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1 Review

# 2 Branched-chain amino acids and insulin resistance, from pro- 3 tein supply to diet-induced obesity

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9 **Abstract:** For more than a decade, there has been a wide debate about the branched-chain amino  
10 acids (BCAA): leucine, valine, isoleucine, with on the one hand the supporters of their anabolic ef-  
11 fects and on the other hand those who suspect them of promoting insulin resistance. Indeed, the  
12 role of leucine in the postprandial activation of protein synthesis has been clearly established, even  
13 though supplementation studies aimed at taking advantage of this property are rather disap-  
14 pointing. Besides, there is ample evidence of an association between the elevation of their plasma  
15 concentrations and insulin resistance or the risk of developing type 2 diabetes; although, there are  
16 many confounding factors, starting with the level of animal protein consumption. After a summary  
17 of their metabolism and anabolic properties, we analyze in this review the factors likely to increase  
18 the plasma concentrations of BCAAs including insulin-resistance. After an analysis of supple-  
19 mentation or restriction studies in search of a direct role of BCAAs in insulin resistance, we discuss  
20 an indirect role through some of their metabolites: branched-chain keto acids, C3 and C5  
21 acylcarnitine, and hydroxyisobutyrate. Overall, given the importance of insulin in the metabolism  
22 of these amino acids, it is very likely that small alterations in insulin sensitivity are responsible for a  
23 reduction in their catabolism long before the onset of impaired glucose tolerance.

24 **Keywords:** leucine; valine; isoleucine; type 2 diabetes; glucose tolerance; protein consumption;  
25 branched-chain keto acids; acylcarnitine; hydroxyisobutyrate.

26

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27 **Abbreviations** : BAT: brown adipose tissue; BCAAs: branched-chain amino acids; BCAT: BCAA  
28 aminotransferase/transaminase; BCKAs: branched-chain keto acids; BCKD: BCKA dehydrogenase;  
29 BDK: BCKD kinase; BAIBA: beta-amino-isobutyric acid; BT2: BDK inhibitor  
30 3,6-dichlorobenzo(b)thiophene-2-carboxylic acid; DIO: diet-induced obesity; 4E-BP1:  
31 eIF4E-binding protein 1; HF: high fat; HFHS: high-fat-high-sucrose; HIB: hydroxyisobutyrate;  
32 HMB: beta-hydroxy-beta-methylbutyrate; HOMA-IR: homeostasis model assessment-insulin  
33 resistance index ; IRS-1: insulin receptor substrate 1; KIC:  $\alpha$ -ketoisocaproate,  
34 KIV $\alpha$ -ketoisovalerate; KMV:  $\alpha$ -ketomethylvalerate; mTORC1: mechanistic Target Of  
35 Rapamycin Complex 1; NAFLD : nonalcoholic fatty liver disease; p70S6K1: 70-kDa ribosomal pro-  
36 tein S6 kinase; PP2Cm/PPM1K: BCKD phosphatase (mitochondrial matrix-targeted protein phos-  
37 phatase 2C/protein phosphatase Mg<sup>2+</sup>/Mn<sup>2+</sup>-dependent 1K); PGC-1 $\alpha$ : peroxisome prolifera-  
38 tor-activated receptor-gamma coactivator 1 $\alpha$ ; T2D: type 2 diabetes; WAT: white adipose tissue

## 1. Introduction

Branched chain amino acids (BCAAs: leucine, valine, isoleucine) are under close scrutiny since the beginning of the 70's because of their unique role in endogenous metabolism and their critical regulatory properties on protein metabolism. A notable property of this metabolism is its compartmentalization; in humans, at mealtime, while more

44 than 50% of ingested amino acids are taken up by the liver, BCAAs essentially escape  
45 from the splanchnic territory and directly contribute to the postprandial activation of  
46 peripheral/muscle protein synthesis. It was initially thought that the primary role of these  
47 amino acids was restricted to the anabolic or anticatabolic properties of leucine. Indeed,  
48 this rapidly led in the clinical setting, to the development of BCAA-enriched nutritional  
49 formulas 1) for the dietary management of cirrhotic patients (and hypothetically to treat  
50 hepatic encephalopathy, a neurological disorder due to a severe chronic liver disease) (1)  
51 or 2) for the stimulation of anabolism or slowdown of catabolism in injured patients (2).  
52 Already at that time, there were some hints of possible interactions between BCAAs and  
53 insulin signaling. However, it is only around the years 2005-2010 that several studies  
54 suggested, on the one hand, a possible association between type 2 diabetes (T2D) and  
55 increased plasma BCAA levels and, on the other hand, a possible causal link between  
56 BCAAs and insulin resistance. This review is the result of a long-term scientific vigil on  
57 the keywords: branched-chain amino acid OR leucine OR valine OR isoleucine AND  
58 insulin resistance.

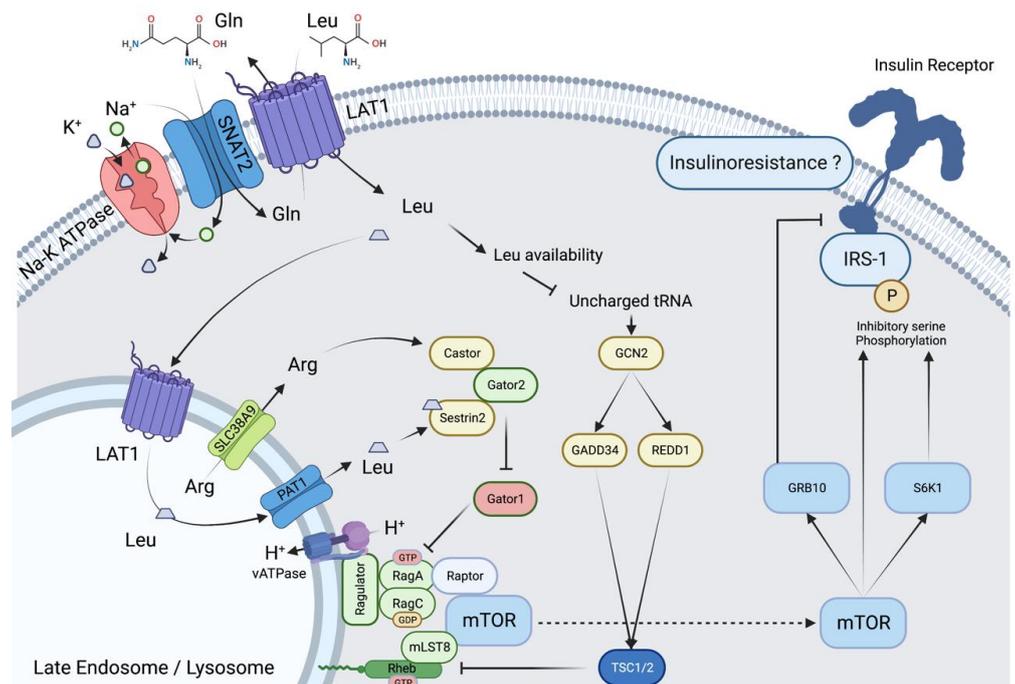
59 In the present article, we will first review the metabolism and properties of BCAAs.  
60 We will then analyze the factors involved in the plasma variations of these amino acids  
61 and in particular their interest as markers of insulin resistance. We will then focus on the  
62 interrelationship between insulin resistance and BCAAs first focusing on its conse-  
63 quences on BCAA metabolism before analyzing the novel properties of BCAAs and their  
64 interactions with insulin effects. We will attempt to elucidate the complex physiological  
65 and species-dependent mechanisms that govern these interactions.

## 66 2. BCAA Metabolism

### 67 2.1. BCAA inter-organ exchanges in the postprandial period

68 From the physiologist's point of view, and leaving aside the digestion and digestive  
69 absorption stages, the metabolism of amino acids and more particularly of essential  
70 amino acids such as BCAAs, starts with inter-organs exchanges in the postprandial pe-  
71 riod. Ten Have et al (3) measured net BCAA and metabolite fluxes across liver, portal  
72 drained viscera, kidney, and hindquarter in a multi-catheterized pig model receiving a  
73 test meal (30% of the daily intake as a bolus). In the postprandial phase, BCAAs were  
74 significantly metabolized by the portal-drained viscera (on average, 57% of leucine, 50%  
75 of isoleucine and 60% of valine), producing through the action of transaminases  
76 branched-chain keto acids (BCKAs) which were then used by the liver. They further ob-  
77 served that BCAAs escaping splanchnic metabolism then underwent an uptake essen-  
78 tially by the hindquarter and the kidneys. One thing to keep in mind about this muscle  
79 peripheral utilization is that it depends in part on the insulin-dependent increase in blood  
80 flow that will condition the peripheral availability of substrates. Also, the relative flux of  
81 BCAAs in liver vs muscle is species dependent. It is important to note that BCAA me-  
82 tabolism significantly differ quantitatively between rodents and humans: in humans,  
83 transaminative and oxidative capacities are higher (66% of total capacities) in the skeletal  
84 muscles. In rats, oxidation is mainly hepatic (60%) and total body oxidative capacity is  
85 nearly 10-fold higher compared to humans (4).

86 The interorgan compartmentation of BCAA metabolism is such that, after a protein  
87 meal, BCAAs represent a major part of the amino acids released by the splanchnic bed.  
88 Indeed, Wahren et al (5) showed in healthy volunteers that, after an oral protein load (3  
89 g/kg), BCAAs accounted for more than half of the amino acids released by the splanchnic  
90 area at 60 min after the protein meal, and were the only amino acids consistently released  
91 at 120-180 min. This was associated with a respective 2.5, 1.9 and 3.3 increase in plasma  
92 leucine, valine, and isoleucine levels.



**Figure 1.** Activation of mTORC1 by leucine in muscle cells.

Activation of mTORC1 in muscle cells involves i) counter-transport in exchange with glutamine and transport into endosomes, localized near the cell membrane, via LAT1, ii) increased leucyl-tRNA charging inhibiting the GCN2 pathway, iii) leucine efflux from the endosome via PAT1 involving inward proton transport via vATPase, iv) interaction of leucine with the protein sestrin2, lifting the inhibitory effect of the GATOR1/2 complex on RAG-GTPases and allowing v) localization of mTORC1 to the endosome and its activating interaction with Rheb.

## 2.2. BCAA cellular metabolism

### 2.2.1. BCAA transport

At the cellular level, different transporters are involved in the distribution and subsequently in the metabolism of BCAAs, with specificities differentiating epithelial and non-epithelial cells. At the subcellular level, there are also differences in transport throughout the different compartments. We will focus here on the heterodimeric transporters associating LAT1 (SLC7A5) or LAT2 (SLC7A8) and the glycoprotein CD98/4F2hc (SLC3A2) involved among others in the uptake of BCAAs in muscle and brain. LAT1 and LAT2 are antiporters that function through an exchange with glutamine. In the skeletal muscle (Figure 1), BCAA transport occurs in a tertiary mode: sodium-dependent uptake of glutamine via the SNAT1/2 transporters provides the glutamine necessary for the function of LAT1 and 2. This is of particular importance when considering the initiating role of glutamine in the activation by leucine of the mechanistic Target Of Rapamycin (mTOR) Complex 1 (mTORC1), the main effector of postprandial protein synthesis (6,7). Note that transport via LAT1 and SNAT2 is influenced by insulin via different mechanisms such as increased protein expression (LAT1, SNAT2) or membrane translocation to the plasma membrane of intracellular storage vesicles (SNAT2) from a subcellular localization similar to that of the glucose transporter GLUT4 (8).

### 2.2.2. BCAA catabolism

Once imported into the cells, BCAAs can be used for protein synthesis. However, leucine, valine, and isoleucine are also metabolized via transamination by BCAA aminotransferase/transaminase (BCAT) into their respective BCKAs:  $\alpha$ -ketoisocaproate

(KIC),  $\alpha$ -ketoisovalerate (KIV) and  $\alpha$ -ketomethylvalerate (KMV) (Figure 2). There are two BCATs isoenzymes, a cytosolic one (BCATc or BCAT1) and a mitochondrial one (BCATm or BCAT2). BCAT1 is mainly found in neuronal tissues where it plays a major role in glutamate synthesis (with  $\alpha$ -ketoglutarate as the amino group acceptor) and nitrogen homeostasis (9). It is also expressed in some immune cells such as macrophages. Of note, BCAT1 seems to be involved in some aggressive tumors and could have a prognostic interest in these situations (10). BCAT2 is found in most other tissues except the liver (explaining the escape from the splanchnic territory); BCAA transamination occurs thus mainly in the mitochondria.

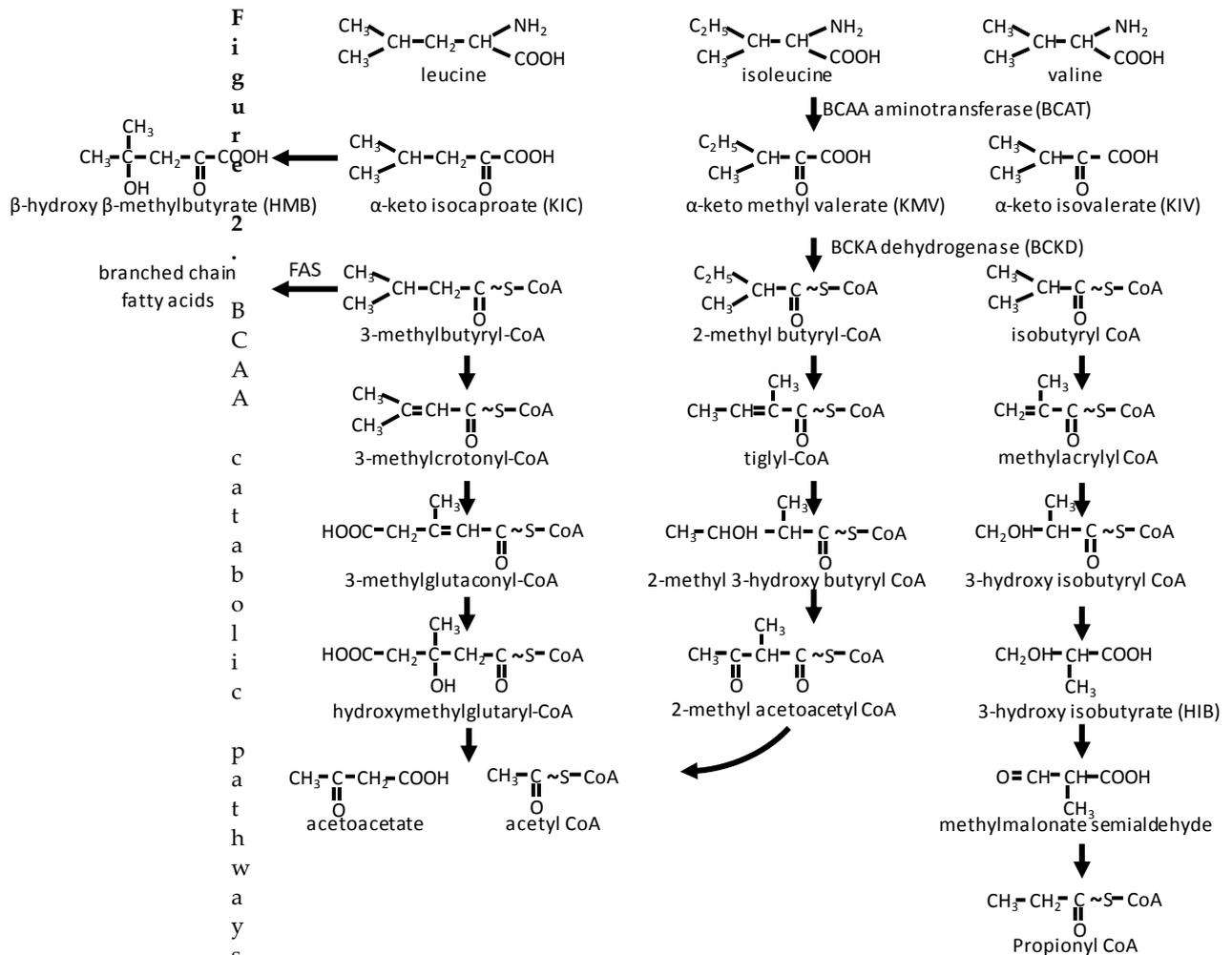
While, in the liver, part of KIC can be converted by a cytosolic dioxygenase into  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB), a ketone body (11), BCKAs undergo mostly oxidative decarboxylation by BCKA dehydrogenase (BCKD) into branched-chain acyl-CoA mainly leading to ketogenic and/or gluconeogenic derivatives. The BCKD complex which shares similarities with pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase complexes (with a common dihydrolipoyl dehydrogenase E3), is associated with the matrix side of the inner mitochondrial membrane (12). It represents the key controlling step of BCAA catabolism. It is subject to inactivating phosphorylation by BCKD kinase (BDK) (13) and activating dephosphorylation by the mitochondrial matrix-targeted protein phosphatase 2C [PP2Cm also known as protein phosphatase Mg<sup>2+</sup>/Mn<sup>2+</sup>-dependent 1K (PPM1K)] (14), the expression of these two enzymes being regulated (respectively decreased and increased) by protein availability (15). Leucine stimulates BCKD activity, promoting its dephosphorylation (16). In fact, the activation of BCKD is rapidly achieved in all tissues through allosteric inhibition of its kinase BDK and activation of its phosphatase PP2Cm by KIC. In this respect, high dietary BCAA intake enables activation of BCKD for rapid disposal of BCAAs and their keto-acids (15,17). Thus, isolated excess leucine could lead to valine and isoleucine deprivation. This could explain why animal experiments have demonstrated that a very high (five-fold) enrichment in plasma leucine leads to a decrease in the level of the other BCAAs (18).

Following their metabolization by BCKD, branched-chain acyl-CoA are mainly degraded by their cognate enzymes into aceto-acetate and acetyl-CoA (leucine), propionyl-CoA and acetyl-CoA (isoleucine) or propionyl-CoA (valine). Note that the penultimate step of leucine degradation is the degradation of 3-hydroxy-3-methylglutaryl-CoA by its lyase, the last enzyme of ketone body formation in the liver. In addition, propionyl-CoA is further metabolized into methylmalonyl-CoA and then into succinyl-CoA feeding into the tricarboxylic acid (TCA) cycle. In addition, some of the intermediates of this metabolism can serve as precursors of branched-chain and odd-chain fatty acids (19) or can escape the mitochondria as acyl-carnitines (C5 and C3 acylcarnitines), as hydroxyisobutyrate (HIB, valine catabolism) or as  $\beta$ -amino-isobutyric acid [BAIBA, a product of the valine derivative methylmalonic semialdehyde] (20).

BCAA degradative metabolic processes are also closely related with energy metabolism. Under stress conditions, secretion of catabolic factors such as counter-regulatory hormones and inflammatory cytokines leads to peripheral protein catabolism. BCAAs released in this process are transaminated, their amino group being used for alanine and glutamine synthesis by the muscle and the BCKAs being oxidized for energy synthesis (21). In highly catabolic states, such as the critically ill intensive care patient, BCAA oxidation in skeletal muscle can provide up to 20% of the energy used by muscles (22). On the other hand, the transamination process confers to BCAAs an important role as nitrogen donors in some tissues/organs, as mentioned above concerning BCAT1. In the brain, BCAAs may be used for the synthesis of glutamate as an excitatory neurotransmitter (23).

**In summary**, BCAAs display a particular profile among amino acids in terms of distribution and metabolism. From a finalistic point of view, this is probably due, apart from their character as essential amino acids, to their importance in postprandial protein

178 synthesis as substrates but also, for leucine, as regulators of this synthesis (see below). In  
 179 addition, they are very good substrates of energy metabolism, either at the muscular  
 180 level or, when their muscular use reaches its limits, at the hepatic level, in particular for  
 181 ketoneogenesis and gluconeogenesis in the post-absorptive period. Finally, the organism  
 182 must take care to limit their plasma concentration, taking into account their potential  
 183 central effects (25): namely their influence on the synthesis of neuromediators (via glu-  
 184 tamate synthesis, or by the competition with the cerebral transport of aromatic amino  
 185 acids via LAT1/2 (8)) or because of their neurotoxicity, with the ultimate situation of  
 186 maple syrup urine disease (MSUD, a BCKD deficiency) (25).  
 187



222 FAS: Fatty acid synthase

223  
 224 **3. Metabolic properties of BCAAs/BCKAs**

225 Since the first demonstration in 1972 by Odessey and Goldberg (26), numerous  
 226 studies have confirmed that BCAAs, and more precisely leucine, can stimulate protein  
 227 synthesis and decrease protein catabolism. Leucine stimulates postprandial protein  
 228 synthesis mainly via activation of mTORC1 and the up-regulation of the cap-dependent  
 229 mRNA translation initiation (Figure 1) (27,28). The core of mTORC1 is formed by mTOR  
 230 and two other proteins, Regulatory associated protein of mTOR (Raptor), and mamma-  
 231 lian Lethal with SEC13 protein 8 (mLST8). To act, mTORC1 must undergo a Rap-

232 tor-mediated translocation to the membrane of lysosomes. Leucine activates this trans-  
233 location by binding to the Sestrin 2 protein enabling Gator 2-Gator 1 interaction, activa-  
234 tion of lysosome membrane-associated proteins (Rag) and binding of Raptor. Note that  
235 this activation requires leucine accumulation in, and efflux from lysosomes, a mechanism  
236 conditioned by nutrient availability and inhibited in starvation conditions (29). As a  
237 consequence, mTOR and the whole mTORC1 complex interacts with the G protein 'Ras  
238 homolog enriched in brain' (Rheb) enabling activation of mTORC1 signaling. Interest-  
239 ingly, it has been shown that under conditions of increased nutrient availability, there is  
240 increased localization of endosomes/lysosomes near the cell membrane and anabolic  
241 signaling (30,31), increased lysosomal expression of LAT1 for leucine entry into lyso-  
242 somes (32), and leucine efflux via the SLC38A9 transporter for mTOR activation (33).

243 Once active, mTORC1 phosphorylates 70-kDa ribosomal protein S6 kinase  
244 (p70S6K1) and eIF4E-binding protein 1 (4E-BP1) resulting in the stimulation of the initi-  
245 ation and elongation steps of protein synthesis. In turn, p70S6K1 also phosphorylates the  
246 inhibiting serine residue of insulin receptor substrate (IRS1), blocking insulin signaling  
247 and thus providing a negative feedback loop on insulin action (34,35). mTORC1 also acts  
248 on the autophagy pathway decreasing protein degradation via inhibitory phosphoryla-  
249 tion of Unc-51-like kinase 1 (ULK1) complex and its downstream effector in  
250 autophagosome formation, the phosphoinositol 3-kinase vacuolar protein sorting 34  
251 (VPS34) complex. Experiments with transaminase inhibitors showed that the leucine ef-  
252 fect on protein catabolism also partially depends on its degradation into KIC; however,  
253 the exact mechanism of this KIC effect is unknown. It should be noted that HMB, derived  
254 from KIC, is also an activator of protein synthesis and an inhibitor of muscle proteolysis,  
255 which has led to its proposed use in the treatment of muscle wasting in situations such as  
256 cancer cachexia, sarcopenia of aging, or critically ill patients (36). However, its endoge-  
257 nous synthesis is far too low to contribute to the effects of leucine.

258 While experimental studies in rodents have clearly shown that leucine can both  
259 stimulate protein synthesis via the mTORC1 pathway and inhibits protein breakdown, its  
260 effectiveness in humans was discussed (37). Indeed, most studies have demonstrated a  
261 decrease in protein degradation but limited effect on protein synthesis (21,37) and only  
262 few studies reported an effect on protein anabolism: for example, Fujita et al (38)  
263 demonstrated that the administration of a single bolus of a leucine-enriched (35%) es-  
264 sential amino acids (0.35 g/kg fat free mass) and carbohydrate (0.5 g/kg fat free mass)  
265 mixture in young healthy volunteers led one hour later to a significant increase in muscle  
266 protein synthesis (4 fold) and the activation of mTOR signaling pathways (mTOR phos-  
267 phorylation, p70S6K1, 4E-BP1). In the elderly, Katsanos et al (39) showed that  
268 leucine-enrichment of an essential amino acid supplement was associated with improved  
269 muscle protein synthesis. Thus, leucine seems to also activate protein synthesis in hu-  
270 mans in few studies.

271 Anabolic effects of BCAAs can also be explained in part through their action on in-  
272 sulin secretion. Indeed, a physiological supply of leucine and isoleucine (1 to 2 g/kg) in-  
273 duces insulin secretion, an effect which decreases with ageing (40). This insulin secretory  
274 effect has been attributed to the activation of glutamate dehydrogenase by leucine in  
275 pancreatic  $\beta$ -cells, thus providing substrates such as  $\alpha$ -ketoglutarate for the citric acid  
276 cycle (anapleurosis) which fuels the production of ATP. An increase of ATP cellular lev-  
277 els impacts the function of several channels at the level of the cell membrane, which ul-  
278 timately leads to  $\text{Ca}^{2+}$  entry and exocytosis of insulin-loaded vesicles. Another  
279 non-exclusive mechanism could be a negative crosstalk between leucine metabolism and  
280 the activity of AMP kinase since AMP kinase has been shown to antagonize amino acid  
281 signaling and to decrease insulin production (41).

282 Another important aspect of these metabolic effects is the interaction between amino  
283 acid and glucose metabolism. Indeed, high physiologic concentrations of amino acids  
284 (and not specifically of leucine), while they stimulate protein synthesis, are associated  
285 with inhibition of glucose metabolism (42). Indeed, in healthy volunteers, euglycemic

hyperaminoacidemic hyperinsulinemic clamp may be associated with reduced whole-body glucose disposal (43).

**In summary**, BCAAs, namely leucine, plays an important regulatory role on protein homeostasis via i) mTORC1 signaling and the activation of postprandial protein synthesis, ii) an only partially elucidated inhibitory effect on proteolysis, and iii) potentiation of insulin secretion. These muscular anabolic properties of leucine have led to the development of supplementation protocols in many conditions of muscle loss, but with varying outcomes (2,28). These broad applications of leucine raise several issues including the risk of developing insulin resistance in people supplemented with BCAAs given the amino acid-induced reduction of whole-body glucose disposal. This question is all the more important because, as we will see now and even if different factors are likely to increase the plasma concentrations of BCAAs, many studies show an association between the increase of these concentrations and insulin resistance or even the risk of insulin resistance.

#### 4. Blood BCAAs as biomarkers of metabolic status

##### 4.1. Physiological and pathological variations in blood BCAAs

Different factors can influence plasma amino acid concentrations and, as with other essential amino acids, BCAA concentrations reflect a balance between inflow (protein intake, proteolysis) and outflow (oxidation and protein synthesis). In fact, at similar exogenous supplies of BCAAs, there is an increase in plasma BCAAs in all conditions associated with a decrease in oxidation and/or protein synthesis or an increase in peripheral protein catabolism, and a decrease in BCAAs in the opposite situations. A number of early studies had shown variations in plasma concentrations of BCAAs in different circumstances. For instance, Felig et al (44), in 1969, observed an increase not only in BCAAs but also in aromatic amino acids, sulfur amino acids, threonine and alanine in subjects with obesity compared to healthy controls. Studies from the same period also showed an increase in the plasma concentration of BCAAs during starvation, both in healthy and obese subjects (45), or in fasting type 1 diabetes patients at distance from the administration of insulin (46). An increase in plasma leucine and isoleucine, albeit to a more limited extent, has been demonstrated in healthy volunteers after 40 minutes of high-intensity exercise (47). Plasma BCAAs also increase after injury, in particular if the injury is severe (48); however, it has been shown to be decreased in severe sepsis. Other clinical situations such as liver or kidney disease, and aging may also be associated with either increased or decreased plasma BCAAs (22).

Even in an apparently healthy population, it is possible to identify different factors affecting BCAA levels. Indeed, the analysis of more than 19,000 participants of the Women's Health Study with no history of diabetes, cardiovascular disease, or cancer, showed that the highest concentrations of BCAAs were associated with higher age, higher BMI, lower physical activity and unbalanced diet (49). In addition, women in the highest quartile of BCAAs had a higher inflammatory status and a poorer plasma lipid profile. An additional cross-sectional analysis (50) indicated that the BMI explained 11.6% of BCAA variability. In a study in 487 obese patients with (homeostatic model assessment insulin resistance index [HOMA IR] > 2.5) or without insulin resistance, Guizar-Heredia et al (51) described an association between plasma BCAAs and age (higher leucine below 30 years old). A meta-analysis of three genome-wide association studies of plasma BCAAs identified five genomic regions, the strongest association being with a sequence upstream of the BCKD phosphatase PPM1K gene. As could have been expected, these increases in plasma BCAAs were associated with decreased oxidation by BCKD (52). Note that in the study of Guizar-Heredia et al (51) in obese patients, the non-common blood BCAA-raising alleles BCAT2 rs11548193 and BCKDH rs45500792 were associated with higher BCAA levels and the difference was maintained after adjustment for BMI and insulin resistance.

338 As mentioned above, a factor potentially responsible for variations in BCAA levels,  
339 highlighted by epidemiological studies, is diet, including protein consumption. Inter-  
340 estingly, some human studies show differences between animal and plant proteins. In the  
341 study of Guizar-Heredia et al (51), BCAA levels were positively correlated with animal  
342 protein intake and negatively with that of vegetable protein. Hamaya et al (50), in their  
343 cross-sectional analysis of the Women's Health Study, also showed an association of  
344 plasma BCAAs with animal but not vegetable protein intake. Note that Rousseau et al  
345 (53) showed higher animal protein intake in their overweight subjects with metabolic  
346 syndrome compared to subjects with normal weight or overweight without metabolic  
347 syndrome. BCAA content of vegetal proteins displays a large variability but is generally  
348 either similar or significantly lower than that of animal protein (Table 1) (54). Thus, the  
349 intake of BCAAs is likely to be somewhat lower with a diet rich in plant proteins. An  
350 additional confounding factor is the association found in these epidemiological studies  
351 between increased BCAAs and poor dietary quality index, i.e., a diet favoring simple  
352 sugars and fats, particularly saturated fats, and thus promoting insulin resistance.

353 **In summary**, several physiological and, most importantly, pathophysiological con-  
354 ditions are associated with an increase in blood BCAA concentrations. While initially the  
355 role of increased blood BCAA concentrations was not clear, important studies were pub-  
356 lished in the late 2000 pointing to a possible causative role of BCAAs in insulin resistance,  
357 in particular the metabolomic study of Newgard et al (55).

#### 358 4.2. Blood BCAAs as markers of insulin resistance

359 Newgard et al (55) carried a study including 73 obese subjects compared to 67 con-  
360 trol subjects; these authors showed a strong association between insulin resistance  
361 (HOMA-IR) and a score based on plasma concentrations of BCAAs, but also methionine,  
362 glutamate, glutamine, phenylalanine, tyrosine, and C3 and C5 acylcarnitines. Similar  
363 associations between BCAAs and impaired glucose tolerance or diabetes have also been  
364 demonstrated by others (56,57). An association between BCAAs and insulin resistance  
365 has been found in obese children as shown in the systematic review of Zhao et al (58) of  
366 10 studies and 2673 children. Interestingly, Menni et al (59) suggested, based on a  
367 nontargeted metabolomics study, that it was probably not the BCAAs themselves that  
368 were associated with diabetes but their metabolism as BCKAs were significantly higher  
369 in subjects with impaired fasting glucose (fasting glucose: 5.6 to 7 mmol/L) or type-2 di-  
370 abetes (T2D). They further showed that methyl-ketovalerate, the BCKA metabolite of  
371 valine, was a strong predictor for impaired fasting glucose. These results point to the  
372 importance of the alterations in the metabolism of BCAAs in this association with insulin  
373 resistance rather than the concentrations of the BCAAs themselves

374 There are however some discrepant observations in the literature. For example,  
375 Hamley et al (60) looked at young (18-35 years), nonobese [body mass index (BMI) < 30  
376 kg/m<sup>2</sup>] Australian adults and measured glucose and insulin response during an OGTT  
377 with [6,6-2H]glucose administration. Individuals were categorized according to their  
378 insulin response and the existence or not of impaired fasting glucose or impaired glucose  
379 tolerance. These authors did not observe any increase in plasma BCAA concentrations in  
380 groups with significantly altered insulin sensitivity. Beyond the fact that, for example,  
381 study conditions may be much less stringent in epidemiological studies than in clinical  
382 ones, a confounding factor may be how insulin resistance is defined (fasting glucose,  
383 HOMA index, IV glucose tolerance test, euglycemic clamp...).

384 mice, a high-fat-high-sucrose diet (HFHS, 17% protein, 43% carbohydrates, 40% fat)  
385 was not associated with change in plasma BCAAs (65,66).  
386

387 **Table 1.** BCAA content of different foods.

		Leu			Val			Ile			BCAAs			
		Energy <sup>a</sup>	Protein <sup>b</sup>	Weight <sup>c</sup>	Energy <sup>a</sup>	Protein <sup>b</sup>	Weight <sup>c</sup>	Energy <sup>a</sup>	Protein <sup>b</sup>	Weight <sup>c</sup>	Energy <sup>a</sup>	Protein <sup>b</sup>	Weight <sup>c</sup>	
389														
390	ANIMAL													
391		Chicken	1.45	7.98	2.50	0.96	5.28	1.66	1.02	5.62	1.76	3.42	18.88	5.92
392		Beef	1.27	8.26	2.12	0.79	5.15	1.32	0.73	4.73	1.21	2.79	18.14	4.65
393		Porc	1.08	8.17	2.31	0.73	5.52	1.56	0.63	4.77	1.35	2.43	18.46	5.21
394		Salmon	1.19	8.43	2.16	0.75	5.34	1.37	0.67	4.78	1.23	2.61	18.54	4.76
395		Egg	0.66	8.55	1.02	0.47	6.10	0.73	0.42	5.46	0.65	1.54	20.10	2.39
396		Whole milk	0.46	8.89	0.28	0.31	6.13	0.19	0.25	4.85	0.15	1.02	19.86	0.62
397	LEGUMES													
398		Peas	0.53	7.50	0.62	0.35	4.94	0.41	0.30	4.32	0.36	1.18	16.76	1.39
399		Lentils	0.62	7.89	0.72	0.43	5.40	0.50	0.37	4.70	0.43	1.42	17.99	1.65
400		Soybeans	0.69	7.70	1.19	0.42	4.72	0.73	0.41	4.58	0.71	1.52	17.00	2.64
401		Black beans	0.57	8.43	0.75	0.37	5.52	0.49	0.32	4.65	0.42	1.26	18.60	1.66
402	GRAINS													
403		Whole wheat	0.29	6.78	0.87	0.19	4.41	0.57	0.15	3.63	0.47	0.63	14.82	1.90
404		Brown rice	0.19	8.28	0.21	0.14	5.85	0.15	0.10	4.22	0.11	0.42	18.35	0.47
405		Oats	0.26	7.76	0.22	0.18	5.45	0.16	0.13	3.98	0.11	0.57	17.20	0.49
406		Quinoa	0.27	7.13	0.32	0.19	5.04	0.23	0.16	4.28	0.19	0.61	16.45	0.75
407	NUTS													
408		Almonds	0.24	6.53	1.42	0.14	3.79	0.82	0.12	3.33	0.72	0.51	13.64	2.96
409		Peanuts	0.29	6.43	1.65	0.19	4.16	1.07	0.16	3.49	0.89	0.64	14.07	3.61
410		Pecans	0.09	6.73	0.63	0.06	4.62	0.43	0.05	3.78	0.36	0.21	15.13	1.42
411		Walnuts	0.18	7.42	1.16	0.11	4.78	0.75	0.10	3.97	0.62	0.39	16.16	2.53
412	VEGETABLES													
413		Potatoes	0.12	6.01	0.10	0.11	5.60	0.09	0.08	4.08	0.07	0.30	15.69	0.26
414		Carrots	0.13	5.95	0.06	0.09	4.03	0.04	0.10	4.49	0.04	0.33	14.47	0.13
415		Butternut	0.15	6.88	0.06	0.12	5.26	0.05	0.10	4.72	0.04	0.37	16.87	0.15
416		Sweet potatoes	0.14	6.19	0.12	0.13	5.77	0.12	0.08	3.67	0.07	0.35	15.63	0.32
417		Green beans	0.41	7.62	0.14	0.33	6.11	0.11	0.24	4.53	0.09	0.98	18.25	0.34
418	FRUITS													
419		Apple	0.03	6.47	0.02	0.03	5.97	0.02	0.02	2.98	0.01	0.08	15.42	0.04
420		Banana	0.10	7.97	0.09	0.07	5.51	0.06	0.04	3.28	0.04	0.21	16.76	0.18
421		Mango	0.10	7.40	0.06	0.08	6.21	0.05	0.06	4.29	0.03	0.24	17.90	0.14
422		Mushrooms	0.83	6.05	0.18	1.60	11.70	0.35	0.52	3.83	0.12	2.95	21.58	0.65

388 Adapted from (54).

389 <sup>a</sup> kcal/100 kcal; <sup>b</sup> g/100 g prot; <sup>c</sup> g/100 g product

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Unfortunately, experimental studies (Table 2) have not clarified this issue. Indeed, She et al (61) in genetically-induced obesity models (ob/ob mice and Zucker rats) only observed a significant increase in plasma BCAA concentrations in the postprandial state or in "random fed" animals but a near normalization in fasted animals. In models of diet-induced obesity, Newgard et al (55) did not observe changes in Wistar rats fed a HF diet (19% protein, 35% carbohydrates, 45% fat) for 13 weeks. In Sprague-Dawley rats, while David et al (62) observed an increase after approximately 7 weeks on a 60% fructose diet (19.3% protein, 64% carbohydrate, 16.7% fat), we never observed changes in BCAAs in Sprague-Dawley rats fed on either a 45%-fructose diet (19.3% protein, 64% carbohydrate, 8.4% fat) or a Western diet (20% protein, 35% carbohydrates, 45% fat diet and 30% fructose in drinking water) (63,64). Last, in several long-term feeding experiments of DIO in C56Bl6 mice, a high-fat-high-sucrose diet (HFHS, 17% protein, 43% carbohydrates, 40% fat) was not associated with change in plasma BCAAs (65,66).

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A number of interventional and experimental studies have investigated the influence of BCAA or protein intake on plasma BCAAs. These data must be analyzed with caution in terms of extrapolation of animal data to humans given the differences in metabolism, also the nature of the experimental models (diet-induced or genetically induced obesity), the type of diet administered (Table 2) or the amount of BCAAs provided (often resulting in a doubling of plasma concentrations, without any relevance to the clinical reality). In the above-mentioned Newgard's study (55), Wistar rats were fed for 13 weeks a conventional or a HF diet supplemented (150% higher content) or not with BCAAs. BCAA supplementation led to a doubling of their plasma concentrations in HF-fed animals but only an approximately 35% increase in the control animals. Solon-Biet et al (67) fed C57Bl6 mice for at least 15 months with standard rodent chow (18% total protein, 64% carbohydrate, 18% fat) but varying BCAA content (0.2, 0.5, 1 or 2-fold control level) and indeed observed that plasma levels increased with intake but reached a plateau at control level supply. Concerning humans, besides the numerous studies that have focused on the anabolic properties of leucine, often within short term trials, Woo et al (68) have performed, in 12 obese patients with prediabetes (fasting blood glucose: 100 to 126 mg/dL or glycated hemoglobin: 5.7 to 6.4%), a randomized cross-over study of supplementation with 20 g of BCAAs or low BCAAs proteins for 4 weeks; they showed that BCAA supplementation did not affect plasma BCAAs. Hattersley et al (69) evaluated the effect of an 18-week increase in the supply of BCAAs given as protein (15, 20-25 or 25-30% of energy intake) with a constant fat intake (30%) in overweight or obese patients. Here also, plasma BCAAs were not modified.

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Nevertheless, keeping in mind the different confounding factors mentioned, a large number of metabolomic studies confirm an association between increased plasma BCAAs and insulin resistance. Interestingly, several cohort studies with follow-ups of 5 to 7.5 years indicate that BCAA levels may be predictive of the deterioration of insulin sensitivity and the development of T2D. Out of the 6244 subjects of the PREVENDE (Prevention of renal and vascular end-stage disease) cohort, 301 developed T2D during a 7.5-year (median) follow-up (70). In this cohort, Flores-Guerrero et al (70) showed that patients in the highest BCAA quartile had an increased risk for developing T2D and that the association was maintained after correction for sex, age, BMI and HOMA-IR. Later on, it was shown in a large subset of the same cohort that both high BCAA levels and fatty liver index (a score based on plasma triglycerides and gamma-glutamyltransferase activity, BMI and waist circumference) were predictive of incident T2D with respective hazard ratio of 1.19 (95% CI 1.03–1.37,  $p = 0.01$ ) and 3.46, (95% CI 2.45–4.87,  $p < 0.001$ ) (71). Lotta et al (52) meta-analyzed the data from 5 prospective studies (with a total of 1.992 incident T2D cases for a population of 6311 subjects) and showed also an increased risk of incident T2D linked to BCAA levels. This association was also true for BCKA but not for metabolites downstream of BCKD in a subset of the Norfolk cohort of European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk). It should be noted that,

here too, various factors can affect this relationship. Indeed, in the above-mentioned study of Guizar-Heredia et al (51), there was an effect of age and insulin resistance on plasma BCAAs, resulting in similar BCAA concentrations between young patients without insulin resistance and those over 30 years with insulin resistance.

In the association between BCAAs and insulin resistance, we are then faced with two non-mutually exclusive possibilities:

- the decrease in insulin sensitivity affects the metabolism of BCAAs and induces an early increase in their plasma concentrations. This phenomenon is well known for other aspect of insulin resistance, for example in the cardiovascular field where alterations of the vascular function can exist many years before the appearance of clinical manifestations (72);
- The BCAAs or some of their derivatives could at certain doses or in certain situations of energy imbalance promote insulin resistance (e.g., through the increased serine phosphorylation of IRS1).

## 5. BCAA metabolism and insulin resistance

### 5.1. BCAA metabolism in insulin resistance situations

It should first be noted that studies on the effects of insulin resistance on BCAA metabolism can sometimes seem inconsistent. It is therefore important to consider the specificities of this metabolism in humans and in rodents, but also the conditions under which the studies were carried out, in the fasted or fed state. Furthermore, although it is well established that there can be significant discrepancies between gene or protein expression, enzymatic activities, and substrate fluxes in vivo, it seems that this is particularly true for the metabolism of AACR (66).

#### 5.1.1. BCAA metabolism and insulin resistance in humans

It has been known for a long time that the peripheral utilization of amino acids in the postprandial period is closely related to the action of insulin. Already in the 1970s, Wahren et al (5) showed in insulin-dependent diabetic patients, in the absence of insulin, both a higher plasma concentration of BCAAs in the basal state and a greater increase in these concentrations in response to a protein meal. The measurement of leg postprandial arteriovenous amino acid differences showed that the postprandial increase in BCAA uptake was much more limited than in control subjects. In a study in 26 subjects either overweight with impaired glucose tolerance (fasting glucose  $\geq 5.6$  mmol/l, 2 h glucose  $\geq 7.8$  mmol/l, or HOMA-IR  $> 2.0$ ) or normal weight with normal glucose tolerance, mRNA-sequencing pathway analysis on subcutaneous white adipose tissue (WAT) and skeletal muscle biopsies indicated decreased post-prandial BCAA metabolism in both tissues, improved by a 12 week-supervised exercise intervention (73).

In contrast, after an overnight fast, measurement of leucine turnover with 1- $^{13}\text{C}$ leucine or U $^{13}\text{C}_6$ leucine showed a 9 to 50% increase in leucine flux and a 20 to 50% increase in leucine oxidation in subjects of increasing insulin resistance (HOMA-IR: 2.57 to 4.89) and normal to morbidly increased weight (BMI: 24.4 to 38.5) (74–76). This suggests that, over the course of the day, the increase in leucine oxidation during fasting fails to compensate for the defect in postprandial utilization, which could lead to increased fasting plasma levels. Note that, in the Glynn et al study (74), neither plasma BCAAs nor leucine flux were affected after a 6 month-training program including aerobic and whole-body resistance training, despite a significant improvement in insulin-sensitivity (mean 54% increase in glucose infusion rate during a hyperinsulinemic-euglycemic clamp). Similarly, plasma BCAAs and related metabolites were not affected by a 12 week-supervised intensive exercise intervention (73). On the other hand, when leucine oxidation was measured during a 2-step hyperinsulinemic-euglycemic clamp in obese subjects, while it increased as a function of insulin level in all subjects, it was lower in

495 DT2 patients compared to non-DT2 ones (77). In the liver, the decrease in hepatic BCAA  
496 oxidation is such that it is exploited in a breath test using  $^{13}\text{C}$ -KIC to evaluate the defect in  
497 hepatocyte mitochondrial function (78). Indeed, Grenier-Larouche et al (79) showed that  
498 the association between BCKA and nonalcoholic fatty liver disease (NAFLD), the hepatic  
499 manifestation of insulin resistance and metabolic syndrome, is the result of the defect in  
500 hepatic BCKA metabolism. In WAT, studies showed either preserved or decreased ex-  
501 pression of enzyme involved in BCAA catabolism in subcutaneous or visceral WAT in  
502 obese subjects compared to normal weight individuals or in insulin-resistant obese  
503 compared to non-insulin-resistant patients (52,74). Bariatric surgery was associated with  
504 increased BCKD expression. It can be noted, however, that measurements of BCAAs and  
505 BCKA fluxes from arteriovenous differences in subcutaneous adipose tissues in humans  
506 do not show any difference with obesity and/or insulin resistance (78); but this is not  
507 surprising given the small amplitude of the variations and the inter-individual variabil-  
508 ity.

#### 509 5.1.2. BCAA metabolism in experimental models of insulin resistance

510 The defect in postprandial BCAA utilization is confirmed experimentally in models  
511 of insulin resistance with a more marked increase in post-prandial plasma BCAAs  
512 compared to controls (61). Note that, in one study (52), the expression of the enzymes of  
513 BCAA catabolism in the fed state was not affected in the skeletal muscle but decreased in  
514 subcutaneous WAT; oxidation, only measured in WAT, was also decreased.

515 In the fasted state, experimental studies do not demonstrate any changes in protein  
516 expression of BCATm, BCKD E1 $\alpha$  subunit, BDK, and BCKD E1 phosphorylation ratio  
517 (61,80), or BCKD activity (65) at the muscle level in models of genetic obesity (ob/ob mice,  
518 zucker rats) (61,80) or diet-induced (HF or HFFS) obesity (65,66). In the liver, experi-  
519 mental data are discordant with, depending on the studies and models, normal or de-  
520 creased expression of BCKD E1 subunit, normal or increased expression of BDK, and  
521 normal or increased BDKE1 phosphorylation ratio (61,65,78). However, both She et al  
522 (61) and White et al (81) observed a reduction in valine oxidation (using  $\alpha$ -keto-[1- $^{14}\text{C}$ ]  
523 isovalerate) in liver sample from ob/ob mice or zucker fatty rats respectively. In WAT,  
524 studies are rather concordant. They globally observe a reduction in BCAA metabolism  
525 whether in terms of enzyme expression or oxidation activity in subcutaneous or visceral  
526 adipose tissue in experimental models of genetic or diet-induced obesity (61,65,80,81).  
527 Interestingly, treatment with thiazolidinediones in db/db mice was associated with an  
528 increase in protein expression of BCKD suggesting a normalization of this metabolism.  
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530 Taken together, based on the data of whole-body leucine turnover and of organ  
531 BCAA metabolism (enzyme activities or expression), it seems that in **fasting** insu-  
532 lin-resistant individuals, BCAA metabolism is characterized by i) increased muscle ox-  
533 idation and ii) decreased oxidation in adipose tissue and perhaps in the liver. This is fur-  
534 ther illustrated by the study by Neinast et al (82) extrapolating the contribution of dif-  
535 ferent organs to BCAA metabolism from the distribution of  $^{13}\text{C}$  after administration of  
536  $^{13}\text{C}$ -labeled BCAAs in mice. These authors show in models of insulin resistance in fasting  
537 animals, a redirection of the oxidation of BCAAs to the benefit of the muscles at the ex-  
538 pense of WAT, and to a lesser extent of the liver.

#### 539 5.1.3. BCAA metabolism in brown adipose tissue

540 An interesting point highlighted by the above-mentioned work of Neinast et al (82)  
541 as well as by that of Yoneshiro et al (83) is the contribution of the brown adipose tissue  
542 (BAT) in the oxidation of BCAAs. Long considered to be limited to hibernating mam-  
543 mals, various studies over the last two decades have highlighted the presence of a sig-  
544 nificant number of thermogenic adipocytes (brown or beige adipocytes) in humans and  
545 their potential contribution to energy metabolism. Indeed, Ouellet et al (84) showed in  
546 healthy subjects exposed to cold, in conditions of limited shivering, an increase of up to  
547 80% of resting energy expenditure largely attributable to the brown adipose tissue. If,

548 until recently, it had been shown that this activation of BAT was associated with a sig-  
549 nificant oxidation of glucose and fatty acids, the work of Yoneshiro et al (83) highlights  
550 an increased use also of BCAAs by the BAT in these conditions. In particular, these au-  
551 thors demonstrated an inverse relationship between BAT activity (assessed by  
552 <sup>18</sup>F-Fluorodeoxyglucose positron emission tomography imaging) and plasma leucine and  
553 valine concentrations in healthy individuals. They further showed in mice the im-  
554 portance of BAT in the oxidation of BCAAs for thermogenesis and the maintenance of  
555 plasma concentrations of BCAAs. Finally, in a model of BCKD invalidation specifically in  
556 BAT, they showed that the defect in BCAA oxidation of the BAT was associated with  
557 weight gain and fat mass gain, increase in hepatic triglyceride content and insulin re-  
558 sistance. Conversely, diet-induced obesity (DIO) in mice was associated with a decrease  
559 in BCAA catabolism in BAT (85). Note that, in DIO mice, pharmacological stimulation of  
560 BCAA oxidation by 3,6-dichlorobenzo(b)thiophene-2-carboxylic acid (BT2) or by oral  
561 administration of some specific probiotics (*Bacteroides dorei* and *Bacteroides vulgatus*) im-  
562 proved BCAA catabolism by the BAT and mice metabolic status (85).

### 563 5.2. Do BCAAs promote insulin resistance?

564 As already mentioned, leucine through its interaction with Sestrin 2 promotes the  
565 activation of mTORC1 leading in particular to the activating phosphorylation of p70S6K1  
566 and inhibiting one of 4E-BP1 thus participating in the postprandial induction of protein  
567 synthesis. In essence, this system involves other regulatory elements or systems, for  
568 example, the availability of different substrates (e.g., via the level of amino acylation of  
569 tRNAs and General control nonderepressible 2 [GCN2]) or the level of restoration of  
570 protein pool. In terms of insulin signaling, through mTORC1 and p70S6K1, leucine in-  
571 duces a feedback inhibition via serine phosphorylation of IRS1. However, whether this  
572 feedback control causes insulin resistance is controversial. Alternatively, it has been  
573 proposed that it is not the BCAAs themselves but their metabolites that impair insu-  
574 lin-sensitivity.

#### 575 5.2.1. BCAA-induced insulin resistance?

576 While some experimental works seemed to support an effector role of BCAAs in  
577 insulin resistance, the results are quite inconsistent. In the above-mentioned Newgard et  
578 al study (55), Wistar rats were fed for 13 weeks a conventional (C) or a HF diet supple-  
579 mented (150% higher content) or not with BCAAs, BCAA supplementation leading to a  
580 doubling of their plasma concentrations. HF/BCAA-fed rats had lower food intake and  
581 lower weight gain than HF fed rats but had similarly impaired glucose tolerance while  
582 C/BCAA-fed animals exhibited weight gain and glucose tolerance similar to those of  
583 C-fed rats. The authors concluded to the responsibility of the BCAA/HF-diet combina-  
584 tion.

585 These results contrast with, for example, those of Zhang et al (86) in mice receiving  
586 for 15 weeks a doubled daily supply of leucine with either a control or a HF diet. They  
587 show that in HF-fed animals, a leucine-induced increase in energy expenditure was re-  
588 sponsible for a reduction in weight gain and adiposity and was associated with improved  
589 glucose tolerance and lower insulin resistance. Indeed, leucine prevented the increase in  
590 blood glucose observed from the 4th week on the HF diet and improved insulin sensi-  
591 tivity throughout the study. In a more recent work (65), mice received an equivalent  
592 supplement of the 3 BCAAs (but half the amount of leucine) with an HF (12 weeks) or an  
593 HFHS (32 weeks) diet. Under these conditions, the BCAA supply did not affect glucose  
594 tolerance. A potential confounding factor could be the role of BCAAs in the regulation of  
595 food intake. In the Solon-Biet et al study cited above (67), in which mice were fed a  
596 standard diet with variable BCAA intake, a double intake of BCAAs was associated with  
597 hyperphagia; this hyperphagia was related to reduced central serotonin synthesis and  
598 corrected by tryptophan supplementation. In addition, some of the differences between  
599 these studies, apart from the animal species, can be accounted for by different nutritional

600 regimen (Table 2) with for example, according to the studies, quantitatively (40, 45 or 60%  
601 of the energy intake) and qualitatively (lard, milk fat, soybean oil) different lipid supplies  
602 or the supply of BCAAs individually or in mixture, in addition to the protein supply or in  
603 substitution of a part of the protein supply (55,67,86).

604 In humans, previous studies showed a decrease in glucose utilization during amino  
605 acid infusion. Hyperinsulinemic euglycemic clamp tests have since been used to try to  
606 clarify these observations. For example, Boden and Tappy (87) investigated glucose me-  
607 tabolism in healthy volunteers receiving an infusion of a parenteral amino acid formula  
608 leading to a 5- to 6-fold increase in their plasma concentrations during hyperinsulinemic  
609 euglycemic clamps associated with somatostatin and glucagon (0.25 and 3 g/kg/min). No  
610 changes in glucose metabolism were observed. On the contrary, under similar conditions,  
611 Krebs et al (43) showed a 25% reduction in whole body glucose disposal. In fact, at least  
612 in short-term experiments, the responsibility of the global protein intake rather than  
613 specifically of BCAAs seems likely. Indeed, both Smith et al (88) and Harris et al (89) have  
614 compared in sedentary obese women during hyperinsulinemic euglycemic clamps the  
615 effect of a protein supply or of an isomolar amount of leucine. Ingestion of proteins, but  
616 not leucine, significantly decreased insulin-stimulated glucose disposal. However, the  
617 previous studies were based on short-term evaluation. In longer-term studies, we also  
618 find this difference between the effect of the global protein intake and the effect of  
619 BCAAs. The type of proteins administered is involved: indeed, Pal et al (90) compared  
620 the effects of a 12-week supplementation with 27 g of casein, whey (rich in BCAAs) or  
621 glucose (control) in overweight patients and observed an improvement of the insulin  
622 sensitivity with the whey supplementation. More specifically, essential amino acids do  
623 not seem to affect insulin sensitivity by themselves (91). Finally, Woo et al (68) compared  
624 in a crossover study the effects of a 4-week supplementation of 20 g of BCAAs or rice  
625 protein (low in BCAAs) in 12 obese prediabetic subjects and showed a trend towards  
626 improved glucose utilization with BCAAs.

627 Contrary to a possible BCAA-induced insulin resistance, several studies have  
628 demonstrated or suggested, particularly in the short term, an anabolic effect of BCAAs  
629 and more particularly of leucine. This notion, which has made them successful among  
630 sport enthusiasts in search of muscle gain, is based on the demonstration of an acute but  
631 transient activation of muscle protein synthesis. In the clinical setting, past studies have  
632 evaluated the possible benefit of BCAA supplementation in injured or cancer patients,  
633 however with limited success owing probably to methodological problems such as very  
634 inadequate parenteral nutrition mixtures not providing all the essential amino acids  
635 (2,22). Since then, various studies have focused on the geriatric population with the aim  
636 of preventing or treating age-associated loss in muscle mass and function, i.e. sarcopenia.  
637 If short-term studies are globally positive, studies on prolonged periods are rather dis-  
638 appointing (28). Interestingly, in healthy older adults ( $69.1 \pm 1.1$  yr) in a situation of pro-  
639 longed bed rest (7 days of bed rest and 5 days of rehabilitation), leucine supplementation  
640 tended to preserve insulin sensitivity and muscle mitochondrial metabolism (92). It  
641 should be noted that, since the various strategies taken individually have only limited  
642 effectiveness, a multimodal approach of sarcopenia is currently favored by combining  
643 leucine with another nutrient, such as vitamin D, or with resistance training exercise. For  
644 example, it has been shown in healthy elderly subjects that the intake of leucine and  
645 vitamin D for 6 weeks was associated with a significant increase in muscle protein syn-  
646 thesis and a gain in muscle mass (93). The studies that evaluated insulin sensitivity in this  
647 context showed an improvement; however, this could have been expected given the im-  
648 provement in body composition and in muscle mass, an important determinant of glu-  
649 cose disposal; therefore, it is difficult to relate it to a direct effect of leucine (94,95).

650 If BCAAs are responsible for insulin resistance, we can imagine that, conversely, a  
651 reduction in plasma BCAA concentrations could be associated with an improvement in  
652 insulin sensitivity. It should be noted that the studies on this subject are carried out in the  
653 context of the evaluation of the effects of caloric restriction and, more specifically, protein

654 restriction, with very significantly reduced protein intake, lower than 50% of standard  
655 levels. It is clearly established, mainly on the basis of experimental work, that protein re-  
656 striction improves metabolic health and longevity. Part of the effects seems related, at the  
657 endocrine level, to the decrease in the secretion of IGF-1 and the increase in the secretion  
658 of FGF-21 (96), a fasting and metabolic stress-induced peptide hormone that regulates  
659 energy substrates utilization (97). At the cellular level, this involves the response of the  
660 mTOR and GCN2 pathways to the decrease in the global or specific availability of certain  
661 amino acids (96). In Solon-Biet et al study (67), median lifespan was similar between  
662 control and BCAA-restricted (at 20 or 50% level of standard supply) mice but the 20%  
663 group had lower hepatic steatosis, and lower basal insulin level with normal basal glu-  
664 cose. Moreover, Yu et al (98) showed that a 2/3 reduction in protein supply or specifically  
665 in BCAA supply was associated with an improvement in glucose tolerance and a slowing  
666 down of weight gain after 3 weeks. Surprisingly, only an equivalent reduction specifi-  
667 cally in isoleucine supply reproduced similar effects while leucine restriction had no ef-  
668 fect. The authors showed that the effects of isoleucine restriction were independent of the  
669 activation of the mTORC1 and GCN2 pathways in the liver and were associated with  
670 increased FGF-21 secretion, activation of ketogenesis and increased energy expenditure.  
671 This was related to a specific, isoleucine deprivation-associated increase in nuclear  
672 FOXA2 (Forkhead box protein A2), a transcription factor that activates lipid metabolism  
673 and ketogenesis and whose nuclear localization is blocked by insulin (99). The effect of  
674 BCAA restriction has been reproduced in T2D patients receiving a one-week supply of  
675 proteins and amino acids (1 g/kg body weight) restricted (-60%) or not in BCAAs (98);  
676 BCAA restriction was associated with an increase in the plasma concentration of FGF-21  
677 and in the "oral glucose sensitivity index", calculated during a "mixed meal tolerance  
678 test".

679 In summary, the results of the restriction studies show a specific response to BCAAs  
680 deficiency that illustrates the importance of these amino acids in both protein synthesis  
681 and ketogenesis and the necessary adaptation of the metabolism for a better management  
682 of energy sources. Observations in excess situations are very contrasted beyond the sim-  
683 ple postprandial regulation of substrate excess, the multiple confounding factors (animal  
684 species, nature of the associated diet, activity level of the animals ...) suggest that the di-  
685 rect role of BCAAs in insulin resistance is quite limited.

#### 686 5.2.2. BCAA metabolites and insulin sensitivity

687 From metabolomic or epidemiological studies, some authors hypothesized that it  
688 was probably not BCAAs themselves but their metabolites that could contribute to insu-  
689 lin resistance. Indeed, She et al (101) observed that global inactivation of BCATm, which  
690 induces a blockade of BCAA degradation and an increase in their plasma concentrations,  
691 was associated with a reduced weight and fat mass gain, improved insulin sensitivity  
692 and resistance to the obesogenic effect of a HF diet. In the same way, telmisartan, an an-  
693 giotensin II receptor blocker with unique BCATm inhibitory properties, favors adipose  
694 tissue browning and improves body weight, glucose tolerance and insulin sensitivity in  
695 HF-fed mice (102). Of course, these data must be interpreted with caution as  
696 BCAAs/BCKAs are significant contributors to energy metabolism in situations where  
697 glucose/glycogen availability is limited and significant regulators of protein homeostasis.  
698 The blockade of their degradation must therefore be compensated by metabolic adapta-  
699 tion as already mentioned in situations of severe protein or BCAA restriction. Anyway,  
700 the metabolism of BCAAs could be necessary to allow their negative effects on energy  
701 metabolism. Indeed, some metabolomic and epidemiological studies showed associa-  
702 tions between plasma concentrations of certain metabolites and insulin resistance or the  
703 risk of developing insulin resistance. The presence of some of these metabolites, mainly  
704 BCKA, C5 and C3 acylcarnitines and HIB, exacerbated in experimental models during  
705 concomitant administration of BCAAs and HF diet, has been interpreted as an indication  
706 of incomplete oxidation of BCAAs and it has been suggested that these metabolites are

707 potentially responsible for insulin resistance. Note that, as in the case of fatty acid oxida-  
708 tion and the appearance of even acyl-carnitines (103), increased BCAA derivatives levels,  
709 particularly odd acyl-carnitines, may not be indicative of incomplete oxidation but may  
710 simply indicate an increase in metabolic flux. This is well illustrated by the study of en-  
711 ergy substrate utilization during aerobic exercise as a function of glycogen availability.  
712 Under conditions of reduced glycogen availability, the muscle increases the oxidation of  
713 lipid substrates (with no change in exogenous carbohydrate utilization) but also of  
714 BCAAs from protein catabolism (104–106). Metabolomic analysis showed an increase in  
715 fatty acid metabolites such as acyl-carnitine proportionately to the increase in lipid oxida-  
716 tion; similarly, the increase in BCAA metabolites was proportional to the increase in  
717 leucine oxidation (105). In insulin-resistant subjects, as we have seen, BCAAs are metab-  
718 olized more slowly in the postprandial period but their oxidation increases in the  
719 post-absorptive period. Therefore, it does not seem surprising that the plasma concen-  
720 trations of the reactive intermediates increase during these same periods.

721 The negative effect of BCKAs on insulin function is suggested, for example, by the  
722 improvement of glucose tolerance and insulin sensitivity in ob/ob or DIO mice induced  
723 by increasing BCKD activity following BDK inhibition by BT2 (107). In vitro work in  
724 C2C12 or L6 muscle cells gives some indication of a potential direct role of BCKAs in  
725 insulin resistance. In these cell models, BCKAs decrease Akt phosphorylation and thus  
726 insulin signaling and decrease glucose utilization (66,108). More precisely, Biswas et al  
727 (66) showed inhibition of insulin induced activating tyrosine phosphorylation of IRS1 (at  
728 very high KIC concentration) but upregulated protein translation signaling and synthe-  
729 sis. This is similar to the previously mentioned effect of leucine on the Akt-mTORC1  
730 pathway, but this conclusion remains controversial. In addition, it has been shown that  
731 valsartan, an angiotensin II receptor blocker that displays BDK inhibitory properties  
732 (109), elicited only limited effects on insulin sensitivity in human. Indeed, the Navigator  
733 study on 9306 patients with impaired glucose tolerance and cardiovascular disease  
734 showed very limited effect of valsartan on glucose tolerance (110). In fact, another  
735 mechanism may explain some of the experimental effects of BDK inhibitors. Indeed, the  
736 effect of BDK inhibition by BT2 is already observed after one week of treatment in Zucker  
737 rats and associated with a shift in respiratory exchange ratio suggesting decreased fatty  
738 acid storage and increased oxidation (111). White et al (111) showed that BDK and  
739 PPM1K also control ATP-citrate lyase phosphorylation, and thus de novo lipogenesis  
740 and fat storage. Interestingly, in a mouse model of nonalcoholic steatohepatitis (NASH),  
741 pharmacological inhibition of ATP-citrate lyase reduced hepatic steatosis, fibrosis, in-  
742 flammation, and blood glucose, and triglycerides (110), suggesting that the beneficial ef-  
743 fect of BT2 may be also ascribed to this ATP-citrate lyase inhibition rather than to BCKD  
744 activation.

745 Data in support of a role for acylcarnitines are quite limited (103). In C2C12  
746 myotubes, Aguer et al (113) evaluated the effects of C4 acylcarnitines on insulin signaling  
747 and show a significant reduction in Akt phosphorylation, which contrasts with the pre-  
748 sumed effects of BCAAs that lead to hyperactivation of the Akt/mTORC1 pathway. On  
749 the contrary, and although this evidence is indirect, various studies show rather absent or  
750 inverse relationships. Experimentally, in HFHS or HF-fed mice, BCAA supplementation  
751 is associated with increased C3 and C5 acyl-carnitine with no impact on insulin re-  
752 sistance. From a clinical point of view, in the follow-up of patients with NAFLD, C5  
753 acylcarnitine concentrations decreased with the progression of liver damage (112). In the  
754 metabolic syndrome, Remchak et al (115) characterized the chronotype of their patients  
755 and showed, in the early chronotype group (“early birds”) compared to the late  
756 chronotype group (“night owls”), a better insulin sensitivity and an increase in plasma  
757 concentrations of most acylcarnitines.

758 **Table 2.** Nutritional conditions in some of the cited experimental studies.

Reference	Diet type	Lipids	Protides	Carbohydrates
(61)	SC	18% 100% soybean oil	24% 100% soybean protein	58.0% 100% wheat/corn
(55)	HF	45% 12.3% soybean oil 87.7% lard	19% 53% casein 47% free amino acids	35% 21% corn starch 29% maltodextrin 50% sucrose
	HF/BCAA	43% 12.3% soybean oil 87.7% lard	23% 41% casein 58% free amino acids including BCAA supplement	34% 21% corn starch 29% maltodextrin 50% sucrose
	SC	10% of kcal 55.5% soybean oil 44.5% lard	19% of kcal 53% casein 47% free amino acids	71% of kcal 45% corn starch 5% maltodextrin 50% sucrose
	SC/BCAA	10% 55.5% soybean oil 44.5% lard	23% 41% casein 58% free amino acids including BCAA supplement	67% 45% corn starch 5% maltodextrin 50% sucrose
(62)	SC or FD	16.7% 100% soybean oil	19.3% 100% casein	64.0% 100 % corn starch or fructose
(63)	SC	8.4% 100% soybean oil	19.3% 100% casein	72.4% 66% corn starch 33% maltodextrin
	FD	8.4% 100% soybean oil	19.3% 100% casein	72.4% 8% corn starch 92% fructose
(64)	HFHS	45% 20% soybean oil 80% lard	20% 100% casein	35% 73% rice starch 27% sucrose
(65)	SC	16.8% 100% lard	+ 30% fructose in drinking water 26.8% Mixed sources	56.4% 93% starch 7% sucrose
	HFHS	40% 95% milk fat 5% corn oil	17% 100% casein	43% 10% corn starch 20% maltodextrin 70% sucrose
	HF	54.8% 94.5% hydrogenated vegetable oil 5.5% corn oil	21.2% 100% casein	24% 69.6% corn starch 30.4% sucrose
(66)	SC	13.5% 100% lard	+ BCAAs in drinking water when added 28.5% mixed sources	58% 84.8% starch 9.8% sucrose 5.5% lactose
	HFHS	45% 87.7% lard 12.3% soybean Oil,	20% 100% casein	35% 20.8% corn starch 28.6% maltodextrin 50.6% sucrose
(67)	SC	18% 100% soybean oil	18% 100% casein	64% 63.5% wheatstarch 20.7 % maltodextrin 15.7% sucrose
(86)	SC	BCAA adapted by reducing casein and adding free amino acids with or without BCAAs 13.1% 100% soybean oil	24.5% mixed sources	62.4% 91.2% starch 8.8% sucrose
	HF	60% 10% soybean oil 90% lard	20% 100% casein	20% 64% maltodextrin 36% sucrose
			+ BCAAs in drinking water when added	

759 expressed as % energy and as % of each macronutrient

760 FD: fructose diet; HF: high fat diet; HFHS: high fat high sucrose diet; SC: standard chow

761 The last candidate is HIB (103). Note that HIB-dehydrogenase deficiency, a rare  
762 metabolic disease with inability to metabolize HIB, does not seem associated with severe  
763 insulin-resistance (116). Moreover, efforts to improve insulin sensitivity by increasing the  
764 flux through the BCKD are a priori accompanied by an increase in the availability of HIB  
765 by simple flux effect! The hypothesis of a detrimental effect of HIB is based on the work  
766 of Jiang et al (117) showing that conditioned-medium from C2C12 myoblasts overex-  
767 pressing peroxisome proliferator-activated receptor-gamma coactivator 1 $\alpha$  (PGC-1 $\alpha$ )  
768 contained increased levels of HIB and that this HIB increased trans-cellular transport of  
769 fatty acids by HUVECs (human umbilical vein endothelial cells) for subsequent oxida-  
770 tion. However, in vivo, although mice supplemented with high doses of HIB displayed  
771 delayed glucose disposal, they presented a normal basal blood glucose. These data are all  
772 the more puzzling that PGC-1 $\alpha$  activation has been associated with improved metabolic  
773 status. In this respect, Roberts et al (118), in myocyte overexpressing PGC-1 $\alpha$ , showed an  
774 increase in BAIBA release and that this BAIBA induced adipocyte browning. In addition,  
775 Hatazawa et al (119) did not observe any increase in muscle HIB in PGC-1 $\alpha$  transgenic  
776 mice but an increase in BAIBA content.

777 **In summary**, there is really very limited data to support a role for acyl-carnitines or  
778 HIB in the development of insulin resistance. With respect to BCKAs, the control of  
779 ATP-citrate lyase by the same kinase and phosphatase that control BCKD requires fur-  
780 ther study, particularly in terms of the interaction between these two metabolic pathways  
781 upon exposure to a diet high in both lipids and BCAAs.

## 782 6. Conclusions

783 A chronic increase in plasma concentrations of BCAAs is a predictive marker for the  
784 risk of developing insulin resistance and subsequently T2D. The fact that this can be ob-  
785 served long before the deterioration of glucose tolerance testifies to the existence of early  
786 asymptomatic insulin resistance and the role of insulin in the metabolism of BCAAs. The  
787 fact that there is a competition between amino acids and glucose to the detriment of  
788 glucose in situations of excess availability of amino acids is probably a testimony to the  
789 limited capacity of the organism to store amino acids, a limit reached as soon as the pro-  
790 tein pool is restored. The priority then becomes the elimination of these excess amino  
791 acids and the conservation of glucose.

792 Insulin resistance will only worsen in populations that are increasingly exposed to a  
793 sedentary lifestyle, poor dietary balance, and environmental insults. Excessive energy  
794 supply (even if limited, but over the long term) and sedentary lifestyle will reinforce this  
795 defect in BCAA utilization due to the progressive reduction of their oxidation in adipose  
796 tissue and compromised muscle mass and the lack of oxidation by brown adipose tissue.  
797 The reduced postprandial utilization of BCAAs, due to insulin resistance and the reduc-  
798 tion of the anabolic response to the meal, is partly compensated by an increased oxida-  
799 tion in the post-absorptive period, especially as these amino acids are very good  
800 ketogenic substrates. The consequence is an increase in plasma concentrations of inter-  
801 mediates of BCAA degradation in the post-absorptive period. The evidence for a possible  
802 role of these intermediates on insulin resistance is at this stage quite limited.

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