAs Supplementation

BCAA supplementation is clinically used for patients with chronic liver diseases. In a single-arm short period administration study [19] (Fig. 18.1), energy metabolism and glucose tolerance were evaluated using an indirect calorimeter and 75 g oral glucose tolerance test (75 g OGTT) before and after 1 week of oral supplementation with a BCAA nutrient (Livact® 4.15 g/pack, Ajinomoto Pharma, Tokyo, Japan) three times a day (after breakfast, dinner, and before sleep) in patients with liver cirrhosis (hepatitis B virus (HBV) in 2 cases, hepatitis C virus (HCV) in 26 cases, alcohol in 1 case and unknown cause in 1 case, complicated with HCC in 19 cases). Nonprotein respiratory quotient (npRQ) as well as branched chain amino acid/tyrosine ratio (BTR) showed significant improvement, especially in patients with a marker for skeletal muscle volume, creatinine height index (CHI, calculated as 24-h urine creatinine multiplied by 100 over the expected 24-h urine creatinine for height) greater than 80. npRQ after 1 week of BCAA supplementation significantly correlated with the CHI (Fig. 18.1a). The patients with CHI greater than 80 and those with borderline pattern assessed by 75 g OGTT showed significant improvement in impaired glucose tolerance [19] (Fig. 18.1b). Authors concluded that liver cirrhosis patients with CHI greater than 80 suggestive of more skeletal muscle volume are the first candidates for BCAA supplementation; these patients show improvement in energy metabolism, BTR, and glucose tolerance [19].

In another single-arm small scale study [20], 12 patients with chronic viral liver disease were given a BCAA-enriched supplement after breakfast and at bedtime for 90 days (Fig. 18.2). BCAA-enriched supplement significantly decreased an insulin resistance index HOMA-IR only in the male group (left panel), in which baseline HOMA-IR levels were significantly elevated compared with the female group (right panel) [20] (Fig. 18.2b). This suggests that BCAA-enriched supplement may be useful in ameliorating insulin resistance in male patients or in severely insulin resistant patients with chronic liver disease.

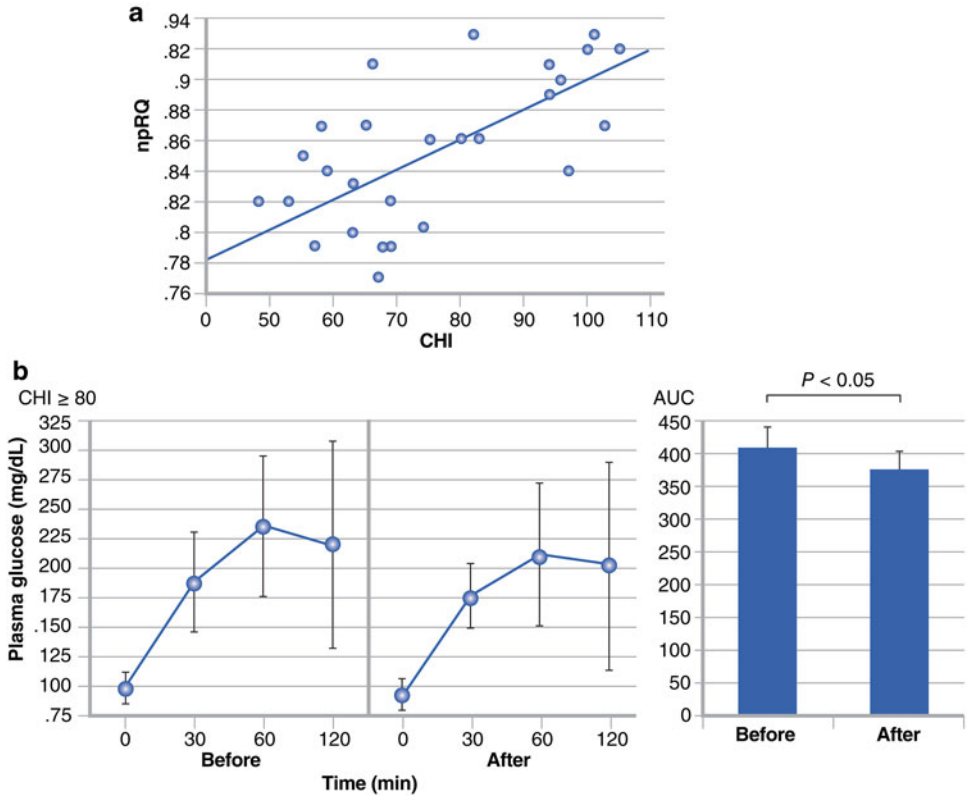

Fig. 18.1 The effect of 1-week supplementation with BCAAs in patients with liver cirrhosis (modified from [19]). (**a**) Significant correlation between the creatinine height index (CHI) and nonprotein respiratory quotient (npRO) value after 1-week supplementation with BCAAs in liver cirrhosis patients. $n=30$, $r=0.644$, $P<0.01$. (**b**) Change of blood glucose level in 75 g OGTT after 1-week supplementation with BCAAs in liver cirrhosis patients with a CHI ≥ 80. AUC, area under the curve made at four points by 75 g OGTT

In a crossover human study [21] (Fig. 18.3), it was examined whether an 8-h infusion of BCAAs or saline enhances maximal mitochondrial ATP production rate (MAPR) in skeletal muscle biopsy samples of 12 healthy young (23.0 ± 0.8 years) and 12 healthy elderly (70.7 ± 1.1 years) participants matched for sex and body mass index. In young participants, MAPR with the substrates glutamate plus malate (GM, supplying electrons to complex I) (Fig. 18.3a) and succinate plus rotenone (SR, complex II) (Fig. 18.3b) increased in response to BCAAs infusion, relative to a decline in MAPR in response to the saline infusion. In contrast, MAPR was unaffected by BCAAs infusion in the elderly participants [21] (Fig. 18.3). These findings suggest that BCAAs increase muscle mitochondrial function in the young but not in the elderly.

Based on the experimental findings discussed above, we hypothesized that BCAAs play a dual role in glucose metabolism in skeletal muscle, enhancing glucose uptake under normoinsulinemic conditions while causing insulin resistance under hyperinsulinemic conditions. In this regard, the effects of BCAA administration on glucose metabolism depend on the balance between insulin-dependent and insulin-independent BCAA signaling pathways [10]. We tested this hypothesis in our open-label, randomized, controlled crossover intervention trial [22] that addressed the effects of BCAAs on glucose tolerance and insulin sensitivity in patients with chronic hepatitis C and insulin resistance. Eligible participants were randomly assigned to the BCAAs group or a control group, and were then

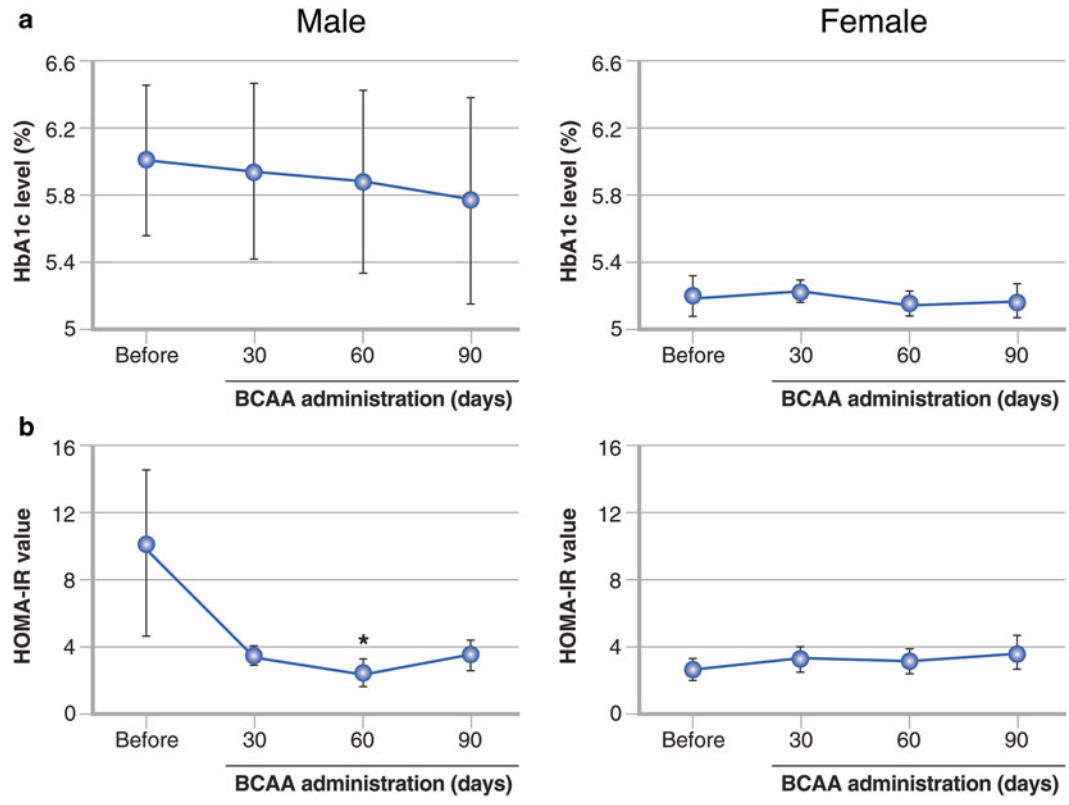

Fig. 18.2 The effects of BCAAs-enriched supplementation on HbA1c levels (**a**) and HOMA-IR values (**b**) in the male (*left panel*, $n=5$) and female (*right panel*, $n=7$) patients with chronic liver disease. Statistical comparisons between before and after 30, 60, or 90 days of the administration was performed by Wilcoxon's test. *$P<0.05$. Modified from [20]

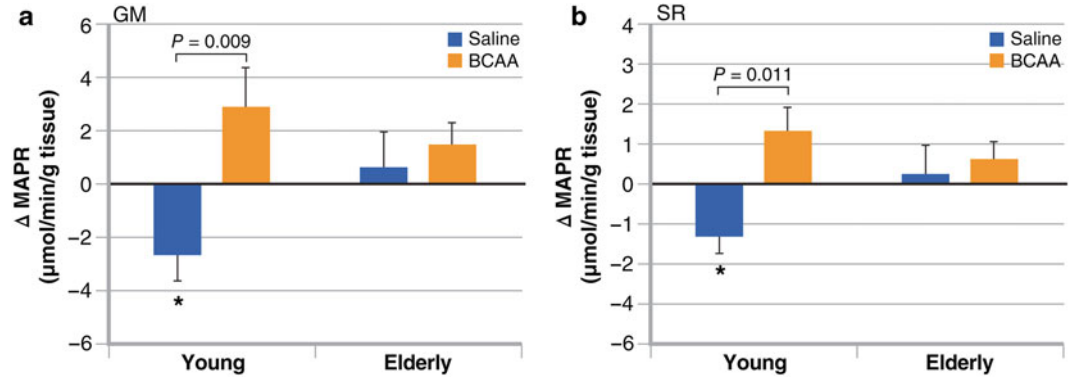

Fig. 18.3 The effect of BCAAs on skeletal muscle mitochondrial function in young and elderly adults (reprinted from [21]). (**a** and **b**) Change in maximal mitochondrial ATP production rate (MAPR) in response to saline (*open bars*) or BCAA (*filled bars*) normalized to tissue weight. MAPR was significantly lower in the elderly participants than the young when GM (complex I) was used (**a**) and a trend when SR (complex II) was used (**b**). Data presented as mean ± SEM. Mixed-effects ANOVA was used to test the main effects of age and treatment and their interaction

Table 18.1 Effects of BCAAs on body composition, protein, lipid and glucose metabolism in chronic hepatitis C patients with insulin resistance

	BCAA group	Control group	*P* value
Body weight (kg)	61.1 ± 1.9	60.5 ± 1.9	N.S
Waist circumference (cm)	87.8 ± 1.7	88.9 ± 1.7	N.S
BMI (kg/m^2)	24.9 ± 0.5	24.7 ± 0.4	N.S
FPG (mg/dL)	96.6 ± 2.1	96.2 ± 2.0	N.S
HbA1c (%)	4.9 ± 0.1	5.0 ± 0.1	N.S
IRI (IU/L)	17.8 ± 3.6	21.2 ± 4.6	N.S
HOMA-IR	4.5 ± 1.1	5.3 ± 1.3	N.S
TC (mg/dL)	157.6 ± 7.2	164.9 ± 7.3	N.S
TG (mg/dL)	78.3 ± 6.3	80.9 ± 5.3	N.S
HDL-C (mg/dL)	49.4 ± 2.9	50.0 ± 2.7	N.S
AST (IU/L)	61.2 ± 6.9	46.0 ± 3.0	*P* = 0.047*
ALT (IU/L)	68.3 ± 11.0	44.2 ± 4.8	*P* = 0.049*
BUN (mg/dL)	14.6 ± 1.0	12.9 ± 0.7	N.S
HpT (%)	86.8 ± 4.2	91.4 ± 5.7	N.S
Total protein (g/dL)	7.5 ± 0.1	7.6 ± 0.1	N.S
Albumin (g/dL)	4.0 ± 0.1	4.0 ± 0.1	N.S
BCAA (μmol/L)	472.6 ± 27.0	403.5 ± 21.0	*P* = 0.050*
Matsuda index	2.9 ± 0.3	2.5 ± 0.2	N.S
H-IR × 10^6	6.6 ± 0.1	6.2 ± 0.6	N.S
MCR (mg/kg/min)	9.8 ± 0.8	10.3 ± 1.0	N.S

Modified from [22]

All data are expressed as the mean and standard error. Statistical comparisons between data from before administration of the BCAAs and data obtained after 12 and 24 weeks were performed using a Wilcoxon matched-pairs signed rank test. *BMI* body mass index, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin, *HOMA-IR* homeostasis model assessment of insulin resistance, *H-IR* hepatic insulin resistance index, *MCR* glucose metabolic clearance rate

*Difference between the BCAA group and control group. *P* values of <0.05 were considered significant

crossed over to the other treatment for a further 12 weeks. Those in the BCAAs group were prescribed oral supplementation with three packs of a BCAAs nutrient (Livact® 4.15 g/pack), taken three times a day: after breakfast, after dinner, and before sleep for 12 weeks. Clinical features, laboratory markers, fatty acid levels, and insulin sensitivity, assessed with 75 g OGTT and a hyperinsulinemic euglycemic clamp (metabolic clearance rate, MCR), were examined before and 12 and 24 weeks after the beginning of the study (Table 18.1). Of the 27 patients who completed the study (ages 61.3 ± 2.1 years, 8 men and 19 women, BMI 24.6 ± 2.0 kg/m^2, mean ± standard error), the plasma amino acid component that showed the strongest difference between the BCAAs and control groups was the combination of leucine, valine, phenylalanine, threonine, and proline ($P < 0.05$) (Table 18.2). BCAA therapy did not have significant adverse or beneficial effects on glucose tolerance, insulin sensitivity, or lipid profiles in patients with chronic hepatitis C and insulin resistance (Table 18.1). These trends were similar in subgroups stratified by glucose tolerance (as diabetes, borderline glucose intolerance, and normal glucose tolerance). Additionally, unlike previous reports that suggested that BCAA-mediated improvements in insulin resistance are only observed in male patients [20], there were no significant sex differences in the effects of BCAA. HbA1c values were improved in 10 patients (37.0 %) and worsened or remained unchanged in 17 patients (63.0 %). As shown in Table 18.3, the only predictive variable for change in HbA1c was the baseline Matsuda index (1.8 ± 0.2 in the HbA1c improved group, 2.9 ± 0.3 in the HbA1c non-improved group, $P = 0.014$) calculated from 75 g OGTT. Furthermore, the percentage change in HbA1c tended to be correlated with the percentage change in the Matsuda index ($r = -0.405$, $P = 0.069$): the lower the index, the greater the improvement in HbA1c values [22].

Table 18.2 Effects of BCAAs on amino acids levels in chronic hepatitis C patients with insulin resistance

	Baseline	BCAA	Control	*P* value*
BCAA (μmol/L)	465.3±99.4	461.4±120.2	418.6±99.6	N.S.
Phenylalanine	103.7±10.9	112.8±23.9	105.5±14.7	*P*=0.014
Threonine	139.7±26.1	121.3+31.6	132.3+28.4	*P*=0.016
Proline	193.5+59.7	172.8+49.1	200.7±86.0	*P*=0.024
Leucine	140.3±28.6	144.2±40.8	129.5±31.4	*P*=0.025
Valinp	753.9±56.0	747.5+61.8	719.7+48.6	*P*=0.040
Glycine	334.5+46.2	306.6+49.7	376.6+272.5	N.S
Tryptophan	64.9±11.9	64.9±13.9	62.2±12.9	N.S
Arginine	135.5±31.9	125.9±36.5	136.6±37.5	N.S
Ornithine	124.8±27.2	114.0+28.9	122.6+37.1	N.S
Isoleucine	71.2±17.4	69.7+21.4	69.3+23.8	N.S
Methionine	32.1±11.5	35.5±9.7	34.6±7.5	N.S
Glutamine	277.2+169.5	365.2+191.2	406.5±157.4	N.S
Lysine	20.8.7+37.7	194.9+41.2	193.3+44.9	N.S
Histidine	96.3+10.7	92.7+13.4	90.8+12.3	N.S
Citrulline	41.3±11.9	40.9+11.6	40.5+11.1	N.S
Asparagine	37.5±10.4	43.2+11.2	44.1+7.4	N.S
Alanine	485.5+84.9	441.7+78.5	463.4+152.1	N.S
Threonine	139.7±26.1	121.3±31.6	132.3±28.4	N.S
Tyrosine	131.5±27.0	129.6+24.9	130.3+22.3	N.S

Modified from [22]

All data are expressed as the mean and standard error. Statistical comparisons between data from before administration of the BCAAs and data obtained after 12 and 24 weeks were performed using a Wilcoxon matched-pairs signed rank test

*Difference between the BCAA group and control group. *P* values of <0.05 were considered significant

Table 18.3 Baseline clinical features and laboratory markers associated with changes in HbA1c after 12 weeks of administration of BCAAs in chronic hepatitis C patients with insulin resistance

	HbA1c non-improved (*N*=μ17)	HbA1c improved (*N*=10)	*P* value*
Body weight (kg)	61.6±2.4	57.6±2.2	N.S
Waist circumference (cm)	87.7±1.8	87.6±1.5	N.S
BMI (kg/m^2)	24.7±0.6	24.4±0.3	N.S
FPG (mg/dL)	97.6±2.7	97.9±3.1	N.S
HbAlc (%)	4.9±0.1	5.0±0.1	N.S
IRI (IU/L)	14.0±1.6	29.0±11.1	N.S
HOMA-IR	3.5±0.4	7.4±3.1	N.S
TC (mg/dL)	160.7±11.4	146.8±7.7	N.S
TG (mg/dL)	91.6±9.0	70.3±7.2	N.S
HDL-C (mg/dL)	48.8±4.1	45.1±4.0	N.S
AST (IU/L)	49.1±3.9	60.3±8.7	N.S
ALT (IU/L)	43.9±5.1	60.2±17.1	N.S
BUN (mg/dL)	13.9±1.1	13.1±1.4	N.S
HpT (%)	89.5±6.5	84.1±3.8	N.S
Total protein (g/dL)	7.6±0.2	7.4±0.2	N.S
Albumin (g/dL)	4.1±0.1	3.9±0.1	N.S
BCAA (μmol/L)	412.3±21.4	412.8±40.0	N.S
Matsuda index	2.9±0.3	1.8±0.2	*P*=0.014*
H-IR×l0^6	5.5±0.6	6.7±1.0	N.S
MCR (mg/kg/min)	10.1±1.0	8.6±1.3	N.S

Modified from [22]

All data are expressed as the mean and standard error. Differences between the two groups were analyzed by a Mann–Whitney *U* test. *BMI* body mass index, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin, *HOMA-IR* homeostasis model assessment of insulin resistance, *H-IR* hepatic insulin resistance index, *MCR* glucose metabolic clearance rate

*Difference between the HbAlc-non-improved group and the HbAlc-improved group. *P* values of <0.05 were considered significant

On the other hand, serum immunoreactive insulin (IRI), hepatic insulin resistance index (H-IR), and HOMA-IR, which are thought to be hepatic insulin resistance indices, did not significantly predict changes in HbA1c values (Table 18.3.). Therefore, BCAA supplementation therapy did not have adverse effects on glucose tolerance or insulin sensitivity in patients with chronic hepatitis C or insulin resistance. BCAAs did not significantly improve overall glycemic control in the present study. However, contrary to our initial hypothesis, BCAA therapy may exert a beneficial effect on HbA1c values in patients with marked insulin resistance in the skeletal muscle.

Conclusions

BCAAs supplementation therapy, together with their metabolites and their target mTOR-related signaling pathway, may modulate insulin signaling and glucose metabolism depending on their dosage, site of action, duration, and synergies with background lipid profiles, which follow a U-shaped curve, where too little or too much has a negative impact on systemic metabolism.

Toward comprehensive understanding of clinical BCAAs supplementation therapy on glucose metabolism, future clinical trials should also evaluate the effect of BCAAs in patients with type 2 diabetes.

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Chapter 19
Leucine Supplementation and Insulin Resistance

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Key Points

- Leucine, one of the three branched chain amino acids (BCAAs) with isoleucine and valine, is an essential amino acid that is able to stimulate insulin release in both human and rodents.
- Leucine-induced insulin release in healthy humans does not necessarily represent physiological effects on skeletal muscle regarding protein synthesis.
- It appears that the therapeutic and/or deleterious effects of leucine supplementation on glucose metabolism occur only in situations characterized by disruption in glucose homeostasis.
- Obese individuals should not increase BCAAs/leucine intake before having previous and significant changes in body composition and food intake.
- Leucine supplementation appears to have no effect on glucose homeostasis of type 2 diabetic subjects (taking hypoglycemiants) which consume high or normal-protein diets.

Keywords Branched chain amino acids • Leucine • Insulin resistance • Glucose transporter 4 • Glycemia

Abbreviations

α-KIC	α-Ketoisocaproate
ACCα	Acetyl-alpha-coAcarboxilase
ADP	Adenosine diphosphate
Akt/PKB	Protein kinase B
AMPK	Adenosine monophosphate kinase
AS160	Akt substrate of 160 kDa
ATP	Adenosine triphosphate
BCAAs	Branched chain amino acids
BCKDH	Branched chain amino acid dehydrogenase complex

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BCKDHA	Gene that encodes the E1-alpha subunit of the BCKDH
BCKDHB	Gene that encodes the E1-beta subunit of the BCKDH
BMI	Body mass index
Ca^{2+}	Calcium
FABP1	Fatty acids-binding protein 1
GDH	Glutamate dehydrogenase
GLUT4	Glucose transporter 4
HOMA-IR	Homeostasis model of assessment of insulin resistance
K_{ATP}	ATP-sensitive potassium channel
LDL-C	Low density lipoprotein cholesterol
mRNA	Messenger RNA
mTOR	Mammalian target of rapamycin
p70S6k	70 kDa ribosomal protein S6 kinase
PPAR-γ	Peroxisome proliferator-activated receptor gamma
REE	Resting energy expenditure

Introduction

Leucine, one of the three branched chain amino acids (BCAAs) with isoleucine and valine, is an essential amino acid that has emerged due its potential ergogenic and therapeutic role in both exercise (especially resistance exercise) and some diseases (i.e., cancer, muscle disuse, and sarcopenia), respectively. Besides its role in energy provision as a nitrogen donor for muscle alanine synthesis, leucine is also a trigger amino acid that can modulate some cellular pathways involved in skeletal muscle protein synthesis and degradation, innate immune system (inflammation), and insulin secretion.

Since the study of Floyd et al. [1] leucine has been described as one of the amino acids able to stimulate insulin release in both human and rodents. Therefore, it is common to associate leucine intake with glucose homeostasis and even with insulin resistance. However, caution should be taken in the interpretation of such evidences. All hormones can undergo significant increases in its plasma concentration by endogenous or exogenous stimuli, and this is determined by statistical methods. However, each tissue requires an optimal threshold of plasmatic increase to promote a biological effect. Thus, leucine-induced insulin release is sufficient to alter glucose metabolism in all tissues (in particular skeletal muscle) and conditions? Does leucine promote adverse side effects in all insulin-resistant conditions? Insulin is an anabolic and anticatabolic hormone in rodents but, recently, it was demonstrated that is just anticatabolic in humans. Finally, leucine metabolism also differs between rodents and humans. Can animal data regarding leucine and insulin metabolism be extrapolated to humans?

Based on the questions described, this brief chapter aims to narratively review the whole-body leucine metabolism and its specific action in the pancreatic cells; the relation between leucine-induced insulin release and the skeletal muscle responsiveness (considering that it is the major tissue able to uptake and store glucose); and the effects of leucine intake/supplementation on distinct insulin-resistant conditions. Since there are evidences demonstrating that skeletal muscle protein metabolism, leucine metabolism, and insulin role differ between rodents and humans we chose to use only human studies to evaluate the clinical effects. Given the scarcity of human studies with cellular mechanisms, compared to rodents, some animal studies were considered in order to try to elucidate the possible cellular events involved and when human evidences were not available.

Leucine and Insulin Secretion

Since its discovery, leucine has been reported to play an important role in glucose homeostasis by performing acute and chronic effects on liver, muscle, adipose tissue, and pancreatic β cells. In the β cells, it is described that leucine acutely stimulates insulin secretion by certain mechanisms. The first of these relates to its role in regulation of ATP-sensitive potassium channel. It is known that the secretion of insulin from β cells is the result of an increase in the ATP:ADP ratio. This increase in intracellular phosphate inhibits the ATP-sensitive potassium channel (K_{ATP}), leading to membrane depolarization, increase of free cytosolic calcium (Ca^{2+}), and the release of free insulin. In this context, it is believed that leucine, or its product transaminated α-ketoisocaproate (α-KIC), may impact on insulin secretion by direct inhibition of K_{ATP}, which then promotes secretion of insulin [2].

α-KIC-stimulated insulin secretion requires that leucine loses an amine group—initial step happens in its catabolism. On the other hand, leucine-stimulated insulin secretion does not need to occur through degradation reactions, speculating that this stimulus occurs due to activation of the mitochondrial enzyme glutamate dehydrogenase (GDH), as described below [3]. However, Gao and colleagues [4] have shown that leucine and α-KIC exert different effects on the stimulation of insulin secretion by β cells. It has been found that the stimulatory effects of leucine, but not α-KIC, on insulin secretion are completely blocked by glucose [4].

Another mechanism by which it is assumed that leucine is able to stimulate insulin secretion is the allosteric activation of GDH, an enzyme that controls the metabolism of amino acids and ammonia in the liver, brain, and β cells. In β cells, this enzyme oxidizes glutamate to α-ketoglutarate, with the concomitant production of intracellular signals and an increase in the ATP:ADP ratio, which induce insulin secretion [2]. A recent in vivo study with rats fed low-protein diet showed that they had 58 % lower GDH mRNA expression, in comparison with rats fed a normal-protein diet, an effect that was attenuated by 28 % with leucine supplementation. This impaired GDH function was associated with lower insulin release, which was normalized with leucine supplementation [5]. Some studies also shows that leucine is able to activate the protein mammalian target of rapamycin (mTOR), which regulates protein synthesis and metabolism in β cells, thus associated with stimulation gene transcription and protein synthesis in β cells [2] (Fig. 19.1).

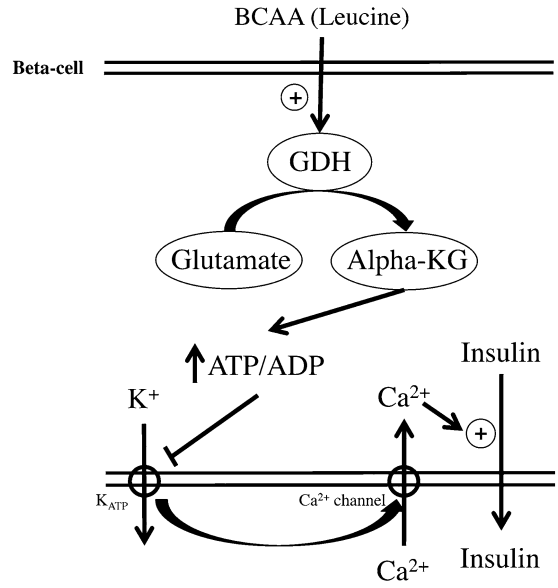

Fig. 19.1 Mechanism linking leucine metabolism with insulin secretion

Therefore, it is plausible to suppose that leucine may play a long-term effect on insulin secretion. Yang and colleagues [6] showed in a study of culture of rat islets that leucine enhances the glucose-stimulated insulin secretion by upregulation of metabolic genes. Few years later, the same authors have shown these equal effects in type 2 diabetic human islets—leucine increased the upregulation of metabolic genes, leading to a glucose-induced increase in cytosolic Ca^{2+} and insulin secretion [7].

The Interaction Between Leucine and Insulin Signaling Pathway in Human Skeletal Muscle

Leucine has been described as a potential insulin secretagogue in humans. Such information has been discussed as a potential mechanism by which leucine could interact with both skeletal muscle anabolism, proteolysis, and glucose uptake. More recently, leucine has been described as an immunomodulator nutrient able to interact with innate immune system and oxidative stress in skeletal muscle.

It is not the focus of this chapter to describe the whole skeletal muscle posttranslational pathways. Briefly, insulin signaling pathway in skeletal muscle is controlled by several mechanisms that include posttranslation modifications (mainly phosphorylation through protein kinases) that are part of non-linear cascades involved in protein synthesis and degradation and glucose uptake (for detailed reviews, please see [8, 9]). Regarding glucose uptake, the protein Akt/PKB (protein kinase B) and its substrate of 160 kDa (AS160) has been described as one of the key regulators of the translocation of glucose transporter 4 (GLUT4) to the sarcolemma. Adenosine monophosphate kinase (AMPK) has also been considered an important modulator of skeletal muscle glucose uptake and appears to be responsive to some amino-like compounds, such as creatine. More recently, the 70 kDa ribosomal protein S6 kinase (p70S6k) was described as a potential mechanism linking inflammation with insulin resistance. All these mechanisms have been extensively studied in order to understand the potential molecular targets to be focused under interventions such as exercise and nutritional programs. However, these mechanisms and their possible interactions with other signaling pathways and metabolic routes still need to be elucidated in humans under several conditions.

The first point to be considered is that insulin is an anticatabolic hormone in humans. Greenhaff et al. demonstrated in humans through amino acids infusion protocol that plasma insulin at 5 mU L^{-1} did not affect neither protein synthesis nor breakdown. However, increasing insulin 30 mU L^{-1} attenuated muscle protein breakdown ($p < 0.05$) with a plateau effect, whereas protein synthesis remained in the same level observer at 5 mU L^{-1}. Therefore, we can assume that leucine-induced insulin release does not have direct effects on human muscle anabolism, but can help to stimulate it indirectly (i.e., increase muscle perfusion). However, plasmatic hormonal threshold can be ineffective to increase protein synthesis but can be enough (or not) to stimulate glucose uptake, attenuate protein degradation, interact with innate immune system, etc. Secondly, it is necessary to evaluate the magnitude of the plasmatic leucine-induced insulin release threshold in humans.

To the best of our knowledge, there is a recent well-controlled study demonstrating the acute effect of leucine intake on plasmatic insulin concentration in humans. Glynn et al. evaluated the effects of two doses of leucine bolus supplementation, as composition of typical high-quality proteins (10 g of essential amino acids), on muscle anabolic signaling in young men and women. In a randomized, double-blind, isonitrogenated, and isocaloric study, subjects received low (1.8 g) or high (3.5 g) doses of leucine after an overnight fasting. Regarding protein expression, it was demonstrated that Akt^{Ser473} did not change after 60, 120, and 180 min of both low and high leucine intake which suggests a possible experimental design bias, because of the temporal response of the phosphorylation, or simply no effect of the interventions. To confirm such hypotheses, the authors demonstrated that plasma insulin concentrations were elevated in both groups 30 min after ingestion when compared to the baseline

(low dose 30 ± 5 to 103 ± 18 pmol L^{-1}; high dose 33 ± 6 to 92 ± 12 pmol L^{-1}; $p < 0.05$). However, 60 min after leucine intake plasmatic insulin concentration remained elevated only in the high dose group (56 ± 8 pmol L^{-1}; $p < 0.05$). When the authors evaluated the physiological response of the intervention (protein synthesis), it was demonstrated that in 10 g of essential amino acids the leucine content of 1.8 g is sufficient to induce a maximal anabolic response.

Of course that other protein could be involved and could have been evaluated, but the physiological result would still be the same. Therefore, it appears leucine-induced insulin release in healthy humans does not necessarily represent physiological effects on skeletal muscle regarding protein synthesis. We cannot assume that such data can be extrapolated to glucose homeostasis since there is no study investigating glucose transporter translocation to the sarcolemma in healthy humans after leucine intake. Even considering the molecular data described previously, Glynn et al. demonstrated a small but statistically significant decrease in blood glucose concentration 120 min after high dose of leucine intake when compared to the baseline (5.0 ± 0.1 to 4.8 ± 0.1 mmol L^{-1}; $p < 0.05$) and 180 min after both low and high dose of leucine intake (low 5.1 ± 0.1 to 5.0 ± 0.1 mmol L^{-1}, $p < 0.05$; high 5.0 ± 0.1 to 4.8 ± 0.1 mmol L^{-1}, $p < 0.05$) but with no significant difference between groups. Thus, it appears that the therapeutic and/or deleterious effects of leucine supplementation on glucose metabolism occur only in situations characterized by disruption in glucose homeostasis. Furthermore, other mechanisms than those described above can elucidate the possible role of leucine in insulin resistance in accordance with the physiological condition as discussed below.

Leucine and Insulin Resistance Conditions

It has been suggested that improving metabolic health is related to protein intake and mediated by specific amino acids such as leucine. We have investigated the effects of leucine intake and resistance on dexamethasone-induced insulin resistance in rats in order to evaluate glucose and skeletal muscle protein metabolism. The results demonstrated that leucine intake aggravated dexamethasone-induced insulin resistance and did not attenuate muscle wasting [10] (Figs. 19.2 and 19.3 and Table 19.1).

Some studies have shown beneficial effects of leucine supplementation on the development of obesity and/or glucose homeostasis, using different experimental models of obesity and type 2 diabetes mellitus [11–14]. High-protein diet has been shown to promote weight loss sparing skeletal muscle mass in obese patients and experimental models. It was also reported that high-protein diets may also increase resting energy expenditure (REE) and fat oxidation [15–18].

Recently, a study showed improvement of hepatic steatosis and plasma lipid profile in nonobese subjects consuming a diet enriched with whey protein [19]. Furthermore, it has been shown that increasing the protein:carbohydrate ratio in a high-fat diet content can attenuate the development of obesity and improved glucose tolerance in rats [20]. The mechanisms attributed to these effects are not yet fully understood. It was suggested that leucine may mediate the metabolic advantages of high-protein diets through influencing the energy balance, lipid metabolism, and skeletal muscle metabolism (synthesis and degradation) [21]. However, there are still many controversies on this topic because not all available results confirm these effects of leucine in other conditions [22, 23].

Zhang et al. [14] published a study supporting the hypothesis that leucine supplementation concurrently may increase postprandial insulin release in order to promote glycemic control. This study involved mice which received drinking water containing 1.5 % leucine with a high-fat diet during 14 weeks. It was observed that there was a 27 % of reduction in total cholesterol and 53 % of low density lipoprotein cholesterol (LDL-C) after supplementation protocol.

A study in mice evaluated if the beneficial metabolic effects of a protein diet are due to leucine intake. Before the experiment, the animals consumed a diet containing 19 % of protein, 4 % of fat, and 50.5 % of carbohydrate. The animals were randomly allocated into three experimental groups ($n = 9$–10)

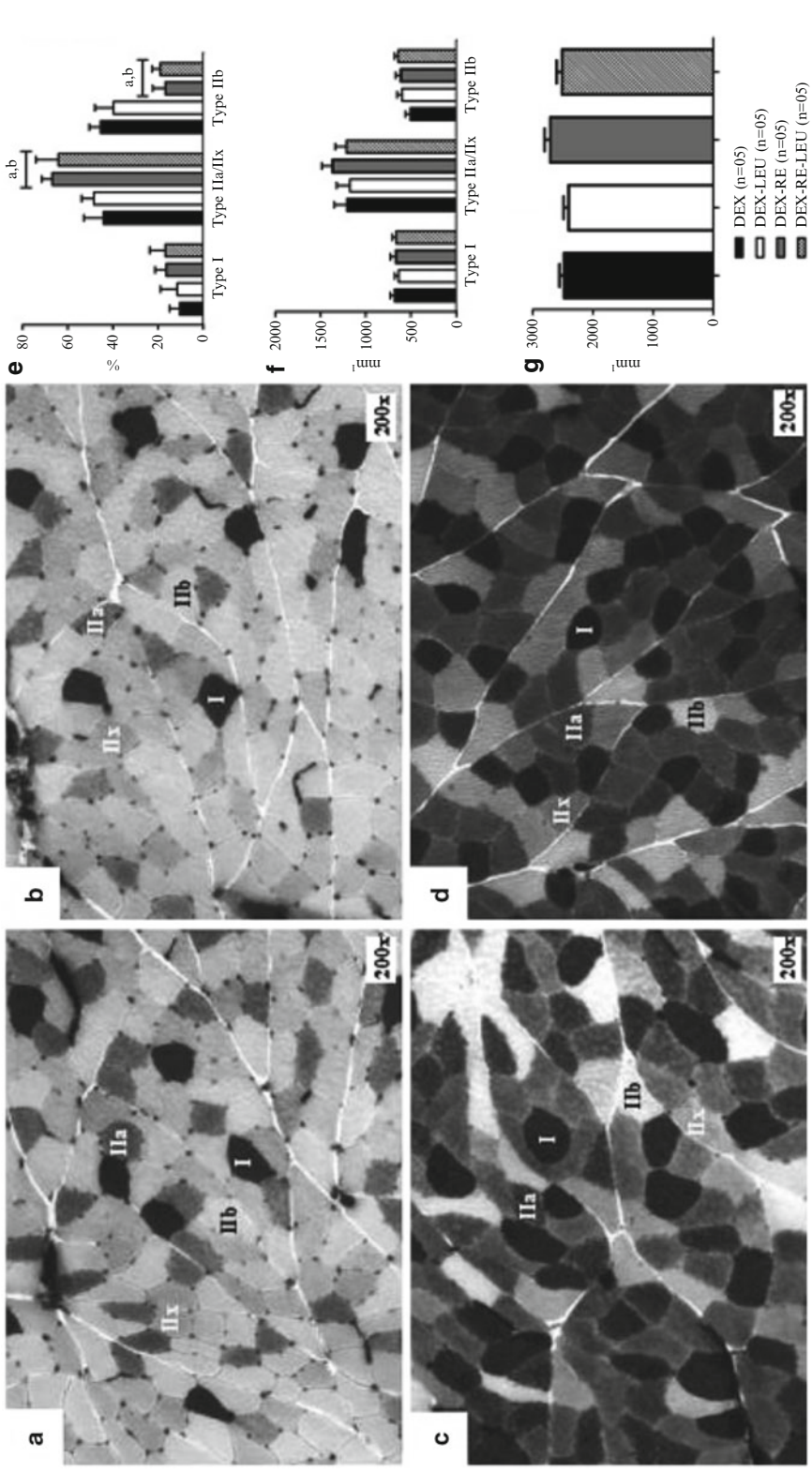

Fig. 19.2 Examples of transverse muscle sections with histochemical staining for myosin adenosine triphosphatase, preincubated at pH 4.6 in plantaris muscles from the (**a**) DEX, (**b**) DEX-LEU, (**c**) DEX-RE, and (**d**) DEX-RE-LEU groups. Fiber types I, IIa, IIx, and IIb were identified. (**e**) Fiber type profile, (**f**) fiber area, and (**g**) muscle cross-sectional. [a]$p<0–0.05$ versus DEX group; [b]$p<0.05$ versus DEX-LEU group. *DEX* dexamethasone, *LEU* leucine supplementation, *RE* resistance exercise. Extracted from Nicastro et al. [10]

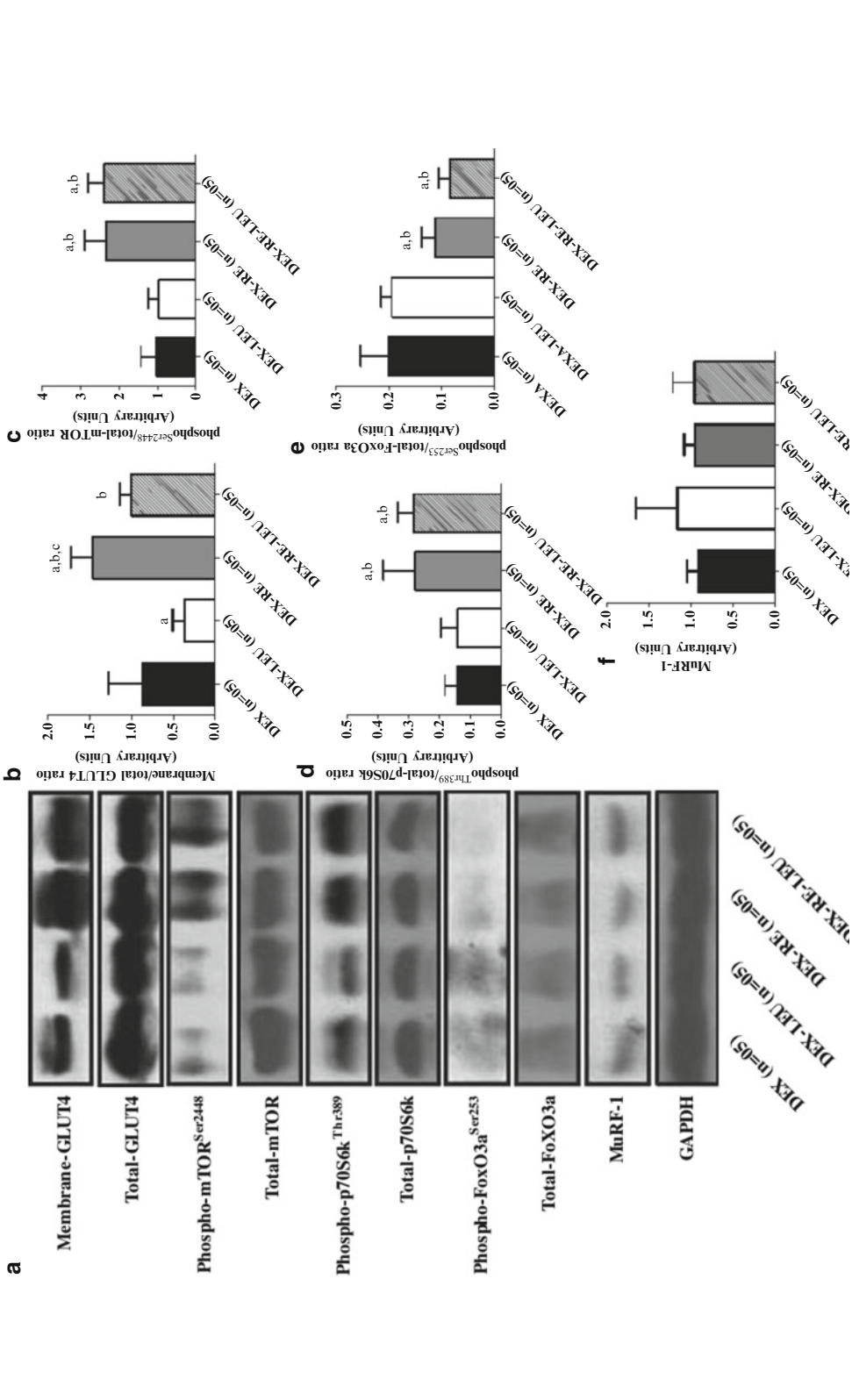

Fig. 19.3 Resistance exercise, but not LEU, improves the expression level of proteins involved in muscle remodeling in the plantaris muscle of DEX-treated rats. (**a**) Representative blots show the effect of RE and LEU on the phospho/total ratio of proteins related to muscle remodeling. (**b**) Plantaris membrane/total ratio of GLUT4, (**c**) phospho/total ratio of mTOR, (**d**) phospho/total ratio of p70S6k, (**e**) phospho/total ratio of FoxO3a, and (**f**) MuRF-1 expression in untrained and RE-trained and DEX-treated rats. $^{a}p < 0.05$ versus DEX group; $^{b}p < 0.05$ versus DEX-LEU group; $^{c}p < 0.05$ versus DEX-RE group; $^{d}p < 0.05$ versus DEX-RE-LEU group. *DEX* dexamethasone, *FoxO3a* Forkhead box protein-3a, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *GLUT4* glucose transporter-4, *LEU* leucine supplementation, *mTOR* mammalian target of rapamycin, *MuRF-1* muscle-specific RINGfinger-1, *p70S6k* 70-kDa ribosomal protein S6 kinase, *RE* resistance exercise. Extracted from Nicastro et al. [10]

Table 19.1 Body weight, skeletal muscle weight, blood glucose, and plasma insulin of each experimental group

Variable	Group			
	DEX ($n=06$)	DEX-LEU ($n=07$)	DEX-RE ($n=05$)	DEX-RE-LEU ($n=05$)
Basal body weight (g)	409 ± 17.9	414 ± 31.3	396 ± 13.4	416 ± 20.1
Final body weight (g)	306 ± 0.9	290 ± 33.5	315 ± 20.1	292 ± 32.6
Plantaris weight (mg)	265 ± 40.2	256 ± 38.3	313 ± 29.1[a, b]	316 ± 35.8[a, b]
Plantaris dry:wet ratio (mg)	0.26 ± 0.04	0.25 ± 0.07	0.25 ± 0.04	0.26 ± 0.04
Plantaris weight/basal BW (mg g^{-1})	0.65 ± 0.09	0.62 ± 0.09	0.79 ± 0.07[a, b]	0.77 ± 0.08[a, b]
Blood glucose (mmol L^{-1})	7.8 ± 1.3[c]	16.1 ± 2.0[a, c, d]	5.3 ± 0.5	7.6 ± 1.8[c]
Plasma insulin (mU L^{-1})	47.7 ± 3.3[c]	65.7 ± 22.4[a, c, d]	25.9 ± 2.9	49.5 ± 11.2[c]
HOMA-IR index (mmol mU L^{-2})	12.7 ± 7.4	47.7 ± 11.0[a, c, d]	6.2 ± 0.2	16.8 ± 3.6[c]

BW body weight. Values are means ± SD
Extracted from Nicastro et al. [10]
[a] $p<0.05$ versus DEX
[b] $p<0.05$ versus DEX-LEU
[c] $p<0.05$ versus DEX-RE
[d] $p<0.05$ versus DEX-RE-LEU

and consumed a high-fat diet (20 %) during 20 weeks. Two of the experimental groups received a normal-protein diet (AP—10 %) or a high-protein diet (HP—50 %) with whey protein and the third group received the same diet of AP group with leucine (AP+L; 6 % of w/w) corresponding to the leucine content of the HP group. The oral glucose tolerance test after 14 weeks showed a significant reduction in fasting plasma insulin of AP+L and HP groups when compared to AP group ($p<0.05$). The levels of GLUT4 mRNA in the epididymal white adipose tissue were 1.6 and 2.8 fold higher in the AP+L and HP groups, respectively, when compared to the AP group ($p<0.01$). In the liver, HP group presented decreased expression of genes related to lipogenesis such as acetyl-alpha-coAcarboxilase (ACCα), fatty acids-binding protein 1 (FABP1), and peroxisome proliferator-activated receptor gamma (PPAR-γ) ($p<0.05$), while AP+L group presented no effect ($p>0.05$). Epididymal white adipose tissue HP diet, but not AP+L, led to increased gene expression of hormone-sensitive lipase ($p<0.05$). These results show that high-dietary protein intake may attenuate weight gain, mainly due to an increase in satiety and diet-induced thermogenesis. It was also found a reduction in body weight gain of 29 and 53 % in AP+L and HP, respectively. This effect can be partially attributed to reduced energy consumption of −7 % (AP+L) and −17 % (HP). Therefore, the authors discuss that leucine can regulate satiety and this occurs due to the activation of mTOR in the hypothalamus. Furthermore, the results suggest an influence of high-protein diets and leucine content in the regulation of body weight in addition to the modulation of food intake. Finally, the researchers concluded that leucine is able to mimic several effects mediated by high-protein diets in preventing the development of obesity and metabolic syndrome, such as attenuation of body weight gain, increased skeletal muscle protein synthesis, as well as prevention of hepatic steatosis and improved insulin sensitivity [24].

There are some studies in humans that corroborate the previous experimental findings. In 2012 was published a cross-sectional cohort study, conducted with 69 children and adolescents (8–18 years), being 17 pre-pubertal or early-pubertal subjects (8–13 years), which were followed in a prospective longitudinal cohort during 18 months. This study aimed to evaluate the possible association between increased plasma fasting BCAAs and obesity and if these factors are independently associated with insulin resistance. The results demonstrated that in cross-sectional cohort, elevations in plasma BCAAs levels were associated with BMI Z-score (Spearman's Rho 0.27, $p=0.03$). In longitudinal cohort, the plasma BCAAs concentrations were significantly correlated with HOMA-IR measured after 18 months ($r^2=0.44$, $p=0.004$) independently of clinical factors such as gender, BMI Z-score, and pubertal stage. These findings demonstrate that elevations in plasma BCAAs levels are substantially correlated with obesity and may predict insulin resistance in children and adolescents [25].

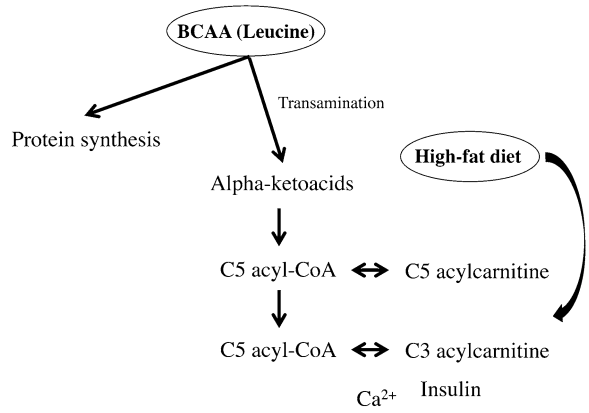

Fig. 19.4 Interplay between BCAA/leucine metabolism, high-fat diet, and insulin resistance (adapted to Newgard et al. [26])

In order to support such evidence, it was recently demonstrated that BCAAs have similar metabolic signatures than that induced by lipid intake. This was clearly demonstrated in the elegant study of Newgard et al. [26] which demonstrates that obese individuals present higher serum levels of BCAAs, glutamate/glutamine, glycine, alanine, phenylalanine, tyrosine, aspartate/asparagine, arginine, citrulline, and proline when compared to lean subjects. Furthermore, it was also observed that obese subjects also present higher serum levels of acylcarnitines such as propyonil (C3), isovaleryl/2-methylbutyryl (C5), hexanoyl (C6), and octenoyl (C8) than lean individuals. Later, Newgard [27] described these acylcarnitines as mediators of mitochondrial stress, which can impair insulin sensitivity. Thus, it is clear that there is a metabolic association between BCAA and lipid metabolism, i.e., high fat diets can increase the generation of acylcarnitines (mainly C3 and C5) that can promote a substantial mitochondrial stress and impair insulin sensitivity and, under this dietetic condition, BCAA intake can synergically aggravate insulin resistance.

Considering that there is a strong association between insulin resistance and plasma BCAAs levels, the question is: why obese individuals present increased plasmatic BCAAs concentration? Is it a result of increased proteolysis? Lackey et al. [28] recently demonstrated that human adipocytes of obese individuals present decreased gene expression (mRNA) of BCKDHA and BCKDHB which are essential components of branched chain amino acid dehydrogenase complex (BCKDH), responsible for amino acids oxidation. Importantly, such decrease is exclusively related to omental adipose tissue and has no relation with subcutaneous body fat. In the same study, the authors also demonstrated in animals that such impairment is dependent of lipid content in the diet (Fig. 19.4).

Therefore, obese subjects' present increased plasma BCAA concentration due to the decreased oxidative capacity in the omental tissue, which contributes to increased acylcarnitines production that may induce mitochondrial stress and aggravate insulin resistance. Such effect is dependent of lipid content in the diet. Therefore, obese individuals should not increase BCAAs/leucine intake before having previous and significant changes in body composition and food intake.

Since there are evidences showing that leucine has the ability to act as an insulin secretagogue, as mentioned previously, it has been suggested that this amino acid may have an effect on glucose homeostasis of type 2 diabetes mellitus subjects [29–31]. In humans, Manders et al. [30] showed in randomized and double-blind design that carbohydrate and protein intake (0.7 g kg d^{-2} of CHO and 0.3 g kg d^{-2} with protein hydrolysate) immediately after breakfast, lunch, and dinner with or without leucine addition (0.1 g kg d^{-2}) decreased postprandial glycemia in individuals with type 2 diabetes when compared to the placebo group ($p<0.05$) receiving metformin and sulfonylureas. Importantly, the therapeutic effect observed on glucose homeostasis was observed independently of leucine addition.

Long-term studies corroborate such results as shown by Leenders et al. [32], who evaluated the effects of leucine supplementation (7.5 g d^{-1}) in elderly people diagnosed with type 2 diabetes

mellitus. Sixty elderly (71 ± 1 years old; BMI 27.3 ± 0.4 kg m^2) were randomly allocated into two groups which received 2.5 g leucine ($n = 30$) three times a day (breakfast, lunch, and dinner) or placebo (500 mg of wheat flour each one) during 6 weeks. Body composition, muscle fibers characteristics, muscle strength, glucose concentration homeostasis, and plasma amino acids and lipids were assessed before, during, and after intervention. The results showed that there was no change in lean mass after leucine supplementation when compared to the placebo group ($p > 0.05$). Also, there was no change in body fat percentage, muscle strength, and muscle fiber type composition ($p > 0.05$). The glycosylated hemoglobin was also not altered after supplementation nor even differed between groups (7.1 ± 0.1 % in leucine group vs. 7.2 ± 0.2 % in the placebo group; $p > 0.05$), suggesting that leucine supplementation by 6 months had no beneficial effect in elderly type 2 diabetics who consume adequate protein through food.

These results demonstrate that leucine supplementation appears to have no effect on glucose homeostasis of type 2 diabetic subjects (taking hypoglycemiants) which consume high or normal-protein diets. Such evidence can be partially explained by the fact the normal or high-protein diets already have sufficient amounts of leucine.

Conclusions

In summary, leucine has significant effects on insulin secretion in both human and rodents. However, the physiological meaning of such plasmatic hormonal increase is variable according to the dietary intake, body composition, and resting plasma concentration of BCAAs. It appears that obese subjects are the main risk population for leucine supplementation since they present elevated resting plasmatic amino acids concentration due to inefficiency of oxidation by the adipose tissue. Furthermore, obese subjects also present strong tendency to consume high-fat diets, which is an aggravating factor in the development of insulin resistance, but more studies are needed.

Future studies should investigate the effects of leucine supplementation associated with different food intake patterns, with and without exercise in both obese and type 2 diabetic subjects in order to confirm which are the limiting and predictive factors associating leucine supplementation with insulin resistance.

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Chapter 20
Weight Loss and Branched Chain Amino Acids and Their Metabolites

Blandine Laferrère, Kirsi Pietiläinen, and Yves Boirie

Key Points

- Circulating branched chain amino acids are strongly associated with insulin resistance, obesity, and type 2 diabetes.
- Circulating branched chain amino acids can predict later development of type 2 diabetes.
- Adipose tissue is not only involved in fat and glucose metabolism but also in protein metabolism and branched chain amino acids oxidation.
- The metabolism of branched chain amino acids in adipose tissue and skeletal muscle can modulate their circulating levels.
- Weight loss and/or physical activity, by modifying insulin resistance and altering the catabolism of branched chain amino acids in adipose tissue and/or muscle, decrease circulating concentrations of branched chain amino acids.

Keywords Amino acids • Obesity • Insulin resistance • Diabetes • Weight loss

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Abbreviations

AA	Amino acids
BCAA	Branched chain amino acids
BMI	Body mass index
T2DM	Type 2 diabetes
RY-GBP	Roux-en-Y gastric bypass
JIB	Jejunoileal bypass
PPARγ	Peroxisome proliferator activated receptor gamma
HPLC	High-performance liquid chromotography
AC	Acyl carnitin
PC	Principal component
HOMA-IR	Homeostatic model assessment-insulin resistance
MZ	Monozygotic
DZ	Dizygotic
SNP	Single-nucleotide polymorphism
GSEA	Gene set enrichment analysis
BCAT	Branched chain amino acid transaminase
BCKDHB	Branched chain keto acid dehydrogenase E1, beta polypeptide
BCKD	Branched chain α-ketoacid dehydrogenase
MCCC	Methylcrotonoyl-CoA carboxylase
AUH	AU RNA binding protein/enoyl-CoA hydratase
HIBADH	3-Hydroxyisobutyrate dehydrogenase
ALDH6A1	Aldehyde dehydrogenase 6 family, member A1
ACAD8	Acyl-CoA dehydrogenase family, member 8
mTOR	Mammalian target of rapamycin
mtDNA	Mitochondrial DNA
CRP	C-reactive protein
hsCRP	High-sensitivity C-reactive protein
IL	Interleukin
NMR	Nuclear magnetic resonance
mRNA	Mitochondrial RNA
AT	Adipose tissue
WAT	White adipose tissue
SAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue
IA	Intraabdominal
GLUT4	Glucose transporter type 4
IR	Insulin resistance

Introduction

The combination of decreased physical activity and over nutrition has resulted in a worldwide increase in the prevalence of obesity and its associated complications. In the United States, about 2/3rd of adults are overweight [with body mass index (BMI) $\geq$25 kg/m^2] and 1/3rd adults are obese (BMI $\geq$30 kg/m^2) [1]. Obesity is often associated with insulin resistance, and with increased incidence of type 2 diabetes (T2DM), cardiovascular disease, hypertension, and cancer [2]. Weight loss reduces

insulin resistance, prevents and/or treats diabetes [3], and decreases cardiovascular risk [4]. The biological factors that link excess weight, insulin resistance, and weight loss with improved health and metabolic status are not completely understood. Studies on insulin resistance and T2DM have focused on disordered glucose and lipid metabolism and insulin signaling, however protein metabolism is also altered in the state of insulin resistance at the whole body level as well as at the muscle level [5]. Elevated amino acid (AA) has long been associated with obesity and insulin resistance [6] and recently, circulating branched chain amino acids (BCAA) have been identified as early biomarker predictors of diabetes risk in obese insulin resistant subjects [7]. This chapter will review the effect of weight loss on BCAA metabolism in obesity and diabetes.

Amino Acid and Insulin Resistance

Altered AA metabolism in obesity was described over 40 years ago, in the late 1960s [6, 8], suggesting a direct or indirect role of adipose tissue (AT) in AA disorders. Obesity and insulin resistance, with or without T2DM, are conditions associated with elevated blood concentrations of BCAA [6]. More recently, these data have been replicated and expanded with the use of metabolomic analysis. Circulating concentrations of BCAA and some of their metabolite acylcarnitin (AC) derivatives have been shown to be elevated in various human cohorts of obese and/or diabetic individuals of different ethnicity [9–14] and strongly associated with insulin resistance [15]. Interestingly, concentrations of BCAA in normoglycemic individuals were recently shown to be a strong predictor of T2DM 12 years later in the offspring of the Framingham cohort [7], underscoring the potential role of AAs early in the pathogenesis of T2DM. The glycemic predictive value of BCAA was also found in cohorts of middle aged Finnish men and women. BCAA and phenylalanine were predictors of both fasting and 2-h glucose at 6.5-year follow-up, suggesting that alterations in BCAA and aromatic AA metabolism precede hyperglycemia [16]. This is interesting as most obese persons with insulin resistance do not develop T2DM, and identifying early biomarker of later risk to develop T2DM could potentially have clinical implications for targeted interventions. Stancáková et al. [17] investigated the association of glycemia and genetic risk variants for hyperglycemia/T2DM with AA levels in 9,369 nondiabetic or newly diagnosed diabetic Finnish men. Increasing fasting and 2-h plasma glucose levels were associated with increasing levels of several AA. Several AA including BCAA, predicted the incidence of T2DM in a 4.7-year follow-up, and effect largely mediated by insulin resistance [17]. Altogether these studies suggest that long-term modulations of plasma BCAA may influence insulin resistance and glucose metabolism, and that changes in fat mass may contribute to glucose improvement through its impact on BCAA metabolism. Inversely, weight loss is a clinical situation where BCAA metabolism may be affected together with improvement of insulin sensitivity and glucose metabolism.

Weight Loss by Caloric Restriction

Circulating concentrations of BCAA can be modified by interventions that modulate insulin resistance, such as thiazolinedione, diet composition, and weight loss.

PPARγ increased insulin sensitivity and expression of mitochondria-associated BCAA degradation pathways in AT of obese rats [18] and 3T3 adipocyte cells [19]. In the presence of a high fat diet, dietary BCAA contributes to the development of obesity associated insulin resistance [9]. The association of BCAA with insulin resistance has led to the hypothesis that over nutrition, particularly dietary fat and protein, could result in incomplete beta-oxidation, accumulation of incompletely

oxidized lipids, mitochondrial stress, and impaired insulin action [20–22], and calorie restriction and weight loss may reverse that phenomenon. This observation clearly explains why some studies have reported opposite results regarding the impact of BCAA especially leucine on insulin sensitivity, since the metabolic impact of BCAA may also depend on the overall energy balance of the individuals. So it can be hypothesized that BCAA may be beneficial for insulin sensitivity in situation of neutral or negative energy balance, and deleterious in the case of high-fat overfeeding.

The decrease of plasma BCAAs after weight loss was first shown by Felig et al. [6] in a small study of three obese subjects participating in a partial "therapeutic starvation" experiment which lead to a 16–32 kg weight loss in 3–4 months' time. In each of the subjects, plasma BCAAs fell by 15–50 % with a concomitant reduction in insulin levels. In one of the subjects, Felig et al. had follow-up data after the patient had regained 13 kg of the 16 kg lost, whereafter the circulating BCAAs concentrations increased back to preintervention levels. More recently, a 6 months diet-induced weight loss was found to be accompanied with improved insulin sensitivity and decreased circulating BCAA and their metabolites in obese adults [23]. Although calorie restriction by diet does not always significantly decrease BCAA [24, 25], the change of AC derivatives is inversely related to fasting insulin during calorie restriction [25].

Surgical Weight Loss

For patients with severe obesity (BMI ≥35 kg/m^2), bariatric surgery is often the treatment of choice, as it results in 20–30 % weight loss, often sustained overtime, and improvement and/or remission of most obesity related comorbitidies. There are different types of surgery. Roux-en-Y gastric bypass (RY-GBP), one of the most frequently performed weight loss surgeries for severe obesity, is a complex procedure that involves the creation of a small gastric pouch, a gastro-jejunal anastomosis, and the shunting of the duodenum and early jejunum. As a result of RY-GBP, the gastro-intestinal transit time is accelerated for liquids, the postprandial release of incretin and other gut hormones is enhanced, and the metabolism of bile acids is impaired, among other significant changes. Clinically, RY-GBP results in significant and sustained weight loss with improvement and /or resolution of most obesity-associated comorbidities, including T2DM [26]. Although weight loss improves insulin sensitivity, the rapidity of diabetes resolution after RY-GBP (within days), well before a large amount of weight loss has occurred, or even without weight loss in patients with lower presurgery BMI, suggests that factors other than weight loss play a role in the improved glucose tolerance observed in response to RY-GBP. These factors have not been fully identified.

It was first noted in 1977 that the circulating concentrations of most AAs were decreased 4 months after jejunoileal bypass (JIB) surgery, compared to preoperative levels, a phenomenon attributed to protein deficiency, frequent after this surgery [27]. JIB is no longer performed, due to high related mortality by liver disease. Subsequently, a decrease of overnight fasted BCAA concentrations, measured by either enzymatic spectrophotometric assay or HPLC-coupled fluorometric method, was reported 17 months after RY-GBP surgery in 16 patients who had lost 55.5 kg [28]. Using relative quantification and principal component analysis (PCA), Mutch et al. [29]. assessed the changes of various compounds in 14 patients, including 5 patients with T2DM. They also showed a decrease in BCAA, particularly of valine and its metabolite, serum beta-amino isobutyric, after 20 and 30 kg GBP weight loss [29]. We applied metabolomic profiling to understand the mechanisms of improved glucose metabolism after RY-GBP in two groups of severely obese patients [25]. Circulating BCAA, AC, and metabolites derived from BCAA oxidation were measured in plasma from fasted subjects by targeted tandem mass spectrometry in two independent cohorts, before and after RY-GBP. Results were very similar in both cohorts. In the first cohort, BCAA decreased by 38 % BCAA after 10 kg

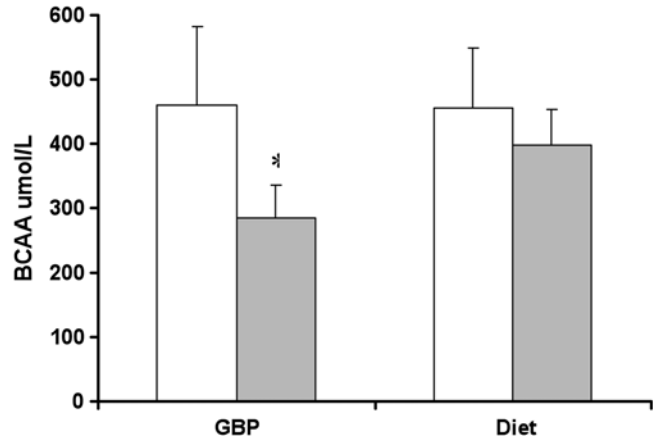

Fig. 20.1 Decrease in plasma BCAA concentrations in response to surgical or dietary interventions. Shown are total circulating (plasma) BCAA concentrations before (*white bar*) and after (*shaded bar*) weight-loss interventions. Data are presented as mean ± standard deviation (SD), * = $P < 0.01$ by Student's paired *t*-test. From Laferrère et al. [25] with permission from Science Translational Medicine [25]

weight loss in severely obese patients with T2DM. PCA identified two major PCs, one comprised almost exclusively of AC (PC1) and another with BCAA and their metabolites as major contributors (PC2). PC1 and PC2 were inversely correlated with proinsulin concentrations, the C-peptide response to oral glucose, and the insulin sensitivity index derived from the oral glucose tolerance test after weight loss, while PC2 was uniquely correlated with levels of insulin resistance (assessed by HOMA-IR). These data suggest that the decrease in circulating AA after GBP in conjunction with negative energy balance may contribute to the improvement in glucose homeostasis observed with this surgical intervention [25]. This observation was replicated in a different cohort of nondiabetic individuals, with 36 % decrease in circulating BCAA, measured by the same laboratory, after a larger 27 kg weight loss by RY-GBP [25]. In an attempt to understand the mechanisms of the superior effect of RY-GBP vs. diet-induced weight loss on glucose metabolism [30], we compared the effect of RY-GBP to that of the effect a matched weight loss by diet. Surprisingly, BCAA and AC derivatives decreased only after RY-GBP but not after diet-induced weight loss, in two independent cohorts [25] (Fig. 20.1). In an elegant study in nondiabetic individuals, Klein's group confirmed that weight loss induced by RY-GBP causes a decline in circulating BCAAs and their C3 and C5 AC metabolites [31]. Plasma BCAA concentration was negatively associated with skeletal muscle insulin sensitivity, measured by insulin clamp [31]. However, the same findings were also seen after a matched ~20 % weight loss by gastric banding, a purely restrictive surgery. The discrepancy between Klein's findings after gastric banding and our data in two different independent cohorts after diet-induced weight loss is puzzling, but could be due to difference in amount and/or rate of weight loss, diet composition, and/or the T2DM status.

Twin Studies

Classical Twin Studies

Twin studies have been traditionally used to estimate the importance of genetic and environmental influences on complex traits such as obesity and insulin resistance. The classical twin design relies on comparing the similarities within monozygotic (MZ) and dizygotic (DZ) twin pairs. While MZ (identical) twins share all of their genes, DZ (fraternal) twins share only about 50 % of them. Both zygocity groups have equal shared (family) environments and the remaining variance for which

neither the MZ nor the DZ cotwins are alike, is explained by unique environmental factors. The latter also includes measurement error.

Based on these assumptions, the heritability estimates of serum BCAA concentrations were 52 % for leucine, 51 % for isoleucine, 45 % for valine, and 55 % for the combination of all BCAAs in a population representative sample of 22–25-year-old Finnish twins [32]. It was interesting to note that the heritability of serum BCAA was similar to that of peripheral insulin sensitivity in an earlier study in Danish twins (53–55 %) [33]. For insulin secretion, the proportion of variance explained by genetic factors has been much higher, (75–84 %) [33] as has been for the development of type 2 diabetes (64–73 %) [34]. This is in line with the current thinking that insulin secretion capacity is more of a genetic trait whereas insulin resistance, for which one marker could be the BCAAs, has a larger environmental component. This thought is also supported by the evidence showing that most of the risk genes found for T2DM influence pancreatic β-cell function [35]. The gene variants associating with BCAAs remain largely unknown. In the same study where the total heritability of circulating BCAAs was calculated to be 55 %, the actual gene variants found associating with them explained only 0.4 % of the variance of BCAAs [32]. This SNP locates in the first intron of SLC1A4 (encoding solute carrier family 1 member 4), a neutral amino acid transporter. Based on the total heritability estimate, there is room to more intensive search for gene polymorphisms and biological pathways underlying BCAA metabolism and circulating BCAA concentrations.

Obesity-Discordant MZ Twins

Twin studies can also provide insights into the role of environmental and lifestyle factors, independent of genes, in trait-discordant MZ cotwins. We have utilized this design and studied rare obesity-discordant MZ twins in their mid twenties collected from five twin birth cohorts (1975–1979) in Finland [36]. In this design, the obese cases and their lean controls are completely matched for genes, age, sex, and family background, which is a powerful advantage in the study of human metabolism. In all pairs, the obese and nonobese cotwins had diverged in their weight development after age 16, a time point when the young adults moved out of their parental home and established more individualistic life styles [36, 37]. The data therefore represents a model of healthy young adults' acquired obesity where both eating habits and physical activity play a role in the etiology of obesity [38, 39]. A number of prediabetic and preatherosclerotic changes were observed in the obese (mean BMI 31 kg/m^2) as compared with their nonobese counterparts (BMI 25.6 kg/m^2): reduced insulin sensitivity in the euglycemic-hyperinsulinemic clamp, increased serum insulin, higher liver fat, mild dyslipidemia, proinflammatory serum and adipose tissue lipidomics profile, and arterial endothelial dysfunction [36]. One of the most interesting findings were observed in the AT Gene Set Enrichment Analysis (GSEA), where the most underexpressed pathway in the obese cotwins was BCAA catabolism, mostly contributed by the genes encoding the mitochondrial components of the pathway [40]. Both genes common to the degradation of all BCAAs (BCAT2, BCKDHB) and those specific for the degradation of leucine (MCCC1, MCCC2, AUH) and valine (HIBADH, ALDH6A1, ACAD8) were downregulated in the obese cotwins. This finding prompted the hypothesis that perhaps the decrease in tissue catabolism of BCAAs, a task mainly dedicated to the mitochondria, is responsible for the increase in circulating BCAA concentrations in obesity and insulin resistant states. Indeed, it has been confirmed in rodents that the decreased rate of AT BCAA oxidation is able to modulate circulating BCAA levels especially when glucose transporter 4 was overexpressed in AT [41]. Clinically and in support of this, the plasma BCAA was higher in obese than in nonobese male cotwins, and the decreased BCAA catabolism activity in AT was paralleled by decreased α-ketoisocaproate (leucine breakdown product) levels in plasma ($r=0.44$, $p=0.027$). Several clinical measures correlated with

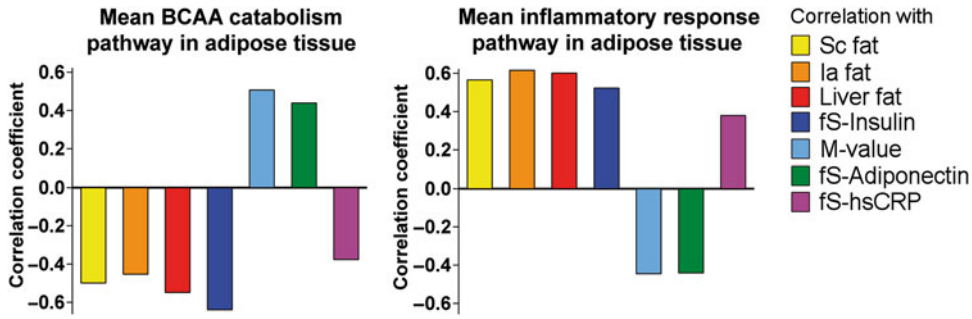

Fig. 20.2 Correlations of BCAA catabolism pathway and inflammatory response pathway expressions in the AT with clinical measures of obesity and insulin sensitivity in 22–27-year-old MZ twin individuals. *Sc* subcutaneous, *Ia* intraabdominal, *M value* whole body insulin sensitivity (from the euglycemic, hyperinsulinemic clamp), *fS* fasting serumM, *hsCRP* high-sensitivity C-reactive protein. Drawn based on data published in Pietilainen 2008 [40]

adipose tissue BCAA catabolism (Fig. 20.2). More active BCAA catabolism in fat was related to lower liver fat, better insulin sensitivity, higher adiponectin, and lower CRP concentrations. On the other hand, the most upregulated pathways in the AT were related to inflammatory cascades, and their correlations with the clinical values were exactly opposite to those of the BCAA catabolism.

In addition to the reduced BCAA catabolism, the obese cotwins' AT also exhibited a decrease in mitochondrial fatty acid oxidation and oxidative phosphorylation pathways and a dramatically (47 %) reduced mitochondrial DNA (mtDNA) copy number [40, 42]. Such reductions had previously been observed in patients with mitochondrial disease, whereby we also sequenced the whole mtDNA to rule out the possibility of mitochondrial mutations in the obese cotwins. There was indeed no evidence of heteroplasmy neither in blood nor in AT as the mtDNA sequence was identical between the MZ cotwins in both of these tissues. Therefore, this suggests that the downregulation in the mitochondrial genes observed in the trascript levels was more likely a functional rather than a primary defect, and consequence rather than a cause of obesity. It is however conceivable that such a drastic reduction in the mitochondrial function may aggravate obesity by decreasing the metabolic efficacy of energy production within the fat tissue. One possible consequence of the decreased catabolism of BCAAs is impaired adipocyte differentiation because mitochondrial metabolism of BCAAs, especially that of leucine, stimulates adipocyte growth and differentiation through activation of the mammalian target of rapamycin (mTOR) signaling [43]. The finding that in our twin studies, low BCAA catabolism cooccurred with decreased adipocyte differentiation pathway and that these were especially well-correlated with increased liver fat [40], argues for the fact that the decreased activity of the BCAA pathway may suppress production of new adipocytes, the "healthy" enlargement of subcutaneous fat, and thereby predisposes to ectopic fat accumulation in obesity.

Physical Activity-Discordant Twins

Another Finnish twin study examined how gene expression profiles of skeletal muscle and AT differed between cotwins discordant for physical activity for more than 30 years [44]. In this study, the GSEA showed that BCAA catabolism was the top second pathway upregulated in the more active cotwins both in the muscle and in the AT, after oxidative phosphorylation in the muscle and IL2B pathway in AT. Increased BCAA tissue gene expression activity was coupled with lower valine and isoleucine concentrations in serum [45]. The researchers then did a meta-analysis of three other population-based cohorts where 1,037 unrelated age-matched and sex-matched pairs with persistent

activity vs. inactivity for more than 5 years were studied for their NMR metabolomic profile. In this meta-analysis, isoleucine, α1-acid glycoprotein, and glucose were lower in the physically active than in the inactive individuals; serum fatty acid composition was shifted toward a less saturated profile; and lipoprotein subclasses were shifted toward lower very-low-density lipoprotein and higher high-density lipoprotein particle concentrations. The findings persisted after adjustment for BMI. Together these findings from the twin studies show that (1) genes and environmental factors are equally important in regulating BCAA metabolism; (2) the polymorphisms underlying individual variations in BCAA levels are yet to be found; (3) acquired downregulation in the mitochondrial activity of BCAA catabolism in the AT in obesity is tightly linked with the development of liver fat and insulin resistance and may prevent its differentiation; (4) and exercise, even independent of BMI, increases mitochondrial enzymatic activity of BCAA metabolism in the insulin sensitive peripheral tissues (AT and muscle), and produces a range of other beneficial metabolic effects.

Impact of Diet on Circulating BCAA Levels

The BCAA: leucine, isoleucine, and valine, three of the nine essential AAs, are relatively abundant in the food supply accounting for 20 % of total protein intake [46] and are a major component of breast milk proteins in many species [47]. The ingestion of dietary protein, of various forms, either as a single meal [48–50] or as chronic supplementation [51], can modify circulating BCAA. Therefore, diet composition and/or the abundance of dietary protein could influence circulating BCAA, and the decreased circulating plasma AA concentration after surgical weight loss could logically result from a decrease in protein intake (and in parallel, essential AAs such as BCAA) [52–54]. Another explanation for changes in BCAA concentrations during weight loss may also come from the balance between BCAA production from dietary intake or from protein breakdown (mainly from muscle) and utilization of BCAA for protein synthesis or AA oxidation. In addition, in the context of over nutrition and high-fat diet, dietary BCAA contributes to the development of obesity associated insulin resistance in rodents [9]. In the context of undernutrition as in the postsurgery situation, it can be hypothesized that part of the BCAA are being used for peripheral energy production to spare glucose for brain utilization. The effect of dietary BCAA supplementation alone or ingested with leucine-rich dietary proteins in health has been studied in the context of liver disease [55], brain neurotransmitters and food intake behavior [56], lactation and mammary gland growth [57, 58], physical activity performance [59, 60], sarcopenia of the elderly [61, 62], and glucose metabolism. It is beyond the scope of this chapter to review this literature.

Metabolism of Amino Acids in Adipose Tissue

The modulation of blood concentration of AAs in the fasted state is affected by the rates of tissue protein catabolism and release of AAs, tissue specific uptake of AAs, oxidative metabolism of AAs, protein synthesis rate, the description of all of which would be beyond the scope of this chapter. In previous studies in obese individuals with insulin resistance, metabolomic analysis coupled with principal components analysis demonstrated that BCAA, other aromatic AAs, and some byproducts of BCAA catabolism form an independent clustered variable, suggesting that the association of IR is not just with BCAA, but with altered metabolic flux of the BCAA catabolic pathway. In contrast to the other 17 AAs, which are predominantly metabolized in the liver, BCAAs are poorly metabolized during their first pass through the liver, as the liver expresses only low levels of the mitochondrial branched chain aminotransferase (BCAT2 or BCATm), the first enzyme in the catabolism of BCAAs

in most peripheral tissues [63]. Therefore BCAA catabolism in the fasted state is primarily done by peripheral tissues (particularly muscle and AT), rather than liver, in three steps: transamination, oxidative decarboxylation, and dehydration (Please see excellent Review) [64]. The capacity of AT and skeletal muscle to catabolize BCAA is estimated to be six to sevenfold higher than in the liver, taking into account the relative masses of different tissues [65, 66]. In the study by Stancáková et al. [17] one of the mechanisms of the relationship between circulating BCAA and altered glucose metabolism, mediated by the degree of insulin resistance, was the catabolism of BCAA in subcutaneous adipose tissue (SAT). In a subgroup of 200 biopsies, these investigators also found an association between insulin sensitivity (measured by the Matsuda insulin sensitivity index) and mRNA expression of SAT genes regulating AA degradation, highlighting the link between insulin resistance, and AA metabolism in adipose tissue [17]. These findings are in line with the earlier mentioned twin study [40] (Fig. 20.1). The decrease in fasted plasma BCAA concentrations after RY-GBP was shown to be associated with an increase of two key BCAA catabolic enzymes, the BCATm and the branched chain α-keto acid dehydrogenase E1 (BCKD E1α) in both SAT and visceral fat depots (VAT) [28]. Data from Sean Adam's group [19] in rodents model and 3T3-L1 adipocytes cell, showed that the expression of the mitochondrial BCAA oxidation check-point, branched chain α-ketoacid dehydrogenase (BCKD) complex, is reduced in obese white adipose tissue (WAT) and regulated by metabolic signals. WAT BCKD protein (E1α subunit) was significantly reduced by 35–50 % in various obesity rodents models (fa/fa rats, db/db mice, diet-induced obese mice), and BCKD component transcripts significantly lower in SAT adipocytes from obese vs. lean Pima Indians. Their results support the hypothesis that a state of obesity and insulin resistance could impair adipose tissue BCAA utilization. Interestingly, crosstissue flux studies comparing lean vs. insulin-sensitive or insulin-resistant obese subjects revealed an unexpected negligible uptake of BCAA from human abdominal SAT. Moreover, the amount of BCAA catabolic enzymes mRNA was markedly reduced in omental (but not SAT) AT of obese subjects with metabolic syndrome compared to weight-matched healthy obese subjects, raising the possibility that VAT, rather than SAT, contributes to the BCAA metabolic phenotype associated with insulin resistance in the overnight-fasted state, in individuals with impaired metabolism. Observation in animal models recently demonstrated that the metabolism of BCAA in AT can exert a direct and significant impact on circulating BCAA levels [41]. The rate of adipose tissue BCAA oxidation per mg of tissue from normal mice is higher than in skeletal muscle. Manipulation of GLUT4 receptor expression in AT downregulates the BCAA metabolizing enzymes selectively in AT, not in muscle, in association with increased circulating BCAA levels. These observations suggest that in situation of lower glucose uptake in muscle as in insulin resistant state, a higher glucose availability for AT may alter BCAA metabolizing enzymes and contribute to higher BCAA concentration. AAs have been shown to be potent modulators of mTOR and its downstream target S6 kinase pathway. Studies in rodent and/or muscle cells found that exposure to high levels of BCAA impairs insulin action, in concert with activation of the mTOR, which causes serine phosphorylation of insulin receptor substrate-1 and disrupts insulin signaling [9, 67–70] and effect is partially inhibited by rapamycin, an inhibitor of mTOR [69]. One study explored BCAA catabolism in muscle following bariatric surgery [31]. In order to test whether the decline in circulating BCAAs after weight loss induced by bariatric surgery was due to decreased signaling through the mTOR, mTOR phosphorylation was assessed before and after surgical weight loss. Contrary to the hypothesis, surgical weight loss by either RY-GBP or gastric banding did not alter muscle mTOR phosphorylation, in spite of an increased peripheral insulin-sensitivity. The implication of these data is that skeletal muscle mTOR pathway may not contribute significantly to the decrease of the concentration of plasma BCAA and C3 and C5 acylcarnitine after weight loss surgery. Further studies on the rates of BCAA catabolism and BCAA turnover to determine their metabolic fate from protein breakdown through AA oxidation or incorporation for protein synthesis will be required to fully understand the preferential decrease in BCAA in RY-GBP versus diet intervention subjects.

Conclusions

Protein metabolism, like glucose and lipid metabolism, is altered in the state of obesity and insulin resistance, and BCAAs have been identified as early biomarker predictors of diabetes risk. However, norms of BCAA concentrations in diseased state have not been determined and it would be premature to use BCAA as clinical index of diseases. In weight loss, particularly surgical weight loss, studies of protein supplementation and turnover, coupled with assessment of adipose tissue and muscle AA metabolism are needed to understand the determinants of protein metabolism.

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Chapter 21
Branched Chain Amino Acid Cocktails and Skin

Hitoshi Murakami

Key Points

- BCAAs, especially leucine, play an important role in stimulating dermal collagen synthesis in wound healing.
- However, single treatments with BCAAs are not effective for stimulating dermal collagen synthesis during protein malnutrition and in response to low-dose UV irradiation.
- Each single amino acid treatment (e.g., glutamine, proline, etc.) is also not effective for stimulating the dermal collagen synthesis.
- Combinations of other specific amino acids, such as glutamine and proline with BCAAs, are necessary to stimulate the dermal collagen synthesis.
- Therefore, BCAA cocktails that include other amino acids (e.g., glutamine and/or proline) are important for improving skin damage.

Keywords Branched chain amino acid cocktail • Dermal collagen synthesis • Glutamine and proline metabolism • Wound healing • UV irradiation • Protein undernutrition

Abbreviations

UV	Ultraviolet
mTOR	Mammalian target of rapamycin
S6K	Ribosomal protein S6 kinase
4E-BP1	Eukaryotic initiation factor-binding protein 1
NO	Nitric oxide
NOS	Nitric oxide synthase
MAPK	Mitogen-activated protein kinase
AP-1	Activating protein-1
TGF-β	Transforming growth factor-β
HMB	β-hydroxy-β-methylbutyrate

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BCAAs	Branched chain amino acids
EAARQ	Essential amino acids + arginine + glutamine
BCAARQ	BCAAs + arginine + glutamine
BCAAR	BCAAs + arginine
BCAAQ	BCAAs + glutamine
BCAAE	BCAAs + glutamate
BCAAP	BCAAs + proline
AAC	Amino acid mixture composed of collagen protein
EAAs	Essential amino acids
RQ	Arginine + glutamine
mTORC	Mammalian target of rapamycin complex
SLC7A5	Solute carrier family 7 member 5
SLC3A2	Solute carrier family 3 member 2
MMPs	Matrix-metalloproteinases

Introduction

Skin Structure and Function

The most important function of the skin is to form a barrier between the inside and outside of body and to protect us from harmful environments. The skin is the largest and heaviest organ in the body, with an external surface of approximately 2 m^2, and represents nearly 6 % of the body weight. Anatomically, skin is a complex organ composed of three main compartments: the epidermis, the dermis, and the subcutaneous tissue. The main components of the epidermis are keratinocytes, and 95 % of epidermal proteins are keratins; other components include melanocytes, Langerhans cells, α-dendritic cells, and Merkel cells. The epidermis is in contact with the external environment; therefore, the main functions of the epidermis are protection against environment changes, desiccation, and exogenous water loss. The dermis determines skin thickness and supports the skin tissue. The dermal properties depend on interactions between the extracellular matrix and fibroblasts and are necessary for maintaining skin structure. Collagen and elastin fibers are the main components of the dermis, and 70 % of the dry skin mass is collagen protein. Numerous blood capillaries are concentrated below the dermal-epidermal junction. The dermis is provided with nutrients by blood capillaries and, therefore, will be more affected by nutritional status than the epidermis. The subcutaneous tissue is the deepest and thickest component, and its functions are to store energy in the adipocytes, to absorb the impact of outer pressure, and to prevent body temperature loss [1].

Skin Aging and Wounds

Skin Aging

Skin aging, especially wrinkling and sagging, is induced by several environmental factors, including ultraviolet (UV) irradiation, malnutrition, dryness, chemical stimulation, and exposure to activated oxygen species [2]. Among these factors, UV irradiation is well known for aggravating skin aging, which is called photo-aging [2, 3]. Many reports suggest that chronic UV irradiation modifies and

decreases skin proteins, such as collagen, and induces wrinkle formation in humans and animals [2, 4–7]. Therefore, maintenance and an increase in the dermal collagen level are key factors for improving skin structure and function during skin aging.

The Signaling Pathways Initiated by UV Irradiation

Some reviews have described the signaling cascade in the skin that is caused by UV irradiation [2, 3, 8]. UV irradiation induces IL-1α secretion from keratinocytes in the epidermis, the release of IL-11α may induce the secretion of other proinflammatory cytokines, such as IL-6 and IL-8. In addition, TNF-α is stored in epidermal mast cells, is produced by keratinocytes, and functions independently of IL-1. IL-1 and IL-8 are secreted by fibroblasts and are associated with the differentiation of T- and B-cells and chemoattraction, respectively [3]. Furthermore, UV irradiation generates oxygen species, such as hydrogen peroxide and reactive oxygen species, and decreases the activity of antioxidant enzymes, inducing the activation of the mitogen-activated protein kinase (MAPK) family. Oxidative stress and inflammation inhibit protein synthesis as a result of decreases in the anabolic signaling pathway. This change indicates a decrease in the activity of the key regulatory enzymes of mammalian target of rapamycin (mTOR) activity, namely 70 kDa ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor-binding protein 1 (4E-BP1) [9], and stimulation of proteolysis through p38 MAPK [10, 11].

The major effector of the MAPK pathway is transcription factor AP-1 (activating protein-1). AP-1 triggers a cellular response following IL-1-mediated stimulation, which is a potent regulator of the NFκB pathway. AP-1 also regulates the transcription of matrix metalloproteinase (MMP) family members, which control the degradation of extracellular matrixes. MMPs are produced primarily by the epidermis rather than the dermis. After initiation by AP-1, MMP-1, -3, and -9 affect the dermis and can degrade type I, III, and IV collagen [3, 12]. In addition, AP-1 negatively regulates transcription of the genes encoding type I collagen, resulting in decreased expression of collagen protein [2, 12]. Therefore, UV irradiation affects protein metabolism, which leads to a decrease in protein synthesis and an increase in protein degradation, and contributes to the decrease in collagen and elastin protein contents. As a result, chronic UV irradiation decreases dermal collagen protein and results in wrinkle formation [4].

The Signaling Pathways Initiated by Undernutrition

Undernutrition also contributes to skin aging. For example, protein malnutrition decreases skin collagen. Dietary protein deprivation decreases wet skin weight and the protein level of newly formed type I and III collagens. In addition, dietary protein deficiency blunts collagen synthesis and degradation by decreasing the protein expression and the mRNA levels of type I and III collagen; a decrease in type III collagen and in the gene expression of collagenase and MMPs in protein malnourished rats has been shown in previous studies [13]. Protein undernutrition also decreases skin collagen content during the early stage of wound healing in rats but does not affect the content during the late stage [14]. The synthesis rate of newly soluble dermal collagen and tropocollagen decreases depending on the dietary protein level in rats [15]. Dietary protein restriction decreases the absolute and fractional synthesis and degradation of acetic acid-soluble collagen proteins; consequently, growing rats exhibit a decreased rate of formation of insoluble collagen [16].

Skin Wounds

Skin wounds are hypermetabolic responses that increase energy consumption and nitrogen loss; therefore, adequate skin collagen synthesis is fundamental for wound healing. The wound healing process has four phases: hemostasis, inflammation, tissue regeneration, and tissue remodeling with scar formation. Among these phases, inflammation is the most fundamental, and nitric oxide (NO) production is also associated with the wound healing. Inflammation affects collagen metabolism, as described above. A low dose of NO regulates collagen formation and cell proliferation, and a high dose of NO regulates bacteriostatic and vasodilatory effects and wound contraction during wound healing [17]. Moreover, inhibiting NO synthase (NOS) reduces collagen synthesis during wound healing [18]. In contrast, UV irradiation can suppress NOS expression, which indicates that the mechanisms involved in the repair of the dermis differ for damage caused by UV irradiation, wounds, and protein undernutrition [19, 20].

The Effect of Amino Acids on Dermal Protein Metabolism

Improving collagen protein metabolism is a fundamental approach to improve skin aging and wound healing. Amino acids are protein substrates and regulators of protein metabolism and treatment with amino acids is safe for human. Most studies that have focused on the effects of amino acids on skin have investigated dermal collagen metabolism in vitro and in vivo.

Glutamine

Glutamine supplementation increases type I and III mRNA levels and collagen synthesis via the specific activities of proline and/or hydroxyproline in in vitro studies using human dermal confluent fibroblast cells. The effect of glutamine supplementation on cultured cells appears at physiological concentrations (e.g., 0.15 mM). Furthermore, this effect is specific because analogs and/or derivatives of glutamine, such as acivicin, 6-diazo-5-oxo-l-norleucine, homoglutamine, and ammonium chloride, do not replicate the effect of glutamine on collagen synthesis. Inhibiting glutamine uptake into cells diminishes the intracellular conversion of glutamine to proline via glutamate, resulting in a reduction of pro-α1 collagen gene transcription [21]. The effect of glutamine on collagen gene expression is independent of transport system A but dependent on transport system L [21]. In addition, the conversion of glutamine to proline and the incorporation of labeled glutamine into procollagen were measured using the radioactivity of proline derived from L-[U-^{14}C] glutamine and the concentration of proline deduced from the radioactivity [22]. Glutamine provides 75 % of the intracellular free proline and 85 % of the procollagen-bound proline in fibroblast cells [22]. Therefore, Bellon et al. suggested that de novo proline synthesis from glutamine is a key factor for collagen synthesis via glutamine supplementation [21, 22]. A potential mechanism underlying glutamine-induced collagen biosynthesis is that pyrroline-5-carboxylate, which is the intermediate during the de novo synthesis of proline from glutamine and/or ornithine, converts to proline in fibroblast cells. Inhibiting glucose-6-phosphate dehydrogenase, which inhibits the enzymatic conversion of proline from pyrroline-5-carboxylate, reduces the stimulatory effect of pyrroline-5-carboxylate during collagen synthesis. Therefore, the pyrroline-5-carboxylate-dependent activation of nucleotide biosynthesis, prolidase activity, and pyrroline-5-carboxylate conversion into proline may stimulate collagen biosynthesis [23].

Arginine

Some studies address the effects of amino acids on wound healing. Arginine [24, 25] and ornithine [13] enhance wound healing; in particular, some studies show that arginine supplementation repairs wounds by increasing the collagen content. The mechanism of arginine in wound healing has been thoroughly reviewed by Stechmiller et al. [24]. Arginine contributes to protein synthesis as a substrate for the urea cycle and functions in differentiation and proliferation. Arginine is also a substrate for NO production. NO activates macrophages induced by the overall cytotoxic wound environment in the early phase of wounds, as described above. NO produced in the fibroblast cells increases collagen formation. Arginine is also metabolized to ornithine by arginase. Ornithine is the precursor of polyamines, which are involved in the regeneration of tissue and have antioxidant functions; these polyamines provide protection from cell damage caused by oxidative stress, which increased during wound healing [26]. In addition, arginine increases the secretion of growth hormone, insulin, and prolactin. Growth hormone stimulates IGF-1, inducing polyamine production [24]. Furthermore, arginine supplementation increases peripheral blood flow in rats [27], and this increase is associated with an increase in protein synthesis. Therefore, arginine accelerates wound healing through the potential mechanisms discussed above.

Amino Acid Mixtures

Amino Acid Mixtures Related to Collagen Protein Structure

Gorsetti et al. indicated that topical application of a dressing containing the amino acids glycine, leucine, and lysine plus sodium hyaluronate improves wound healing in rats [17]. The mixture may increase TGF-β immunolocalisation in the dressed topical area. The induced TGF-β increases fibroblast production and the secretion of eNOS, thus increasing collagen and decreasing inflammation; thereafter, wound healing is enhanced [17]. In that study, the authors used the above composition of amino acid because leucine is fundamental both to promote protein synthesis and to implement elastin synthesis, glycine and proline are the most abundant amino acids in collagen and lysine is necessary to form triple helices. However, the authors did not discuss the interaction between the amino acids in the mixture.

Amino Acid Mixtures Containing Leucine or Its Metabolite

Williams et al. indicated that a mixture of arginine, glutamine, and β-hydroxy-β-methylbutyrate (HMB) improves wound healing in humans [28]. Fourteen days of administration of a mixture of these components (14 g arginine, 3 g HMB, and 14 g glutamine, daily) led to an increase in collagen deposition in polytetrafluoroethylene (PTFE) tubes. Arginine enhances wound healing as described above, and glutamine is essential for the metabolism of cells that are undergoing rapid turn-over, such as lymphocytes and enterocytes. Glutamine is also important as a nitrogen source, especially in the catabolic response to starvation, trauma, and sepsis. HMB is a metabolite of leucine; however, only a minor proportion of leucine (~5 %) is oxidized to HMB. HMB supplementation increases lean body mass and improves skeletal muscle strength, possibly through an increase in the mTOR pathway for protein synthesis and a decrease in the ubiquitin proteasome pathway for proteolysis [29]. Studies have suggested that the combination of the amino acids in the mixture significantly increased collagen in wound healing compared with prior studies using low doses of arginine in humans, and these mixtures provide a safe and effective nutritional means for wound healing [28].

Table 21.1 Effect of single amino acid infusions on the protein synthesis rate of tropocollagen

Synthesis rate (%/h)	Mean	±	SEM
Saline (*n*=6)	1.03	±	0.10
Glutamine (*n*=5)	0.90	±	0.26
Proline (*n*=5)	1.11	±	0.23
Alanine (*n*=5)	1.44	±	0.44
Arginine (*n*=4)	1.19	±	0.25
Glutamate (*n*=4)	1.53	±	0.17
Glycine (*n*=5)	1.35	±	0.26
Aspartate (*n*=4)	1.52	±	0.25
Serine (*n*=5)	1.49	±	0.18
Histidine (*n*=4)	1.54	±	0.47
Lysine (*n*=4)	1.29	±	0.14
Phenylalanine (*n*=4)	0.77	±	0.03
Threonine (*n*=4)	1.53	±	0.21

There were no significant differences between any amino acid and the saline control. The infusion rate of each single amino acid was matched at 2.87 mol/kg/h. The values are presented as the mean ± SEM. With permission from Amino Acids, 2013, 44, 969–976 [15]

Zhang et al. also indicated that an amino acid mixture containing a high level of leucine increased protein synthesis in ear skin wounds in rabbits, but leucine alone and an amino acid mixture with a low level of leucine did not [30]. This amino acid mixture included leucine, isoleucine, valine, lysine, phenylalanine, histidine, threonine, methionine, tryptophan, alanine, arginine, glycine, proline, serine, and tyrosine. The mixture and L-[ring-$^{13}C_6$]-phenylalanine were infused for 4 h to measure protein kinetics in skin wounds 7 days after the ear was scalded. The authors of this study suggested that the combination of amino acids and a high level of leucine had an anabolic effect for protein during wound healing by increasing the availability of additional amino acids [30].

Branched Chain Amino Acid Cocktails

Dermal Collagen Synthesis During Protein Undernutrition

The effect of amino acids on the fractional synthesis of soluble collagen and tropocollagen was investigated in protein malnourished rats [15]. Amino acid solutions and L-[ring-$^{2}H_5$]-phenylalanine as a tracer were infused over a 4-h period into the jugular vein of rats fed on a protein-free diet for 1 week, and the fractional synthesis rate of dermal tropocollagen was evaluated by measuring the stable isotope incorporation rate. As shown in Table 21.1, the infused single amino acids glutamine, proline, alanine, arginine, glutamate, glycine, aspartate, serine, histidine, lysine, phenylalanine, and threonine did not significantly increase the synthesis of tropocollagen. Bellon et al. indicated that glutamine and de novo synthesized-proline are important for collagen protein synthesis in vitro [22]. These authors demonstrated that glutamine provides 75 % of the intracellular free proline and 85 % of procollagen-bound proline [22]. Another study showed that procollagen synthesis was not altered over a wide range of high proline concentrations in fibroblasts [22]. The de novo synthesis of proline and/or pyrroline-5-carboxylate, which is the intermediate of proline, is required for the glutamine-dependent induction of collagen gene expression in fibroblast cells [21, 23]. However, few studies have focused on the interaction between proline and glutamine metabolism in dermal tissue in vivo. Amino acid metabolism occurs through interactions between tissues through the blood. Therefore, it is important to consider the in vivo interactions between glutamine and proline metabolism. Using a constant

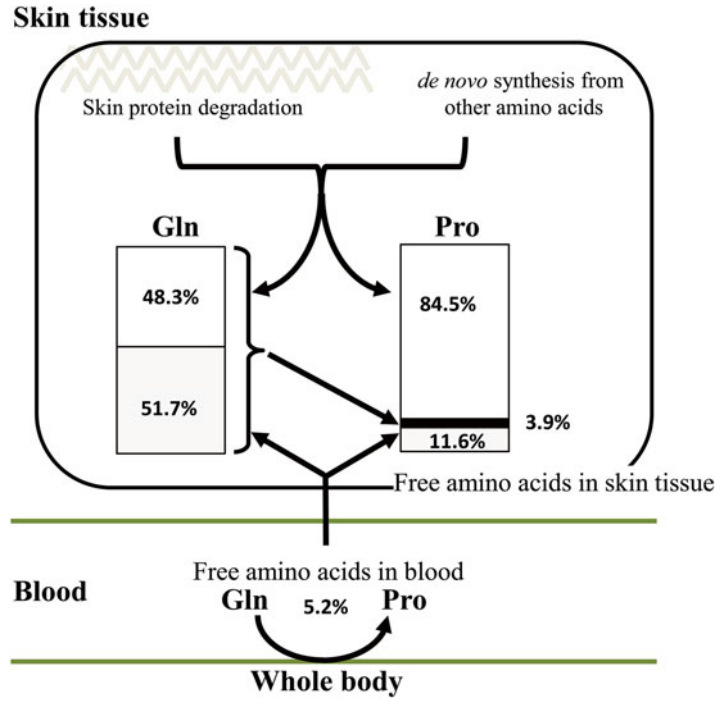

Fig. 21.1 Interaction between proline and glutamine metabolism in the blood and dermal tissue. In total, 3.9 % of free proline in the skin tissue is derived by de novo synthesis from glutamine in the dermal tissue and 11.6 % of free proline in the skin tissue is transported from the blood. Most (84.5 %) of the free proline in the dermal tissue is derived from skin protein degradation and/or de novo synthesis from other amino acids. With permission from Amino Acids, 2013, 44, 969–976 [15]

infusion of the stable isotopes L-d7-proline and L-α-15 *N*-glutamine, a conversion of glutamine to proline was measured during steady-state enrichment in normal rats. The rate of proline conversion from glutamine was 5.2 % in the entire rat body and only 3.9 % in dermal tissue (Fig. 21.1). This result suggests that glutamine contributes less to de novo proline synthesis in dermal tissue in vivo compared with the previous in vitro study [22]. In addition, 11.6 % of free proline in the dermal tissue is supplied from the blood (Fig. 21.1). These results suggest that 84.5 % of free proline in the dermal tissue is supplied from within the dermal tissue in vivo. Therefore, the difference in the contribution of glutamine metabolism in the dermal tissue between in vivo and in vitro studies is one of the reasons why glutamine does not increase collagen synthesis in the in vivo study.

The effect of infusions of amino acid mixtures on dermal tropocollagen during protein malnutrition is shown in Fig. 21.2. Interestingly, the amino acid mixtures essential amino acids + arginine + glutamine (EAARQ; leucine, isoleucine, valine, histidine, lysine, phenylalanine, proline, threonine, tryptophan, methionine, glutamine, and arginine) and branched chain amino acids + glutamine (BCAAQ; leucine, isoleucine, valine, and glutamine) significantly increase tropocollagen synthesis, but BCAAs (leucine, isoleucine, and valine) and amino acid mixtures composed of collagen protein (AAC; leucine, isoleucine, valine, histidine, lysine, phenylalanine, proline, threonine, methionine, alanine, aspartate, glutamate, glycine, serine, and arginine) do not. The amount and composition of BCAAs in the EAARQ, BCAAQ, and BCAAs groups were the same. This result indicates that arginine supplementation and the synergistic effect of glutamine supplementation with BCAAs have no impact on tropocollagen synthesis. BCAAs in particular, but also leucine and its metabolites, modulate mTOR and stimulate phosphorylation of p70S6K and 4E-BP1, thus initiating translation and transcription for protein synthesis [31–35]. Some reports have indicated that glutamine regulates protein synthesis. For example, glutamine restores energy metabolism in cells [36], increases cell swelling [37], and activates GAPP (glutamate-dependent protein phosphatase, which correlates with mTOR activation) [38, 39]. Xu et al. demonstrated that the combination of leucine and glutamine synergistically stimulates S6K activity in pancreatic beta cells [33]. In addition, supplemented glutamine affects

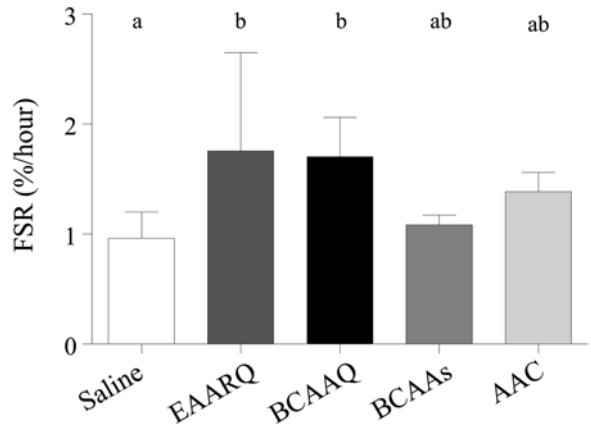

Fig. 21.2 Effect of amino acid mixtures on the fractional synthesis rate of tropocollagen in protein-malnourished rats. Essential amino acids + arginine + glutamine (EAARQ, $n = 4$) and branched chain amino acids + glutamine (BCAAQ, $n = 6$) significantly increased the synthesis rate of tropocollagen, but BCAAs ($n = 5$) and amino acids mixture composed of collagen protein (AAC, $n = 4$) did not affect the synthesis rate. The infusion rate of EAARQ, BCAAQ, BCAAs, and AAC was 0.60, 0.33, 0.22, and 0.60 g/kg/h, respectively, and the concentration and composition of the BCAAs were the same among the groups. The values are presented as the means ± SEM. Statistical analysis was performed using Tukey's test after ANOVA for multiple comparisons. Values with different superscripts are significantly different ($P < 0.05$). With permission from Amino Acids, 2013, 44, 969–976 [15]

BCAA metabolism. A portion of supplemented BCAAs is metabolized to glutamine because BCAAs are essential donors of nitrogen for the synthesis of glutamine in skeletal muscle [40]. Nicklin et al. showed that glutamine imports leucine via the antiporter SLC7A5-SLC3A2 into the cell, leading to mTORC1 activation [41]. In this study, the plasma concentration of each BCAA after a 4-h infusion was significantly higher in rats treated with BCAAQ than with BCAAs. In addition, the increase in protein synthesis depends on the plasma leucine concentration in rats [42]. This result suggests one of the mechanisms by which BCAAQ stimulates the synthesis of dermal tropocollagen to a much greater extent than BCAAs in protein-malnourished rats. In addition, a combination of BCAAs and glutamine may synergistically stimulate dermal tropocollagen protein synthesis based on each amino acid's individual effects on protein synthesis.

Dermal Collagen Synthesis Under UV Irradiation

The acute effect of orally administered amino acids on the fractional synthesis rate of tropocollagen was investigated under low-dose UV irradiation in mice [43]. The synthesis rate of tropocollagen extracted from the dermis that was irradiated with a low dose of UV was measured by the flooding dose method using L-[ring-$^2H_5$]-phenylalanine. Single amino acids, such as glutamine, proline, and arginine, did not increase the synthesis of tropocollagen (Fig. 21.3). In addition, a combination of BCAAs, RQ (arginine and glutamine), and BCAAE (BCAAs and glutamate) also did not increase synthesis. Interestingly, amino acid mixtures, such as BCAARQ (BCAAs, arginine, and glutamine), BCAAQ, BCAAP (BCAAs and proline), and the essential amino acid mixture (EEA; leucine, isoleucine, valine, methionine, histidine, lysine, phenylalanine, and tyrosine), significantly increased the synthesis of tropocollagen independently of insulin secretion (Fig. 21.4) [43]. This result coincides with the results shown for the protein undernutrition studies. In addition, tropocollagen synthesis did not increase when precursors of proline (glutamine, arginine, and RQ) were administered with the amino acids but did increase with BCAAP. In addition, plasma proline concentrations (Table 21.2)

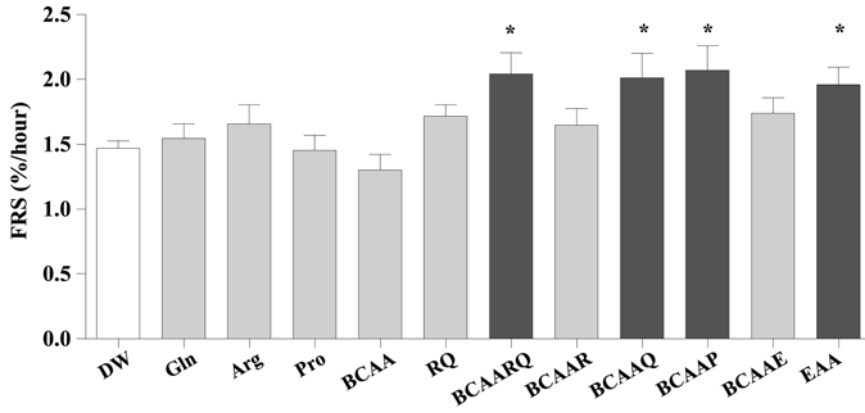

Fig. 21.3 Effect of orally administered amino acids on the fractional synthesis rate of dermal tropocollagen in UVB-irradiated mice. Branched chain amino acids + arginine + glutamine (BCAARQ, $n = 11$), BCAA + glutamine (BCAAQ, $n = 7$), BCAA + proline (BCAAP, $n = 10$), and Essential amino acids (EAA, $n = 8$) significantly increased the synthesis rate of dermal tropocollagen 30 min after oral administration, but single amino acids and some mixtures [BCAAs ($n = 7$), arginine + glutamine (RQ, $n = 9$), BCAA + arginine (BCAAR, $n = 11$), and BCAA + glutamate (BCAAE, $n = 8$)] did not increase tropocollagen synthesis. BCAAs and glutamine and/or proline must be included in amino acid mixtures to increase the synthesis of skin tropocollagen. Amino acids were orally administered at a dose of 1.0 g/kg for each group. The values are presented as the means ± SEM. Comparisons with the control group (DW: distilled water, $n = 14$) were conducted with a Dunnett's test after ANOVA for multiple comparisons (*: $P < 0.05$). With permission from Amino Acids, 2012, 42, 2481–2489 [43]

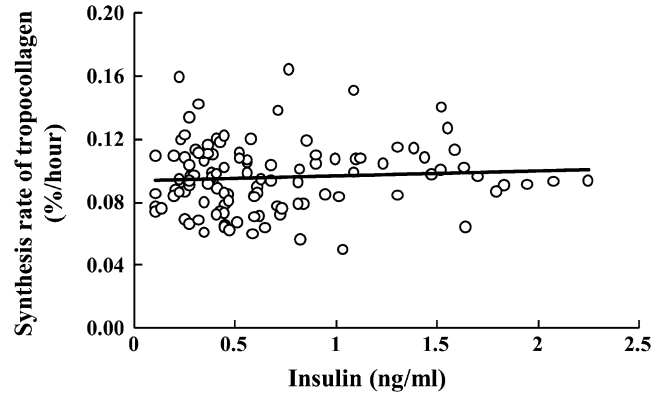

Fig. 21.4 Correlation between the synthesis rate of tropocollagen and plasma insulin. Multivariate correlations were calculated for the synthesis rate of tropocollagen and plasma insulin concentration. There was no significant correlation between the variables ($r^2 = 0.004$). With permission from Amino Acids, 2012, 42, 2481–2489 [43]

were not increased by collagen synthesis-stimulating amino acid mixtures containing proline precursors (BCAAQ, BCAARQ) but were slightly increased by RQ and arginine, neither of which stimulates tropocollagen synthesis. These results also indicate that de novo proline synthesis may not be the main cause of the increased collagen protein synthesis. Proline constitutes one-third of the amino acid residues of collagen. However, there is little information on the effect of proline supplementation on dermal skin collagen synthesis. Further study is needed to understand the effect of the combination of BCAAs and proline on the synthesis of tropocollagen. EAAs increase the synthesis of skeletal muscle protein [44]. In a previous study, EAAs were the only amino acid combination that increased the synthesis of skeletal muscle protein (data not shown). Thus, an improvement of tropocollagen synthesis resulting from EAAs is associated with an improvement of whole-body protein metabolism.

Table 21.2 Plasma amino acid concentration 30 min after oral amino acid administration in UV-irradiated mice

	DW	Gin	Arg	Pro	BCAAs	RQ	BCAARQ	BCAAR	BCAAQ	BCAAP	BCAAE	EAA
Taurine	66.5±12.3	74.9±11.9*	83.8±2.9	95.7±32.5*	78.5±12.3	71.6±10.7	73.1±8.4	65.8±7.3	63.2±9.5	78.0±17.1	71.1±10.4	60.8±16.4
Aspartic acid	2.7±0.7	3.3±1.6	2.9±2.1	2.8±1.0	1.4±1.0	1.7±2.2	2.0±1.3	2.3±1.2	2.2±1.6	1.6±0.6	4.5±2.9	1.8±1.4
Hydroxyproline	1.7±1.1	1.1±0.9	2.1±0.9	0.9±0.3	0.8±0.2	1.5±0.8	1.7±11	0.8±0.5	0.8±0.7	1.0±0.4	0.8±0.8	1.6±1.0
Threonine	15.9±2.5	13.1±2.2	13.6±0.8	15.0±4.9	13.5±3.6	14.3±2.6	11.9±3.0*	11.3±2.5*	11.0±2.0*	11.9±1.2*	11.7±1.7*	69.4±16.2*
Serine	12.9±2.2	11.8±2.2	12.2±0.0	12.6±3.8	10.6±1.4	13.3±4.0	10.0±1.7	8.3±0.9*	9.0±2.2*	9.9±1.0*	9.8±1.8*	10.5±2.3
Asparagine	4.3±0.6	3.4±1.0	2.7±1.3	3.8±1.2	2.7±1.8	3.4±1.4	3.5±1.7	3.2±0.6	3.5±1.3	3.2±1.2	2.8±0.8	3.3±1.5
Glutamic acid	8.5±1.7	9.1±2.1	11.1±1.3	11.6±4.0	8.7±1.9	9.4±1.6	8.7±3.0	7.0±1.2	7.1±1.9	8.5±2.6	32.6±6.4*	6.7±2.3
Glutamine	43.2±8.2	60.5±9.4*	43.1±6.0	50.3±14.7	54.1±8.4*	48.8±5.8	50.7±3.5	44.4±7.8	53.3±9.6	59.0±9.9*	51.2±3.3	46.0±9.0
Proline	7.0±1.4	7.1±1.7	11.8±1.9	350.0±126.8*	6.2±1.5	11.1±1.9	7.9±1.2	7.0±1.0	6.2±1.6	111.4±32.6*	6.1±1.8	5.9±1.4
Glycine	22.3±2.8	20.0±2.6	22.6±2.5	23.6±7.0	16.8±2.9	20.2±4.1	16.9±2.8	15.0±1.9	15.2±3.3	17.4±2.7	17.9±3.6	14.2±3.7
Alanine	23.8±6.1	21.7±3.6*	26.8±1.8	38.0±10.3*	20.7±5.3	28.1±6.8	25.9±5.5	21.8±4.2	23.7±4.5	27.0±6.1	31.1±4.4	28.4±8.2
Citrulline	3.1±0.6	5.1±1.3*	3.1±0.5	3.1±1.0	4.4±0.9*	4.2±1.0	6.5±1.3*	5.3±1.1*	5.8±1.1*	4.1±0.3	4.2±0.6	3.5±1.5
Valine	25.7±3.7	29.0±31.8	22.7±1.6	20.5±5.1	250.6±39.4	23.6±4.2	110.5±19.4*	103.2±18.9*	108.6±18.5*	110.5±14.3*	104.0±11.5*	91.2±20.1*
Cystine	0.1±0.1	0.2±0.0	0.2±0.1	0.2±0.2	0.1±0.2	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1	0.0±0.1	0.3±0.1	0.3±0.2
Methionine	5.9±1.2	5.8±1.1	5.6±0.1	5.3±1.5	4.3±1.1	6.6±2.3	4.0±1.0	3.4±0.6	3.6±1.3	4.0±0.7	3.8±1.6	20.9±4.5*
Isoleucine	11.7±1.8	12.3±13.8	10.8±1.4	8.1±1.6	167.3±43.2	10.2±2.2	54.0±13.2*	49.5±9.5*	51.6±13.6*	56.9±9.6*	49.2±7.8*	38.6±11.3*
Leucine	18.6±3.0	21.3±26.4	17.8±3.0	13.0±3.3	235.1±55.2	18.0±3.8	100.7±24.5*	92.5±17.6*	93.1±23.6*	101.7±17.2*	88.7±14.5*	66.6±18.7*
Tyrosine	27.1±6.7	27.5±5.4	29.7±8.9	26.2±8.0	19.9±1.8*	25.5±6.9	19.7±4.2	19.6±4.2*	20.8±4.8	24.1±4.7	19.1±6.0*	30.7±5.6
Phenylalanine	138.6±30.9	131.4±24.8	161.2±40.0	130.4±39.6	158.5±26.5	115.3±49.6	128.2±48.6	133.1±36.3	152.9±34.9	146.4±10.8	143.5±12.0	158.4±37.1
Tryptophan	8.1±1.3	7.7±1.2	7.2±2.3	7.3±2.8	5.6±2.0*	6.2±1.3	5.4±1.6*	6.5±1.4	7.2±1.5	6.3±1.3	7.2±1.8	5.3±2.3*
Omithine	9.9±2.6	9.9±2.3	135.1±13.7*	11.1±3.8	6.0±1.8	85.4±32.3*	68.9±17.1*	115.3±29.3*	22.8±43.3	7.8±0.7	8.7±2.8	7.8±3.3
Lysine	23.4±4.0	21.5±3.6	24.9±1.9	17.3±4.7	21.2±4.5	30.1±7.7	20.5±3.3	20.9±3.3	18.4±2.9	18.0±2.6	19.3±3.7	61.8±20.4*
1Methylhistidine	0.2±0.1	0.2±0.1	0.1±0.2	0.1±0.1	0.1±0.1	0.2±0.0	0.1±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1
Histidine	5.4±0.7	6.4±1.2	4.7±0.3	5.3±1.3	4.3±0.7	5.9±1.2	4.4±1.3	4.0±0.7	4.7±0.8	4.4±0.5	4.4±0.7	8.1±2.7*
3Methylhistidine	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.2	0.5±0.1	0.6±0.2	0.5±0.2	0.4±0.1	0.5±0.2	0.6±0.1	0.3±0.1	0.5±0.2
Arginine	12.3±3.0	17.0±6.4	53.2±5.1*	10.2±2.4	16.7±4.1	33.4±11.5*	31.7±9.9*	45.4±11.7*	18.2±10.6	12.1±1.8	15.0±7.7	13.6±3.3

Plasma branched chain amino acids (BCAA, $n=7$) concentrations increased by approximately five times in the BCAA + arginine + glutamine (BCAARQ, $n=11$), BCAA + arginine (BCAAR, $n=11$), BCAA + glutamine (BCAAQ, $n=11$), BCAA + proline (BCAAP, $n=10$), BCAA + glutamate (BCAAE, $n=8$), and Essential amino acids (EAA, $n=8$) groups compared with the distilled water (DW, $n=14$) group. Plasma tryptophan, histidine, tyrosine, threonine, serine, and glycine concentrations decreased in the groups that received solutions containing BCAAs. The plasma proline concentration increased in the arginine ($n=7$) and RQ groups. The values are presented as the means ± SD. Comparisons with the DW group were conducted with a Dunnett's test after ANOVA for multiple comparisons (*: $P<0.05$). With permission from Amino Acids, 2012, 42, 2481–2489 [43]

While BCAAs are important for skin tropocollagen synthesis, other specific amino acids, such as glutamine or proline, are also necessary to stimulate dermal tropocollagen synthesis under low-dose UV irradiation.

Estimation of the Effective Amount of Branched Chain Amino Acid Cocktails on Collagen Synthesis in Humans

Some human studies have shown an effect of arginine or amino acid mixtures on wound healing [24, 28]; unfortunately, no study in humans has examined the effect of BCAA cocktails on dermal collagen metabolism. As described above, the increase in protein synthesis depends on the plasma leucine concentration in rats [42]. Therefore, plasma amino acid concentrations may be used to estimate the amount of amino acids that improves dermal collagen synthesis in humans. The plasma leucine concentration increased 3.8 times from basal levels after the intake of 5 g of BCAAs in young Japanese men (22–25 years old), and this increase depended on the amount of BCAA intake [45]. In an animal study, the plasma leucine concentration in the BCAAQ group was five times higher than the control group [43]. Therefore, 6.5 g of BCAAs is needed to reach a plasma concentration that is five times higher than the basal level in human; for example, approximately 13 g of BCAAQ may be needed to achieve the acute effect of BCAAQ on the synthesis of dermal tropocollagen protein in humans. However, there are no studies on the effect of chronic intake of BCAA cocktails, such as BCAAQ, on dermal collagen synthesis or the amount of these cocktails required to counteract wound healing, undernutrition, and UV irradiation. Amino acids requirement changes in each condition such as UV irradiation and undernutrition. Further study is necessary to understand the optimal conditions, such as the amount and the timing of intake, for BCAA cocktails to have an effect on dermal collagen.

Conclusions

Many studies on the relationship between skin and amino acids focus on high anabolic conditions, such as cultured cells and wound healing. In contrast, few studies have focused on the ability of amino acids to restore dermal collagen synthesis under common situations, such as malnutrition and low-dose UV irradiation. A single treatment with glutamine increases the collagen content in human dermal fibroblast cultures but does not increase collagen protein synthesis in vivo. In addition, arginine is a potent accelerator of wound healing. Moreover, BCAAs, especially leucine, are important stimulators of dermal collagen synthesis in wound healing, during protein undernutrition and in response to low-dose UV irradiation. However, a single treatment with BCAAs is not effective for dermal collagen synthesis, and other specific amino acids are necessary to stimulate synthesis. BCAA cocktails that include other amino acids, such as glutamine and/or proline, are important for reversing skin damage.

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Chapter 22
Branched Chain Amino Acids in Inherited Muscle Disease: The Case of Duchenne Muscular Dystrophy

Jamshid Davoodi, Susan M. Hutson, and Robert W. Grange

Key Points

- Duchenne Muscular Dystrophy (DMD) is a human muscle wasting disease caused by the absence of the protein dystrophin.
- Dystrophin is typically thought to provide structural and signaling functions for the sarcolemmal membranes of skeletal muscle fibers.
- When dystrophin is absent, the membrane weakens, signaling is disrupted, and fiber function is severely compromised.
- DMD patients exhibit enhanced cycles of fiber degeneration and regeneration accompanied by increased reactive oxygen species (ROS) and reduced nitric oxide.
- Each of these deleterious effects might be countered by Branched chain Amino Acids (BCAA) in the diet.
- BCAAs, especially leucine, induce protein synthesis through activation of mammalian target of rapamycin complex 1 (mTORC1) and inhibit protein degradation by reducing E3 ubiquitin ligases.
- In addition, BCAA supplementation in combination with other essential amino acids increases the level of enzymes that attenuate ROS, and increases nitric oxide.
- BCAAs and other essential amino acid interventions may be an appropriate dietary treatment for DMD to slow loss of muscle mass until a cure is found.

Keywords Branched chain amino acids • Leucine • Isoleucine • Valine • Duchenne muscular dystrophy • Protein synthesis • Protein degradation

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Abbreviations

4EBP-1	4E-binding protein-1
AICAR	5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside
AMPK	AMP kinase
BCAA	Branched chain amino acids
CHO	Carbohydrates
DMD	Duchenne muscular dystrophy
DGC	Dystrophin glycoprotein complex
EDL	Extensor digitorum longus
LPD	Low-protein diet
mTORC1	Mammalian target of rapamycin complex 1
MuRF1	Muscle-specific RING finger protein 1
ROS	Reactive oxygen species
TA	Tibialis anterior

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder affecting boys. The disease is characterized by progressive loss of muscle mass and gain of body fat [1]. Steroids (e.g., prednisone) to ameliorate the symptoms of the disease are the only treatment available at this time, but they exacerbate accumulation of body fat, among other unwanted side effects. Given the dramatic changes in body composition, nutritional strategies are attractive alternatives to improve quality of life in DMD patients, but surprisingly, there have been few systematic studies conducted. Branched chain amino acids, especially leucine, induce protein synthesis, inhibit protein degradation, and induce few to no side effects. Consequently, supplementation of DMD patients with BCAAs is a potential and logical approach to reduce the rate of disease progression. Increased muscle mass could increase strength and mobility of these patients. The current chapter describes the potential benefits of BCAA supplementation for DMD patients.

Duchenne Muscular Dystrophy (DMD)

DMD is a lethal, X-linked recessive, muscle-wasting disease caused by mutations in the dystrophin gene, located on chromosome Xp21 [2]. The symptoms of the disease appear prior to 3 years of age, with a life expectancy of 20–30 years. One in every 3,500 boys is affected [3, 4]. Genetic mutations in the dystrophin gene result in frame shifts which introduce premature stop codons. A less severe form of muscular dystrophy known as Becker muscular dystrophy is caused by other dystrophin gene mutations that yield truncated proteins. In DMD, the frame shift results in absence of the 427 kDa dystrophin protein, which then impairs the link between the F-actin cytoskeleton and the extracellular matrix protein laminin 2 via the membrane-bound dystrophin glycoprotein complex (DGC, Fig. 22.1) [4, 5]. There is no current cure for DMD and palliative and prophylactic interventions to improve the quality of life of patients remain limited. Initial open-label trials of prednisone and deflazacort showed clear improvement of DMD symptoms which led to the prescription of prednisone in many countries [6, 7]. Nonetheless, prolonged treatment of DMD patients with prednisone causes numerous undesirable side effects including weight gain, hypertension, and bone demineralization. In fact, in some

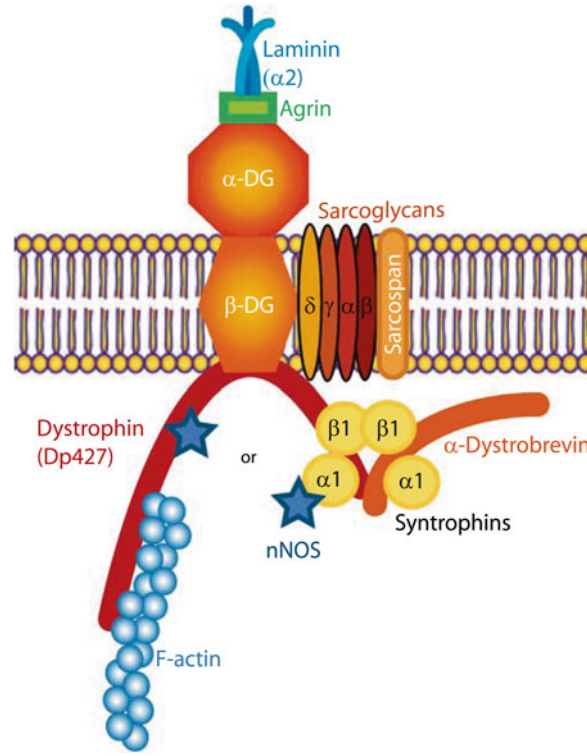

Fig. 22.1 Dystrophin-associated glycoprotein complex at mammalian muscle. Dystrophin binds to actin, β-dystroglycan (β-DG), and α-dystrobrevin2 through its N-terminus, cysteine-rich region, and C-terminus, respectively. β-DG is linked to the extracellular α-dystroglycan (α-DG) which links lamin α2 along the sarcolema. β-DG is also bound to δ-sarcoglycan. In addition, neuronal nitric oxide synthase (nNOS) can be recruited to the DGC through syntrophins or directly bind to dystrophin. Adopted from reference 5 with permission (License NO. 3134790914139)

cases the treatment was stopped due to the severity of the symptoms. Consequently, until a cure is available, discovery of potential interventions to improve quality of life for DMD boys is warranted.

Although the overall rates of protein synthesis and degradation in DMD patients are similar to healthy controls, because of the lower muscle mass in patients, normalization of protein turnover to total muscle mass reveals a two- to threefold higher relative protein turnover [8, 9]. Progressive and ultimately fatal rounds of skeletal muscle degeneration and regeneration are hypothesized to result from either a fragile or weakened skeletal muscle membrane [10] or altered cell signaling, which ultimately cause necrotic and apoptotic cell death. Destruction of the muscle cells is accompanied by fibrosis as well as deposition of fat tissue, which cause pseudohypertrophy of the muscles [11]. Beyond these general hypotheses, the specific cellular mechanisms and the temporal progression of the dystrophic process are still not clear.

Branched Chain Amino Acids and Protein Synthesis and Degradation

Nutritional supplements such as BCAAs (leucine, isoleucine, and valine) are widely used by individuals to improve physical performance and sufficient evidence exists in the literature that suggests under the appropriate conditions, leucine can stimulate protein synthesis and inhibit protein degradation in vivo [12, 13]. In addition, the Society for Sarcopenia, Cachexia, and Wasting Disease [14] recommended that essential amino acid supplementation, especially leucine, was appropriate for those suffering from sarcopenia. Sarcopenia is defined by a loss of muscle mass and function due to normal aging processes or other pathological conditions [14]. The Society also suggested protein supplementation to enhance or maximize the effects of anabolic stimuli in sarcopenic individuals.

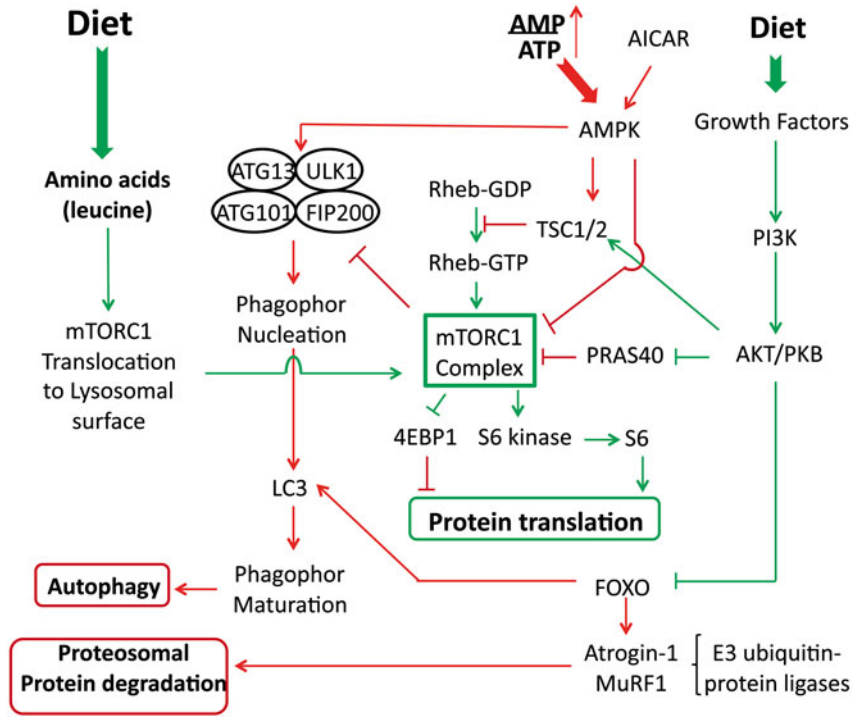

Fig. 22.2 Upregulation of mTORC1 activity and suppression of protein degradation by amino acids. Food intake activates mTORC1 through two separate signaling pathways: secretion of growth factors activating the PI3K-AKT pathway and amino acid-mediated translocation of mTORC1 to the lysosomal surface where Rheb is present. AKT inactivates PRAS40, an inhibitor of mTORC1, and FOXO by preventing its translocation to the nucleus. Translocation of FOXO to the nucleus upregulates the expression of a number of proteins involved in protein degradation including LC3, required for phagophor maturation, and the E3 ubiquitin protein ligases Atrogin-1 and MuRF1, which are involved in the ubiquitin proteasome system. Starvation, which decreases ATP and increases AMP, or pharmacological intervention by AICAR activate AMKP. AMPK activates the initiation step of autophagy and suppresses mTORC1 activity. Induction of autophagy provides nutrients for basal mTORC1 activity needed for cell survival under starvation conditions. Red lines indicate actions leading to protein degradation and prevention of protein synthesis and the green lines indicate the induction of protein synthesis

These recommendations clearly support the beneficial effects of leucine in muscle wasting conditions. The BCAAs induce mammalian target of rapamycin complex 1 (mTORC1) activity and stimulate protein synthesis in selected tissues (Fig. 22.2) [15, 16]. mTORC1 is considered the main sensor for the availability of nutrients to induce anabolic processes [17]. Although a number of essential amino acids have been shown to induce mTORC1, leucine is the primary activator. mTORC1 is the central regulator of cell growth, survival, and death [18]. Increased activation of mTORC1 enhances anabolic pathways causing enhanced protein and fat synthesis. However, decreased activity of mTORC1 is characterized by diminished cell growth and activation of survival pathways, especially autophagy [19]. The diversity of cellular events controlled by mTORC1 makes it an attractive target to prevent or treat diseases associated with growth or atrophy (wasting). Induction of protein synthesis by mTORC1 that tips the balance to net protein gain (protein synthesis > protein degradation) could be exploited for the treatment or at least amelioration of muscle wasting (protein degradation > protein synthesis). Consequently, BCAA in combination with other essential amino acids have been studied to determine their efficacy as nutritional supplements to improve health and physical performance.

To induce protein accretion in muscle, net protein synthesis must exceed protein degradation. Induction of global protein synthesis depends on activation of the regulatory master switch, mTORC1. This protein complex becomes active when growth factors, especially insulin, and amino acids, mainly leucine, are abundant. Therefore, abundance of nutrients and growth factors signals the cells primarily through mTORC1, that the conditions for growth are present [20]. Conversely, scarcity of nutrients leads to diminished mitochondrial ATP production. Reduced ATP availability increases the AMP over ATP ratio. The rise of AMP is sensed by AMP Kinase (AMPK) causing its phosphorylation and activation [21]. Activated AMPK inhibits mTORC1-stimulated anabolic pathways indirectly through the activation of TSC, an mTORC1 inhibitor, and directly through inactivation of mTORC1 [22]. Consequently, three conditions must be met to induce maximal protein synthesis through mTORC1: (1) availability of amino acids represented by leucine, (2) availability of nutrients reflected by the secretion of growth factors; and, (3) sufficient energy for protein synthesis [20]. As long as all three conditions are met, supplementation of leucine in the diet should tip the balance towards protein synthesis rather than degradation. mTORC1 activation by leucine inactivates 4E-binding protein-1 (4EBP-1; Fig. 22.2) and activates ribosomal protein S6 (S6 protein), thereby enhancing global protein synthesis [23–25]. Activated mTORC1 has the added benefit of blocking protein degradation through autophagy. Activated mTORC1 phosphorylates ATG13 preventing the initiation step in autophagosome formation. Finally, the presence of growth factors activates the Akt/PKB pathway which results in mTORC1 activation and FOXO inactivation. Inactivation of FOXO dampens both autophagy and protein degradation through the proteosomal system [26]. Activation of the PI3K-mTORC1 signal transduction pathway results in both acute (i.e., occurring in minutes to hours) and chronic (i.e., occurring in hours to days) upregulation of protein synthesis through the initiation of mRNA translation and ribosome biogenesis, respectively, promoting cell growth, which in turn promotes muscle hypertrophy [27].

Although the details of amino acid signaling have been delineated in cell culture models, numerous studies support the notion that amino acid and protein supplementation lead to enhanced protein synthesis and reduced protein degradation in vivo. For example, leucine supplementation attenuated loss of soleus muscle mass in immobilized rat hind limbs. This effect was caused by reduction of two E3 ubiquitin-protein ligases, MuRF1 (Muscle-specific RING finger protein 1), and atrogin-1 expression [28] and not due to increased protein synthesis. The authors suggested that leucine supplementation attenuated muscle wasting induced by immobilization by minimizing gene expression of E3 ligases. Consistently, incubation of isolated rat soleus and extensor digitorum longus (EDL) muscles in the presence of 10 mM leucine resulted in a decreased proteolytic rate as measured by the release of tyrosine into the incubation medium. The effects of leucine were also associated with a decreased activity of lysosomal proteases and a decreased expression of the genes of the ATP-ubiquitin-dependent proteolytic pathways [29]. It appears that the inhibitory effects of leucine on protein degradation are not limited to the fed state, as leucine also aids in the retention of lean mass in a hypocaloric state [30]. Finally, studies in rats that compared induction of protein synthesis by leucine alone versus carbohydrates (CHO) alone revealed increased insulin levels in the CHO-fed rats but not the leucine-fed rats. However, despite the absence of any change in insulin levels, protein synthesis-induced by leucine alone was 89 % of the freely fed rats (i.e., complete diet) suggesting that leucine stimulates protein synthesis in skeletal muscle independent from increases in serum insulin. Induction of protein synthesis by carbohydrates alone, likely due to an insulin-dependent mechanism, was only 65 % of the freely fed rats [16, 31].

Hypertrophic effects of leucine and protein supplementation are also observed in humans. For instance, 14 weeks of a high protein diet when compared to an isoenergetic carbohydrate diet resulted in a considerable increase in muscle size (18 and 26 % hypertrophy of type I and type II muscle fibers, respectively) and a modest increase in muscle strength when combined with resistance exercise in human subjects [32]. Supplementation of healthy men with leucine and whey protein enhanced the

acquisition of strength beyond that achieved with resistance training and a carbohydrate placebo [33]. Enhanced protein synthesis due to supplementation of young males with essential amino acids (histidine, 8 %; isoleucine, 8 %; leucine, 35 %; lysine, 12 %; methionine, 3 %; phenylalanine, 14 %; threonine, 10 %; and valine, 10 %) was associated with signaling proteins upstream and downstream of mTORC1. Increased protein synthesis was attributed to enhanced translation initiation and signals promoting translation elongation [34]. Taken together, these observations indicate that amino acid supplementation induces protein synthesis and prevents protein degradation under in vivo conditions. Consistently, leucine supplementation helped to maintain muscle mass in animal models of muscle-wasting disorders such as sepsis and cachexia [35–39]. Therefore, on the basis of these findings, it is reasonable to assume that BCAA supplementation would have similar effects in DMD. Surprisingly, there have been only a few studies designed to test the beneficial effects of BCAA in DMD.

The Effects of BCAAs on Protein Turnover in DMD

The rates of protein synthesis and degradation have been studied in DMD patients (see Table 22.1). These and other studies led to the conclusion that DMD patients display higher rates of protein synthesis and degradation compared to normal individuals. However as the disease progresses, net protein loss occurs due to an insufficient rate of protein synthesis compared to degradation. It is therefore logical to apply nutritional and pharmacological interventions to tip the balance toward protein synthesis. It is in this context that amino acid supplementations have been attempted.

Studies on plasma glutamine levels in DMD boys revealed lower glutamine concentrations compared to healthy boys [46]. This decrease in glutamine was accompanied by a higher leucine breakdown. Oral supplementation with glutamine decreased whole body protein breakdown in DMD boys [47]. However, a recent clinical trial on glutamine supplementation for a period of 4 months, which was completed in 2009, did not produce a functional benefit for the DMD boys [48]. Given the high activity of branched chain aminotransferase in muscle, BCAAs are the main nitrogen donors for glutamine and alanine in skeletal muscle [49]. BCAAs contribute amino nitrogen through transamination with α-ketoglutarate to form glutamate [50]. BCAAs therefore appear to substitute for protein breakdown as a nitrogen source. Therefore, BCAA supplement should provide the required nitrogen and replace protein breakdown as a source, thus sparing the dystrophic muscle.

Activation of mTORC1 appears to represent a reasonable strategy to ameliorate DMD symptoms. For example, valproic acid was used to activate the Akt/mTORC1 pathway in mdx mice to induce hypertrophy of myotubes and inhibit apoptosis [51]. Notably, valproic acid treatment increased sarcolemmal membrane integrity and Akt activity concurrent with reduced fibrosis in the mdx muscles. Another study applied Wnt7a treatment to induce satellite cell expansion and myofiber hypertrophy in the muscles of mdx mice through the Akt/mTORC1 pathway. Importantly, Wnt7a treatment resulted in a 1.9-fold greater force output in the EDL and reduced contractile damage [52]. Given the activation of mTORC1 is the common theme in these treatments and leads to an improved phenotype, it can be hypothesized that activators of mTORC1, such as leucine, should be beneficial to DMD. Consistent with this notion is the recent observation that the decrease in maximal force and muscle mass of older mdx mice coincided with reduced phosphorylation of Akt (also known as protein kinase B) and ribosomal S6 protein, both of which are indicative of reduced activity of the mTORC1 signaling pathway [53]. Pharmacological intervention combined with amino acid supplementation has also been beneficial for mdx mice. For example, combined deflazacort plus L-arginine treatment spared mdx dystrophic limb muscle from exercise-induced damage and induced a persistent functional improvement in distance run [54]. In this case, the effects of L-arginine were attributed to the increased production of nitric oxide, but the possible effects of L-arginine on mTORC1 activity were not investigated. Recent evidence suggests mTORC1 can be activated by nitric oxide [26].

Table 22.1 Early studies on protein turnover in Duchenne muscular dystrophy

Authors	Year	System	Conclusion
Ionasescu et al. [40]	1976	Primary muscle culture of DMD patients	50 % decrease in total protein synthesis
Ionasescu et al. [41]	1979	Primary muscle culture from DMD patients	The decrease in protein synthesis is countered by diphenylhydantoin and orgotein
Ballard et al. [8]	1979	DMD patients	Increased turnover of muscle contractile proteins in DMD
Boule et al. [42]	1979	Fibroblast culture from DMD patients	Reduced rate of protein synthesis
Haymond et al. [43]	1978	DMD patients	Accelerated protein degradation in the muscles of DMD patients
Warnes et al. [6]	1981	DMD patients	Increased rates of myofibrillar protein breakdown
Mussini et al. [44]	1984	DMD patients	Increased myofibrillar protein degradation as assessed by 3-methylhistidine excretion in the urine
Rodemann et al. [45]	1986	Skin fibroblasts of DMD patients	Enhanced rate of protein degradation

BCAA and Mitochondrial Biogenesis and Attenuation of ROS

A recent study by D'Antona et al. showed that an amino acid supplement composed primarily of BCAAs increased the average life span of middle aged mice with C57BL/6J and 129S1/SvImJ backgrounds which was characterized by increased mitochondrial biogenesis, enhanced physical endurance, upregulation of reactive oxygen species (ROS) defense system genes, and reduction of ROS production. Interestingly, all of the BCAA-mediated effects were strongly attenuated in endothelial nitric oxide synthase null mutant mice. These data reveal an important antiaging role of BCAAs mediated by mitochondrial biogenesis in mammals [55]. Given the observation that L-arginine supplementation attenuates pathophysiology associated with DMD through nitric oxide production [54], this observation suggests BCAA supplementation should be beneficial for DMD patients through a similar mechanism. For example, it has been shown that production of ROS has deleterious effects on various cellular components and is considered one of the hallmarks of DMD [56–58]. Consistently, treatment of Duchenne Dystrophy with reducing agents like *N*-acetyl cysteine [59] or green tea extract [60] has improved the DMD phenotype. Therefore, it can be hypothesized that induction of mitochondrial biogenesis and reduction of ROS by BCAA supplementation should improve the condition of DMD patients.

Autophagy and DMD

Cellular components are degraded through two major pathways: the ubiquitin proteasome and lysosomal systems [61]. It is thought that macroautophagy, simply known as autophagy, is responsible for the majority of intracellular protein degradation in mammalian cells, particularly during starvation-induced proteolysis. Autophagy is responsible for the digestion of large cellular components like mitochondria to remove damaged organelles or to provide nutrients for normal cellular functions during starvation. Successful autophagy requires the cooperation of two distinct cellular organelles: autophagosomes and lysosomes [62]. Autophagy has attracted considerable attention due to its involvement in various

pathological conditions. Importantly, removal of damaged mitochondria is carried out by autophagy, a process known as mitophagy. Accumulation of defective mitochondria increases ROS causing further damage to the mitochondria itself and other cellular components. Two recent observations suggest the beneficial effects of inducing autophagy in mdx mice [63, 64]. These beneficial effects come as a surprise given the enhanced rate of protein degradation in mdx mice (see Table 22.1). In the first study, the LC3II level, which is an indicator of autophagy, was lower in both mdx mice as well as DMD muscle biopsies. In addition, Akt and 4EBP1 proteins, which are located upstream and downstream of mTORC1, respectively, were present in highly phosphorylated forms suggesting over-activation of this pathway [63]. Intriguingly, only a long-term low-protein diet (LPD) but not 15 h starvation could suppress mTORC1 and enhance autophagy in mdx mice. The LPD treatment led to normalization of Akt and mTORC1 signaling and significant reduction of muscle inflammation and fibrosis accompanied by significant recovery of muscle function. The authors did not assess the effects of LPD on utrophin levels. Given the structural similarity of utrophin with dystrophin and its partial functional replacement of dystrophin, effects on utrophin expression should be explored. In the second study [64], autophagy was induced in mdx mice by 4 weeks of treatment with the AMPK agonist, AICAR (5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside) (Fig. 22.2). Activation of AMPK, which suppresses mTORC1 activity, potently triggered autophagy in the diaphragm without inducing muscle fiber atrophy. This was accompanied by both improved mdx diaphragm histopathology and force-generating capacity. However, utrophin levels were not changed suggesting that the beneficial effects were not linked to upregulated utrophin expression. To the best of our knowledge, these are the only two published studies that suggest beneficial effects of autophagy in DMD and harmful effects of mTORC1 activation. Nevertheless, a recent study of mdx mice did not exhibit consistent activation of mTORC1 in different muscle types [65]. The authors reported no difference in the phosphorylation status of mTOR protein from extracts of tibialis anterior (TA) muscle of mdx mice compared to that of age-matched wild-type C57BL/10 (B10) mice. The phosphorylation of mTOR protein in diaphragm muscle was age-dependent, only modestly higher at 6 weeks of age and significantly lower at 12 weeks of age when compared to B10 mice. In addition, other reports underscore the beneficial effects of mTORC1 activation in response to growth factors or high amino acid and high-protein diets (see previous section).

DMD features upregulation of protein turnover. Given the inhibitory effects of BCAA supplementation (leucine in particular) on protein degradation even under starvation conditions, [16, 28, 30] during which mTORC1 is inactivated and AMPK is activated, argues for the beneficial effects of BCAAs in DMD. A recent observation of increased mitochondrial biogenesis in skeletal muscles, reduced ROS, and increased life expectancy by amino acid mixture supplementation (mainly composed of BCAAs) suggests the absence of defective mitochondrial accumulation in leucine supplemented animals [55]. If BCAA supplementation resulted in abnormal mTORC1 activation and excessive suppression of autophagy, defective mitochondria would have accumulated causing increased ROS which did not occur. Finally, nitric oxide-dependent mechanisms increase the expression of genes involved in antioxidant defense in endothelial cells [66]. BCAAs also induce eNOS upregulating antioxidant defense genes [55]. Consequently, BCAA should be beneficial to DMD patients to attenuate the increased levels of ROS in these individuals.

Conclusions

Nutritional recommendations for DMD patients are poorly described in the literature [60, 67, 68]. The only clinical trial with leucine was conducted by Mendell et al. [69], which consisted of 44 patients on placebo and 47 patients on 0.2 g/kg/day of leucine. No side effects were observed from the leucine treatment. Initial results indicated significant improvement in strength of the DMD boys compared to the controls following 1 month of leucine supplementation. However, a longer treatment of 1 year failed

to show any benefit from leucine due to an unexpected drop in the strength of the placebo-treated DMD boys. As authors point out, an important caveat of this study was the absence of blood leucine measurements throughout the trial to verify compliance. No clinical trial has been conducted since, possibly because the outcome discouraged other groups from attempting one. Nevertheless, since this initial clinical trial, convincing evidence that supports induction of protein synthesis and prevention of protein degradation by BCAAs, especially leucine has been reported. As noted above, the Society for Sarcopenia, Cachexia, and Wasting Disease recommended inclusion of essential amino acids, specifically leucine, in the diet of patients suffering from these muscle-wasting conditions. The collective data to date suggest it may be appropriate to conduct another clinical trial in DMD patients.

Although there are promising treatment approaches being tested clinically for DMD [3], no cure has yet been demonstrated, nor is it yet clear that a single treatment will be adequate for full recovery of dystrophic muscle. In the absence of an effective individual treatment, it appears that nontoxic leucine and other essential amino acid interventions (e.g., arginine) that could promote protein synthesis and diminish protein breakdown, may be an appropriate intermediate and potentially supplementary treatment approach for DMD.

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Chapter 23
Use of Branched Chain Amino Acids (BCAA) During Radiotherapy

Jinsil Seong and Ik Jae Lee

Key Points

- Most patients referred for radiation therapy have chronic disease and a state of protein-calorie malnutrition.
- Similarly, most hepatocellular carcinoma patients referred for radiotherapy have cirrhotic liver and their lesions at an advanced stage.
- In addition, there is a risk for radiation-induced liver disease.
- Oral supplementation with a branched chain amino acid (BCAA) preparation seems to help HCC patients undergoing radiotherapy by increasing BCAA concentrations in these patients.

Keywords Branched chain amino acid • Hepatocellular carcinoma • Radiotherapy • Liver cirrhosis • Malnutrition

Abbreviations

BCAA	Branched chain amino acids
HCC	Hepatocellular carcinoma
3D-CRT	3-Dimensional conformal radiotherapy
AAA	Aromatic amino acids
TACE	Transarterial chemoembolization
BMI	Body mass index
CTV	Clinical target volume
GTV	Gross tumor volumes
HBV	Hepatitis B virus
HCV	Hepatitis C virus
ITT	Intention-to-treatment
PP	Per-protocol

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Introduction

Branched chain amino acids (BCAA) constitute approximately 40 % of the essential amino acids present in the human body. In contrast to other amino acids that are metabolized in the liver, most BCAA are utilized in muscles and peripheral tissues. The major clinical effects of BCAA that have been reported include awakening effect on hepatic encephalopathy, nutritional supplementation on liver toxicity, and chronic renal failure (Fig. 23.1) [1]. Moreover, randomized controlled studies have demonstrated that parenteral or oral nutritional support with BCAA-enriched preparations in patients undergoing hepatic resection for hepatocellular carcinoma (HCC) significantly improves their postoperative nutritional status and reduces morbidity and length of hospital stay [2, 3]. Hepatocellular carcinoma represents one of the critical health issues globally. Cancer statistics list this disease as the third most common cause of cancer-related deaths worldwide [4]. According to guidelines of the American Association for the Study of Liver Diseases (AASLD), potentially curative therapies can treat the very early and early stages of the disease. However, less than 30 % of HCC patients are detected with the disease in those stages [5]. Since HCC is unresectable in the majority of patients at the time of the first diagnosis, patients are often directed to nonsurgical treatments. Several types of loco-regional treatments are applied for unresectable HCC patients involving chemoembolization, intraarterial infusion chemotherapy, cryosurgery, percutaneous ethanol injection, radioimmunotherapy, external radiotherapy, and radiofrequency ablation [6]. Localized unresectable cases of HCC could be candidates for radiotherapy of HCC. Radiotherapeutic modality has long been overlooked, because the entire liver cannot tolerate a high dose of radiation [7, 8]. However, recent development of radiotherapeutic technology such as 3-dimensional conformal radiotherapy (3D-CRT) and stereotactic body radiotherapy allows increasing application of these technology to HCC management with substantial success [9–16].

Most patients referred for radiation therapy have chronic liver disease, and their lesions are at an advanced stage that is frequently associated with a state of protein-calorie malnutrition. In addition, radiation might induce toxicities such as radiation-induced liver disease. General fatigue, nausea, and vomiting also remain as a challenge to achieving successful radiotherapy [17–19]. These adverse effects may further aggravate the patients' nutritional status. However, there has been little study on nutritional support in such patients undergoing radiotherapy for liver tumor. In this chapter, we would like to review the results about the nutritional supplement in liver cirrhosis and our clinical trial data about the evaluation of the biochemical and amino acid profiles including BCAA in liver tumor patients undergoing radiotherapy [20].

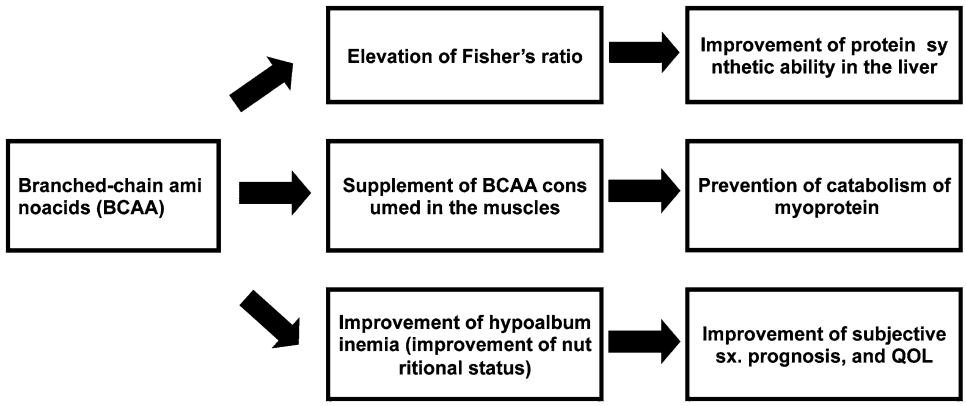

Fig. 23.1 The effect of administration of branched chain amino acids (BCAA) for the metabolism of protein, amino acids, and albumin. Fischer's ratio is determined by serum BCAA/aromatic amino acid molar ratio

Nutritional Supplement with Branched Chain Amino Acids in Liver Cirrhosis

Liver cirrhosis is defined as a state of "Protein-calorie malnutritioin" with increased catabolism, and it has been reported that nutritional state influences survival in patients with decompensated liver cirrhosis [21]. Protein malnutrition, as manifest for example by reduced skeletal muscle mass and hypoalbuminemia, might exist in patients with cirrhosis despite apparent adequate food consumption. In preclinical study, Kajiwara et al. investigated the effect of supplementation of BCAA for carbon tetrachloride-induced cirrhotic rat. They showed that BCAA-supplementation significantly preserved plasma albumin concentrations ($p<0.05$) and inhibited significantly the occurrence of ascites and hyperammonemia ($p<0.05$) [22]. Yoshida et al. evaluated the cumulative survival rates among groups of patients with liver cirrhosis and the effect of long-term oral supplementation with BCAA on the prognosis of liver cirrhosis [23]. They showed that oral supplementation with BCAA for 6 months or more brought about significant increase of plasma BCAA concentration, BCAA/Aromatic amino acids (AAA) molar ratio, and serum albumin concentration. Furthermore, these patients showed significantly higher survival rate during 2–4 years as compared to the control cases matched for age, sex, and involvement of hepatitis B virus. They concluded that long-term supplementation with BCAA raises or maintains nutritional status and improves the survival of patients with decompensated cirrhosis. Muto et al. conducted a multicenter, randomized, and nutrient intake–controlled trial on the comparative effects of BCAA. The event free survival events were significantly improved in the BCAA group as compared with the control group ($p=0.015$). Serum albumin concentration also increased significantly in the BCAA group as compared with the control group ($p=0.018$, Table 23.1). The "general health perception" domain in Short Form-36 measures was also improved ($p=0.003$) [24]. BCAA has been considered beneficial for nutritional improvement because BCAA can be used as a calorie source or accelerate protein synthesis in muscles as well as suppress protein degradation and accelerate protein synthesis in the liver. Marchesni et al. [25] conducted a multicenter randomized study comparing 1 year of nutritional supplementation with BCAA in 174 patients with advanced cirrhosis. The average time of hospital admission was lower in the BCAA arm compared with the control treatments ($p=0.006$ and $p=0.003$, respectively). In addition, nutritional parameters, liver function tests, and the Child–Pugh scores improved in patients treated with BCAA ($p=0.013$). However, long-term compliance with taking BCAA was poor. Our group conducted a prospective study investigating the efficacy of orally administered BCAA in cirrhotic patients. By dividing patients into two groups, BCAA administered group (BCAA group) and control, we found that there was a significant increase in plasma levels of BCAA in the BCAA group ($p<0.001$) [26].

Patients with severe liver failure often manifest symptoms of hepatic encephalopathy. Plasma and brain accumulation of aromatic amino acids may in fact cause a severe impairment of brain neurotransmitter synthesis, in turn causing hepatic encephalopathy [27, 28]. The impairment of liver

Table 23.1 Hazard ratios of BCAA supplementation to diet therapy for events of primary end point

Event	Hazard ratio	95 % Confidence interval	*P* value
Overall	0.67	0.49–0.93	0.015
Hepatic failure	0.45	0.23–0.88	0.016
Liver cancer	0.76	0.50–1.15	0.197
Rupture of varices	0.83	0.32–2.15	0.696
Death	1.18	0.41–3.41	0.76

From Muto Y, Sato S, Watanabe A, et al. Effects of oral branched chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clinical gastroenterology and hepatology* : the official clinical practice journal of the American Gastroenterological Association. Jul 2005;3(7):705–13, with permission

function induces a profound alteration of the plasma amino acid pattern characterized by an elevation in aromatic amino acid and a reduction in BCAA. These patterns are expressed as a Fischer ratio [29]. The Fischer ratio can be calculated by dividing the BCAA by the aromatic amino acids. This ratio shows an excellent correlation with the grade of encephalopathy, whereas increased levels of aromatic amino acids have been correlated with an increased mortality [30]. Rossi et al. evaluated the efficacy of BCAA in two consecutive clinical studies in patients with severe hepatic encephalopathy [31]. In the preliminary uncontrolled study 19 patients with grade 3–4 hepatic encephalopathy were given an intravenous solution containing leucine 11 g/l, isoleucine 9 g/l, and valine 8.4 g/l in 20 % dextrose. A complete recovery of mental state was obtained in all patients in a mean time of 20.5 h. In a subsequent controlled study 40 patients with grade 3–4 hepatic encephalopathy were randomly assigned to receive intravenous BCAA in 20 % dextrose (group A) or oral lactulose (group B). Twelve patients (70–6 %) in group A and eight (47 %) in group B regained consciousness in a mean time of 27–6 and 31.5 h, respectively. The difference in the recovery rate between the two groups, although evident, was not significant. Intravenous branched chain amino acids are thus at least as effective as lactulose in reversing hepatic coma. Plauth et al. reported the safety and efficacy of long-term oral supplementation with BCAA as an adjunct to conventional therapy in patients with stable cirrhosis and latent encephalopathy [32]. Latent encephalopathy was diagnosed by psychometric testing, used to assess automobile driving capacity. Seventeen patients with impaired driving capacity received either BCAA or placebo for 8 weeks before being crossed over to the other regimen for an equal period. BCAA but not placebo significantly improved psychomotor disturbances ($p<0.01$) and driving capacity ($p<0.002$). They conclude that long-term BCAA supplementation is well tolerated and effective in the treatment of impaired automobile driving capacity associated with latent porto-systemic encephalopathy.

Nutritional Supplement with BCAA in Hepatocellular Carcinoma

Until recently, hepatic resection of primary or secondary liver tumors has become a primary choice. However there are some risks for morbidity including inflammatory response syndrome, hepatic failure, disseminated intravascular coagulation, severe infections, and hepatic encephalopathy [33]. Most patients with HCC also have underlying liver cirrhosis, and hepatic resection must be performed under rather unfavorable conditions. In addition, liver regeneration after hepatectomy in patients with liver cirrhosis is thought to be poor [34].

A decrease in Fisher's ratio after resection of liver tumors correlates with the duration of postresection hepatic failure [35]. Togo et al. evaluated the usefulness of BCAA after hepatectomy for liver cancer. They prospectively randomized and assigned to one of two groups: those who received BCAA supplementation and a control group. Those in the BCAA group drank 4.74 g of Livact (Ajinomoto Pharma Company, Tokyo, Japan) three times a day in addition to their meals for 1 year. For patients who had liver cancer complicated by liver cirrhosis and who underwent surgery, serum albumin levels recovered earlier in the BCAA group (Fig. 23.2) [36]. In the BCAA group, hyaluronic acid and type IV collagen 7S improved significantly sooner than in the control group ($p=0.045$ and $p=0.008$). For the patients undergoing hepatic resection, it has been demonstrated that long-term oral administration of BCAA after resection of HCC significantly increases the serum albumin level and reduces the incidence of ascites or peripheral edema in other prospective randomized trial [37].

Nutritional support may also be important in patients undergoing transarterial chemoembolization (TACE). Patients with inoperable HCC undergoing locoregional therapy have either more advanced tumors or more severe cirrhosis than those undergoing hepatic resection, and thus are more likely to suffer from malnutrition. Poon et al. conducted a prospective randomized trial to evaluate any benefit

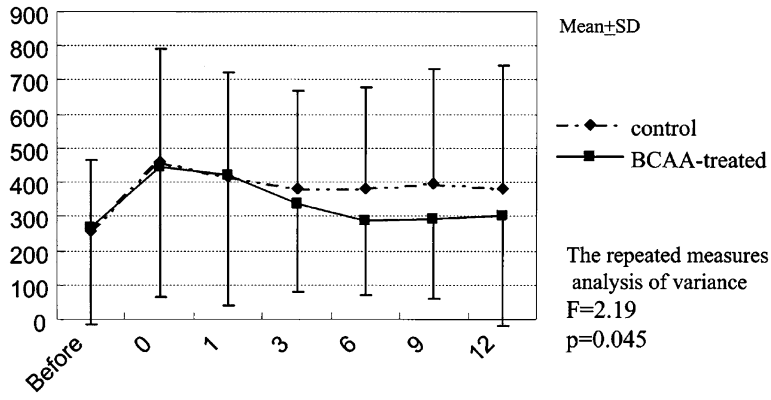

Fig. 23.2 Serum albumin returned to its preoperative value 9 months after surgery in the control group but took only 6 months in the BCAA group. (Adapted from Togo S, Tanaka K, Morioka D, et al. Usefulness of granular BCAA after hepatectomy for liver cancer complicated with liver cirrhosis. *Nutrition*. Apr 2005;21(4):480–86)

of long-term oral supplementation with BCAA is patients with unresectable HCC undergoing TACE. The administration of BCAA resulted in a lower morbidity rate compared with the control group (17.1 % vs. 37.2 %, $p=0.039$). In particular, the group given BCAA showed a significantly lower rate of ascites (7.3 % vs. 23.2 %, $p=0.043$) and peripheral edema (9.8 % vs. 27.9 %, $p=0.034$). Significantly higher serum albumin, lower bilirubin, and a better quality of life were observed after TACE in the group given BCAA [38].

Nutritional Supplement with BCAA During Radiotherapy for Hepatocellular Carcinoma

Irradiation can induce breakdown of skeletal muscle that results in the release of amino acids from irradiated muscle into the blood stream [39]. Kurohara et al. suggested that this alteration in protein metabolism is probably the principal pathogenic factor of radiation myopathy, fatigue, and muscle wasting in patients receiving radiation therapy [40]. Holecek et al. evaluated the effect of alanyl-glutamine administration on the metabolism of proteins in irradiated rats [41]. An increase in whole-body leucine oxidation and insignificant changes in whole-body proteolysis and in protein synthesis were observed after irradiation. They observed a decrease in muscle glutamine concentration, a decrease in protein synthesis in jejunum, colon, and heart, and an increase in synthesis of proteins of blood plasma and spleen after irradiation. They concluded that irradiation induces metabolic derangements which are associated with increased oxidation of essential BCAA and that these disturbances can be ameliorated by administration of alanyl-glutamine in animal study. In clinical situation, nutritional supplement is important particularly before radiotherapy. The patients presenting advanced tumors as well as severe cirrhosis are more likely to suffer from malnutrition. Therefore, oral BCAA support may play an important role in the management of patients who receive radiation therapy for HCC. However, there has been a few studies on nutritional support in patients undergoing radiotherapy for HCC. Our group conducted a double-blind, randomized, placebo-controlled study to evaluate whether BCAA improves biochemical and amino acid profiles of HCC patients undergoing radiotherapy (Fig. 23.3) [20]. Primary endpoints were serum biochemical and amino acid profiles as a result of receiving the BCAA supplement, the number of patients that reverted to normal serum

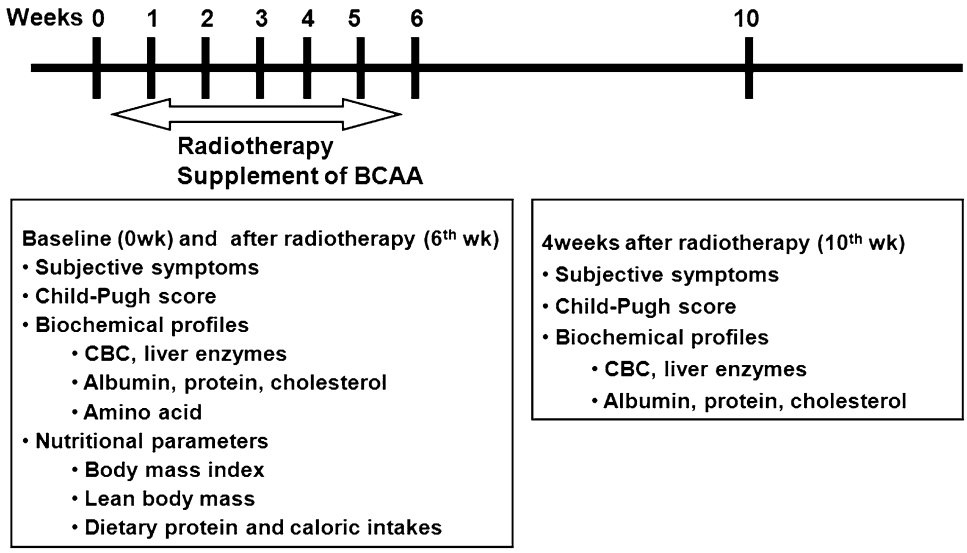

Fig. 23.3 Trial design. Subjects were randomized into branched chain amino acid (BCAA) and placebo groups. Subjective symptoms, Child-Pugh score, biochemical profiles, and nutritional states were evaluated. (Adapted from Lee IJ, Seong J, Bae JI, You SH, Rhee Y, Lee JH. Effect of Oral Supplementation with Branched Chain Amino Acid (BCAA) during Radiotherapy in Patients with Hepatocellular Carcinoma: A Double-Blind Randomized Study. *Cancer Res Treat*. Mar 2011;43(1):24–31)

albumin values. Secondary end points included: subjective symptoms and nutritional parameters. The BCAA group was given a sachet (4.74 g) of BCAA, which contained 952 mg of L-isoleucine, 1,904 mg of L-leucine, and 1,144 mg of L-valine, orally three times a day after meals during radiotherapy (5–6 weeks). The placebo group was given placebo on the same schedule.

For radiotherapy planning and treatment, patients were positioned supine with their arms above their head. All gross tumor volumes (GTV) were contoured on intravenous contrast-enhanced lesions. For a better delineation of the tumor volume, a hepatic angiographic image was used as a reference. A minimum of 5 mm around the GTV was included in the clinical target volume (CTV). In designing the planning target volume (PTV), the margins were individualized by observing the position of the liver as well as liver movement at the time of simulation. Radiation therapy was administered using a 6 or 10 MV linear accelerator (Varian Medical Systems, Palo Alto, CA, USA). The radiation dose to the target volume was determined depending on the functional reserve of the liver and chosen to be in the range of dose prescription guidelines at each $V_{50\%}$ category (Fig. 23.4). For the guidelines from Yonsei University, if the percentage of nontumor liver volume receiving 50 % of the isocenter dose was < 25 %, the total dose was increased to 59.4 Gy; if 25–50 %, the dose was 45–54 Gy; if 50–75 %, the dose was 30.6–41.4Gy; and if >75 %, no treatment was administered [42]. A total of 45 Gy was usually prescribed in 25 fractions of 1.8 Gy over 5 weeks. A multiport combination of two or more ports was adopted, depending on the tumor location, and the median total dose was 46.8 ± 9.7 Gy. We analyzed 37 HCC patients in order to conduct an evaluation with a more homogenous group. Of these, 20 patients were assigned to the BCAA group, and 17 to the placebo group. Among them, 30 patients completed the entire protocol. We enrolled all patients with liver failure and evaluated them using the intention-to-treat (ITT) and per-protocol (PP) analysis. Except for two patients, hepatic function received a Child-Pugh class A score. None had hepatic encephalopathy before enrollment in this study. All had hepatitis B virus (HBV) infection except for three cases (3 cases of hepatitis C virus [HCV] infection in the placebo group).

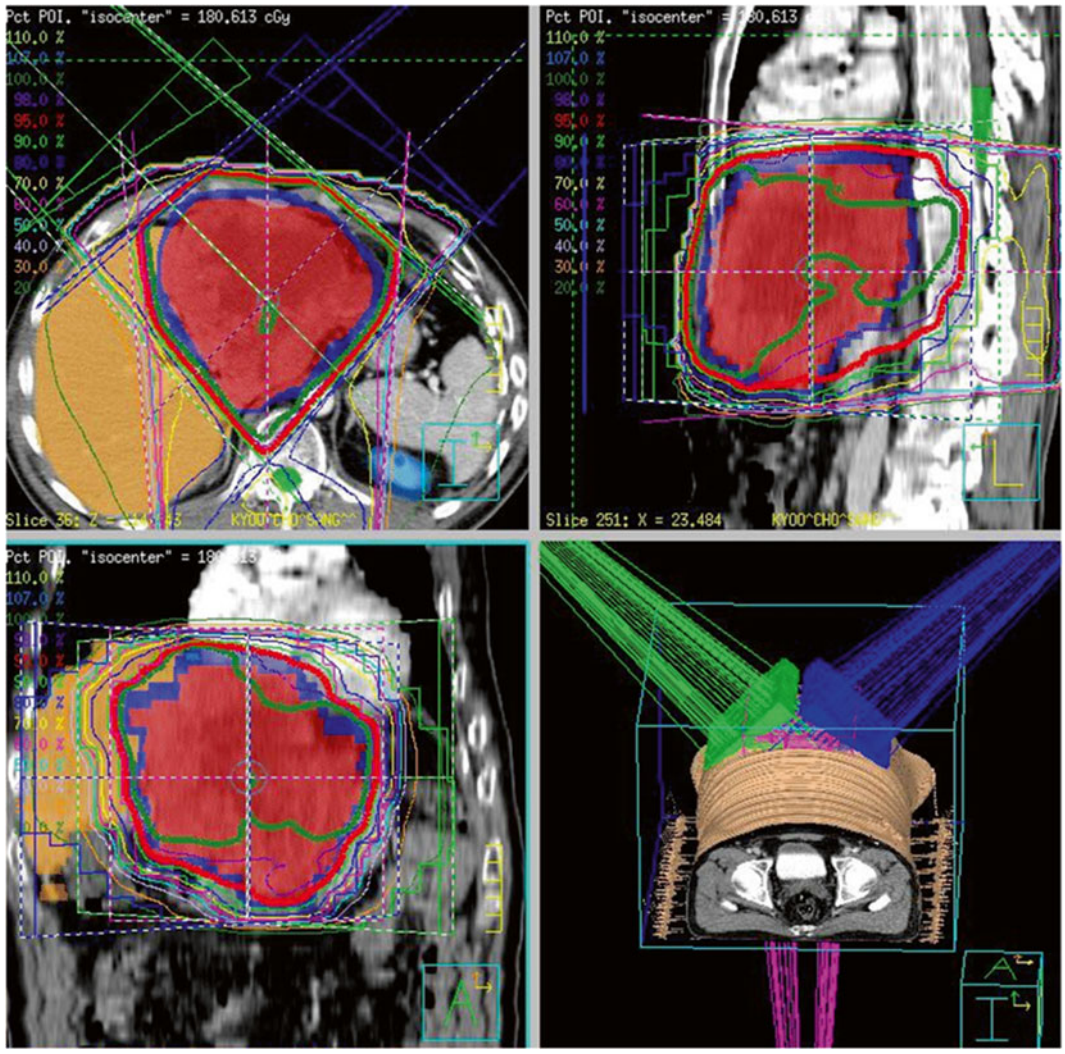

Fig. 23.4 3-Dimensional view of the planned beams and isodose curve of 3-dimensional conformal radiotherapy plan for an example patient

Effect of BCAA on Biochemical Profiles and Amino Acids

Biochemical profiles, such as mean values of serum total cholesterol, triglycerides, total protein, and bilirubin were not significantly different between the two groups for ITT or PP analyses. No differences were also seen in hemoglobin concentration and platelet counts. However, there was a slightly higher tendency in the BCAA group for the cases to return to normal serum albumin levels. In particular, between the period of 3 and 10 weeks after the administration of BCAA, the serum albumin levels of seven patients (of 17 patients; 41.18 %) returned to normal values, whereas only 1 (of 12 patients; 8.33 %) in the placebo group returned to normal. These results showed that the proportion of patients in the BCAA group whose serum albumin recovered to normal levels significantly increased from 3 to 10 weeks ($p=0.043$; Fig. 23.5). Changes in plasma amino-acid profiles showed no substantial differences between the BCAA and placebo groups. However, BCAA supplementation slightly increased the Fisher's ratio.

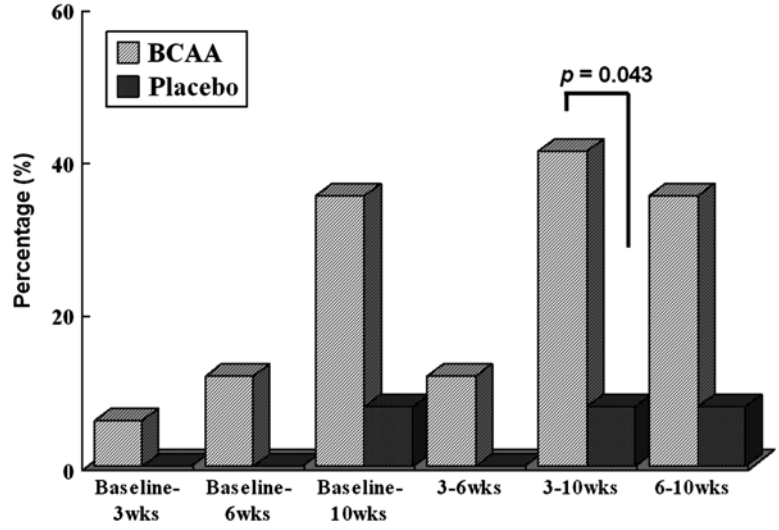

Fig. 23.5 Percentage of patients whose serum albumin returned to a normal level. Serum albumin levels recovered from 3 to 10 weeks in the BCAA treatment group ($p = 0.043$). (Adapted from Lee IJ, Seong J, Bae JI, You SH, Rhee Y, Lee JH. Effect of Oral Supplementation with Branched Chain Amino Acid (BCAA) during Radiotherapy in Patients with Hepatocellular Carcinoma: A Double-Blind Randomized Study. *Cancer Res Treat*. Mar 2011;43(1):24–31)

In our study, the oral supplementation was administered only during the period of radiation therapy (5–6 weeks). Furthermore, the number of patients recruited was small due to difficulty in enrollment. A larger scale study seems necessary to further elucidate the role of nutritional support in radiotherapy for HCC.

Conclusions

Most patients referred for radiation therapy have chronic disease and a state of protein-calorie malnutrition. Oral supplementation with a BCAA preparation seems to help patients undergoing radiotherapy to liver by increasing BCAA concentrations in these patients.

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Chapter 24
Oral Branch Chain Amino Acids and Encephalopathy

Lise Lotte Gluud, Gitte Dam, Niels Kristian Aagaard, and Hendrik Vilstrup

Key Points

- Hepatic encephalopathy is a serious complication to severe liver disease.
- Interventions to prevent episodes of hepatic encephalopathy and improve manifestations of the disease are essential considering the high morbidity and mortality.
- Although a number of high-quality randomized controlled trials on BCAA for hepatic encephalopathy are available, none of the individual trials are able to provide definite evidence to support treatment recommendations.
- A Cochrane systematic review on nutritional interventions for patients with liver disease found no definite evidence to support or refute the use of BCAA based on meta-analyses including some of the available trials.
- Meta-analyses of BCAA for hepatic encephalopathy found that enteral administration may be more beneficial than intravenously administered BCAA.
- A systematic review found a clear beneficial effect of oral BCAA on manifestations of chronic recurrent hepatic encephalopathy based on analyses of outcomes recalculated based on individual patient data.
- Oral BCAA should be considered in the treatment of patients with hepatic encephalopathy.
- The role of BCAA in relation to other interventions for hepatic encephalopathy (in particular rifaximin and nonabsorbable disaccharides) should be assessed.

Keywords Branched chain amino acids • Hepatic encephalopathy • Cirrhosis • Portal systemic encephalopathy • Systematic reviews • Meta-analysis • Randomized controlled trials

Abbreviations

BCAA Branched chain amino acids
CI Confidence intervals

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Introduction

Hepatic encephalopathy is a metabolic neuro-psychiatric syndrome of cerebral dysfunctions due to severe chronic or acute liver disease. The condition can occur in the clinical course of liver failure and is often precipitated by clinical events such as infection, gastrointestinal bleeding, electrolyte derangements, or insertion of a transjugular intrahepatic portal-systemic shunt (TIPS). The manifestations of hepatic encephalopathy range from minor symptoms with personality changes and altered sleep patterns to deep coma. Characteristic signs include shortened attention span and asterixis (flapping tremor). The clinically evident stages of hepatic encephalopathy are classed as overt whereas subtle stages identified through specific tests are classed as minimal [1]. Diagnosing hepatic encephalopathy is paramount because it has deleterious effects on the patient's and the caregivers' lives and is in most cases treatable [2]. Prevention of hepatic encephalopathy as well as correct and early administration of evidence-based interventions is essential.

How the Intervention Might Work

The branched chain amino acids (BCAA) consist of the three essential amino acids valine, leucine, and isoleucine. Patients with cirrhosis have a low plasma concentration of BCAA so that the ratio between the aromatic amino acids and the BCAA is increased. The *relatively* higher concentration of the aromatic amino acids is reported to lead to cerebral neurotransmitter synthesis disturbances. Therefore, treatment with nutritional supplements containing BCAA was initially developed to normalize the ratio between aromatic amino acids and BCAA in cirrhosis patients with hepatic encephalopathy. The results of subsequent experimental and clinical studies showed that that the role of BCAA supplements in cirrhosis was much more complex involving several organs including a beneficial effect on the building of muscles that assist the liver in ammonia removal. Several clinical trials have evaluated the effects of BCAA supplements in patients with cirrhosis and hepatic encephalopathy [3–8]. The results of individual trials vary considerably with the trial setting and design and the patients' inclusion criteria. None of the individual trials were able to provide a definite conclusion regarding the potential effects of BCAA for hepatic encephalopathy. Due to the differences between trials, combined analyses that allow for the differences and adjust for the variation between different trials are necessary to assess the role of BCAA for the treatment of hepatic encephalopathy.

Oral or Intravenous BCAA Supplements

BCAA and Other Nutritional Interventions for Patients with Liver Disease

In a recent comprehensive Cochrane review Koretz and colleagues included randomized clinical trials on any type of nutritional support for any type of liver disease [9]. A meta-analysis from the systematic review included 18 randomized controlled trials on the effect of nutritional therapy versus no nutritional therapy for liver disease in a medical (nonsurgical) setting. The meta-analysis found no effects on mortality (parenteral supplements risk ratio 0.67; 95 % CI 0.28–1.62 and enteral supplements risk ratio 0.81; 95 % CI 0.50–1.33). Meta-analyses of randomized controlled trials on the effects of nutritional supplements on the prevention of hepatic encephalopathy found no detrimental or beneficial effects when analyzing medical trials or trials on patients undergoing surgery. A subgroup analysis of trials on nutritional supplements containing a high concentration of BCAA found no difference between the intervention and control group regarding the risk of developing hepatic

encephalopathy (risk ratio 0.82; 95 % CI 0.63–1.09). A fixed effect meta-analysis of six small trials including a total of 119 participants found that nutritional supplements have a beneficial effect on resolution of hepatic encephalopathy (risk ratio 2.10; 95 % CI 1.18–3.72). The effect was more pronounced in the two trials on BCAA-enriched nutritional interventions (risk ratio 7.48; 95 % CI 1.87–29.94) than in the remaining trials that evaluated "standard" amino acid nutritional supplements (risk ratio 1.13; 95 % CI 0.62–2.07). The subgroup analysis on the effect of BCAA-enriched interventions for the resolution of hepatic encephalopathy included two trials with a total of 33 patients randomized to the BCAA-enriched supplements and 29 patients to the control groups [10, 11]. One of the trials by Calvey and colleagues [11] was published in 1985 and included patients with acute alcoholic hepatitis. The trial compared BCAA versus "conventional" protein supplements administered orally, nasogastrically, or intravenously depending on the severity of the underlying disease. The trial found no differences between the BCAA and control groups regarding mortality, hepatic encephalopathy, or nutritional parameters. The second trial by Hayashi and colleagues [10] was published in 1991 and included 67 patients with cirrhosis who were randomized to a BCAA versus a control diet. The BCAA-enriched supplement and the control supplements were administered orally or via an enteral feeding tube. The trial found a clear beneficial effect of BCAA on improvement of clinically overt hepatic encephalopathy (relative risk 6.40; 95 % CI 1.58–26.00). Potential bias was identified in both trials including selection, ascertainment, and attrition bias [9]. Therefore, the result of the meta-analysis based on the two trials may also be biased. Considering the limited number of patients included and limitations related to the trial design and available data, Koretz and colleagues did not find the evidence strong enough to recommend BCAA-enriched supplements or any other type of nutritional intervention for patients with hepatic encephalopathy or for prevention of hepatic encephalopathy [9]. However, it may be argued that the evidence is promising. Furthermore, the Cochrane review only included two of the available trials on BCAA. The exclusion of larger high-quality trials, published after the two trials that were included in the Cochrane Systematic Review, may provide additional essential information on the influence of oral or intravenous BCAA on manifestations of hepatic encephalopathy. To evaluate the potential effects of BCAA-enriched interventions, separate analyses of intravenous and orally administered supplements are needed to determine the potential beneficial and harmful effect in different settings.

Intravenous BCAA

A number of trials have evaluated the effect of intravenous BCAA supplements [12–15]. Three of the earliest trials were published in 1985 [13–15]. One of the trials was conducted by Fiaccadori and colleagues [14]. The trial included 48 patients with cirrhosis and overt acute or chronic intermittent hepatic encephalopathy. Included patients were randomized to one of two BCAA-enriched solutions that were depleted in aromatic amino acids versus isocaloric glucose. All intervention groups received lactulose. Based on clinical assessments, the number of patients who survived and had complete recovery from hepatic encephalopathy was higher in the BCAA than the control groups (relative risk 1.55; 95 % CI 1.06–2.28). Michel and colleagues found less encouraging results in a similar randomized controlled trial comparing BCAA-enriched versus "standard" amino acid infusion for 70 patients with cirrhosis and acute hepatic encephalopathy [13]. The trial found no difference between the allocation groups regarding any of the clinical outcome measures assessed. In particular, the proportion of patients with improved manifestations of hepatic encephalopathy was similar in the BCAA and control group (relative risk 1.13; 95 % CI 0.56–2.27). No difference in survival was detected. In the third trial from 1985 [13], Michel and colleagues reached a similar result. The trial included 70 patients with cirrhosis and acute hepatic encephalopathy. Included patients were randomized to 5 days of treatment with a BCAA-enriched solution or an isonitrogenous, isocaloric control. None of the patients received nonabsorbable disaccharides. No differences were seen between the intervention and control group regarding

improvement of hepatic encephalopathy manifestations, mortality at the end of follow-up, or mortality 1 month after the end of treatment. Vilstrup and colleagues reached a similar result in a trial from 1990 [12]. The trial compared infusion of a BCAA versus glucose for acute hepatic encephalopathy in patients with cirrhosis. Included patients were randomized to an amino acid mixture (1 g per kg of body weight per day) with 40 % BCAA or isocaloric glucose for a maximum of 16 days. Both intervention groups received lactulose. The number of patients who died and the number of patients with improved hepatic encephalopathy were similar in the two groups. However, the negative nitrogen balance at baseline reversed in the amino acid, but not in the glucose group.

Three trials published from 1986 to 1998 evaluated the effects of BCAA, lactulose, and antibiotics [16–18]. Rossi-Fanelli and colleagues included 40 patients with cirrhosis severe (at least grade 3) acute HE and found that BCAA was more effective than lactulose in the improvement of hepatic encephalopathy manifestations. Mortality was similar in the two groups. Strauss and colleagues [18] compared the effect of BCAA versus neomycin administered orally or as enemas in 29 patients with cirrhosis and acute hepatic encephalopathy. Some of the patients were included more than once. Irrespective of whether each patient was included only once in the analysis or whether the analysis was based on the number of episodes of hepatic encephalopathy (i.e., patient were included more than once), no differences were identified between allocation groups regarding improvement of hepatic encephalopathy manifestations or mortality. A similar result was reached by Hwang and colleagues who evaluated the effect of neomycin and lactulose alone or with a BCAA-enriched solution in 55 patients (60 episodes of hepatic encephalopathy) with acute hepatic encephalopathy associated with acute liver failure [16].

In conclusion, only two of seven trials found a potential benefit of intravenous BCAA on clinical outcome measures and one trial found a potential benefit on nutritional parameters. The individual trials provide little evidence to support the use of intravenous BCAA-enriched solutions for acute episodes of hepatic encephalopathy in clinical practice. On the other hand, the trials were small and the statistical power was weak, which could mean that clinically relevant intervention effects may be overlooked. Furthermore, the trials were published several decades ago, which means that the collateral interventions and supportive care for the underlying liver disease did not follow current recommendations. The prognosis for patients with cirrhosis and acute hepatic encephalopathy is considerably improved, which means that the benefit of intravenous BCAA-enriched solutions may still need to be assessed in relation to current practice.

Oral BCAA Supplements

Eight trials have evaluated the effect of orally administered BCAA for hepatic encephalopathy [3, 4, 7, 8, 19–21]. The definitions and assessments used in the diagnosis of hepatic encephalopathy varied between trials (Table 24.1). All trials included patients with cirrhosis. The first two trials were published as early as 1984 [21] and 1985 [19]. Both trials included a control group randomized to an isonitrogenous control. Egberts and colleagues included 22 patients with alcoholic liver disease (86 %) or viral hepatitis (14 %) [19]. Horst and colleagues [21] included a total of 37 patients (38 % with alcoholic liver disease and 8 % with viral hepatitis). None of the trials found a beneficial effect of BCAA on improvement of hepatic encephalopathy manifestations or mortality at the end of treatment. In a larger randomized controlled trial from 1990, Marchesini and colleagues included patients with cirrhosis and chronic latent hepatic encephalopathy [3]. The trial compared BCAA (30 patients) versus an isonitrogenous control diet containing casein (34 patients). Only three patients were lost to follow-up. The trial found a clear beneficial effect of BCAA on improvement of hepatic encephalopathy manifestations after 3 months of follow-up (relative risk 2.27; 95 % CI 1.39–3.70), but no difference

Table 24.1 Table of diagnostic criteria and methods used in included trials

Hayashi [10]	Portalsystemic encephalopathy index, Trail Making Test Part A, serial sevens test, and plasma ammonia
Horst [21]	Asterixis, portalsystemic encephalopathy index, Trail Making Test Part A, electroencephalography, and ammonia
Marchesini [3]	Portalsystemic encephalopathy index, a complete neuropsychological examination, Trail Making Test Part A, electroencephalography, and fasting venous ammonia
Marchesini 2003 [4]	Encephalopathy score and Trail Making Test Part A
Muto [7]	West-Haven criteria grade 1–4
Plauth 1993 [20]	The digit symbol test, Trail Making Test Part A, number revision test, motor performance test battery, and the Vienna Reaction Time Apparatus
Egberts [19]	Culture Fair intelligence test, Wechsler Adult intelligence test, Digit Symbols, Multiple Choice Vocabulary Test, The visual retention test, Number Revision Test, Attention Stress Test, Attention Diagnostic Method, Motor Performance Test Battery, and Vienna Reaction Time Apparatus
Les [8]	Trail Making Test part A, Symbol Digit Test (oral version), Grooved Pegboard Test (dominant hand), and Barthel's autonomy index

in mortality between allocation groups. Hayashi and colleagues performed a similar trial that was published in abstract form in 1991 [10]. The trial included a total of 67 patients and found that BCAA increased the proportion of patients with improved manifestations of hepatic encephalopathy at the end of treatment compared with an isonitrogenous control group (relative risk 6.40; 95 % CI 1.58–26.00). The effect of BCAA on mortality was not described.

Three trials have evaluated the effect of oral BCAA for patients with cirrhosis and an increased risk of developing clinically overt hepatic encephalopathy [4, 7, 8]. The trials were published between 2003 and 2011. The first trial by Marchesini and colleagues [4] compared 12 months of treatment with BCAA ($n=33$) versus isonitrogenous and isocaloric supplements ($n=79$). The proportion of patients with minimal hepatic encephalopathy at inclusion was 74 %. The trial found a beneficial effect of BCAA on a composite outcome measure that included mortality and clinical deterioration. When analyzing patients with minimal hepatic encephalopathy at baseline, no benefit of BCAA was detected (relative risk 1.31; 95 % CI 0.74–2.32). In a subsequent trial, Muto and colleagues reached a similar result [7]. The trial included 646 patients who were randomized to a standard diet with or without BCAA. In agreement with Marchesini and colleagues, Muto and colleagues found that the BCAA supplement had a beneficial effect on a composite outcome measure that included mortality and deterioration of the underlying liver disease. When analyzing patients with clinically overt hepatic encephalopathy at baseline ($n=39$), BCAA had no beneficial effect on hepatic encephalopathy manifestations (relative risk 1.08; 95 % CI 0.62–1.89). Les and colleagues performed a randomized controlled trial on 116 patients with cirrhosis and at least one previous episode of clinically overt hepatic encephalopathy [8]. Included patients were randomized to a standard diet alone or with a BCAA supplement. The duration of follow-up was 56 weeks. The number of patients who developed clinically overt hepatic encephalopathy and the number of patients who survived were similar in the BCAA and control group. Among patients with minimal hepatic encephalopathy at baseline, no improvements in hepatic encephalopathy manifestations were identified.

The results of individual trials on oral BCAA supplements vary considerably. Some trials found a potential benefit of oral BCAA on hepatic encephalopathy manifestations. Other trials found the

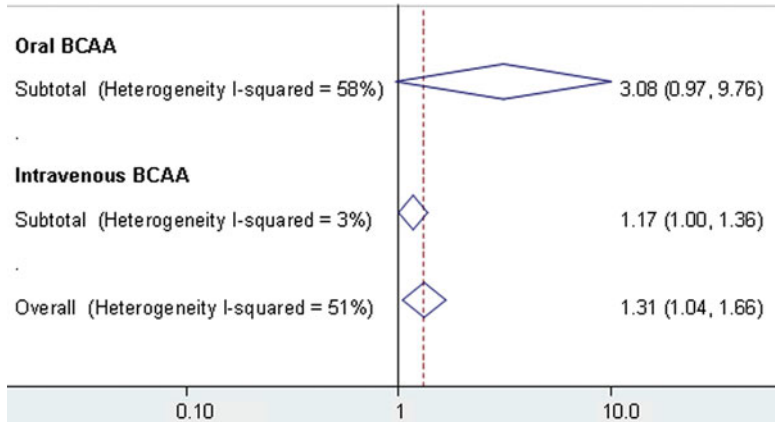

Fig. 24.1 Random effects meta-analysis on branched chain amino acids for patients with hepatic encephalopathy. Random effects model meta-analysis of randomized controlled trials on branched chain amino acids (BCAA) versus placebo, no intervention, control diets, or other interventions for hepatic encephalopathy. The outcome measure is improvement of manifestations of hepatic encephalopathy. The analyses are stratified for subgroups of trials on intravenous or orally administered BCAA. The results of the meta-analyses are presented as risk ratios (RR) with 95 % confidence intervals (CI)

opposite result. None of the trials found a benefit on mortality, but a potential benefit cannot be excluded when evaluating the result of composite clinical outcome measures. Based on the differences between trials and trial outcomes, systematic reviews with meta-analyses of available trials are needed.

Cochrane Systematic Reviews

In a Cochrane Review from 2003, the results of individual randomized clinical trials on BCAA were evaluated [22]. The review included eleven randomized trials on BCAA versus placebo, no intervention, control diets, or other interventions (including lactulose or antibiotics) for hepatic encephalopathy. Due to the inclusion criteria, the trials by Marchesini and colleagues was excluded from the review [4]. Trials were included regardless of blinding, language, or publication status. The maximum duration of follow-up was 30 days. Nine of the trials reported data on improvement of manifestations of hepatic encephalopathy. The remaining trials did not report data that allowed an assessment of this outcome measure. A random effects meta-analysis of the nine trials (Fig. 24.1) showed that BCAA had a beneficial effect on hepatic encephalopathy compared with control interventions (risk ratio 1.34; 95 % CI 1.12–1.61). The result was confirmed in fixed effect meta-analysis (Fig. 24.2). There was no difference between trials in which the control group received an isonitrogenous diet or a nonisonitrogenous diet (test for subgroup differences $P=0.19$). No differences were seen between trials using a high or low dose of BCAA (test for subgroup differences $P=0.50$) or trials with a duration of follow-up that was less than 3 months or at least 3 months BCAA (test for subgroup differences $P=0.37$).

There were no beneficial or detrimental effects on mortality, quality of life, or adverse events. A subgroup analysis was performed to evaluate the potential influence of the mode of administration on the estimated intervention benefit. The subgroup analysis found some evidence of a beneficial effect of intravenous BCAA in random effects model meta-analysis (risk ratio random effects model 1.17; 95 % CI 1.00–1.36; Fig. 24.1. The fixed effect model meta-analysis confirmed the findings (risk ratio 1.21; 95 % CI 1.02–1.43; Fig. 24.2). There was no difference between trials in which patients in the

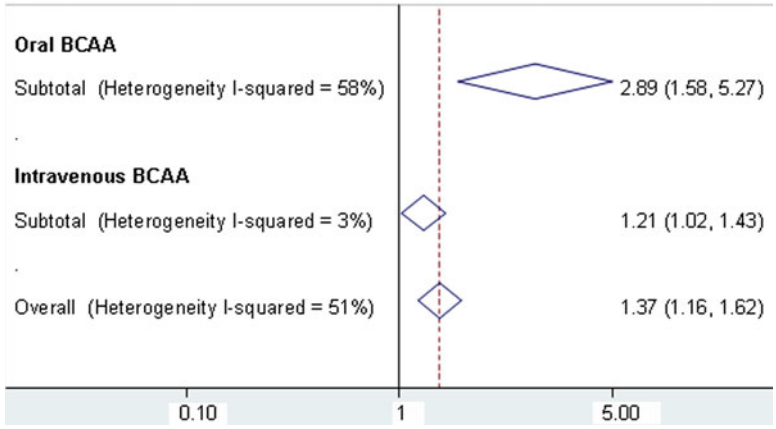

Fig. 24.2 Fixed effect meta-analysis on branched chain amino acids for patients with hepatic encephalopathy. Fixed effect model meta-analysis of randomized controlled trials on branched chain amino acids (BCAA) versus placebo, no intervention, control diets, or other interventions for hepatic encephalopathy. The outcome measure is improvement of manifestations of hepatic encephalopathy. The analyses are stratified for subgroups of trials on intravenous or orally administered BCAA. The results of the meta-analyses are presented as risk ratios with 95 % confidence intervals (CI)

control groups received standard diets, neomycin, or lactulose (test for subgroup differences $P = 0.79$). When analyzing trials on oral BCAA there was a potential benefit on manifestations of hepatic encephalopathy when the meta-analysis was performed using a fixed effect model (relative risk 2.89; 95 % CI 1.58–5.27). The beneficial effect was not confirmed in random effects meta-analysis (relative risk 3.08; 95 % CI 0.97–9.76). However, the analysis of oral BCAA supplements only included two trials with a total of 41 patients [3, 10]. Considering that the Cochrane review did not include the large high-quality trials by Marchesini and colleagues [4] or the more recent trials on oral BCAA supplements (Muto and colleagues and Les and colleagues [7, 8]), an updated meta-analysis is needed to determine the strength of the overall evidence.

Updated Meta-Analysis on Oral BCAA Supplements

Updated meta-analyses were performed in order to include the evidence from all available randomized controlled trials [5, 6]. One meta-analysis included trials on oral or intravenous BCAA [5] whereas the second meta-analysis focused on oral BCAA and excluded trials in which intravenously administered BCAA solutions were used [6]. Based on extensive searches, the meta-analysis in the Cochrane Review [22] was updated with inclusion of data from the three randomized controlled trials on oral BCAA [5]. The updated meta-analysis on intravenous or oral BCAA included data from a total of fourteen trials. Seven trials assessed the effect of intravenous BCAA formulations for patients with acute episodes of overt hepatic encephalopathy and seven trials assessed the effect of oral BCAA supplements for recurrent/minimal hepatic encephalopathy. Data on the outcome measures that were assessed were recalculated based on individual patient data for four of the included trials [3, 4, 7, 8]. The included trials were small (Fig. 24.3). In most trials, less than 40 patients were randomized to BCAA versus control groups. Accordingly, the effect of BCAA versus control interventions on hepatic encephalopathy manifestations was not statistically significant in individual trials. However, BCAA had a clear beneficial effect on hepatic encephalopathy manifestations when the results of the trials were combined in a meta-analysis, regardless of whether a random effects model (risk ratio 1.35; 95 % CI 1.11–1.64; Fig. 24.4) or fixed effect model was used (relative risk 1.41; 95 % CI

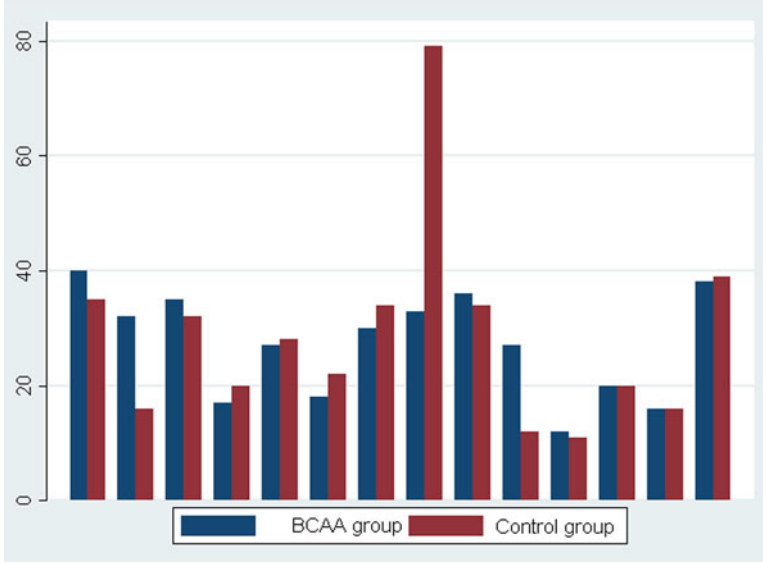

Fig. 24.3 Included patients in randomized controlled trials on branched chain amino acids for hepatic encephalopathy. Sample size in randomized controlled trials on branched chain amino acids (BCAA) versus placebo, no intervention, control diets, or other interventions for the treatment of hepatic encephalopathy. The figure shows the number of patients with hepatic encephalopathy in the allocation groups at the time of randomization

1.22–1.63; Fig. 24.5). The updated meta-analysis revealed a clear difference between subgroups of trials on oral or intravenous formulations of BCAA (test for subgroup differences $P=0.023$). The beneficial effect or oral BCAA was confirmed in meta-analyses using a random or fixed effect model (random effects relative risk 1.41; 95 % CI 1.22–1.63 and fixed effect relative risk 1.84; 95 % CI 1.41–2.39, respectively). Additional subgroup analyses of the trials on oral BCAA found no difference between subgroups of trials stratified by the control intervention (test for subgroup differences $P=0.13$). All patients in the trials on oral BCAA had cirrhosis and most used the standard dose of BCAA. Accordingly, there was very little intertrial heterogeneity (Fig. 24.4). No evidence of bias was identified and the results were robust for multiple comparisons [23]. No beneficial or detrimental effects were identified when assessing mortality (Fig. 24.6). The analysis of losses to follow-up showed some evidence that the proportion of patients who were withdrawn or dropped out after randomization was higher in the BCAA than the control group (Fig. 24.6). However, the difference in losses to follow-up was only statistically significant in the fixed effect meta-analysis (relative risk 0.46; 95 % CI 0.22–0.95) and not when a random effects model was used (relative risk 0.49; 95 % CI 0.23–1.02). The combined evidence suggests that BCAA should be considered in the treatment of patients with cirrhosis and hepatic encephalopathy. The evidence on oral BCAA is more promising than intravenous BCAA solutions. In a meta-analysis that focused on oral BCAA, a similar overall result was achieved [6]. The meta-analysis was based on outcomes recalculated based on individual patients' data from four trials ($n=255$ patients) and data extracted from published trial reports from four trials ($n=127$ patients). Included patients had cirrhosis and recurrent clinically overt hepatic encephalopathy or minimal hepatic encephalopathy. Seven trials reported the proportion of patients with improved manifestations of hepatic encephalopathy (all trials with individual patient data and three trials with published data). Improved manifestations of hepatic encephalopathy were seen in 87 of 172 patients in the BCAA group and 56 of 210 patients in the control (random effects model relative risk 1.71; 95 % CI 1.17–2.51). The corresponding number needed to treat was 5 patients. The effect of BCAA was associated with the type of hepatic encephalopathy (minimal or clinically overt) at baseline (test for subgroup differences $P=0.04$). In random effects meta-analysis the relative effect

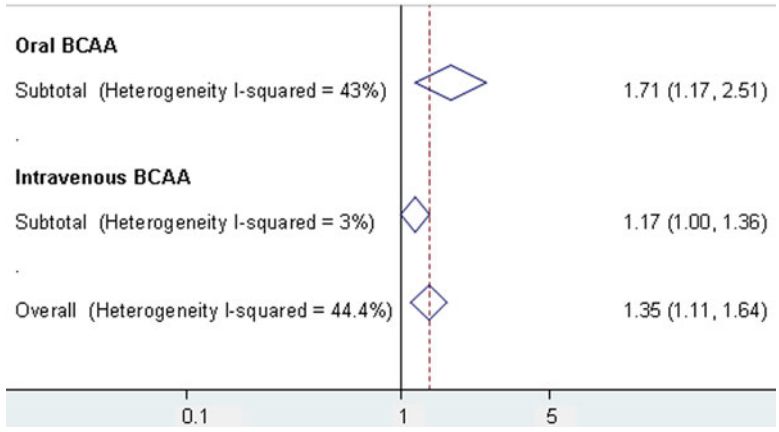

Fig. 24.4 Updated random effects subgroup meta-analysis of oral or intravenous branched chain amino acids for improvement of manifestations of hepatic encephalopathy. Updated random effects meta-analysis of randomized controlled trials on branched chain amino acids (BCAA) versus placebo, no intervention, standard diets, or other active interventions for improvement of manifestations of hepatic encephalopathy. The analyses are stratified for subgroups of trials on orally administered BCAA versus placebo or control diets and trials on intravenous BCAA versus placebo, no intervention, control diets or other interventions. The results of the meta-analysis are presented as risk ratios with 95 % confidence intervals (CI)

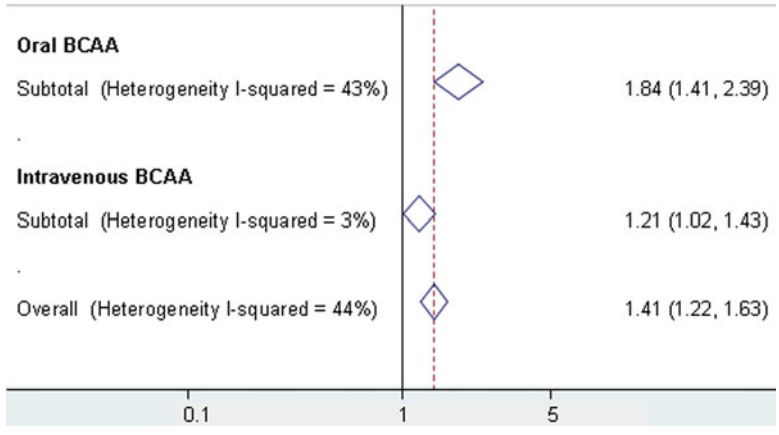

Fig. 24.5 Updated fixed effect subgroup meta-analysis of oral or intravenous branched chain amino acids for improvement of manifestations of hepatic encephalopathy. Updated fixed effect meta-analysis of randomized controlled trials on branched chain amino acids (BCAA) versus placebo, no intervention, standard diets, or other active interventions for improvement of manifestations of hepatic encephalopathy. The analyses are stratified for subgroups of trials on orally administered BCAA versus placebo or control diets and trials on intravenous BCAA versus placebo, no intervention, control diets, or other interventions. The results of the meta-analysis are presented as risk ratios with 95 % confidence intervals (CI)

of BCAA was different in patients with clinically overt hepatic encephalopathy (relative risk 3.26; 95 % CI 1.47–7.22) and patients with minimal hepatic encephalopathy (relative risk 1.32; 95 % CI 0.97–1.79). A similar result was seen when a fixed effect model was used (clinically overt hepatic encephalopathy relative risk 3.11; 95 % CI 1.93–5.01 and minimal hepatic encephalopathy relative risk 1.30; 95 % CI 0.95–1.79). No evidence of bias was identified. No beneficial or harmful effects on remaining clinical outcome measures (including mortality) were identified.

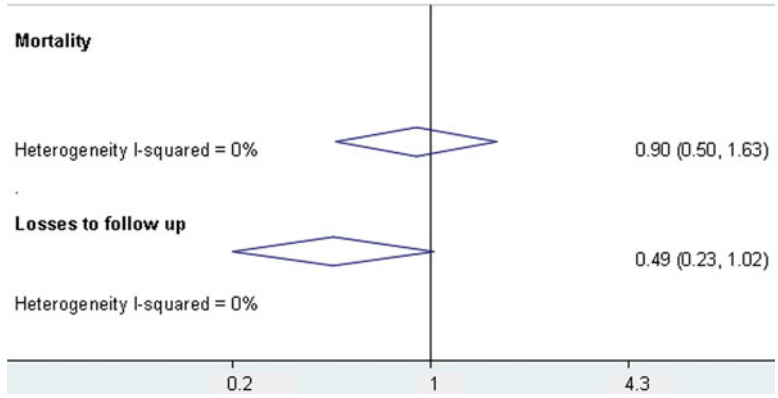

Fig. 24.6 Random effects subgroup meta-analysis on mortality and losses to follow-up in randomized controlled trials on oral branched chain amino acids. Updated random effects meta-analyses of randomized controlled trials on oral branched chain amino acids (BCAA) versus placebo or standard diets for patients with hepatic encephalopathy. The outcomes measures are mortality (all-cause) and losses to follow-up (including all withdrawals and dropouts). The results of the meta-analyses are presented as risk ratios with 95 % confidence intervals (CI)

The Dose of BCAA

The evidence concerning the optimal dose of BCAA supplements in liver disease is weak. Dose finding studies are based on measurements in healthy controls and pathophysiological assessments or plasma concentrations of BCAA and aromatic amino acids in patients with liver disease. In a randomized controlled trial from 1993 [24], thirty patients with cirrhosis and severe hepatic encephalopathy (coma) were randomized to a standardized BCAA-enriched solution with or without additional valine. The results of the trial showed no added benefit of valine when evaluating the course of the disease or mortality. The updated meta-analysis included trials on several different doses of oral BCAA supplements. At present the recommended dose of oral BCAA is 0.25 g per kg of bodyweight per day. Several trials included in the meta-analysis evaluated this recommended dose [3, 19]. For a patient with a body weight of 70 kg, this corresponds to 17.5 g of BCAA per day. Three of the included trials used a relatively low dose of BCAA, 7.2 g to 12 g [4, 10]. In other trials [8, 21], the daily dose of BCAA was set higher than recommended to 20 or 29 g. In subgroup analyses, the beneficial effect of BCAA was confirmed in all three subgroups of trials. BCAA had a beneficial effect on hepatic encephalopathy when administered at a dose of 20–29 g per day (relative risk 2.15; 95 % CI 1.33–3.49), 0.25 mg per day (relative risk 2.15; 95 % CI 1.33–3.49), or 7.2–12 g per day (relative risk 1.65; 95 % CI 1.11–2.45). The difference between subgroups was not statistically significant (test for subgroup differences $P=0.69$). Considering that the beneficial effect of oral BCAA on hepatic encephalopathy is established, future trials should consider the assessment of the optimal dose.

The Effect of BCAA in Relation to Other Interventions

At present, the nonabsorbable disaccharides lactulose and lactitol are recommended as the first-line intervention in hepatic encephalopathy. A systematic review and meta-analysis from 2004 evaluated the effect of the disaccharides [25]. The review included 22 randomized controlled trials. The primary outcome measure was “lack of improvement of hepatic encephalopathy.” The overall analyses showed

a potential beneficial effect of the disaccharides (lactulose and lactitol) compared with placebo or no intervention. The findings were not confirmed in subgroup analyses of trials with a low risk of bias (relative risk 0.92; 95 % CI 0.42–2.04). Several randomized controlled trials have evaluated the effect of lactulose after the systematic review was published [26–30]. One of the trials compared the effect of lactulose versus no intervention. The trial also included intervention groups receiving probiotics or L-ornithine-L-aspartate. The included patients had cirrhosis and minimal hepatic encephalopathy. The results showed that all interventions improved manifestations of hepatic encephalopathy compared with no intervention. Other randomized trials have evaluated the preventive effect of lactulose. One trial included patients with cirrhosis and no previous episodes of hepatic encephalopathy [31]. The results showed that lactulose reduced the proportion of patients who developed hepatic encephalopathy episodes (lactulose 11 % versus no intervention 28 %; $P=0.02$). A similar trial on secondary prevention of hepatic encephalopathy episodes in cirrhosis reached a similar result [30]. In a recent trial on patients with cirrhosis and gastrointestinal bleeding [26], lactulose also prevented hepatic encephalopathy. When updating the meta-analysis from 2004 with recent trials [5], the disaccharides have a beneficial effect on hepatic encephalopathy manifestations (improvement of manifestations) in both random effects (relative risk 1.99; 95 % CI 1.14–3.48) and fixed effect meta-analysis (relative risk 2.21, 95 % CI 1.60–3.05). The benefit of nonabsorbable disaccharides for prevention of hepatic encephalopathy was also established (random effects model relative risk 1.27; 95 % CI 1.17–1.39 and fixed effect model relative risk 1.23; 95 % CI 1.05–1.44).

A large high-quality randomized controlled trial evaluated the effect of the addition of rifaximin to lactulose in patients who did not respond to lactulose alone [32]. The trial found that rifaximin reduced the risk of developing hepatic encephalopathy compared with placebo (hazard ratio 0.42; 95 % CI 0.28–0.64). A meta-analysis of trials on rifaximin versus lactulose showed a clear benefit of rifaximin. The benefit was confirmed in a trial on minimal hepatic encephalopathy [33]. When comparing rifaximin versus lactulose for clinically overt HE, rifaximin increases the proportion of patients with improved manifestations (relative risk 1.57; 95 % CI 1.03–2.39).

Unlike nonabsorbable disaccharides and rifaximin, there is no evidence to support the use of BCAA for prevention of hepatic encephalopathy. Based on the results of individual metaanalyses, the size of the effect of the three interventions is similar. Only one trial on patients with overt hepatic encephalopathy has compared the effect of BCAA versus lactulose [17]. The trial found no statistically significant difference between the two intervention groups (71 % vs. 47 %; $P>0.05$). The trial was small, the duration of follow-up was short and the design entailed a considerable risk of bias. The benefit of BCAA compared with other interventions for hepatic encephalopathy is not established. Additional trials comparing these interventions may be considered. However, the potential benefit of combining interventions is equally or maybe even more important. In a recent randomized trial on 5–6 months of treatment with BCAA versus BCAA and zinc supplements for patients with liver cirrhosis [34]; the trial included 40 patients with blood albumin levels of $\leq$3.5 g/dL and zinc levels of $\leq$70 μg/dL. The trial found that the addition of zinc to BCAA improved nitrogen metabolism. No effects on clinical outcome measures were identified.

Suggestions for Future Research

Additional evidence is still needed to determine the effects of BCAA for both clinically overt and minimal hepatic encephalopathy. Future trials should use a randomized design and evaluate the effect of interventions on symptoms and manifestations as well as the prevention of hepatic encephalopathy episodes. The dose of BCAA should be established to optimize the management of patients with hepatic encephalopathy.

Conclusions

The combined evidence supports the use of oral BCAA as treatment for patients with hepatic encephalopathy. The evidence supporting the use of oral BCAA includes several large, high-quality randomized controlled trials. There is no convincing evidence on the use of intravenous BCAA for acute or chronic hepatic encephalopathy. The value of combining BCAA with other recommended interventions focusing on lactulose and rifaximin remains unresolved albeit promising.

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Chapter 25
Web-Based Resources, and Suggested Readings

Rajkumar Rajendram, Vinood B. Patel, and Victor R. Preedy

Key Points

- Branched chain amino acids have significant clinical value in modern medicine.
- This chapter lists the most up-to-date resources on the regulatory bodies, journals, books, professional bodies and websites that are relevant to an evidence-based approach to the use of branched chain amino acids.

Keywords Branched chain amino acids • Evidence • Resources • Books • Journals • Regulatory bodies • Professional societies

Introduction

Leucine, isoleucine and valine are the branched chain amino acids (BCAA). Branched chain amino acids are essential amino acids that cannot be synthesised within the body. There are subtle but important differences in their structure, function and metabolism which mean that it is important to consider the BCAA as separate entities.
Examples of the applications of branched chain amino acids can be found in this book and also via the recommended resources in the tables below.

Tables 25.1, 25.2, 25.3, 25.4, and 25.5 list the most up-to-date information on the organizations and regulatory bodies interested in branched chain amino acids (Table 25.1), professional bodies (Table 25.2), journals (Table 25.3), books (Table 25.4) and websites (Table 25.5) that are relevant to an evidence-based use of branched chain amino acids in health and disease.

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Table 25.1 Regulatory bodies and organisations

American Association for Cancer Research
www.aacr.org
American Society for Parenteral and Enteral Nutrition (ASPEN)
www.nutritioncare.org
American College of Sports Medicine
www.acsm.org
British Association for Cancer Research
www.bacr.org.uk
Centers for Disease Control and Prevention
www.cdc.gov
Department of Health and Human Services
www.hhs.gov
European Commission DG Health and Consumers; Food and Feed Safety
ec.europa.eu/food/food/labellingnutrition/claims
European society for clinical nutrition and metabolism (ESPEN)
www.espen.org
Food and Drug Administration
www.fda.gov
Institute of Cancer Research
www.icr.ac.uk
International Life Sciences Institute (ILSI)
www.ILSI.org
National Cancer Institute
www.cancer.gov
National Institutes of Health
www.nih.gov
Nutrition
www.nutrition.gov
Sociedade Brasileira de Nutrição Parenteral e Enteral (SBNPE)
www.sbnpe.com.br
US National Library of Medicine and National Institutes of Health
www.ncbi.nlm.nih.gov/pubmed
World Health Organization
www.who.int/en

This table lists the regulatory bodies and organisations with interests in branched chain amino acids

Table 25.2 Professional societies

American Association for the Study of the Liver (AASLD)
www.aasld.org
American Diabetes Association
www.diabetes.org
American Heart Association
www.heart.org/HEARTORG
American Physiological Society
www.the-aps.org
American society for biochemistry and molecular biology
www.asbmb.org
American Society for Nutrition
www.nutrition.org
American Thoracic Society
www.thoracic.org
Australasian society for inborn errors of metabolism

(continued)

Table 25.2 (continued)

www.hgsa.org.au/asiem
Brazilian Society of Food and Nutrition
www.sban.com.br
British Heart Foundation
www.bhf.org.uk/#&panel1-1
European Association for the Study of the Liver (EASL)
www.easl.eu
European Federation of Neurological Societies
www.efns.org
European Respiratory Society
www.ersnet.org
Federation of American Societies for Experimental Biology (FASEB)
www.fasebj.org
International Council on Amino Acid Science (ICAAS)
www.icaas-org.com
International Society for Hepatic encephalopathy and nitrogen metabolism (ISHEN)
www.ishen.org
Japanese Society for Amino Acid Sciences
www.asas.or.jp/jsaas/english/index.html
Japanese Society of Parenteral and Enteral Nutrition (JSPEN)
www.jspen.jp/top.html
Japanese Society of Clinical Nutrition
Japanese Society of Internal Medicine
www.naoka.or.jp
Japan Gastroenterological Endoscopy Society
www.jges.or.jp
Japanese Society of Gastroenterology
www.jsge.or.jp
Japan Society of Hepatology
www.jsh.or.jp
Japan Society of Nutrition and Food Science
www.jsnfs.or.jp/english
Japan Society for the Promotion of Science
www.jsps.go.jp/english/index.html
Johns Hopkins School of Public Health
www.jhsph.edu
Korean Association for the Study of the Liver
www.kasl.org
Korean Cancer Association
www.cancer.or.kr
Northeast ALS Consortium (NEALS)
www.alsconsortium.org
Nutrition and metabolism society
www.nmsociety.org
Nutrition Society
www.nutritionsociety.org
Society for inherited metabolic disorders
www.simd.org
SEBBM
www.sebbm.es/EN
Society for Nutrition Education and Behavior
www.sneb.org
Society for the study of inborn errors of metabolism
www.ssiem.org

This table lists the professional societies with interests in branched chain amino acids

Table 25.3 Journals that publish articles on branched chain amino acids

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration
informahealthcare.com/journal/afd
American Journal of Clinical Nutrition
ajcn.nutrition.org
American Journal of Gastrointestinal and Liver Physiology
ajpgi.physiology.org
American Journal of Physiol Endocrinol Metab
ajpendo.physiology.org
American Journal of Psychiatry
www.ajp.psychiatryonline.org/journal.aspx?journalid=13
American Journal of Respiratory and Critical Care Medicine
www.atsjournals.org/journal/ajrccm
Amino Acids
www.link.springer.com/journal/726
Biosci Biotechnol Biochem
www.jstage.jst.go.jp/browse/bbb
BioMed Central Cancer
www.biomedcentral.com/bmccancer
Cancer Cell
www.cell.com/cancer-cell
Cancer Immunology, Immunotherapy
www.springer.com/medicine/oncology/journal/262
Cancer Research
cancerres.aacrjournals.org
Cancer research and treatment
www.cancerresearchandtreatment.org
Cell Metabolism
www.cell.com/cell-metabolism
Circulation Journal
www.j-circ.or.jp/english/circulation_journal/circulation_journal.html
Clin Dev Immunol
www.hindawi.com/journals/cdi/2012/749189
Clinical Hemorheology and Microcirculation
www.iospress.nl/journal/clinical-hemorheology-and-microcirculation
Clinical and Molecular Hepatology
www.e-cmh.org
Clinical Nutrition
www.journals.elsevier.com/clinical-nutrition
Critical Care Medicine
journals.lww.com/ccmjournal
Diabetes
diabetes.diabetesjournals.org
European Heart Journal
eurheartj.oxfordjournals.org
European Respiratory Journal
erj.ersjournals.com
European Wound management (EMWA) Journal
ewma.org/english/publications/ewma-journal/latest-issues.html
Hepatology
onlinelibrary.wiley.com/journal/10.1002/%28ISSN%291527-3350
Hepatology Research

(continued)

Table 25.3 (continued)

www.jsh.or.jp
International Journal of Diabetes and Metabolism
ijod.uaeu.ac.ae
Journal of Biological Chemistry
www.jbc.org
Journal of Clinical Investigation
www.jci.org
Journal of hepato-biliary-pancreatic sciences
www.editorialmanager.com/jhbp
Journal of Hepatology
www.jhep-elsevier.com
Journal of Immunology
jimmunol.org
Journal of Infectious Diseases
jid.oxfordjournals.org
Journal of Inherited Metabolic Disease
www.springer.com/?SGWID=5-102-0-0-0
Journal of Neurochemistry
onlinelibrary.wiley.com/journal/10.1111/(ISSN)1471-4159
Journal of Neurotrauma
www.liebertpub.com/overview/journal-of-neurotrauma/39
Journal Nuclear Medicine
jnm.snmjournals.org
Journal of Nutrition
jn.nutrition.org
Journal of Nutrition and Metabolism
www.hindawi.com/journals/jnume/2011/617597
Journal of Nutritional Biochemistry
www.jnutbio.com
Journal of Nutritional Science and Vitaminology
editors.capj.or.jp/~jnsv_web/index.html
Journal of Parenteral and Enteral Nutrition
pen.sagepub.com
Journal of Physiology and Pharmacology
www.jpp.krakow.pl
Journal of Sports Sciences
www.tandfonline.com/loi/rjsp20
Medicine and science in sports and exercise
journals.lww.com/acsm-msse/pages/default.aspx
Mediterranean Journal of Nutrition and Metabolism
www.springer.com/food+science/journal/12349
Metabolic Brain Diseases
link.springer.com/journal/11011
Molecular Genetics and Metabolism
www.journals.elsevier.com/molecular-genetics-and-metabolism
Muscle and Nerve
onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4598
Nature Genetics
www.nature.com/ng/index.html
Neurotoxicology
www.journals.elsevier.com/neurotoxicology/CachedSimilar

(continued)

Table 25.3 (continued)

Nuclear Medicine and Biology
www.sciencedirect.com/science/journal/09698051
Nutrition
www.nutritionjrnl.com
Nutricion Hospitalaria
www.nutricionhospitalaria.com
Orphanet Journal of Rare Disease
www.ojrd.com
Proceedings of the National Academy of Sciences of the United States of America (PNAS)
www.pnas.org
Toxicological Sciences
www.bioxbio.com/if/html/TOXICOL-SCI.html

This table lists the journals publishing original research and review articles related to glutamine

Table 25.4 Relevant books

Berg JM, Tymoczko JL, Stryer L. Biochemistry, 5th edition. W H Freeman, 2002, USA
Chynober LA. Metabolic and therapeutic aspects of amino acids in clinical nutrition. CRC Press, 2004, USA
Hall JE. Guyton and Hall Textbook of Medical Physiology. Saunders, 2011, USA
Mahan LK, Escott-Stump S, Raymond JL. Krause's Food & the Nutrition Care Process. Saunders, 2011, USA
Mullen KD, Prakash RK. Hepatic Encephalopathy. Springer, 2010, USA
Okabayashi T, Kozuki A, Sumiyoshi T, Shima Y. Clinical benefits for liver regeneration of oral supplementation with carbohydrates together with branched chain amino acids. Advances in Medicine and Biology. Volume 63 Nova Science Publishers, Inc., USA; 2013
Okabayashi T, Shima Y. Management of early gastric carcinoma. Gastric carcinoma –new insights into current management-; Section 1; Chapter 2: p41-51 (University Campus STeP Ri, InTech Europe, Croatia, 2013
Tirapegui J. Nutrição, Metabolismo e Suplementação na Atividade Física (Nutrition, Metabolism and Supplementation on Physical Activity). Atheneu, 2012, Brazil
Tirapegui J. Nutrição - Fundamentos e Aspectos Atuais (Nutrition fundamentals and current aspects). Atheneu, 2006, Brazil.

This table lists books on branched chain amino acids

Table 25.5 Relevant internet resources

British Nutrition Foundation
www.nutrition.org.uk
Cardiogenetics
cardiogenomics.med.harvard.edu/home
Canadian Clinical Practice Guidelines
www.criticalcarenutrition.com
Chemistry of Amino Acids
home.nas.net/~dbc/cic_hamilton/amino.html
Complementary Medicine
www.umm.edu/altmed/articles/glutamine-000307.htm
Critical Care Nutrition at the Clinical Evaluation Research Unit, Kingston General Hospital, Canada
www.Criticalcarenutrition.com

(continued)

Table 25.5 (continued)

DISEASES
diseases.jensenlab.org
European portal for rare diseases and orphan drugs
www.orpha.net
Genetics home reference-MSUD
ghr.nlm.nih.gov/condition/maple-syrup-urine-disease
GenMANIA
www.genemania.org
Global Initiative for Chronic Obstructive Lung Disease (GOLD)
www.goldcopd.org
HighWire
highwire.stanford.edu
Human Protein Reference Database
www.hprd.org
Italian ALS Association
www.arisla.org
International Alliance of ALS/MND Associations
www.alsmndalliance.org
Mitomap
www.mitomap.org/MITOMAP
National Institutes of Health grants:
www.nih.gov
www.ncbi.nlm.nih.gov/pubmed
Strength and Fitness UK
www.strengthandfitnessuk.com/branched-chain-amino-acids
United States Olympic Committee
www.teamusa.org
United States ALS Association
www.alsa.org
UpToDate
www.uptodate.com/home

This table lists some internet resources on branched chain amino acids

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