Amino Acids (2012) 43:171–181
DOI 10.1007/s00726-011-1088-7

REVIEW ARTICLE

Insulin resistance and the metabolism of branched-chain amino acids in humans

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Received: 6 April 2011 / Accepted: 15 September 2011 / Published online: 8 October 2011
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Abstract Peripheral resistance to insulin action is the major mechanism causing the metabolic syndrome and eventually type 2 diabetes mellitus. The metabolic derangement associated with insulin resistance is extensive and not restricted to carbohydrates. The branched-chain amino acids (BCAAs) are particularly responsive to the inhibitory insulin action on amino acid release by skeletal muscle and their metabolism is profoundly altered in conditions featuring insulin resistance, insulin deficiency, or both. Obesity, the metabolic syndrome and diabetes mellitus display a gradual increase in the plasma concentration of BCAAs, from the obesity-related low-grade insulin-resistant state to the severe deficiency of insulin action in diabetes ketoacidosis. Obesity-associated hyperinsulinemia succeeds in maintaining near-normal or slightly elevated plasma concentration of BCAAs, despite the insulin-resistant state. The low circulating levels of insulin and/or the deeper insulin resistance occurring in diabetes mellitus are associated with more marked elevation in the plasma concentration of BCAAs. In diabetes ketoacidosis, the increase in plasma BCAAs is striking, returning to normal when adequate metabolic control is achieved. The metabolism of BCAAs is also disturbed in other situations typically featuring insulin resistance, including kidney and liver dysfunction. However, notwithstanding the insulin-resistant state, the plasma level of BCAAs in these conditions is lower than in healthy subjects, suggesting that these organs are involved in maintaining BCAAs blood concentration. The pathogenesis of the decreased BCAAs plasma level in kidney and liver dysfunction is unclear, but a decreased afflux of these amino acids into the blood stream has been observed.

Keywords Insulin resistance · Branched-chain amino acids · Leucine · Isoleucine · Valine

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Introduction

Obesity, the metabolic syndrome, and diabetes mellitus are presently frequent disorders affecting the Western civilization. The metabolic syndrome and diabetes mellitus are associated with increased cardiovascular risk and other devastating complications, including nephropathy and retinopathy. Obesity-associated resistance to the peripheral action of insulin is a major underlying mechanism causing the metabolic syndrome and ultimately type 2 diabetes. Metabolic disturbance associated with insulin resistance is widespread, involving not only carbohydrate and fat metabolism, but also protein metabolism. The branched-chain amino acids (BCAAs) are particularly sensitive to insulin action and their metabolism has been observed to be profoundly altered in insulin-resistant states.

Branched-chain amino acids metabolism

Leucine, isoleucine, and valine are referred to as BCAAs. The side chain in leucine and isoleucine is an isobutyl

group and the branching occurs on the γ- and on the β-carbon, respectively. Valine side chain is an isopropyl group branched on the β-carbon (Fig. 1).

The first step in the metabolism of the BCAAs is a reversible transamination reaction to their respective branched-chain α-keto acids (BCKAs), catalyzed by the enzyme branched-chain amino acid aminotransferase (BCAT). The reversibility of this step implies that the BCAAs may be either transformed into their cognate BCKAs or synthesized from them. The transamination reaction takes place with the partner pair α-ketoglutarate/glutamate. When the direction of the reaction is toward the formation of the BCKAs, α-ketoglutarate receives the amino group of the BCAAs and glutamate is formed. At the same time, L-leucine yields α-ketoisocaproate (KIC), L-isoleucine renders α-keto-β-methylglutarate (KIM), and L-valine produces α-ketoisovalerate (KIV). When the transamination reaction occurs in the opposite direction, the BCKAs receive the amino group of glutamate to generate BCAAs and α-ketoglutarate. Therefore, the BCAT reaction performs the reversible conversion between glutamate and BCAAs (Fig. 2). In the plasma of healthy subjects, the BCAAs are much more abundant than their corresponding keto acids. KIC and KIM are present in the blood in an amount which is approximately one-fifth (20%) of the circulating level of leucine and isoleucine, and the plasma level of KIV is only about 5% of valine plasma concentration. Valine is the most abundant plasma BCAA, whereas its keto acid is the least abundant BCKA in plasma (Schauder et al. 1984a, b)

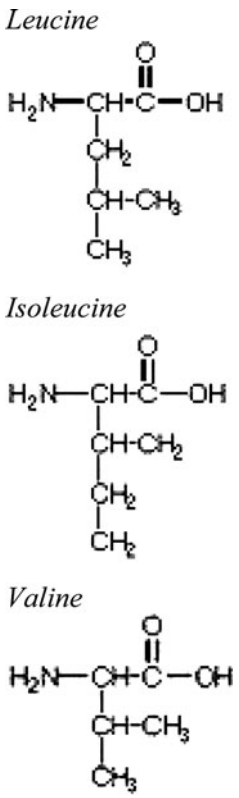

Fig. 1 The branched-chain amino acids

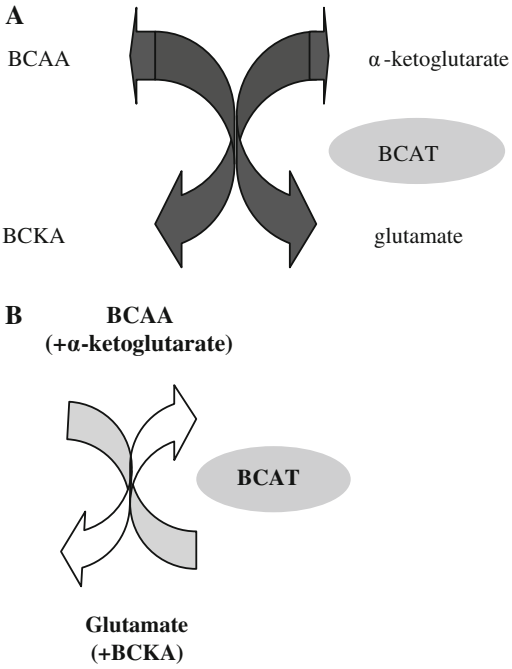

Fig. 2 The branched-chain amino transferase (BCAT) reaction

Two human genes, *BCT1* (localized to the short arm of chromosome 12) and *BCT2* (situated in chromosome 19) encode the expression of two molecular isoforms of BCAT, cytosolic (BCATc) and mitochondrial (BCATm), respectively (Naylor and Shows 1980). BCAT activity in human tissues is highest for kidney, followed by brain, stomach, and heart. A lesser and similar grade of activity is present in liver, small intestine, and colon while the lowest activity is showed by muscle and adipose tissue (Suryawan et al. 1998). BCAT activity has been also detected in human pancreas in a small study (Goto et al. 1977). In the tissues analyzed, the activity of the cytosolic BCAT isoenzyme in human beings is restricted to the brain (Suryawan et al. 1998)

The second step in the BCAAs catabolism is the oxidative decarboxylation of the BCKAs by the branched-chain keto acid dehydrogenase (BCKD) complex, an inner mitochondrial membrane enzyme. This enzymatic step is irreversible and commits the BCKAs to oxidation, producing ultimately NADH, CO_2, and different end-products depending on the BCKAs being oxidized. Leucine renders acetoacetate and acetyl-CoA, isoleucine yields propionyl-CoA and acetyl-CoA, and the end product of valine catabolism is propionyl-CoA. Propionyl-CoA formed as the result of isoleucine and valine catabolism is successively converted into methylmalonyl-CoA and succinyl-CoA (Fig. 3).

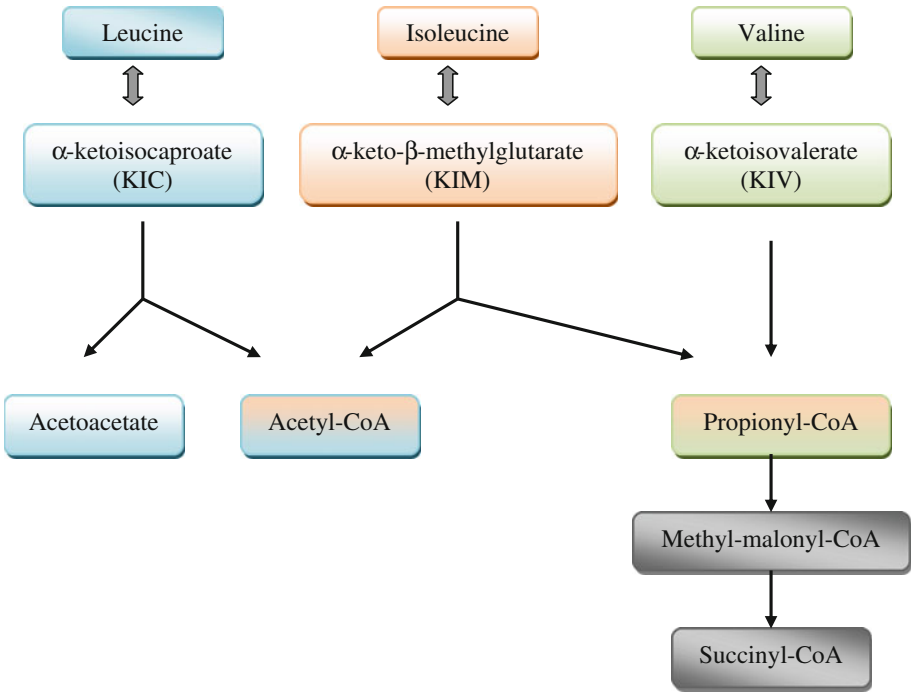

Fig. 3 End products of the BCAAs/BCKAs metabolism

The human BCKD complex possesses three subunits: a branched-chain α-ketoacid decarboxylase/dehydrogenase, a dihydrolipoyl transacylase, and a dihydrolipoamide dehydrogenase, which are called E1, E2, and E3, respectively. The E1 and E2 components are specific for the BCKD complex, whereas E3 is shared with other mitochondrial multienzyme complexes, the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex. BCKD complex framework is organized around the E2 component, to which E1 and E3 subunits are attached. BCKD kinase and BCKD phosphatase join the structure to complete the multiprotein complex (Chang et al. 2006). The activity of the BCKD complex is tightly regulated by phosphorylation and dephosphorylation at the Ser293 residue of E1, catalyzed by a specific kinase and phosphatase, respectively. Phosphorylation catalyzed by the specific kinase inhibits the enzymatic activity of the BCKD complex, whereas it becomes activated when the Ser293 residue is dephosphorylated by a specific phosphatase whose molecular identity has recently been established in cultured stable cell lines. In these cells, a mitochondrial matrix-targeted phosphatase, PP2Cm, specifically binds the BCKD complex and induces dephosphorylation of Ser293 in the presence of BCKD substrates. In the same cultured cells, the loss of PP2Cm completely abolishes substrate-induced E1 dephosphorylation. PP2Cm may be the human endogenous BCKD phosphatase required for substrate-mediated activation of the BCKD complex. At least five human single-nucleotide polymorphisms in the PP2Cm coding sequence have been listed in GenBank, suggesting that mutations leading to PP2Cm dysfunction might be responsible for some types of maple syrup urine disease (Lu et al. 2009).

The highest enzymatic activity of the human BCKD complex has been found in kidney, followed by liver, brain, and heart. Muscle, stomach, and colon had similar and lesser activity of the enzyme. Adipose tissue and small intestine had the lowest BCKD complex activity. The highest concentration of human BCKD kinase (responsible for the inactivation of the complex) has been found in skeletal muscle while kidney possesses the lowest activity state (Suryawan et al. 1998). Tissue distribution of the putative human BCKD complex phosphatase has not been reported.

As mentioned, BCAT and the BCKD complex are broadly expressed in human tissues, indicating wide tissue ability for BCAAs transamination and oxidation. Taking into account the organ weight as a proportion of body weight, the skeletal muscle has the highest quantitative capacity for both BCAAs transamination and oxidation (Suryawan et al. 1998). However, the BCKD kinase concentration is highest in muscle tissue, denoting the capacity of skeletal muscle to restrain the oxidation of BCKAs as well. Additionally, the BCAT has been reported to be more active than the BCKD complex in skeletal muscle, pointing out the high transamination capacity of this tissue (Matthews et al. 1981). Of note, while the kidney possesses the highest BCKD complex activity in qualitative terms, it holds the lowest ability to inhibit its enzymatic action, suggesting that this organ may be a major site where BCKAs are oxidized with little restraint. Peripheral human lymphocytes possess both BCAT and BCKD complex

activities, but the total capacity for leucine transamination is about five times greater than for oxidation of KIC (Schauder and Schäfer 1987). Adipose tissue contribution to BCAA metabolism in humans has been recently highlighted by the significant down-regulation of mitochondrial BCAA catabolism found in the adipose tissue of obese co-twins in a study performed on monozygotic twins discordant for obesity. Genes common to the degradation of all BCAAs including the BCATm and the BCKD E1 β-polypeptide are down-regulated in the obese co-twins. Specific genes for the catabolism of leucine and valine are also down-regulated in adipose tissue of obese compared to healthy co-twins (Pietilainen et al. 2008). These findings confirm a previous report revealing increased BCATm and BCKD complex enzymatic activity in omental and subcutaneous adipose tissue following surgical weight loss intervention (She et al. 2007).

Some factors are known to influence BCKD complex activity and BCAAs oxidation. In normal subjects, insulin suppresses leucine (KIC) oxidation and this effect is blunted in diabetes mellitus (Tessari et al. 1986; Keller et al. 2002). The administration of long-chain fatty acids, unlike medium-chain triglycerides, also decreases leucine oxidation compared with controls (Keller et al. 2002). In contrast, leucine oxidation is activated in healthy subjects by glucagon, (Pacy et al. 1990) glucocorticosteroid therapy (Zimmerman et al. 1989), and ammonium chloride-induced metabolic acidosis (Reaich et al. 1992; Straumann et al. 1992). Acute exercise induces activation of BCKD complex in human skeletal muscle, but after training the activity of this enzyme is reduced, along with an increase in BCKD kinase protein (Howarth et al. 2007). In a small randomized crossover study, the activity of BCAT and BCKD complex in skeletal muscle has been found similar in young and elderly healthy persons and there was no difference in the activity of these enzymes during short-term ingestion of a high-protein diet compared with usual protein dietary content (Walrand et al. 2008).

Branched-chain amino acids kinetics

Postprandial period

After the ingestion of an animal protein-rich meal, there is a striking rise in the plasma concentration of BCAAs, reaching approximately 200% of fasting values. Even though the BCAAs constitute only around 20% of the total amino acid content in meat, they represent the majority of the amino acids entering the systemic circulation following a meat meal and a disproportionate elevation of BCAA plasma concentration takes place in this situation (Aoki et al. 1973; Felig 1975; Aoki et al. 1976; Wahren et al. 1976; DeFronzo and Felig 1980; Elia and Livesey 1983). The dramatic rise in the systemic levels of BCAAs following a meat meal probably reflects transamination ability besides intestinal uptake. This notion is supported by studies demonstrating that enterally delivered KIC is predominantly converted to leucine by the splanchnic bed (Matthews et al. 1999). Further, only 12% of the enterally administered glutamate enters the systemic circulation and a transfer of nitrogen from glutamate to BCAAs, alanine, and proline has been detected in the splanchnic bed (Matthews et al. 1993). Also, in response to an animal protein-rich meal, blood levels of BCAAs increase significantly more than BCKAs in healthy volunteers (Schauder et al. 1984a, b)

During the postprandial period after the consumption of a meat meal by healthy individuals, the BCAAs are removed by the muscle tissue (Aoki et al. 1973; Felig 1975; Aoki et al. 1976; Wahren et al. 1976; DeFronzo and Felig 1980; Elia and Livesey 1983). Similarly, after the ingestion of L-leucine meals, leucine flux into muscle increases (Aoki et al. 1981). The excess amino nitrogen extracted by muscle from leucine is subsequently released mainly as glutamine and approximately 2 h after meat ingestion, release of glutamine from muscle is observed (Elia and Livesey 1983; Aoki et al. 1981).

Intravenous administration of leucine

Skeletal muscle is also the major organ extracting an intravenous load of leucine. In healthy humans in the postabsorptive state (after an overnight fast), the intravenous infusion of leucine results in a rise in leucine arterial concentration followed by an uptake mainly by muscle tissue (55%), although leucine is also extracted by the splanchnic region (25%) and by the brain (10%) (Hagenfeldt et al. 1980). As with the oral administration, glutamine is the major vehicle by which nitrogen leaves muscle tissue after an intravenous leucine load, and the large muscle uptake of leucine is followed by a muscle outflow and a rise in the blood concentration of glutamine in healthy individuals (Elia and Livesey 1983). Similarly, after an intravenous infusion of amino acids not containing glutamine, the skeletal muscle removes approximately 65–70% of BCAAs, followed by a significant increase of the leg efflux of glutamine in normal persons (Gelfand et al. 1986).

Postabsorptive state

In healthy subjects in the postabsorptive condition, there is a net release of amino acids from the skeletal muscle, with alanine and glutamine making the greatest contribution, whereas glutamate is released by splanchnic tissues and

extracted by skeletal muscle. A lesser amount of BCAAs is released from muscle (Felig 1975; Hagenfeldt et al. 1983). Alanine and glutamine are generated in excess of their content in muscle protein, accounting for 60–80% of the amino acids released from skeletal muscle in the postabsorptive condition, denoting that they mostly derive from "de novo" synthesis rather than from proteolysis. Deamination of BCAAs is the major source of nitrogen for the synthesis of alanine and glutamine in postabsorptive humans (Haymond and Miles 1982; Darmaun and Déchelotte 1991).

Branched-chain amino acid metabolism in insulin-resistant conditions

As the BCAAs are very responsive to the circulating level and cellular action of insulin, BCAA metabolism has been consistently observed altered in association with insulin deficiency or tissue resistance to the hormone action. Disturbances of BCAA metabolism have been described in obesity, diabetes mellitus, and other insulin-resistant states such as kidney and liver dysfunction.

In healthy subjects, the ingestion of a meat meal or the oral provision of leucine increase serum insulin levels, perhaps via L-leucine action as positive allosteric modulator of glutamate dehydrogenase (Stanley et al. 1998). In turn, insulin lowers glucose and amino acid content of plasma in the postprandial period facilitating their entry into peripheral tissues, including muscle. The decline in plasma amino acid concentration is most marked for the BCAAs, tyrosine, phenylalanine, and methionine (Felig 1975; Aoki et al. 1976, 1981; Elia and Livesey 1983). In the postabsorptive state, insulin inhibits muscle protein degradation and the rate of net proteolysis is inversely related to insulin level. The BCAAs are particularly responsive to the inhibitory action of insulin on amino acid release by muscle tissue (Pozefsky et al. 1969; Louard et al. 1992), and consequently only a small amount of BCAAs are liberated from muscle in the postabsorptive state (Felig 1975; Hagenfeldt et al. 1983). Similarly, exogenous insulin infusion to healthy postabsorptive individuals induces consistent declines in muscle release of amino acids, particularly BCAAs, tyrosine, phenylalanine, threonine, and glycine, while alanine release is not significantly affected (Felig 1975; Aoki et al. 1976; Elia and Livesey 1983; Hagenfeldt et al. 1983; Pozefsky et al. 1969). Exogenous insulin diminishes also blood concentration of BCKAs in the postabsorptive condition (Schauder et al. 1983).

Diabetes mellitus and the metabolism of BCAAs

Insulin deficiency and insulin resistance in uncontrolled diabetes mellitus results in the breakdown of muscle protein leading to muscle wasting while insulin treatment improves nitrogen balance. In diabetes mellitus there is a marked increase in the plasma concentration of BCAAs compared with healthy individuals, particularly in patients with deficient control of the disease (Elia and Livesey 1983; Felig et al. 1969a, b; Wahren et al. 1972; Berger et al. 1978; Vannini et al. 1982; Szabó et al. 1991; Manders et al. 2006). The striking elevation in the plasma level of BCAAs present during diabetic ketoacidosis promptly improves after initiation of insulin therapy and the adequate control of the disease results in normalization of the plasma BCAAs level (Vannini et al. 1982; Szabó et al. 1991). The 24-h urinary excretion of BCAAs is also elevated in diabetic patients before insulin treatment and normalizes after initiation of insulin (Sasaki et al. 1988; Szabó et al. 1991). Early starvation (within 36 h of fasting) in healthy subjects is also accompanied by elevated blood concentration of BCAAs and BCKAs, coinciding with a period of maximal fall in serum insulin (Adibi 1968; Felig et al. 1969a, b; Rudolph et al. 1981; Elia and Livesey 1983; Schauder et al. 1985).

Obesity and the metabolism of BCAAs

It has been long recognized that obesity is associated with resistance to insulin action on skeletal muscle and therefore with impaired glucose disposal. The majority of the studies demonstrate the existence of an insulin-resistant state pertaining to protein metabolism as well (Felig et al. 1969a, b; Forlani et al. 1984; Trevisan et al. 1986; Chevalier et al. 2005, 2006). In obese patients, a much greater increase in insulin secretion than in controls is required to produce a comparable fall in amino acid concentration, especially BCAAs, after glucose infusion (Felig et al. 1969a, b). During euglycemic insulin infusion, insulin reduces plasma BCAA levels less efficiently in obese subjects compared with controls, although no significant difference was observed in the basal BCAAs values (Forlani et al. 1984). During a hyperinsulinemic, euglycemic, isoaminoacidemic clamp, obese women required significantly less exogenous amino acid infusion to maintain BCAAs level than did the lean women (Chevalier et al. 2005). Additionally, gluconeogenesis is less suppressed and the fraction of gluconeogenesis from phosphoenolpyruvate is higher in obese than in lean subjects, while gluconeogenesis from glycerol is similar, indicating that the major substrates being used to the endogenous glucose production are amino acids and lactate rather than glycerol (Chevalier et al. 2006).

Accordingly with the occurrence of an insulin-resistant state, postabsorptive serum insulin levels in obesity have been consistently found elevated compared with healthy controls and insulin has been observed to be less effective in blocking the postabsorptive outflow of amino acids from

muscle, particularly the BCAAs, phenylalanine, and tyrosine (Felig et al. 1969a, b; 1974; Forlani et al. 1984; Caballero and Wurtman 1991; Solini et al. 1997; Chevalier et al. 2005; She et al. 2007). Consequently, the postabsorptive plasma concentration of BCAAs, phenylalanine, and tyrosine has been detected elevated in obese patients compared with nonobese individuals (Felig et al. 1969a, b; Caballero and Wurtman 1991; Solini et al. 1997; Chevalier et al. 2005, 2006; She et al. 2007; Newgard et al. 2009; Huffman et al. 2009). Recently, the elevated concentration of BCAAs in overweight and obese patients has been independently associated with insulin resistance and linearly related to the homeostasis model assessment (HOMA) index of insulin resistance (Newgard et al. 2009; Huffman et al. 2009). Additionally, weight loss results in a fall in serum insulin and a concomitant reduction in the concentration of BCAAs, phenylalanine, and tyrosine (She et al. 2007). Further association between obesity-related insulin resistance and BCAA metabolism is provided by the results of a study on monozygotic twins discordant for obesity disclosing a close correlation between insulin resistance and adipose tissue down-regulation of mitochondrial BCAA catabolism in the obese co-twins, who show significantly lower whole body insulin sensitivity and higher plasma insulin concentrations than their healthy co-twins. The liver accumulation of fat is also increased in the obese twins (Pietilainen et al. 2008).

Insulin-resistant state in healthy individuals and the metabolism of BCAAs

The results of recent investigations disclose a clinical association between BCAA metabolism and insulin resistance in apparently healthy persons. In a study aimed to investigate the correlates of insulin sensitivity in two groups of healthy individuals measuring 191 metabolites by mass spectrometry, multivariate analysis reveals that reduction in glycerol and BCAAs (leucine/isoleucine) together during the oral tolerance test provide the strongest predictor of insulin sensitivity. The decline in BCAAs plasma level is blunted in insulin-resistant subjects during the oral glucose tolerance test (Shaham et al. 2008). Further, in normal weight subjects, a definite association between increased blood levels of BCAAs and insulin resistance has been detected, suggesting that the increased concentration of BCAAs found in obese subjects is related to insulin resistance itself rather than obesity. Other amino acids elevated in persons with high HOMA value are phenylalanine, tyrosine, and methionine (Tai et al. 2010). A nested case–control study performed in the Framingham Offspring Study shows that the BCAAs and two aromatic amino acids (tyrosine and phenylalanine) have a highly significant association with future diabetes, displaying a predictive value for the development of new-onset diabetes up to 12 years after the baseline examination (Wang et al. 2011).

Chronic kidney disease and the metabolism of BCAAs

It has been long known that chronic kidney disease (CKD) is an insulin-resistant state (DeFronzo et al. 1981) and that metabolic acidosis induces insulin resistance to glucose disposal (DeFronzo and Beckles 1979). The insulin-resistant state induced by metabolic acidosis may be the cause of the increased muscle protein catabolism observed in this situation. Patients with CKD feature a protein catabolic state linked to the occurrence of metabolic acidosis. The insulin-induced inhibition of skeletal muscle proteolysis is blunted in patients with CKD and metabolic acidosis, but net muscle protein degradation is not increased in CKD patients provided that metabolic acidosis is corrected. In CKD patients, muscle net proteolysis has been reported to be inversely related to arterial bicarbonate levels (Tizianello et al. 1977; Garibotto et al. 1996; Boirie et al. 2000). In children with CKD, the leucine rate of appearance is markedly higher and the net leucine balance tends to be more negative when metabolic acidosis is present (Boirie et al. 2000). The increased muscle protein breakdown associated with metabolic acidosis is also present in hemodialysis patients who show greater BCAAs efflux from skeletal muscle during acidosis in an open crossover study (Löfberg et al. 2006). Correction of metabolic acidosis decreases protein catabolism in both CKD and dialysis patients (Löfberg et al. 2006; Graham et al. 1997).

Notwithstanding the protein catabolic state existing in CKD patients with metabolic acidosis, the postabsorptive plasma concentration of BCAAs is decreased in these patients and correction of metabolic acidosis induces an increment in the plasma concentration of BCAAs. The fasting plasma concentration of BCAAs, particularly valine, has been consistently detected lower in CKD patients than in healthy controls (DeFronzo and Felig 1980; Jones and Kopple 1978; Deferrari et al. 1981; Tizianello et al. 1982, 1983; Garibotto et al. 1993a, b, 1994a, b; Deferrari et al. 1985; Alvestrand et al. 1988; Fürst et al. 1992; Mak 1999; Canepa et al. 2002). The reduction in plasma BCAA level persists in hemodialysis patients (Mak 1999; Bergström et al. 1990; Riedel et al. 1992; Kooman et al. 1997; Małgorzewicz et al. 2008). In patients with CKD, fasting plasma valine concentration correlates with venous pH (Mak 1999). Prospective studies show that the correction of metabolic acidosis with sodium bicarbonate increases the fasting plasma concentration of BCAAs in hemodialysis patients, while there is no change following sodium chloride administration (Mak 1999; Kooman et al. 1997).

The muscle intracellular concentration of BCAAs, particularly valine, is also markedly reduced in CKD and hemodialysis patients compared with controls, and there is a significant positive correlation between the predialysis plasma bicarbonate level and the muscle valine concentration in hemodialysis patients (Fürst et al. 1992; Bergström et al. 1990). Furthermore, the correction of metabolic acidosis induces an increase in the muscle intracellular levels of BCAAs in hemodialysis patients (Löfberg et al. 1997). In uremic children, the red blood cell intracellular concentration of valine and isoleucine has been reported diminished compared to controls (Canepa et al. 2002).

The cause of the reduced plasma and intracellular concentration of BCAAs associated with the uremic state is elusive, but it has been shown that both the kidney and the skeletal muscle liberate smaller amounts of BCAAs into the circulation in uremic patients than in normal persons. In healthy individuals, the kidney releases into the circulation amounts of leucine approximately equal to one-third of the total leucine production. In CKD patients, the renal release of leucine per 100 ml of glomerular filtration rate (GFR) appears markedly increased in comparison with controls, striving to preserve the leucine release to the blood stream (Tizianello et al. 1980, 1983; Tessari et al. 1996; Garibotto et al. 1997). Muscle release of leucine and valine in the postabsorptive state is also significantly reduced in CKD patients compared with controls, being directly correlated with the GFR. Despite the presence of an insulin-resistant state, these amino acids are added to peripheral blood in lower amounts in CKD patients than in healthy subjects (Tizianello et al. 1983; Deferrari et al. 1985; Garibotto et al. 1992; Garibotto et al. 1994a, b). CKD and hemodialysis patients also display significantly lower plasma levels of BCKAs than healthy subjects in the postabsorptive state (Riedel et al. 1992; Schauder et al. 1980). A positive correlation has been observed between plasma KIC level and the GFR (Walser et al. 1989) and between arterial KIC concentration and arterial bicarbonate level (Garibotto et al. 1993a, b). In CKD patients, KIC is released in reduced amounts than in healthy subjects into the forearm vein (Garibotto et al. 1994a, b).

Liver failure and the metabolism of BCAAs

The occurrence of an insulin-resistant state involving both glucose and BCAAs metabolism has been described in liver disease. Basal plasma insulin levels are higher and the insulin effect on carbohydrate and BCAAs metabolism are reduced in cirrhotics compared with controls (Marchesini et al. 1983a, b). In patients with liver failure, the plasma concentration of BCAAs has been regularly reported lower compared with healthy subjects (Elia and Livesey 1983; Hagenfeldt et al. 1983; Marchesini et al. 1983; Plauth et al. 1990). The plasma level of BCKAs is also reduced (Schauder et al. 1984a, b). The decreased BCAA plasma concentration in cirrhosis may be at least in part related to diminished afflux of BCAAs into plasma. Following intravenous infusion of these amino acids to cirrhotic patients and controls, the endogenous basal appearance rate of BCAAs is lower in cirrhotics, whereas the plasma clearances of the three BCAAs were near normal despite hyperinsulinemia (Marchesini et al. 1987). The muscle intracellular concentration of BCAAs in cirrhosis has been reported altered heterogeneously, with a reduction of valine and isoleucine, whereas the intracellular concentration of leucine remains similar to controls (Plauth et al. 1990).

In contrast to obesity and diabetes and in spite of being insulin-resistant states, both liver and kidney dysfunction show low plasma concentration of BCAAs, suggesting that these organs are involved in maintaining circulating levels of BCAAs. In both disorders, the whole body afflux of the BCAAs into the bloodstream is lower than in normal individuals. In CKD the intracellular muscle concentration of BCAAs is also lower than in healthy persons, whereas in liver failure, reduction in the muscle concentration of valine and isoleucine has been reported.

L-leucine and m-TOR

The intracellular biochemical pathways and gene transcription modifications beyond the insulin action upon the metabolism of BCAAs are not well defined. Oral administration of L-leucine enhances insulin secretion. In turn, insulin promotes muscle uptake of amino acids in the postprandial period and prevents amino acid release from muscle during the postabsorptive period. After meals, the afflux of amino acids and the effect of insulin activate an anabolic response and protein synthesis is enhanced. The cellular mechanisms involved in the regulation of protein synthesis in human muscle are mostly unknown. Both leucine and insulin seem to activate different signaling pathways that converge at the mammalian target of rapamycin (mTOR), which is a serine threonine kinase involved in protein synthesis, among other cellular processes. mTOR may be integrated in at least two multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is characterized by the presence of a regulatory protein called RAPTOR (regulatory-associated protein of mTOR), whereas mTORC2 possesses RICTOR (rapamycin-insensitive companion of TOR). While mTORC1 is sensitive to rapamycin, mTORC2 displays a limited inhibition by this drug. Furthermore, the two complexes differ in their primary cellular function: activation of mTORC1 stimulates protein synthesis and cell growth, whereas mTORC2 is involved in cytoskeleton organization and cellular

Table 1 mTORC1 and mTORC2

	mTORC1	*mTORC2*
Associated regulatory protein	Raptor	Rictor
Rapamycin sensitivity	Rapamycin-sensitive	Limited inhibition
Cellular function	Protein synthesis and cell growth	Cytoskeleton organization and cell proliferation

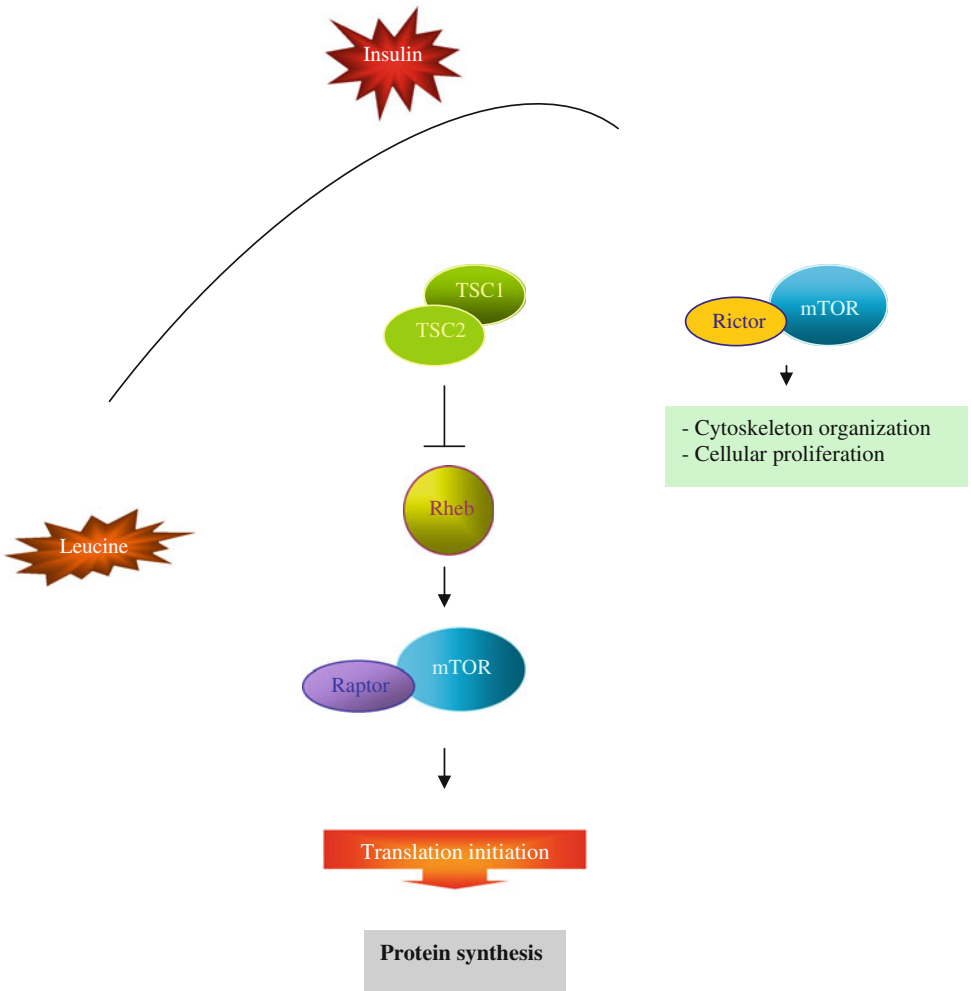

Fig. 4 Simplified mTOR signaling pathway

proliferation (Table 1). The signaling pathway through mTOR in humans and its interaction with other cellular communication channels to regulate protein synthesis and other cell functions is not fully understood. Activation of mTORC1 is mediated by a small GTPase called Rheb (Ras homolog enriched in brain), which in turn is controlled by TSC2 (tuberin) and its binding partner, TSC1 (hamartin). The TSC1-TSC2 complex acts upon Rheb promoting the conversion of Rheb-GTP to Rheb-GDP and consequently induces Rheb inactivation which in turn represses mTORC1 activity. Signaling through mTORC1 is enhanced by insulin and leucine at least in part through repression of the activity of the TSC1-TSC2 complex and consequent inhibition of the GTPase activity of Rheb. Upon activation, mTORC1 phosphorylates downstream components ultimately activating translation initiation (Fig. 4). mTORC1 signaling is also increased by exercise in humans through an incompletely determined mechanism (Dickinson and Rasmussen 2010).

In summary, the BCAAs are particularly sensitive to the effects of insulin and therefore their metabolism is affected in clinical conditions featuring insufficient insulin action related either to insulin deficiency or to cellular resistance to the hormone effect. Elevated BCAA plasma concentration and a blunted response to insulin have typically been reported in obesity and diabetes mellitus. Recent studies reveal similar metabolic alterations in healthy subjects in

whom insulin resistance is demonstrated and emerging evidence shows that elevated plasma levels of BCAA may occur years before than diabetes mellitus is diagnosed, suggesting a significant predictive value. BCAA metabolism and plasma concentration are surfacing as reliable and useful markers of insulin action in healthy individuals, obesity, and diabetes mellitus. Disturbance of BCAA metabolism in other insulin-resistant conditions such as kidney and liver dysfunction and its relationship with metabolic acidosis are not well defined.

Acknowledgments There was no financial support for this work.

Conflict of interest None.

References

Adibi SA (1968) Influence of dietary deprivations on plasma concentration of free amino acids of man. J Appl Physiol 25:52–57

Alvestrand A, Defronzo RA, Smith D et al (1988) Influence of hyperinsulinaemia on intracellular amino acid levels and amino acid exchange across splanchnic and leg tissues in uraemia. Clin Sci (Lond) 74:155–163

Aoki TT, Muller WA, Brennan MF et al (1973) Blood cell and plasma amino acid levels across forearm muscle during a protein meal. Diabetes 22:768–775

Aoki TT, Brennan MF, Müller WA et al (1976) Amino acid levels across normal forearm muscle and splanchnic bed after a protein meal. Am J Clin Nutr 29:340–350

Aoki TT, Brennan MF, Fitzpatrick GF et al (1981) Leucine meal increases glutamine and total nitrogen release from forearm muscle. J Clin Invest 68:1522–1528

Berger M, Zimmermann-Telschow H, Berchtold P et al (1978) Blood amine acid levels in patients with insulin excess (functioning insulinoma) and insulin deficiency (diabetic ketosis). Metabolism 27:793–799

Bergström J, Alvestrand A, Fürst P (1990) Plasma and muscle free amino acids in maintenance hemodialysis patients without protein malnutrition. Kidney Int 38:108–114

Boirie Y, Broyer M, Gagnadoux MF et al (2000) Alterations of protein metabolism by metabolic acidosis in children with chronic renal failure. Kidney Int 58:236–241

Caballero B, Wurtman RJ (1991) Differential effects of insulin resistance on leucine and glucose kinetics in obesity. Metabolism 40:51–58

Canepa A, Filho JC, Gutierrez A et al (2002) 9 Free amino acids in plasma, red blood cells, polymorphonuclear leukocytes, and muscle in normal and uraemic children. Nephrol Dial Transplant 17:413–421

Chang CF, Chou HT, Lin YJ et al (2006) Structure of the subunit binding domain and dynamics of the di-domain region from the core of human branched chain α-ketoacid dehydrogenase complex. J Biol Chem 281:28345–28353

Chevalier S, Marliss EB, Morais JA et al (2005) 9 Whole-body protein anabolic response is resistant to the action of insulin in obese women. Am J Clin Nutr 82:355–365

Chevalier S, Burgess SC, Malloy CR et al (2006) The greater contribution of gluconeogenesis to glucose production in obesity is related to increased whole-body protein catabolism. Diabetes 55:675–681

Darmaun D, Déchelotte P (1991) Role of leucine as a precursor of glutamine alpha-amino nitrogen in vivo in humans. Am J Physiol 260(2 Pt 1):E326–E329

Deferrari G, Garibotto G, Robaudo C et al (1981) Brain metabolism of amino acids and ammonia in patients with chronic renal insufficiency. Kidney Int 20:505–510

Deferrari G, Garibotto G, Robaudo C et al (1985) Leg metabolism of amino acids and ammonia in patients with chronic renal failure. Clin Sci (Lond) 69:143–151

DeFronzo RA, Beckles AD (1979) Glucose intolerance following chronic metabolic acidosis in man. Am J Physiol 236:E328–334

DeFronzo RA, Felig P (1980) Amino acid metabolism in uremia: insights gained from normal and diabetic man. Am J Clin Nutr 33:1378–1386

DeFronzo RA, Alvestrand A, Smith D et al (1981) Insulin resistance in uremia. J Clin Invest 67:563–568

Dickinson JM, Rasmussen BB (2010) Essential amino acid sensing, signaling, and transport in the regulation of human muscle protein metabolism. Curr Opin Clin Nutr Metab Care 14:83–88

Elia M, Livesey G (1983) Effects of ingested steak and infused leucine on forelimb metabolism in man and the fate of the carbon skeletons and amino groups of branched-chain amino acids. Clin Sci (Lond) 64:517–526

Felig P (1975) Amino acid metabolism in man. Annu Rev Biochem 44:933–955

Felig P, Marliss E, Cahill GF Jr (1969a) Plasma amino acid levels and insulin secretion in obesity. N Engl J Med 281:811–816

Felig P, Owen OE, Wahren J et al (1969b) Amino acid metabolism during prolonged starvation. J Clin Invest 48:584–594

Felig P, Wahren J, Hendler R et al (1974) Splanchnic glucose and amino acid metabolism in obesity. J Clin Invest 53:582–590

Forlani G, Vannini P, Marchesini G et al (1984) Insulin-dependent metabolism of branched-chain amino acids in obesity. Metabolism 33:147–150

Fürst P, Alvestrand A, Bergström J (1992) Branched-chain amino acids and branched-chain ketoacids in uremia. Contrib Nephrol 98:44–58

Garibotto G, Russo R, Sala MR et al (1992) Muscle protein turnover and amino acid metabolism in patients with chronic renal failure. Miner Electrolyte Metab 18(2–5):217–221

Garibotto G, Paoletti E, Fiorini F et al (1993a) Peripheral metabolism of branched-chain keto acids in patients with chronic renal failure. Miner Electrolyte Metab 19:25–31

Garibotto G, Paoletti E, Fiorini F et al (1993b) Peripheral metabolism of branched-chain keto acids in patients with chronic renal failure. Miner Electrolyte Metab 19:25–31

Garibotto G, Russo R, Sofia A et al (1994a) Skeletal muscle protein synthesis and degradation in patients with chronic renal failure. Kidney Int 45:1432–1439

Garibotto G, Russo R, Sofia A et al (1994b) Skeletal muscle protein synthesis and degradation in patients with chronic renal failure. Kidney Int 45:1432–1439

Garibotto G, Russo R, Sofia A et al (1996) Muscle protein turnover in chronic renal failure patients with metabolic acidosis or normal acid-base balance. Miner Electrolyte Metab 22(1–3):58–61

Garibotto G, Tessari P, Robaudo C et al (1997) Leucine metabolism and protein dynamics in the human kidney. Contrib Nephrol 121:143–148

Gelfand RA, Glickman MG, Jacob R et al (1986) Removal of infused amino acids by splanchnic and leg tissues in humans. Am J Physiol 250(4 Pt 1):E407–E413

Goto M, Shinno H, Ichihara A (1977) Isozyme patterns of branched-chain amino acid transaminase in human tissues and tumors. Gann 68:663–667

Graham KA, Reaich D, Channon SM et al (1997) Correction of acidosis in hemodialysis decreases whole-body protein degradation. J Am Soc Nephrol 8:632–637

Hagenfeldt L, Eriksson S, Wahren J (1980) Influence of leucine on arterial concentrations and regional exchange of amino acids in healthy subjects. Clin Sci (Lond) 59:173–181

Hagenfeldt L, Eriksson LS, Wahren J (1983) Amino acids in liver disease. Proc Nutr Soc 42:497–506

Haymond MW, Miles JM (1982) Branched chain amino acids as a major source of alanine nitrogen in man. Diabetes 31:86–89

Howarth KR, Burgomaster KA, Phillips SM et al (2007) Exercise training increases branched-chain oxoacid dehydrogenase kinase content in human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 293:R1335–R1341

Huffman KM, Shah SH, Stevens RD et al (2009) Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 32:1678–1683

Jones MR, Kopple JD (1978) Valine metabolism in normal and chronically uremic man. Am J Clin Nutr 31:1660–1664

Keller U, Turkalj I, Laager R et al (2002) Effects of medium- and long-chain fatty acids on whole body leucine and glucose kinetics in man. Metabolism 51:754–760

Kooman JP, Deutz NE, Zijlmans P et al (1997) The influence of bicarbonate supplementation on plasma levels of branched-chain amino acids in haemodialysis patients with metabolic acidosis. Nephrol Dial Transplant 12:2397–2401

Löfberg E, Wernerman J, Anderstam B et al (1997) Correction of acidosis in dialysis patients increases branched-chain and total essential amino acid levels in muscle. Clin Nephrol 48:230–237

Löfberg E, Gutierrez A, Anderstam B et al (2006) Effect of bicarbonate on muscle protein in patients receiving hemodialysis. Am J Kidney Dis 48:419–429

Louard RJ, Fryburg DA, Gelfand RA et al (1992) Insulin sensitivity of protein and glucose metabolism in human forearm skeletal muscle. J Clin Invest 90:2348–2354

Lu G, Sun H, She P et al (2009) Protein phosphatase 2Cm is a critical regulator of branched-chain amino acid catabolism in mice and cultured cells. J Clin Invest 119:1678–1687

Mak RH (1999) Effect of metabolic acidosis on branched-chain amino acids in uremia. Pediatr Nephrol 13:319–322

Małgorzewicz S, Debska-Slizień A, Rutkowski B et al (2008) Serum concentration of amino acids versus nutritional status in hemodialysis patients. J Ren Nutr 18:239–247

Manders RJ, Koopman R, Sluijsmans WE et al (2006) Co-ingestion of a protein hydrolysate with or without additional leucine effectively reduces postprandial blood glucose excursions in type 2 diabetic men. J Nutr 136:1294–1299

Marchesini G, Bianchi G, Zoli M et al (1983a) Plasma amino acid response to protein ingestion in patients with liver cirrhosis. Gastroenterology 85:283–290

Marchesini G, Forlani G, Zoli M et al (1983b) Effect of euglycemic insulin infusion on plasma levels of branched-chain amino acids in cirrhosis. Hepatology 3:184–187

Marchesini G, Bianchi GP, Vilstrup H et al (1987) Plasma clearances of branched-chain amino acids in control subjects and in patients with cirrhosis. J Hepatol 4:108–117

Matthews DE, Bier DM, Rennie MJ et al (1981) Regulation of leucine metabolism in man: a stable isotope study. Science 214:1129–1131

Matthews DE, Marano MA, Campbell RG (1993) Splanchnic bed utilization of glutamine and glutamic acid in humans. Am J Physiol 264(6 Pt 1):E848–E854

Matthews DE, Harkin R, Battezzati A et al (1999) Splanchnic bed utilization of enteral alpha-ketoisocaproate in humans. Metabolism 48:1555–1563

Naylor SL, Shows TB (1980) Branched-chain aminotransferase deficiency in Chinese hamster cells complemented by two independent genes on human chromosomes 12 and 19. Somatic Cell Genet 6:641–652

Newgard CB, An J, Bain JR et al (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9:311–326

Pacy PJ, Cheng KN, Ford GC et al (1990) Influence of glucagon on protein and leucine metabolism: a study in fasting man with induced insulin resistance. Br J Surg 77:791–794

Pietilainen KH, Naukkarinen J, Rissanen A et al (2008) Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. PLoS Med 5:e51

Plauth M, Egberts EH, Abele R et al (1990) Characteristic pattern of free amino acids in plasma and skeletal muscle in stable hepatic cirrhosis. Hepatogastroenterology 37:135–139

Pozefsky T, Felig P, Tobin JD et al (1969) Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. J Clin Invest 48:2273–2282

Reaich D, Channon SM, Scrimgeour CM et al (1992) Ammonium chloride-induced acidosis increases protein breakdown and amino acid oxidation in humans. Am J Physiol 263(4 Pt 1):E735–E739

Riedel E, Hampl H, Nündel M et al (1992) Essential branched-chain amino acids and alpha-ketoanalogues in haemodialysis patients. Nephrol Dial Transplant 7:117–120

Rudolph HJ, Gerbitz KD, Michal G et al (1981) Enzymic determination of branched-chain amino acids. Clin Chem 27:431–433

Sasaki M, Sato K, Maruhama Y (1988) Rapid changes in urinary serine and branched-chain amino acid excretion among diabetic patients during insulin treatment. Diabetes Res Clin Pract 5:219–224

Schauder P, Schäfer G (1987) Oxidation of leucine in human lymphocytes. Scand J Clin Lab Invest 47:447–453

Schauder P, Matthaei D, Henning HV et al (1980) Blood levels of branched-chain amino acids and alpha-ketoacids in uremic patients given keto analogues of essential amino acids. Am J Clin Nutr 33:1660–1666

Schauder P, Schröder K, Matthaei D et al (1983) Influence of insulin on blood levels of branched chain keto and amino acids in man. Metabolism 32:323–327

Schauder P, Schröder K, Langenbeck U (1984a) Serum branched-chain amino and keto acid response to a protein-rich meal in man. Ann Nutr Metab 28:350–356

Schauder P, Schröder K, Herbertz L et al (1984b) Evidence for valine intolerance in patients with cirrhosis. Hepatology 4:667–670

Schauder P, Herbertz L, Langenbeck U (1985) Serum branched chain amino and keto acid response to fasting in humans. Metabolism 34:58–61

Shaham O, Weil R, Wang TJ et al (2008) Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol 214:1–9

She P, Van Horn C, Reid T et al (2007) Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am J Physiol Endocrinol Metab 293:E1552–E1563

Solini A, Bonora E, Bonadonna R et al (1997) Protein metabolism in human obesity: relationship with glucose and lipid metabolism and with visceral adipose tissue. J Clin Endocrinol Metab 82:2552–2558

Stanley CA, Lieu YK, Hsu BY et al (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. N Engl J Med 338:1352–1357

Straumann E, Keller U, Küry D et al (1992) Effect of acute acidosis and alkalosis on leucine kinetics in man. Clin Physiol 12:39–51

Suryawan A, Hawes JW, Harris RA et al (1998) A molecular model of human branched-chain amino acid metabolism. Am J Clin Nutr 68:72–81

Szabó A, Kenesei E, Körner A et al (1991) Changes in plasma and urinary amino acid levels during diabetic ketoacidosis in children. Diabetes Res Clin Pract 12:91–97

Tai ES, Tan ML, Stevens RD et al (2010) Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia 53:757–767

Tessari P, Nosadini R, Trevisan R et al (1986) Defective suppression by insulin of leucine-carbon appearance and oxidation in type 1, insulin-dependent diabetes mellitus. Evidence for insulin resistance involving glucose and amino acid metabolism. J Clin Invest 77:1797–1804

Tessari P, Garibotto G, Inchiostro S et al. (1996) Kidney, splanchnic, and leg protein turnover in humans. Insight from leucine and phenylalanine kinetics. J Clin Invest 15(98):1481–1492

Tizianello A, De Ferrari G, Gurreri G et al (1977) Effects of metabolic alkalosis, metabolic acidosis and uraemia on whole-body intracellular pH in man. Clin Sci Mol Med 52:125–135

Tizianello A, De Ferrari G, Garibotto G et al (1980) Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. J Clin Invest 65:1162–1173

Tizianello A, Deferrari G, Garibotto G et al (1982) Renal ammoniagenesis in an early stage of metabolic acidosis in man. J Clin Invest 69:240–250

Tizianello A, Deferrari G, Garibotto G et al (1983) Branched-chain amino acid metabolism in chronic renal failure. Kidney Int Suppl 16:S17–S22

Trevisan R, Nosadini R, Avogaro A et al (1986) Type I diabetes is characterized by insulin resistance not only with regard to glucose, but also to lipid and amino acid metabolism. J Clin Endocrinol Metab 62:1155–1162

Vannini P, Marchesini G, Forlani G et al (1982) Branched-chain amino acids and alanine as indices of the metabolic control in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic patients. Diabetologia 22:217–219

Wahren J, Felig P, Cerasi E et al (1972) Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. J Clin Invest 51:1870–1878

Wahren J, Felig P, Hagenfeldt L (1976) Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. J Clin Invest 57:987–999

Walrand S, Short KR, Bigelow ML et al (2008) Functional impact of high protein intake on healthy elderly people. Am J Physiol Endocrinol Metab 295:E921–E928

Walser M, Jarskog FL, Hill SB (1989) Branched-chain-ketoacid metabolism in patients with chronic renal failure. Am J Clin Nutr 50:807–813

Wang TJ, Larson MG, Vasan RS et al (2011) Metabolite profiles and the risk of developing diabetes. Nat Med 17:448–453

Zimmerman T, Horber F, Rodriguez N et al (1989) Contribution of insulin resistance to catabolic effect of prednisone on leucine metabolism in humans. Diabetes 38:1238–1244